HYPOTHALAMIC β-ENDORPHIN, BODY WEIGHT, AND FOOD INTAKE IN OVARIAN STEROID TREATED RATS

by

Amy C. Brown

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Human Nutrition and Foods

APPROVED:

S. J. Ritchey, Chairman

J. A. Driskell

M. K. Korslund

J. L. Walberg

D. M. Denbow

August 21, 1986

Blacksburg, Virginia

HYPOTHALAMIC β-ENDORPHIN, BODY WEIGHT, AND FOOD INTAKE IN OVARIAN STEROID TREATED RATS

by

Amy C. Brown

S. J. Ritchey, Chairman

Human Nutrition and Foods

(ABSTRACT)

To test the hypothesis that hypothalamic β-endorphin levels were related to the body weight changes occuring with ovarian steroid treatments, 80 adult female Sprague-Dawley rats were ovariectomized and allowed to recover for 7 days. Four treatment groups of 20 each were subjected to daily injections for 14 days with A) oil, B) β-estradiol benzoate (2μg), C) β-estradiol benzoate (2μg) plus progesterone (5mg), or D) progesterone (5mg). Weight gain was significantly ($p < 0.01$) lower in the estrogen group when compared to the control, estrogen/progesterone, or progesterone groups. The estrogen/progesterone-treated group gained significantly less weight than either the control or progesterone group. A significant ($p < 0.05$) decrease in food intake was also observed in the estrogen and estrogen/progesterone groups when compared to the control and progesterone groups, but not between each other. The progesterone-treated group was not significantly different from the controls in either weight gain or food intake. Hypothalamic β-endorphin (ng/mg protein) concentrations were significantly ($p < 0.05$) higher in the estrogen- and estrogen/progesterone-treated groups compared to the control and progesterone groups. Again, much like the weight gain, food intake, and hypothalamic β-endorphin (ng/mg protein), the estrogen and estrogen/progesterone groups had significantly ($p < 0.05$) heavier adrenal weights when compared to the controls and progesterone groups. There was no significant difference in plasma corticosterone levels between any of the groups.

In this study, hypothalamic β-endorphin (ng/mg protein) appeared to be effected by ovarian steroid hormone treatment. Whether this is related to the weight gain also observed with the treatments remains to be determined.

Acknowledgements

My sincere appreciation goes out to each person on my committee, their dedication to science and polished professionalism.

I am grateful to Dean S.J. Ritchey for the calm guidepost of strength that was a constant source of reassurance. His generosity and supportive attitude will long be remembered. To Dr. J. Driskell I extend deep gratitude for her wish that I do well which was expressed in her many unofficial talks and timely doses of advice. I am grateful to my parents making these last three years possible because their presence has permeated every single year of my life.

Appreciation beyond words is extended to the One above who is teaching, guiding, and expanding my horizons to ever widening degrees.

Table of Contents

List of Illustrations

List of Tables

chapter I

Introduction

The pituitary is the body's "master gland", but the hypothalamus has been called the "master gland's" master. Shally et al.(242) proposed that the hypothalamus, lying at the base of the brain, may be the link between the central nervous system and the endocrine system. These are the two major communication systems of the body and they mesh to regulate numerous functions -- one of these is food intake.

Many peptide hormones modulate appetite. Bray (27) stated that most of the classical hormones also play a role in body fat metabolism -- insulin, growth hormone, gonadal hormones, thyroid hormones, and adrenal corticoids. For instance, the hypercorticodism observed in Cushing's disease is associated with weight gain, while the hypocorticodism in Addison's disease is related to weight loss. The influence of ovarian hormones on food intake and weight has long been known; progesterone is associated with weight gain, while estrogen is related to weight loss. Changes in weight following natural ovarian steroid fluctuations occur during pregnancy, pseudopregnancy, the reproductive cycle, and oral contraceptive use (10,99,250). These conditions have also been related to varying β-endorphin levels (30,192,267,279)

β-endorphin was discovered in 1975 after a race to find the natural body opiates that react with opiate receptors known to bind with drugs. The word, endorphin, is derived from "endogenous morphine" and the peptide has been implicated in numerous studies in appetite regulation. Morley (186) stated that the primary site of action for both exogenous and endogenous opiates appears to be the hypothalamus. Rats subjected to fasting were reported by Gambert et al. (82) to have decreased hypothalamic β-endorphin levels. Harsing et al. (108) injected rats with D-fenfluramine, an anorexic drug that releases serotonin, and reported increased hypothalamic β-endorphin content. Another drug known to suppress appetite, amphetamine, was administered for 3 weeks by Schultz et al. (245) who observed that the hypothalamic β-endorphin increased by 36 percent. Pentobarbitol, an opiate antagonist and sedative which inhibits adrenocorticotropic hormone release and occasionally depresses appetite, also increases hypothalamic β-endorphin (147). Naloxone, the widely used opiate antagonist generally known to decrease appetite, was reported by Lee et al. (147) to increase hypothalamic β-endorphin.

The possible link between the ovarian steroid influence on weight and hypothalamic β-endorphin content has not been reported in the literature. The objective of this study was to test the hypothesis that hypothalamic β-endorphin levels were related to the body weight changes occuring with ovarian steroid treatments.

chapter II

REVIEW OF THE LITERATURE

β-*ENDORPHIN*

Description of β-Endorphin

During the early 1970's, brain opiate receptors were discovered. Since opiate receptors in the brain do not exist primarily to bind to opium, the race was on to isolate and identify the natural opiates of the body (93,104).

In 1975, endogenous opioid peptides were discovered. One of these peptide's, endorphin was named after "endogenous morphine" because of its ability to mimic the pharmacological effects of the drug. Endorphin is actually one of several types of opioid peptides ranging from 5-31 amino acids in length. The majority of opioid peptides originate from three family systems that each have their own different subtypes:

1. Proopio-melanocortin (POMC) system -- yields α, β, and γ endorphins.

2. Proenkephalin system -- yields enkephalins.

3. Prodynorphin system -- yields dynorphins.

The major opioid peptides include β-endorphin, leu-enkephalin, met-enkephalin, and dynorphin. Another source of opioid peptides has been isolated from foods, specifically casein and gluten, and are called exorphines (306). The most studied endogenous opioid (302) is β-endorphin. It consists of the 61-91 peptide of POMC (104), is 10-100 times more potent than morphine, and has a half-life of about 30 minutes (104,73). β-endorphin's structural origin is depicted in Figure 1 (104,185,156). β-endorphin has been called a neurotransmitter, neuromodulator, and hormone (23). Neurotransmitters are molecules that transmit information between neurons and act at points extremely close to their origin.

Location of β-Endorphin

β-endorphin concentrations are high in the hypothalamus (257,47) and pituitary (Figure 2). As one of the oldest parts of the brain, the hypothalamus regulates life supporting systems such as heartrate, body temperature, metabolism, reproduction, and sleep. The hypothalamus regulates such functions by sending hormone releasing factors to the pituitary which then regulate release of various pituitary hormones. The pituitary, known as the "master gland", is governed by the hypothalamus. Some 50,000 nerve fibers descend from the brain into the pituitary gland which is the size of a pea. Measuring β-endorphin in the hypothalamus and pituitary is complicated by the fact that β-endorphin levels vary within these units substructures. The hypothalamus has numerous nuclei that differ in the amounts of β-endorphin they contain (Figure 3), with the arcuate nucleus appearing to be the major brain source of β-endorphin (38,58). The pituitary is segmented into the

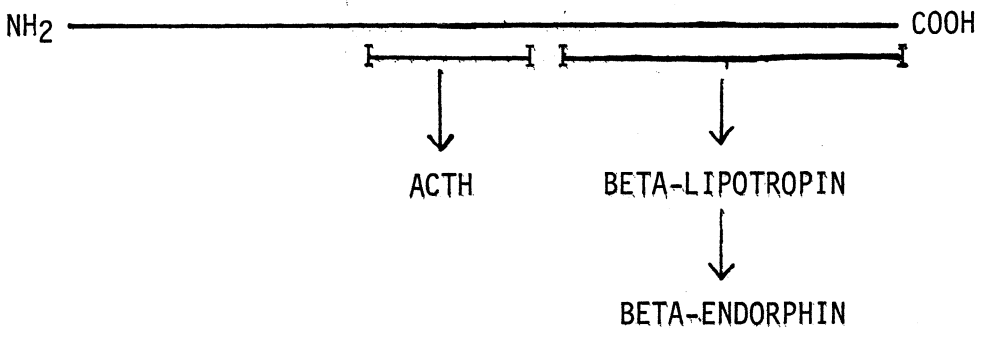


Figure 1. Beta-endorphin's structural origin (104, 181).

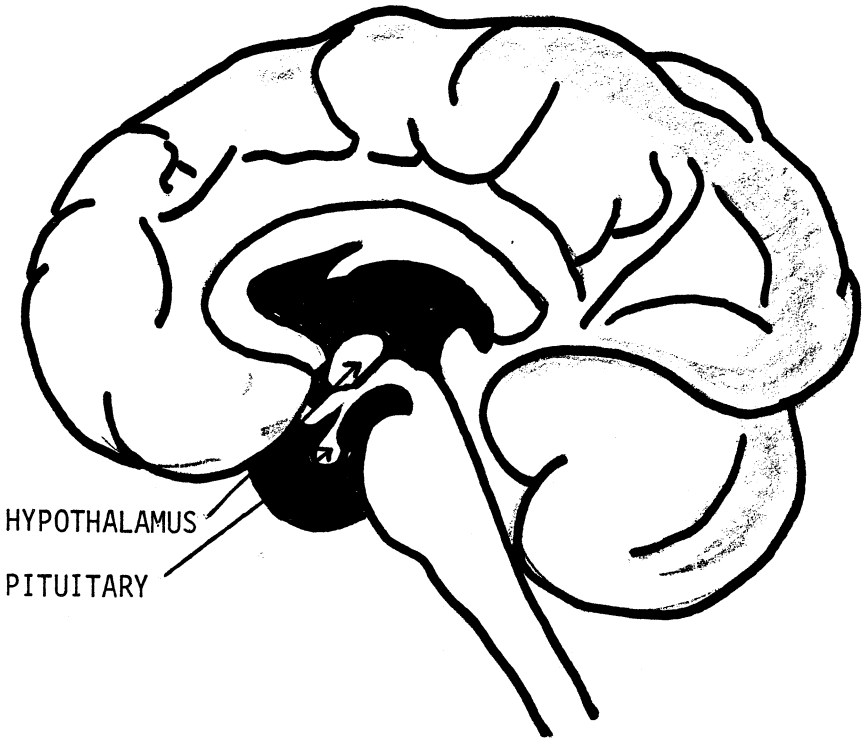


Figure 2. Beta-endorphin concentrations are high in the hypothalamus and pituitary (47).

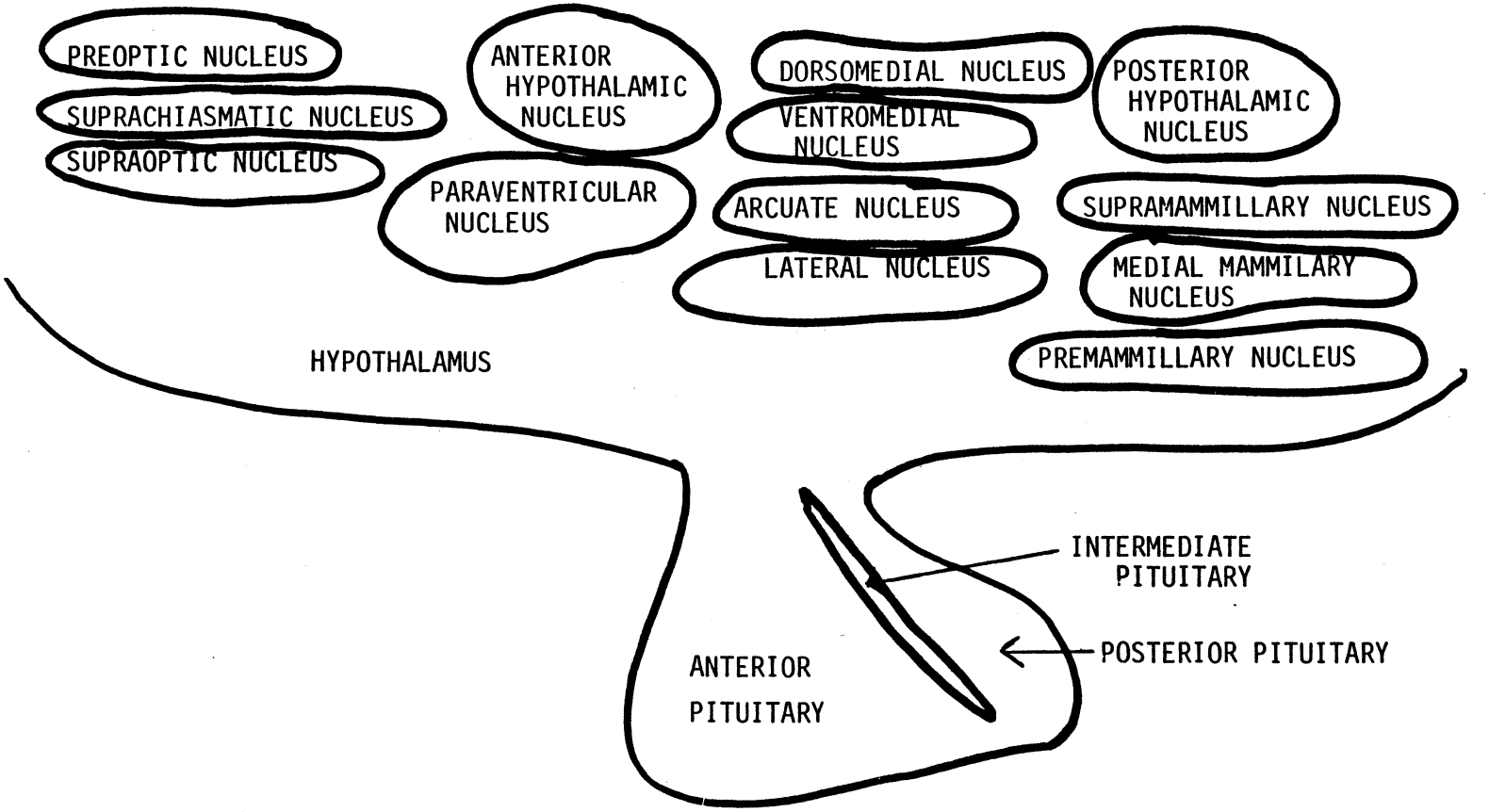


Figure 3. The hypothalamus has numerous nuclei that differ in the amounts of beta-endorphin they contain.

anterior lobe, posterior lobe, and a tiny section called the intermediate lobe (Figure 3). Copolov and Helm (47) reported that β-endorphin is primarily produced from cells in the anterior and intermediate lobe of the pituitary. Pro-opiomelanocortin, β-endorphin's precursor, is synthesized in the anterior and intermediate pituitary and in the arcuate nucleus of the hypothalamus (95). The source of circulating β-endorphin, adrenocorticotropin, and β-lipotrophin appears to be the anterior pituitary which is under corticotropin releasing factor (CRF) control (140).

Regional differences also exist in the cleavage of pro-opiomelanocortin to β-endorphin and its various peptides (38). Krieger and Martin (140) reported that in the anterior pituitary, the precursor molecule is processed predominantly to adrenocorticotropin hormone and β-endorphin. These end products are further processed to α-melanocyte-stimulating hormone and β-endorphin like material, respectively, in the intermediate lobe (β-endorphin, N-acetyl β-endorphin, β-endorphin 1-27, and N-acetyl β-endorphin 1-27), while in the hypothalamus the major form is authentic β-endorphin.

β-endorphin has been measured in areas other than the brain and pituitary. The plasma and cerebrospinal fluid (CSF) are often measured due to their relative ease of access. The pancreas (31), placenta (141), and adult human (not rat) adrenal gland (65) have also been reported to contain β-endorphin.

Location of opioid peptides can be deduced from the location of opiate receptors. Both β-endorphin and opiate receptors are found at many sites within and ouside the central nervous system (197). The limbic system, long known to be linked to the emotions, is rich in opiate receptors. Other sites reported to have opiate receptors include the stomach, ileum, vas deferens, certain peripheral smooth muscle systems (164), liver, pancreas, kidney, heart, lungs, and spleen (207,224).

Existence of an opiate receptor does not always mean β-endorphin will be present. Several different receptor subtypes exist (mu, delta, kappa, sigma, and epsilon) and they bind differently to various opioid peptides (185).

Functions Related to β-Endorphin

β-endorphin is involved in so many physiological systems that no single theory exists to encompass all of its actions. Endogenous opioid peptides, of which β-endorphin is one, may act not only as neurotransmitters and neuromodulators at synaptic junctions (123), but possibly mediate hormonal response (218). Such a description may explain the wide variety of functions attributed to opioid peptides (Table 1).

β-*ENDORPHIN RELATED TO FOOD INTAKE*

The relationship between central opioid peptides and feeding is demonstrated in five ways.

1. Central nervous system administration of opioids.

2. Peripheral administration of opioids.

3. Narcotic administration.

4. Opiate antagonist/agonist administration.

5. β-endorphin content in the a) hypothalamic b) cerebrospinal fluid, c) pituitary, and d) plasma.

TABLE 1

SOME HYPOTHESIZED EFFECTS OF ENDOGENOUS OPIOID PEPTIDES

- Analgesia/sedation
- Pain perception
- Euphoria
- Respiratory depression
- Blood pressure
- Appetite regulation
- Temperature regulation
- Immune function regulation
- Reproduction
- Meiosis
- Memory
- Decreased gut motility
- Ulcers
- Hormonal effects
    - Increased anterior pituitary release of:
        - Adrenocorticotropic hormone (ACTH)
        - Growth hormone (GH)
        - Prolactin (PRL)
    - Decreased anterior pituitary release of:
        - Thyroid-stimulating hormone (TSH)
        - Follicle-stimulating hormone (FSH)
        - Luteinizing hormone (LH)
    - Increased corticosterone secretion from isolated adrenals

Source: Compiled from Gold et al. (93), Mundler et al. (162), Meites et al. (177), Morley et al. (183), and Shanker and Shama (148).

Central Nervous System Administration of Opioids

β-endorphin injected directly into the brain stimulates feeding (55,98,150,173,175,235,273). The mode of β-endorphin administration strongly influences the outcome. Peripheral injection of high β-endorphin doses in rats does not generally cause analgesia and so β-endorphin is not believed to cross the blood brain barrier. Cerebrospinal fluid injections bypass the blood brain barrier and results in an analgesia lasting several hours in rodents,but not monkeys. The effect was blocked by naloxone, an opiate antagonist (104). Foley et al. (73), who intravenously injected β-endorphin into humans, reported no detectable levels of β-endorphin in the cerebrospinal fluid and suggested that β-endorphin poorly penetrates the blood brain barrier, if at all. It appears that β-endorphin must reach central sites in adequate amounts to produce an effect.

Peripheral Administration of Opioids

Much of the evidence linking β-endorphin to food intake is from its action at the central instead of peripheral level. Peripherally injected β-endorphin in rats was reported to result in increased intake of sweet solutions and increased consumption of fat over carbohydrate (116).

Narcotic Adminstration

A large proportion of β-endorphin's role in food intake stems indirectly from drugs thought to act at opiate receptors. Many psychotropic drugs function by mimicking, antagonizing, or enhancing the central effects of natural neurohormonal substances (264). In fact, drugs are synthetic compounds that are analogues of natural neurotransmitters that lock onto receptors in the body (45).

In humans, Schneider et al. (244) reported that narcotic addicts lost weight while addicted and gained what they lost when entering steady-state methadione maintenance or complete abstinance. Giugliano (89) mentioned that narcotic addicts are thought to have a craving for sweets, that heroin users are prone to diabetes mellitus, and that flat glucose test curves occur after narcotic administration. β-endorphin immunoreactivity was reported by Ho et al. (115) to be decreased in chronic male opiate addicts. Facchinetti et al. (67) reported on the impairment of adrenergic-induced pro-opiomelanocortin related peptide release in heroin addicts.

Reduced brain β-endorphin levels also occurred in rats chronically treated with morphine (115). Several studies report increased feeding in animals both peripherally and centrally administered with narcotics (237). Morphine injected into the rat hypothalamus stimulated increased feeding (176,268). However, chronic morphine and heroin are both known to decrease body weight in rats (269,270).

At least six influences can contribute to cause of conflicting results reported from research investigating opioids and food intake.

1. Inverted-U response curve -- dopamine antagonists and agonists both decrease food intake (Figure 4). (253).

2. Biphasic action; morphine at low doses initially suppresses feeding which is then followed by increased intake (302).

3. Acute vs. chronic doses; an acute morphine dose stimulates adrenocorticotropin hormone and corticosterone release while a chronic dose inhibits the release of adrenocorticotropin hormone and corticosterone (177).

4. Low and high doses can produce different responses.

5. Tolerance can develop.

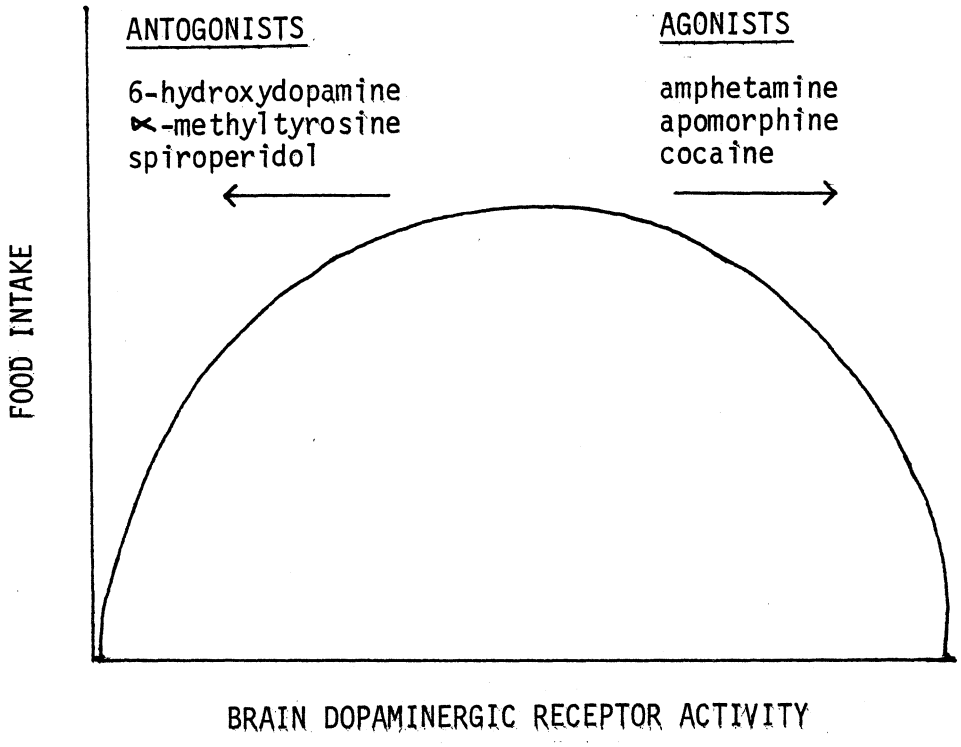


Figure 4. Proposed effects of brain dopaminergic activity on food intake. Dopaminergic antagonists and agonists both reduce intake in food-deprived rats (253).

6. Species difference -- naltrexone's action to decrease food intake and body weight was not as successful in humans as it was in squirrel monkeys (110).

These phenomenom must be considered when investigating if opioid peptides are related to food intake.

Administration of Opiate Antagonists/Agonists

Antagonists:

Naloxone and naltrexone are the two major opioid antagonists (Table 2). One of the strongest and perhaps overly depended upon arguments for β-endorphin's role in food intake is that naloxone blocks opioid stimulated food intake. Naloxone was first synthesized in 1960 and has a very short duration action that makes it suitable for treatment of acute narcoticism. Naloxone's effect peaks at 20 minutes and lasts 1-4 hours (94).

Numerous animal studies have demonstrated the suppressive effect of naloxone on food intake (26,29,79,120,130), with a greater decrease occurring in obese mice (249). Naloxone also decreases the feeding observed in tail-stressed satiated rats (157). Schultz et al. (246) observed a brief anorectic action of naloxone in guinea pigs, but they resumed normal food intake by the 4th day.

In humans, opioid antagonists have had mixed results. Hourly infusions of naloxone decreased food intake in obese subjects by 29% compared to controls. The same treatment was not effective in lean subjects (14). However, other studies (42,272) reported an effect in lean subjects. Kyriakides et al. (144) administered naloxone to three Prader-Willi syndrome patients; food intake decreased in the two males, but no effect occurred in the female. Krotkiewski et al. (143) reported

TABLE 2

OPIATE ANTAGONIST CHARACTERISTICS

OPIATE ANTAGONIST	SYNTHESIZED	ADMINISTRATION	ACTION
Naloxone	1960	injection	short
Naltrexone	1965	injection/oral	long

Source: Goland et al. (91)

a decrease in food intake in 2 female twins given naloxone. Thompson et al. (269) observed that the increased food intake induced by 2-deoxy-D-glucose injection in humans was blocked by naloxone. Atkinson (14) administered naloxone in obese humans and reported no effect on the quanties of a meal ingested 30 minutes later.

Naltrexone differs from naloxone in that it is more potent, longer lasting, and can be administered orally. In humans, naltrexone decreased food intake in obese (14) and nonobese subjects (93). Sternbach et al. (260) administered natrexone to former addicts and reported weight losses of 2.3-5.0 kg after 2-6 weeks. Naltrexone was reported by Hollister et al. (117) to decrease food intake according to the subjects' food dairies, although food intake and body weight were not measured. Atkinson (13) administered naltrexone and placebo to 60 obese subjects and reported no significant weight loss. However, when females were analyzed alone, they had a small loss of 1.7 kilogram compared to controls was recorded. Negative results reported by Malcolm et al. (160) who administered naltrexone to 41 obese subjects for 8 weeks with no significant difference in weight. Maggio et al. (159) reported that naltrexone treatment in 8 obese men also failed to yield significant weight loss.

In animals, Mandenoff (161) reported an anorectic effect of naltrexone only in normal rats offered a palatable diet, but not in rats fed standard laboratory chow.

Limitations:

Investigators in the early research of endogenous opioids adapted the naloxone response as a criterion of an opioid effect. This indirect assumption may have some serious limitations. Most of the studies demonstrating a decreased food intake with naloxone have measured intake for only several hours. None of the above studies evaluated the long term effects of naloxone on body weight. Naloxone's relatively short duration time allows food intake to return to control levels within 4 hours after administration (237). Often not addressed in the results obtained with naloxone is that

acute naloxone doses decrease feeding, but chronic doses increase intake (249). Shimomura et al. (249) reported that lean rats increased consumption 140-200 percent above controls while obese rats elevated their intakes by 200 percent. Sanger (237) reported that repeated naloxone doses increased intake by 60 percent.

Questions also exist concerning the interaction of naloxone with opiate receptors. Naloxone doses are normally given at levels in which the receptors' specificity is non-existent (244). Sanger (237) reported that naloxone blocks opiate receptors, but the doses required to decrease food intake are higher than the doses required to completely block other drugs such as morphine. Naloxone is also not equally specific for the different receptor types (95).

Agonists:

Conflicting results exist concerning agonists. Butorphanol tartrate, a kappa agonist, decreased caloric intake when administered to normal weight humans and this effect was reversed by naloxone (183). Opposite results were obtained using a lower dose (181). Lowly and Lim (154) and Sanger and McCarthy (238) administered opiate agonists to rats and reported increased food intake.

β-Endorphin Levels

β-endorphin has been related to food intake by investigators who have measured β-endorphin levels in the hypothalamus, cerebrospinal fluid, pituitary, and plasma during 1) various conditions, 2) drug treatments and 3) surgery.

Hypothalamus

Various Conditions:

The main site of action for opiates appears to be in the hypothalamus. Genetically obese mice were reported by Greenberg et al. (101) to have no significant change in hypothalamic, pancreas, or adrenal β-endorphin levels. Margules et al. (166) also detected no change in hypothalamic β-endorphin concentrations between ob/ob mice and lean littermates. Rats subjected to fasting were reported by Gambert et al. (82) to have decreased hypothalamic β-endorphin levels. Dum et al. (62) observed lower hypothalamic β-endorphin concentrations in rats fed palatable diets. Animals with protein malnutrition were reported to have decreased brain and pituitary β-endorphin (206).

Drug Treatments:

Naloxone, the widely used opiate antagonist generally known to decrease appetite, was reported by Lee et al. (147) to increase hypothalamic β-endorphin, however Stein et al. (258) reported opposite results. Pentobarbitol, an opiate antagonist and sedative which inhibits adrenocorticotropin hormone and occassionally depresses appetite, also increased hypothalamic β-endorphin (147). Another drug known to suppress appetite, amphetamine, was administered for 3 weeks by Schultz et al. (245) who observed hypothalamic β-endorphin levels increase by 36 percent. Harsing et al. (108) injected rats with with D-fenfluramine, an anorexic drug that releases serotonin, and reported increased hypothalamic β-endorphin content. Chronic treatment with haloperidol, a drug that decreases food intake, increased levels of hypothalamic β-endorphin (118).

Acute or chronic doses of drugs often have different results. Nohtomi et al. (194) reported that a single injection of sulpiride, a dopamine receptor antagonist, decreased β-endorphin in the arcuate nucleus, paraventricularis, and median eminence. β-endorphin concentrations in these same areas increased with repeated sulpiride injections.

Although not a drug, thyroxine was reported by Lee et al. (147) to increase hypothalamic β-endorphin. Brain catecholamines inhibit the synthesis/release of hypothalamic corticotrophin releasing factor (CRF) and therefore adrenocorticotropin hormone. Decreased β-endorphin secretion may be due to catecholamine-mediated secretion of corticotrophin releasing factor (274).

Surgery:

The weight gain observed in ovariectomized rats (103,147,179,259) and genetically obese rats is abolished by adrenlectomy (303). Lee et al. (147) reported that hypothalamic β-endorphin (&plasma) levels increased in adrenalactomized rats. Contrasting these results was Rossier (231) who reported no change in brain β-endorphin levels of adrenalectomized or hypophysectomized rats. Hypophysectomy was reported by Ogawa et al. (195) to result in a 90 percent decrease in brain β-endorphin. This may occur if pituitary β-endorphin passes into the brain by the retrograde vascular network (196).

Cerebrospinal Fluid

Various Conditions:

Although not part of the brain itself, the cerebrospinal fluid represents part of the central nervous system. Measuring β-endorphin levels in the cerobrospinal fluid or plasma, rather than brain, is often necessary when using human subjects.

Kral et al. (138) reported a significant increase of β-endorphin in the fraction II portion of the cerebrospinal fluid of obese humans when compared to controls. An obese 17 year old male was reported by Fraioli et al. (76) to have elevated opioid activity in the cerebrospinal fluid. Krotkiewski et al. (143) found significantly higher β-endorphin concentrations in fraction II of the cerebrospinal fluid of a pair of twins with Prader-Willi syndrome compared to lean controls. An

association between severly underweight anorexia nervosa patients and cerebrospinal fluid opioid activity was reported by Kaye (137).

Pituitary

Various Conditions:

Genetically obese mice were reported by Margules et al. (166) to have double the pituitary β-endorphin levels when compared to non-obese controls. The pituitary also contained 14 times more adrenocorticotropin hormone and weighed 20 times less than the pituitary of lean mice. Weight gain was abolished following pituitary removal. Greenberg et al. (101) also reported increased pituitary β-endorphin in ob/ob mice. Margules et al. (166) concluded that the increased pituitary β-endorphin may play a role in the hyperphgia and obesity. Rosseir et al. (230) questioned Margules findings when they reported that the elevated pituitary β-endorphin occurred 3 months after birth which is after the greatest percentage of weight gain occurs in ob/ob mice.

Stress is often associated with food intake and was reported to increase synthesis/release of β-endorphin from the intermediate lobe (2). Decreased pituitary β-endorphin in rats subjected to footshock was demonstrated by Perry et al. (206).

Surgery:

Adrenalectomy was reported by Lee et al. (147) and Rosseir et al. (231) to increase pituitary levels. Adrenalectomized males were reported by Lee et al. (147) to have decreased pituitary β-endorphin while no difference was observed in ovariectomized females. Lee et al. (147) paralleled this difference between the sexes to their different pituitary responses to naloxone. Ventromedial

hypothalmus (VMH) lesions were reported by Matsumura et al. (167) to result in elevated pituitary β-endorphin.

Plasma

Plasma is the most accessible and practical tissue to measure β-endorphin levels. As a result, most studies originally measured this source and researchers may have been misled from the origin and ultimate control of plasma β-endorphin concentrations.

Plasma β-endorphin concentrations often differ from brain β-endorphin levels. Blood β-endorphin levels do not always reflect what is going on in the brain. Changes in β-endorphin levels may reflect modifications in synthesis, degradation, release, and or a combination of these processes. Increased plasma β-endorphin levels may reflect increased release and therefore decreased hypothalamic β-endorphin content. Increased hypothalamic β-endorphin synthesis/turnover may increase these brain levels or have them remain "unchanged" as the excess overflows to the blood to elevate plasma β-endorphin levels. This is also true for the pituitary β-endorphin levels which can differ from hypothalamic and plasma β-endorphin levels. Much room exists for speculation and interpretations may easily be tangled in an intricate web of interdependencies.

Plasma β-endorphin can even exist in different forms. Opioid activity does not occur unless the β-lipotropin amino group of tyrosine-61 is free. Goldstein (95) stated that a small portion of plasma β-endorphin demonstrates opioid activity, however, the amount released due to stress is not enough to significantly activate known opioid receptors.

Despite serious limitations, peripheral opiates have been implicated in numerous initial studies to stimulate feeding (116).

Various Conditions:

Stress has been implicated in elevated plasma β-endorphin levels and increased food intake. Footshock administered to rats resulted in increased plasma β-endorphin (105). Obese subjects have also been reported to have hyperendorphinemia (66). However, Wallace (288) stated that little evidence exists for the role of circulating β-endorphin in the control of food intake in the rat. McLaughlin and Baile (174) immunized rats against plasma β-endorphin which resulted in elevated food intakes and body weight gains. These results led McLaughlin to conclude that β-endorphin plays a role in satiety. In humans, Awoke et al. (15) reported that plasma β-endorphin levels in 15 obese humans were not significantly higher than controls. Fasting plasma levels of obese children and adolescents were reported by Genazzani et al. (84) to be twice that of lean controls. The obese adolescents also did not have the normal β-endorphin increase observed with insulin-induced hypoglycemia. Matsumura et al. (168) reported increased β-endorphin-like immunoreactivity (BELI) in the plasma of subjects following a test meal, duodenal acidification and tetragastrin injection. Margules and Inturrisi (163) reported no significant difference in the plasma β-endorphin of 6 patients with Prader-Labhart-Willi syndrome compared to 7 of their normal siblings.

Drugs:

Petraglia et al. (210) reported that drugs enhancing brain serotonin neurotransmission increased plasma β-endorphin. Serotonin neurons stimulate release of β-endorphin from the anterior pituitary, but probably not from the neural intermediate lobe or hypothalamus. Destruction of brain serotonin neurons decreases plasma β-endorphin levels but increase with drugs enhancing these brain neurons. Serotonin antagonists decrease plasma β-endorphin (31). Sapun-Malcolm et al. (240) reported serotoninergic-induced release of β-endorphin is blocked by the synthetic glucocorticoid, dexamethasone.

Increased plasma β-endorphin was referred in the article by Petraglia and associates (210) to occur with vasopressin, drugs activating serotonin neurotransmission, haloperidol (dopamine receptor

antagonist), metyrapone, lysine, physostigmine, clonidine, insulin hypoglycemia, and testosterone propionate.

Surgery:

There was no significalt difference in the plasma β-endorphin of intact and adrenalectomized rats treated with dexamethasone. Adrenalectomized rats administered with deoxycorticosterone experienced increased plasma β-endorphin.

Ventromedial hypothalamic lesions which result in obesity were reported by Matsumara et al. (167) to increase plasma and pancreatic β-endorphin while decreasing duodenum β-endorphin levels. Petraglia et al. (209) reported that gonadectomy in rats resulted in depressed plasma β-endorphin and suggested that it was from the decreased synthesis/release of β-endorphin from the pituitary.

MECHANISMS RELATING β-ENDORPHIN TO FOOD INTAKE

History of Hypotheses

The central control of appetite remains hidden in a black box. Much of the work on obesity has emphasized peripheral factors because of their relative ease of accessability. Experiments range from studying appetostatic theories (glucostatic, lipostatic, aminostatic, thermostatic, purinostatic), brown adipose tissue, taste, palatability, food variability, stomach distension/rate of emptying, nutrient apsorption rate, gastrointestinal hormones (cholecystokinin, glucagon, bombesin, somatostatin), fat cell size, heredity, and environment.

Studies determining the central control of appetite, thought mainly to be in the hypothalamic, involve a complex mass of neurotransmitters that are not only difficult to detect, but regulate numerous other body functions. Analogous to this situation is the way acetylcholine leaves neuron cell bodies to signal muscle movement. The chemical is known, but not how the body distinguishes which electro-chemical signals will lead to specific physical responses. β-endorphin, as one of these chemicals, acts as a signal along a cascade of intertwined events.

Catecholamines

Opiates are known to interact with the catecholamine system (203) which is involved in self-stimulation reward (300). Leibowitz (148) reported that brain monoamines and neuropeptides act in the regulation of eating behavior. β-endorphin levels are high in the hypothalamus and may interact with brain monoamines to influence food intake. The catecholamines, norepinephrine and dopamine, are seen as major components of self-stimulation, while the minor roles involve serotonergic, cholinergic, endogenous brain opioids, and other peptide reward systems. Overactivity of brain dopamine may result from underactivity of gabaminergic, serotonergic, noradrenergic, or opioid systems (203). Opiate binding sites have been discovered on terminals of dopaminergic neurons (69).

Morley et al. (182) suggested that food intake is via a tonic dopaminergic-opioid mechanism in the lateral hypothalamic, one of the brain's most potent "reward/pleasure" centers. Naloxone decreases rates of responding reinforced by electrical stimulation of the brain. Belluzi and Stein (18) suggested that opiate drugs may be important substrates for physiological reward. Despite evidence for the involvement of catecholamines in weight regulation, Orosco et al. (200) reported that the obesity of female Zucker rats occurred before any monoamine alterations.

Addiction

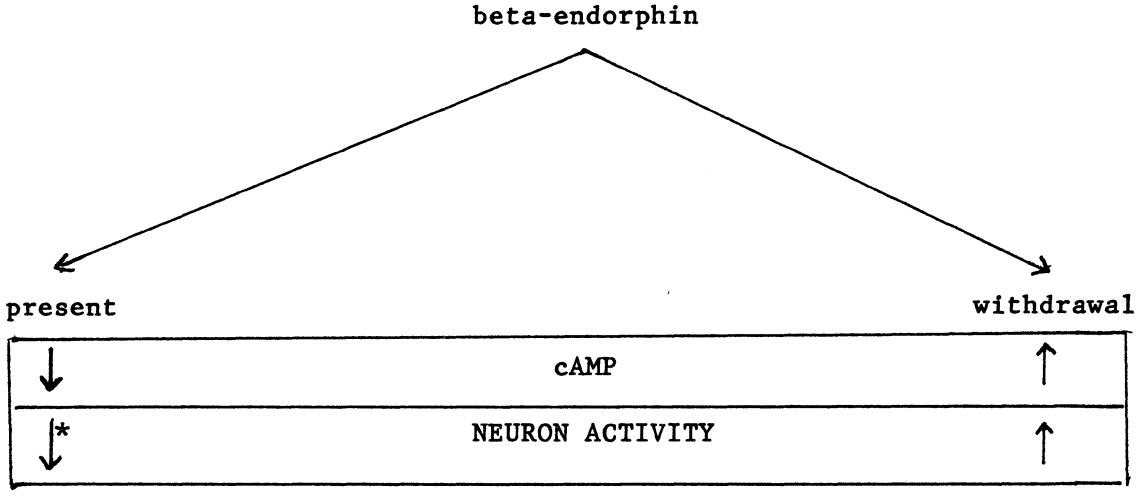
Opiate receptors in the brain have led McCloy and McCloy (169) to hypothesize that obesity is a form of autoaddiction to endogenous opiates. McCloy and McCloy (170) stated that endorphins and enkephalins are as addictive as morphine. The hypothesized purpose of enkephalins is to inhibit enkephalinergic neurons to switch off feeding in the brain. Shimomura et al. (249) also suggested that endogenous opioids and opioid receptors may play a role in signaling satiety. Decreased appetite is common among morphine addicts.

Increased levels of opioids may lead to tolerance and therefore larger amounts of food are required to satisfy the opioid level needed to reach the "reward" state. Excess opioids after meals, like uniform doses of morphine, soon cease to satisfy.

Pain causes increased firing of the locus coerulus neurons which is blocked by morphine. Studies cited by Gold et al. (93) reveal that endogenous opioid peptides decrease firing in the locus coeruleus, and that naloxone acts to prevent this decrease in firing (94). Theoretically, exogenous opiates could lead to a decrease in the synthesis/release of β-endorphin. Withdrawing these exogenous opiates may weaken the ability of the endogenous system to inhibit the locus coeruleus (94).

cAMP

Addiction was assumed by Collier (45) to involve cellular changes (Figure 5). McCloy and McCloy (169) suggested that opiates inhibit cAMP production and therefore the neuron activity. If inhibition continues, the neuron's capacity to make more cAMP increases and so more opiates are required to suppress it, resulting in tolerance. Withdrawal occurs when the removal of excess opiate unleashes the increased capacity to produce cAMP and this results in neuron hyperactivity.



*Decreased neuron activity stimulates cAMP production in the neuron. Larger opiate amounts are then necessary to decrease cAMP, and tolerance develops.

Figure 5. Hypothesized cellular changes of addiction (169).

Gold et al. (93) supports the role of cAMP in tolerance/dependence. Opiates initially decrease cAMP, but chronic opiate treatments increase cAMP and lead to tolerance. Greater opiate amounts become necessary to decrease cAMP levels. It is known that opioid peptides decrease cerebral calcium content. Calcium and cyclic nucleotide concentrations roughly parallel tolerance and dependence development. Giugliano (89) cited an article in which the opioid peptide-receptor decreases intracellular cAMP levels. This rise was blocked by naloxone, an opiate-receptor blocker. The catecholamine hypothesis of food intake may involve cAMP. Ferland et al. (71) expanded on the interactions between cAMP and dopamine.

Stress-Induced Eating

If β-endorphin does influence catecholamine and/or cAMP levels, it may be related to the increased food intake observed with stress. Rats subjected to tail-pinch stress are reported to have increased food intakes and plasma β-endorphin levels (7,8,185,187)

A clear association exists between stressful conditions and the release of opioid peptides through corticotrophin releasing hormone (105)(Figure 6). Stress is well known to stimulate adrenal secretion of cortisol (corticosterone in the rat) (183). Corticotrophin releasing factor, stimulated by stress, signals release of adrenocorticotropin hormone from the pituitary and other substances altering this hormone's secretion produce a similar effect on β-endorphin (Figure 7) (183). Adrenocorticotropin hormone synthesis/release is subject to corticosteroid negative feedback inhibition in the anterior pituitary.

The opiate antagonist, naloxone, was reported by Lowly et al. (155) to reduce stress-induced feeding. Lymangrover et al. (157) indicated that naloxone indirectly influenced the adrenal cortex and appeared to potentiate the action of adrenocorticotropin hormone. Dexamethasone, the potent

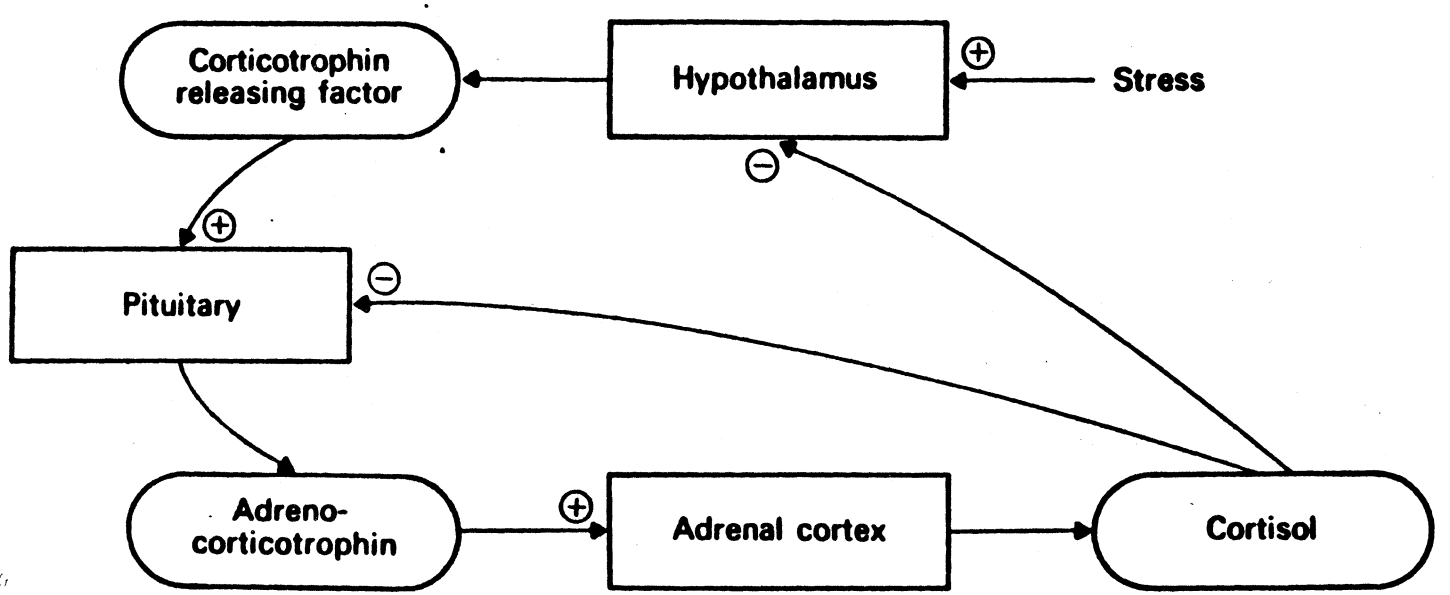


Figure 6. Feedback regulation to the hypothalamus and pituitary in regulating the production of cortisol from the adrenal cortex (191).

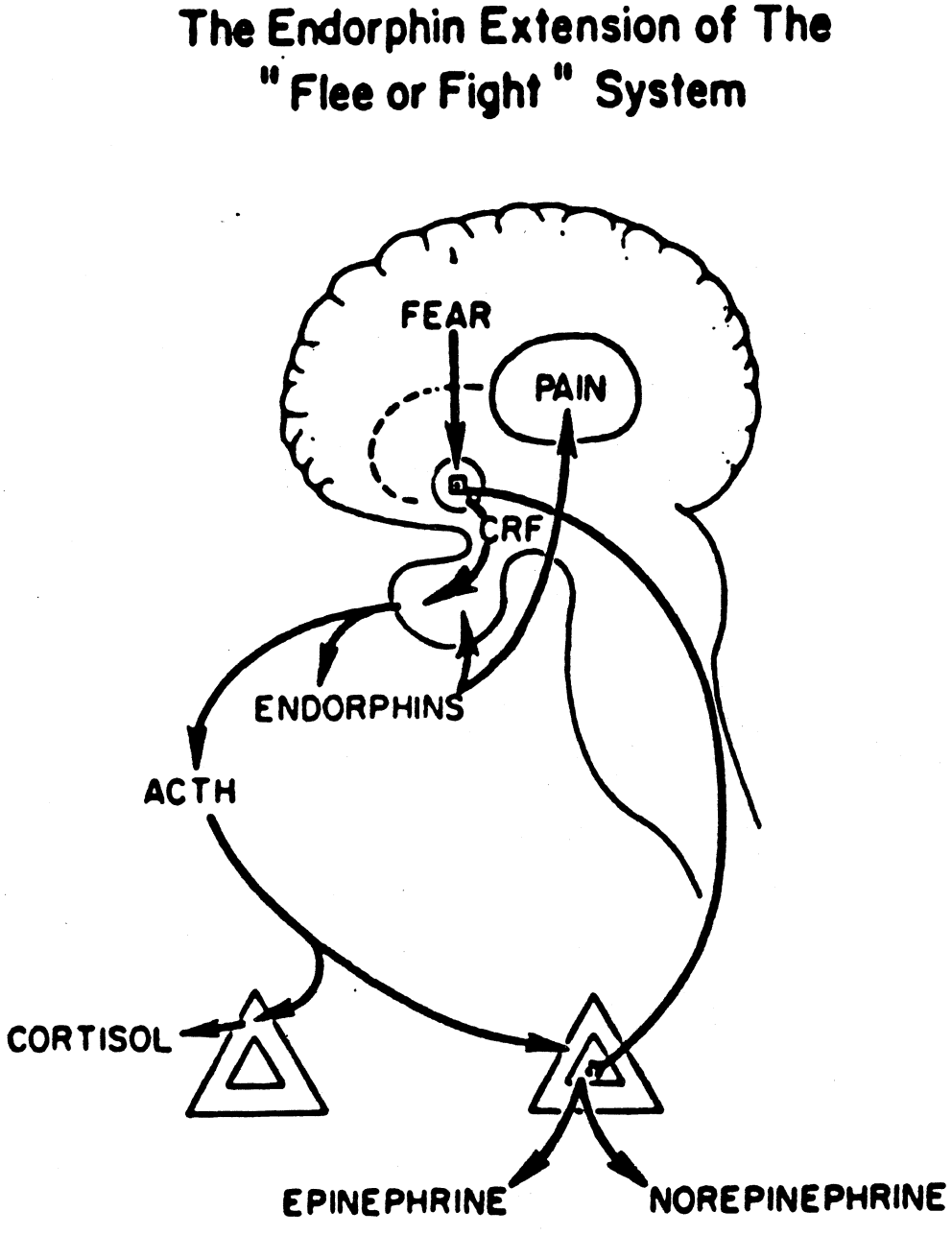


Figure 7. The endorphin extension of the preparation for "fight or flight." The concomitant release of endogenous opiates with adrenocorticotrophin (ACTH) is a logical extension of Seyle's general adaptation system. Stress leads to the release of corticotrophin releasing factor (CRF) that stimulates ACTH and endorphin release from the pituitary (186).

synthetic steroid, decreases tail pinch induced feeding (155) and also reduces the stress induced release of adrenocorticotropin hormone and β-endorphin (229).

Hypothalamic-Pituitary-Adrenal Axis

Observing β-endorphin's role in the hypothalamic-pituitary-adrenal axis (32), raises several questions. Does obesity involve the action of the hypothalamic-pituitary-adrenal axis? Schneider et al. (244) noted that Rossier's genetically obese mice which had elevated plasma β-endorphin levels, were also afflicted with numerous endocrine abnormalities -- including increased adrenocorticotropin hormone and corticosterone levels.

Abnormally low corticosteroid production was reported by Gold et al. (93) to occur in opiate addicts. Excess cortisol is released from the adrenals in Cushings disease and is associated with weight gain. Lack of adrenal cortisol secretion exists in Addison's disease where weight loss occurs. The obesity often seen in middle age was hypothesized by Margules (165) to be a mild form of Cushing's syndrome. Margules et al. (166) suggested that chronic increased activity of β-endorphin/adrenocorticotropin hormone stimulated by stress may be a possible contribution to obesity.

Several other conditions related to β-endorphin levels and the hypothalamic-pituitary-adrenal axis include Cushings disease (119), Addison's disease (261,190), Nelson's syndrome (190), diabetes (75,101) exercise induced amenorrhea (132), alcoholism (114), anorexia nervosa (56,87,137) and bulemia (80,106.

OVARIAN STEROID HORMONES RELATED TO WEIGHT GAIN

Ovarian steroid hormones have long been known to affect body weight (283). Estrogen holds a reputation as an anorexic hormone that decreases food intake, body weight, and adiposity in female rats. The opposite effect is observed with progesterone, nicknamed the "hungry hormone" (10,22,61,99,226,247).

The influence of ovarian hormones in female rats is observed in weight gains occurring during pregnancy, pseudopregnancy, ovariectomy, the reproductive cycle, and oral contraceptive use. Ovariectomy in adult female rats resulted in hyperphagia and rapid weight gain for about one month (179,259,266). In immature rats, ovariectomy had no effect on body weight (285) and estrogen treatment did not decrease food intake as it does in adults (28). Some sort of mechanism not available until maturity is further illustrated by the lack of weight gain observed in immature females subjected to ventromedial hypothalamic lesions (20). Hyperphagia and increased body weight are also more likely to occur in females than males following ventromedial hypothalamic lesioning (111,275). The increased weight gain in adult ovariectomized rats appears to be due to estrogen withdrawal (22). Ovariectomy also increased secretion of progesterone from the adrenal glands (225). Progesterone attenuated estrogen's effect of decreased food intake in ovariectomized rats (226,228,213,282).

Shirling et al. (250) demonstrated that weight gain in pregnant rats could be replicated in non-pregnant rats with progesterone implants. A specific dose-response relating progesterone to weight gain was reported by Hervey and Hervey (113). Male rats treated with progesterone showed no significant weight changes.

Adult male rats experienced only small daily fluctuations in body weight gain whereas females showed large, predictable changes. Females decreased food intake and lost weight at proestrus, only to have increased food intake and weight at diestrus (280,22). Proestrus occurs after estrogen's peak and Morali and Beyer (180) and Dubuc (61) reported that hypophagia was the largest contributing factor in estrogen-induced decreased weight gains. Dalvit (51) reported that women increased food intake and gain weight in the luteal phase of the cycle. Females in Dalvit-McPhilips (52) study ate 500 additional calories/day after ovulation (when progesterone levels rise) than before and consumed more carbohydrates. Weizenbaum et al. (296) reported that females ate more M&Ms and peanuts during the luteal phase of the menstrual cycle when progesterone was high than during the other phases. Increased food consumption during the luteal phase has also been reported in female rhesus monkeys (227). Casper et al. (36) reported a tendency for women on oral contraceptives to eat sweets and gain weight. Berger and Telwar (19) reported increased appetite with progestin based oral contraceptives.

In males, the predomimant steroid hormone is testosterone which was reported to suppress adiposity in rats. Testosterone was also reported to decrease food intake in adult male, but not female hamsters (307). Gentry and Wade (86) reported that body weight can increase or decrease depending on the dose of testosterone propionate. Low doses increased weight while high doses over a 2-6 week period reduced weight gain. Gentry and Wade suggested that if testosterone propionate was aromatized to estrogen it may attenutate food intake. Progesterone acts as an antiandrogen and blocks the conversion of testosterone to estrogen (172). Male adipose tissue contains receptors for estrogen at the same concentrations as those found in females. Nimrod and Ryan (193) reported that adipose tissues contain enzymes that convert testosterone to estrogen.

Despite reported results suggesting an influence of ovarian hormones on weight gain, several questions remain unanswered. Hyperphagia/hypophagia is not always necessary for the weight gain/loss observed with estrogen, progesterone, or ovariectomy treatments (112,250,232). Species differences also exist (158). Unlike other rodents, adult female hamsters have androgens rather than estrogens as their major hormones related to food intake/body weight (262,297). Raible and

Gorzalka (219) reported that unlike rats, female gerbils experience decreased body weight after ovariectomy and increased, rather than decreased, food intake with estrogen treatments.

MECHANISMS RELATING OVARIAN STEROIDS TO WEIGHT GAIN

The mechanism by which ovarian steroids influence weight is unknown. Several influences have been suggested; fat enzymes, taste, insulin, opioids, and central control.

Fat Enzymes

Wade and Gray (281) indicated that lipoprotein lipase levels and therefore adiposity regulation are altered by ovarian steroids. The lipoprotein lipase concentrations were double in the progesterone treated rats, whereas those treated with estrogen had lowered lipoprotein lipase levels. Fat metabolism involves clearing triglycerides from the blood which initially have to be hydrolyzed by two rate limiting lipases present near or on the vascular wall.

1. Hepatic lipase - located in the liver.

2. Lipoprotein lipase - located in the skelatal, heart, lung, mammary, and adipose.

Estrogen increases triglycerides by decreasing lipoprotein lipase and hepatic triglyceride lipase enzymes. Decreased intake may be induced by estrogen's ability to increase triglycerides (281). As an available fuel, the triglycerides act to spare glucose and possibly avoid hypoglycemia (53).

Pecquery (205) reported that estrogen may decrease lipolytic response through a defect in the catalytic subunit of adenylate cyclase.

Taste Modulation

Gender differences exist involving taste (286,308). Wade (283) reported that ovarian hormones alter taste preferences and may therefore influence food intake. In general, females are more sensitive to taste stimuli and have lower identification thresholds for sweets. For instance, female rats have a greater preference for saccharin than males. Composition of the diet has also been reported to vary with fluctuations in ovarian steroid hormone concentrations. The possibility of sweetness perception varying during different stages of the menstraul cycle was also investigated (296,301).

Insulin

Ovarian steroids have been suggested to influence food intake through insulin metabolism. Estrogen treatment has been reported to lower plasma glucose curves after induction of hyperinsulinemia (48). Progesterone administration induces pancreatic hypertrophy and enhanced insulin response to glucose in vitro (134). Kalkhoff et al. (135) administered 300-400 mg of progesterone intramuscularly into five normal human males and two hysterectomized females for six days and reported that progesterone increased basal plasma insulin levels to those observed in pregnancy. Several studies report that progesterone increases insulin secretion following a glucose load (11,12,48,134).

Ashby et al. (11) suggested that insulin might facilitate weight gain by:

1. Increasing hepatic lipogenesis.

2. Increasing lipogenesis.

3. Increasing lipoprotein lipase which increases triglyceride synthesis, and decreases lipolysis.

Opioids

Progesterone may also increase insulin levels by facilitating the release of β-endorphin. Abou-Samra et al. (1) reported that progesterone enhanced the in vitro release of β-endorphin from rat anterior pituitary in primary culture. However, Vale et al. (274) and Simnitov (254) indicated that progesterone inhibited the in vitro secretion of β-endorphin-like immunoreactivities.

Margules (165) stated that the excess β-endorphin concomitantly released with adrenocorticotropin hormone stimulates insulin secretion which promotes lipogenesis in adipose tissue. Recant et al. (221) suggested that tonic opiate overactivity in the pancreatic islets of obese mice may play a role in insulin hypersecretion. In these obese mouse islets, naloxone, an opiate antagonist, inhibited insulin release.

Matsumura et al. (167) suggested that β-endorphin may be involved in the hyperinsulinemia observed in rats made obese by ventromedial hypothalamic lesions. Amir and Bernstein (6) disagreed and felt that endogenous opioids do not appear to play a significant role in the secretion of insulin in vivo. If an influence exists, it may be only partial because Si et al. (252) reported that insulin-induced feeding was divided into an early naloxone-sensitive stage and a later naloxone-insensitive phase. Insulin levels, whether influenced by β-endorphin or not, are a peripheral phenomenom reflecting a central mechanism.

Central Control

The main site of opiate action appears to be in the hypothalamus (186) where food intake is hypothesized to be regulated. The lateral hypothalamus is the "feeding center": it constantly stimulates food intake. The ventromedial hypothalamus represents the "satiety center"; it continuously inhibits the lateral hypothalamus. Destruction of the lateral hypothalamus results in anorexia, while ventromedial hypothalamus damage results in obesity. The ventromedial hypothalamus contains estrogen-sensitive neurons that modulate food intake and Wade (284) suggested that this might be related to the appetite fluctuations seen during pregnancy, pseudopregnancy, and the reproductive cycle. Estrogen is known to directly stimulate ventromedial hypothalamic nerve cells (31). Wade (284) indicated that estrogen may act on the ventromedial hypothalamus to depress appetite through its inhibitory actions on lateral hypothalamic activity. Estrogen's action may be antagonized by progesterone which can interfere with the brain cells' uptake and binding of estrogen (282).

Ovarian hormones acting centrally may involve another mechanism that includes the brain's catacholamine systems. The brain has receptor sites for estrogen, progesterone (172), and β-endorphin. Physiological concentrations of estrogen and progesterone were reported by Wardlaw et al. (292) to alter brain β-endorphin levels. Estrogen and progesterone have been hypothesized to mediate their effect on gonadotropin release through catecholamine systems (212,299). Opiates are also known to interact with the catecholamine system (203) which is involved with self-stimulation reward (300). Opiate receptors have been reported to be localized on dopamine terminals (69). The catecholamines, norepinephrine and dopamine, are seen as major components of self-stimulation, while minor roles involve serotonergic, cholinergic, endogenous brain opioids, and other peptide reward systems (203).

Morley et al. (182) suggested that food intake is regulated via a tonic dopaminergic-opioid mechanism in the lateral hypothalamus. Estrogen has potent anti-dopamine action (128). Dopamine

receptor sensitivity in the rat striatum is altered by estrogen treatment (64,96). Ferland et al. (71) presented data in which 17-β-estradiol increases hypothalamic dopamine release. Part of estrogen's association to dopamine might be due to its ability to lower brain β-dopamine hydroxylase, the enzyme responsible for converting dopamine to norepinephrine. Wise (298) suggested that falling norepinephrine levels are related to satiety. Progesterone, which antagonizes estrogen's effect, is known to increase norepinephrine levels. Morley et al. (184) suggested that progesterone may also decrease opioid receptor sensitivity.

It has been suggested that the dopamine mediated components of feeding could be partially inhibited by estrogen and β-endorphin, in addition to GABA, acetylcholine, serotonin, and norepinephrine systems (203).

OVARIAN HORMONES AND β-ENDORPHIN LINKED TO HEALTH

The mechanism(s) of how the catecholamine system interacts with ovarian steroids and β-endorphin is unknown. What is known is that males and females respond differently to opiates (136). The possibility that estrogen and progesterone interact with β-endorphin to influence the catecholamine system lends support to statement by Labrie and associates (146) that the ovarian hormones may have clinical implications in neurological as well as psychiatric diseases. Estrogen increases the appearance of Parkinson's disease in neuroleptics and improves the tardive dyskinesias observed in the same condition induced by L-dihydroxyphenylalanine (L-DOPA). If β-endorphin interacts with ovarian steroids, then support is given to Pickar et al. (211) who stated that ever since β-endorphin and other endogenous opiates were reported to bind to the brain, they have been im-

plicated as a possible key in the biologic basis of behavior and mental illness. Increased or decreased activity of opioids, according to Way (294) may ultimately lead to pathological states.

Imbalanced activities among transhypothalamic neurochemical circuits may foreshadow major psychiatric disorders (203). Most anti-psychotic drugs are dopamine blockers (116). Numerous conditions involve various brain systems: Schizophrenia (increased or decreased activity of dopamine system)(243), endogenous depression (decreased activity of adrenergic/serotonergic system), mania (increased activity, especially norepinephrine), anxiety (possible increased serotonin activity), Parkinson's disease (dopamine deficiency) (9).

Depression which has been linked to ovarian steroids (83) and β-endorphin (4,43) is more prevelant in women. Tricyclic antidepressants have long been known to influence appetite. β-adrenergic and serotonin receptors are often implicated in antidepression action. Both are decreased by estrogen which is antagonized by progesterone (21). Women are also more prone than men to hypothyroidism and its often accompanying depression.

β-endorphin has been linked to the immune system (72,162,256). Johnson et al. (133) observed that β-endorphin inhibited antibody production in vitro. The immune system was reported by Grossman (102) to be regulated by gonadal steroids, estrogen, androgens, and progesterone. Certain autoimmune disorders were stated by Grossman (102) to be significantly influenced by sex steroid hormones. Some medical problems are more prevalant in women - lupus erythematosus, idiopathic thrombocytopenic purpura (ITP), and rheumatoid arthritis.

Women with Turner's syndrome have been claimed to have an increased risk of obesity (151). Anorexia nervosa is also paradoxically linked to Turner's syndrome (142). Patients with Turner's syndrome have also been reported to have an increased incidence of abnormal glucose tolerance and diabetes (151). Levine (151) cited studies suggesting that Turner's syndrome carries an increased susceptibility to the development of endometrial carcinoma with estrogen treatment. A treatment for metastatic breast cancer includes ovariectomy, hypophysectomy, and adrenalectomy. It has

been hypothesized that adrenalectomy or hypophysectomy causes tumor regression by lowering estrogen production (239). Estrogen may also play a role in the increased body weight and incidence of breast endometrial carcinoma. The percentage of glucuronides as estradiol and estriol is increased, while 2-hydroxyestrone is decreased. Reciprocal changes are observed in anorexia nervosa (255).

Hoyenga and Hoyenga (121) stated that almost all weight surveys from almost every country have found women to be more obese than men at all ages. Twenty-four percent of American women are obese, compared to only fourteen percent of the men. Hoyenga stated that among genetically or dietetically obese rodents, females often get fatter than males. If no steroidal influences existed, the ratio should theoretically be 50:50. The implications of steroids influencing body weight differently according to gender is observed in the weight gain occurring with the onset of puberty. This pulls the topic full circle back to weight gain and the possible link between hypothalamic β-endorphin and ovarian steroids.

OVARIAN STEROID HORMONES RELATED TO β-ENDORPHIN

Introduction

Opiates and steroid hormones are both derivatives of perhydrophenanthrene. Similarities between the two were observed by LaBella (145) who stated that opiates appear to mimic steroid hormones; both act on the hypothalamus and limbic system and show similar withdrawal syndromes.

Steroids have also been reported by Lee et al. (147) to compete for binding on opiate receptors. Kasson (136) suggested that the response in rats to morphine is influenced by gender. Morphine was also reported by Pinsky et al. (213) to be ineffective in gonadectomized rats.

An association between steroid hormones and opioids is also demonstrated by Hahn and Fishman (107) who observed an increased number of brain opiate receptors in castrated male rats. Gender differences are also found in the pituitary β-endorphin content of obese (ob/ob) mice. Govoni and Yang (97), based on Herberg and Coleman's (109) observations, stated that the commonly displayed disorder in carbohydrate metabolism of genetically hyperglycemic mice is more severe in males than females.

Ovarian steroids and β-endorphin both interact with the hypothalamic-pituitary-adrenal axis. Corticosterone from the adrenal cortex signals the hypothalamus to release corticotrophin releasing hormone which stimulates adrenocorticotropin hormone release from the anterior pituitary. β-endorphin is concomitantly released with adrenocorticotropin hormone (Figuire 7). β-endorphin may be linked to ovarian steroids through their dependence on the hypothalamic-pituitary-adrenal axis.

Estrogen has a positive feedback effect on adrenocorticotropin hormone secretion. Progesterone antagonizes the feedback effects of corticosterone on adrenocorticotropin hormone release (24). Abou Samra et al. (1) demonstrated in vitro that progesterone antagonized the corticosterone feedback inhibition of pituitary β-endorphin release. The antiglucocorticoid property of progesterone is illustrated by its ability to bind to glucocorticoid brain receptors (172). Progesterone can also substitute for glucocorticoids in adrenalectomized rats to prolong their lives. It has been suggested that conditions which increase blood glucocorticoids can inhibit pituitary β-endorphin synthesis and therefore decrease plasma β-endorphin.

Opioid peptides, including β-endorphin, modulate the release of pituitary gonadotropins, which in turn, alter sex steroids. The association between ovarian steroid hormones and β-endorphin is

supported by studies generating evidence from ovarian steroid treatment, pregnancy, reproductive cycles, and indirect data.

Ovarian Steroid Treatment

Wardlaw et al. (291) and Wehrenberg et al. (295) suggested that ovarian steroids may be important regulators of β-endorphin content in the brain. Estrogen treatment was reported by Wardlaw et al. (291) to be related to decreased hypothalamic β-endorphin. Another study by Wardlaw et al. (289) confirmed her earlier study, however, results were reported in pmoles and β-endorphin units/unit protein may yield different results since hypothalamic weights differ in rats administered with ovarian steroids. Forman et al. (74) also reported decreased hypothalamic β-endorphin in estrogen treated rats when compared to controls that were in estrous. Frantz et al. (78) administered acute estrogen doses to ovariectomized pigtailed monkeys and did not find any difference in hypophyseal portal blood β-endorphin concentrations. Chronic treatment of estrogen in two out of four of these ovariectomized animals did result in measurable hypophyseal portal blood β-endorphin levels. Two of the animals treated with both estrogen and progesterone were reported to have high hypophyseal portal blood β-endorphin concentrations.

No significant difference was found by Knuth et al. (139) who expected to find higher hypothalamic β-endorphin levels in high estrogen treated rats, although the five day treatment may have been too short. Hulse and Coleman (126) also reported no change in hypothalamic β-endorphin, however, conjugated estrogen was used and the treatment spanned only three days. Wilcox and Roberts (297) reported decreased pro-opiomelanocortin mRNA levels in the hypothalamus of rats after three days of estrogen treatment. Increased levels of mediobasal hypothalamic β-endorphin levels in the estrogen treated rats were reported by Petraglia et al. (209). Barden et al. (17) also observed elevated mediobasal hypothalamus concentrations in ovariectomized rats treated with estrogen.

Tejawani et al. (267) administered ovarian hormones in the form of oral contraceptives to female rats. Acute (five days) or chronic (7 weeks) administration of progesterone (norethindrone) to rats resulted in no significant difference in pituitary or hypothalamic β-endorphin when compared to controls. When given acutely, high doses of progesterone and estrogen (ethinyl estradiol) decreased both pituitary and hypothalamic β-endorphin. Chronic doses of the combined high doses resulted in no significant difference in the pituitary, but decreased hypothalamic β-endorphin levels.

Forman et al. (74) stated that the discrepancies in hypothalamic β-endorphin levels associated with estrogen may be due to whether estrogen treatment was acute or chronic, and how long the animals are ovariectomized. Other possible factors include dose amounts, steroid form, stress, and method or time of sacrifice.

Pregnancy

Natural fluctuating levels of ovarian steroids occur during pregnancy. Research relating β-endorphin to pregnancy is primarily from the measurement of plasma β-endorphin. Most studies report elevated plasma β-endorphin levels during pregnancy (30,49,85,88,91,192,263).

Brain β-endorphin levels during gestation were reported by Wardlaw (290) to be increased in the hypothalamus, mid-brain, and amygdala. After pregnancy, regardless if rats were lactating or not, hypothalamic β-endorphin concentrations decreased. However, controls in this study were female rats killed at random during estrous. Wardlaw concluded that brain β-endorphin levels vary during pregnancy, but questioned whether it was a result of progesterone or of the multiple other hormonal and metabolic changes. Supporting data was reported by Petraglia (208) who reported increased β-endorphin in the plasma, anterior pituitary, neurointermediate pituitary lobe, and mediobasal hypothalamus of pregnant rats. However, β-endorphin levels measured in the same areas of these lactating rats were comparable to non-pregnant controls. Panerai et al. (201) also reported that

hypothalamic β-endorphin decreased in lactating rats. Different hypothalamic β-endorphin levels in these studies may have been due to different sacrifice methods.

Reproductive Cycle

Fluctuations of ovarian steroid hormone levels orchestrate the sequence of events governing the reproductive cycle. Several studies report changes of β-endorphin levels during the estrous cycle. Ferin et al. (70) suggested that endogenous opioids may be related to the cycle through their role in gonadotropin secretion. The four-day estrous cycle in the rat is divided into four phases; estrus, diestrus I, diestrus II, and proestrus. Estrogen peaks at proestrus (8 a.m. to 4 p.m.) while progesterone peaks twice: 1) proestrus (4 p.m. to 8 p.m.), 2) diestrus I & II (180).

β-endorphin during estrous/mensus has been measured both in the plasma and brain. Animal brain levels during the cycle are difficult to decipher because pituitary and hypothalamic β-endorphin levels are not always related and specific substructures of the hypothalamus can simultaneously increase and decrease. The four day rat cycle is quite short and brain β-endorphin levels have been reported to even change from the morning to the afternoon of one day (16).

Pituitary β-endorphin was reported by Raps et al. (220) to be lowest during proestrus, apparently the same day that corticosterones are released. Hulse et al. (124) reported that hypothalamic β-endorphin decreased during estrus and increased with diestrus. No significant difference was reported by Hulse and Coleman (125) between plasma β-endorphin of rats during estrous and diestrus. Barden et al. (16) illustrated different β-endorphin levels in separate brain areas during the same day. On the afternoon of proestrus, β-endorphin decreased by 50 percent in the arcuate nucleus when compared to the mean content of all other days of the cycle. In contrast, β-endorphin increased in the suprachiasmatic nucleus by 100 percent and in the median eminence by 65 percent. Knuth et al. (139) also reported increased β-endorphin in the arcuate nucleus during proestrus.

In primates, Veith et al. (278) reported that mean human plasma β-endorphin did not significantly change throughout the cycle, although the variance did during the ovulatory phase. Takayama et al. (263) reported higher serum β-endorphin in the human luteal phase when compared to other phases. A study by Vrbicky et al. (279) indicated that a preovulatory peak of serum β-endorphin occurred in humans with a post ovulatory trough five days later. The hypophyseal portal blood of Rhesus monkeys was measured for β-endorphin over the cycle and found to be high in the mid to late follicular and luteal phase.

Indirect Data

Several studies implicate a relationship between ovarian steroid hormones and β-endorphin.

Morphine addiction in humans is often accompanied by amenorrhea and sterility (93). Ovulation in rats can be blocked by morphine (202). Cicero et al. (40) reported that morphine acts on the hypothalamic-pituitary axis to decrease luteinizing hormone.

It is known that β-endorphin injected into humans decreases luteinizing hormone and increases prolactin (223). Quigley et al. (217) suggested that increased opioid inhibition of luteinizing hormone occurs in prolactin producing microadenoma. Other studies referenced by Quigley et al. (215) indicate that endogenous opioids influence prolactin release by interacting with opiate receptors on dopamine nerve terminals. Opiate administration was reported by Van Vugt et al. (277) to increase rat prolactin levels through inhibition of tuberoinfundibular dopamine release in the median eminence. Naloxone suppresses this prolactin release. In women, naloxone has been reported to increase luteinizing hormone except during the early follicular phase (216). Cicero et al. (39) administered two narcotic antagonists, naloxone and naltrexone, to male rats and reported elevated luteinizing hormone levels. He suggested that endogenous opioids exist in the brain tissue which normally inhibit activity in the hypothalamic-pituitary-luteinizing hormone axis. Morley et

al. (184) reported that estrogen treated rats were 20 times less sensitive to naloxone's suppressive effects on feeding than ovariectomized rats.

Indirect data from observing luteinizing hormone response and ovarian steroid hormone concentrations to naloxone has led some investigators to suggest an involvement of endogenous opioids (276). Shoupe et al. (251) observed no difference between the plasma β-endorphin levels of pre- and ovariectomized women, but treatment with estrogen and progesterone in these subjects led the researchers to conclude that increased opioid activity occurred. A hypothesis stemming from this research suggested that central β-endorphin may play an important role in the psychological changes of menopausal patients. Casper and Alapin-Rubillovitz (35) indicated that progestins exert their negative feedback effects on luteinizing hormone partially through an opioid-peptide mechanism. Progestin therapy in a dose which improves menopausal flushes was reported to increase endogenous opioid activity. Casper and Alapin-Rubillovitz hypothesized that estrogen withdrawal at menopause results in endogenous opioid peptide withdrawal and may be related to flushes.

Women with polycystic ovarian disease are commonly overweight. Aleem et al. (5) studied patients with this condition in patients who averaged 124 percent of their ideal body weight and reported a significant correlation between plasma β-endorphin levels and body weight. Givens et al. (90) reported elevated plasma β-endorphin levels in eight obese, hirsute females with oligo-amenorrhea.

Just how β-endorphin interacts with the brain and ovarian steroids to possibly influence interdependent loops remains a mystery. What is known is that endogenous opioids inhibit gonadotropin release (maximum at the luteal phase) and Reid et al. (223) suggested a possible association to premenstrual syndrome. Reid indicated that symptoms of fatigue and depression might be related to the opioids ability to inhibit biogenic amine systems and decrease norepinephrine and dopamine release. A withdrawal of endogenous opioid peptide inhibition may lead to rebound hyperactivity of neural pathways. Decreasing endogenous opioid peptides is followed by increased prostaglandin activity. Anti-prostaglandins have been used for treatment of premenstrual syndrome.

The role of β-endorphin between ovarian steroid hormones and various medical conditions may involve the hypothalamic-pituitary-adrenal axis. Feder and Ruf (68) suggested that adrenal hyperactivity stimulated by stress may effect the reproductive cycle. After injecting estrogen-primed rats and guinea pigs with adrenocorticotropin hormone, Feder observed increased progesterone release from the adrenals. Hulse and Coleman (126) also reported adrenal release of progesterone in response to elevated plasma adrenocorticotropin hormone levels stimulated by stress. Increased opioid concentrations observed with stress may disrupt the luteinizing hormone surge and possibly play a role in the stress-induced block of ovulation (127).

Exercise, perceived as a stress on the body, is accompanied by increased peripheral opioid peptide levels (34,46,77). Men have a greater rise in β-endorphin than women exercising at the same percentage of their predicted maximum heart rates (81). Jewelewicz (132) suggested that increased plasma β-endorphin and catechol estrogens may contribute to the menstrual dysfunction occurring with strenuous exercise. Russel et al. (233) suggested that the ability of catechol estrogens and β-endorphin to decrease basal luteinizing hormone levels may partially explain the oligomenorrhea observed in some female athletes.

chapter III

MATERIALS AND METHODS

Animals

Eighty female adult Sprague-Dawley (Charles-River) rats,[1] weighing 278-346 g (x = 313.0 g) were individually housed in a 12:12 light/day cycle (lights on from 6:00-18:00 hours) and maintained at 23 degrees C with ad libitum access to laboratory pelleted Purina rat chow[2] and water. Charles River Sprague-Dawley rats were used because Gray and Greenwood (99) reported that Holtzman Sprague-Dawley rats are less progestin sensitive. Pelleted rather than ground chow was used to ensure accurate food intake measurements. At 17-19 weeks of age, rats were ovariectomized through a flank incision under halothane anesthesia. After 7 days of recovery, rats were divided into four weight-matched groups of 20 each. Figure 8 depicts the experimental design.

---

[1] Charles River Breeding Laboratory, Wilmington, MA.

[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis, MO.

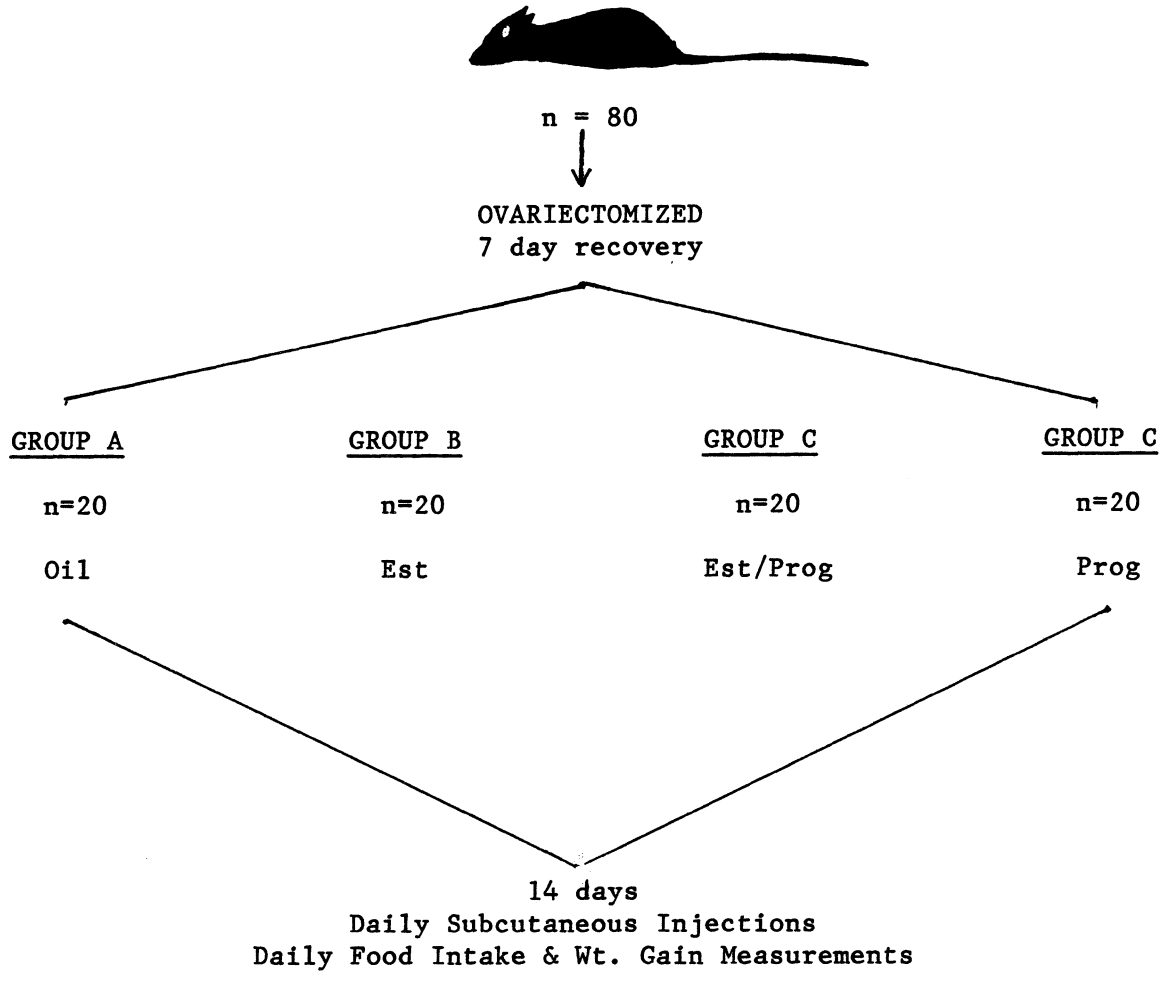


Figure 8. Experimental design.

Hormone Treatments

The four different treatment groups were as follows: A (controls) received corn oil, Group B received 2 ug estradiol benzoate,[3] Group C received 2 ug estradiol benzoate plus 5 mg of progesterone, and Group D received 5 mg of progesterone.[4] All hormones were suspended in a 10 percent corn oil/benzyl benzoate solution. The doses were chosen on the basis of previous studies reporting alterations in food intake and body weight (113,282). 5 mg of progesterone is equivalent to the levels found during pregnancy in the rat (113). Treatment involved daily subcutaneous injections rotated around the body between the hours of 10:00 and 12:00. At this time, body weight and food intake were also measured.

Sacrifice

An outline of the experimental methodology is illustrated in Figure 9. After 14 days, rats were sacrificed by decapitation. The brain was rapidly removed and placed dorsally on a moist kimwipe covered petri-dish lying upside down over ice. Figure 10 depicts where the hypothalamus is located in a cross section of the rat brain. A ventral view is illustrated in Figure 11 (204). Using sharp, slightly curved forceps, the dura was removed before the brain was excised. A forceps cut was made at the anterior end of the hypothalamus underneath the optic chiasm. An incision was made by the mammallary bodies at the most posterior end of the hypothalamus. In a scooping motion, the curved forceps were slid down between the cerebral cortex and lateral sides of the hypothalamus to lift the hypothalamus 2-3 mm out of the brain.

---

[3] Sigma Chemical Co., St. Louis, MO.

[4] The UpJohn Co., Kalamazoo, MI.

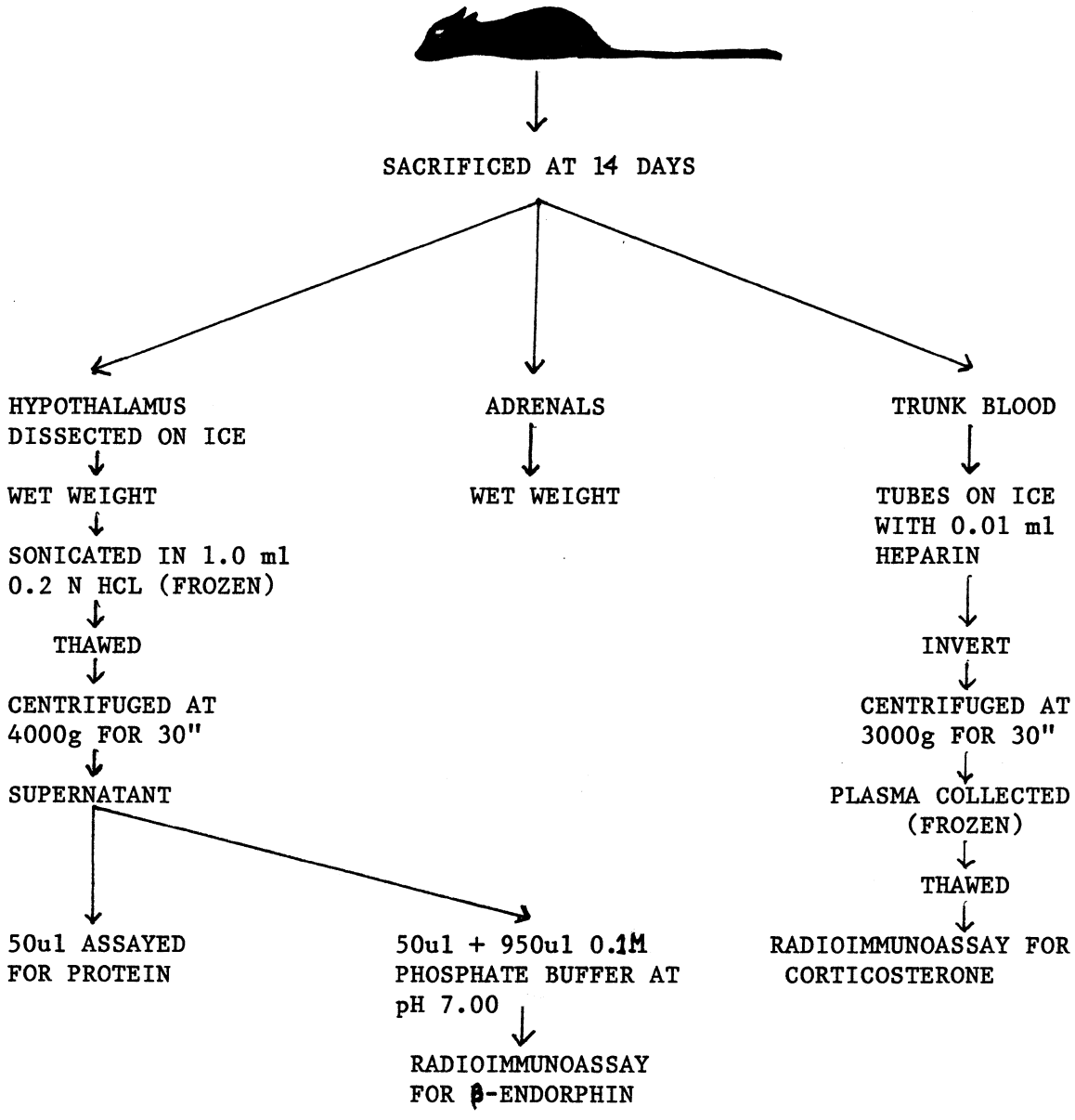


Figure 9. Experimental Methodology.

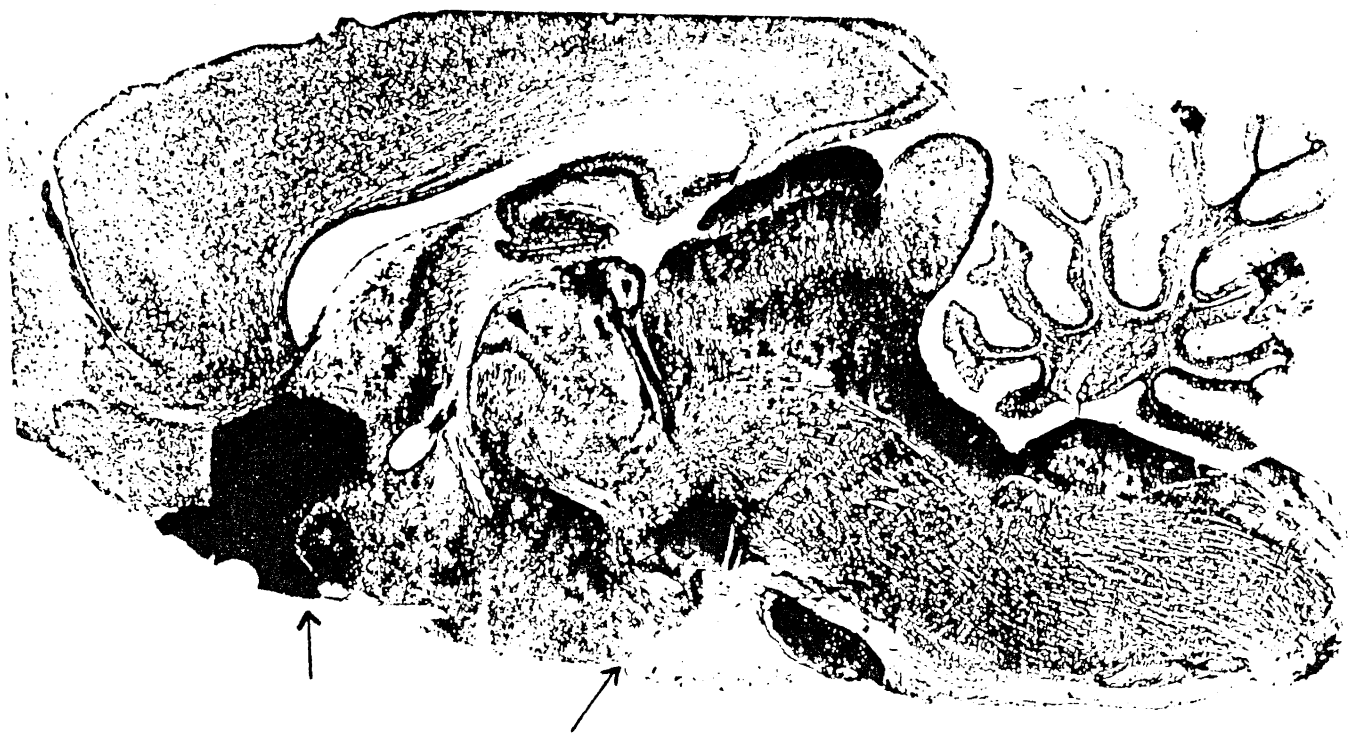
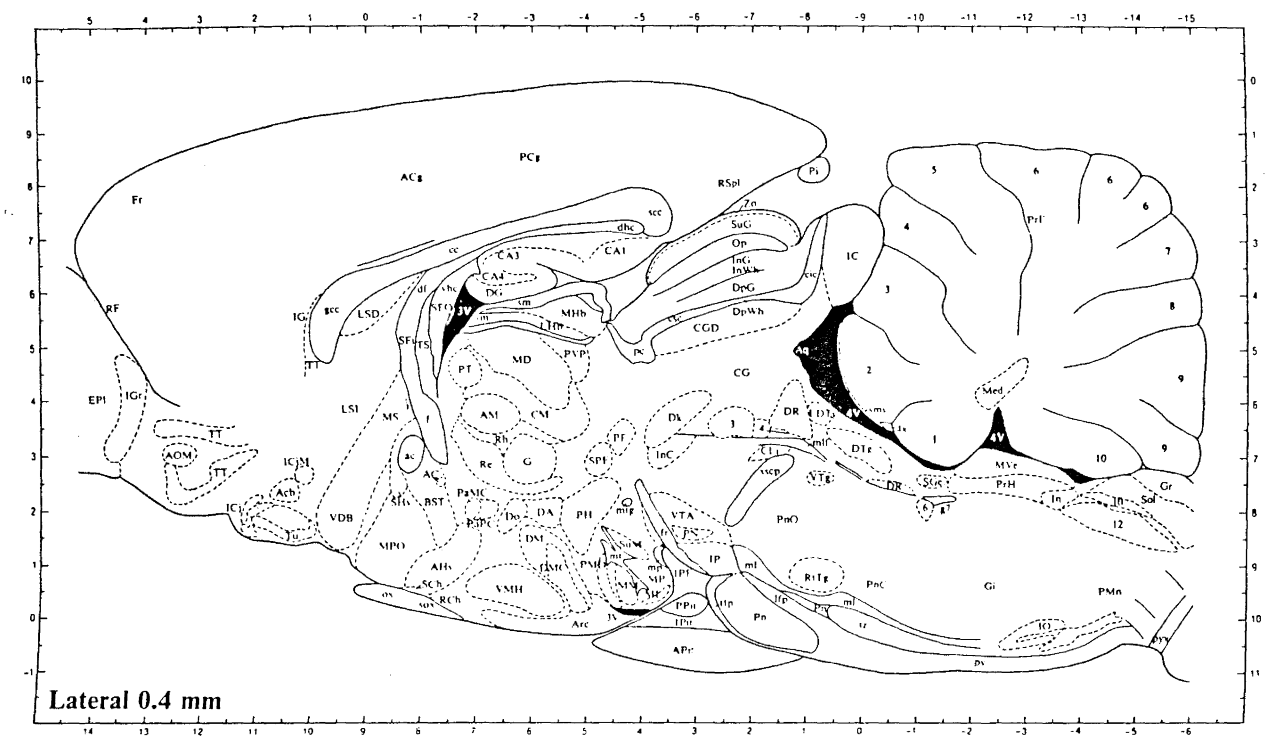


Figure 10. Location of the hypothalamus in a photographic cross-section of the rat brain matched against a diagram of the structures with stereotaxic reference points (204).



3 principal oculomotor nu
3V third ventricle
4 trochlear nu
4V fourth ventricle
4x trochlear decussation
6 abducens nu
10 dors motor nu of vagus
12 hypoglossal nu
AC ant commissural nu
ac ant commissure
Acb accumbens nu
ACg ant cingulate cortex
AHy ant hy area
AM anteromed th nu
AOM ant olf nu, med
APit ant lobe pituitary
Aq cerebral aqueduct
Arc arcuate hy nu
BST bed nu stria terminalis
CA1 field CA1 of Ammon's horn
CA3 field CA3 of Ammon's horn
CA4 field CA4 of Ammon's horn
cc corpus callosum
CG central grey
CGD central grey, dors
cic commissure inf colliculus
CLi caudal linear nu raphe
CM central med th nu
csc commissure sup colliculus
DA dors hy area
df dors fornix
DG dentate gyrus
dhc dors hip commissure
Dk nu of Darkschewitsch
DM dorsomedial hy nu
DMC dorsomedial hy nu, compact
Do dors hy nu
DpG deep grey layer sup colliculus
DpWh deep white layer sup colliculus
DR dors raphe nu
DTg dors tegmental nu
EPl ext plexiform layer olf bulb
f fornix
Fr frontal cortex
fr fasciculus retroflexus
G gelatinosus nu thalamus
g7 genu facial nerve
gcc genu corpus callosum
Gi gigantocellular reticular nu
Gr gracile nu
IC inf colliculus
ICj islands of Calleja
ICjM islands of Calleja, major island
IG induseum griseum
IGr int granular layer olf bulb
In intercalated nu
InC interstitial nu of Cajal
InG intermediate grey layer sup colliculus
InWh intermediate white layer sup colliculus
IO inf olive
IP interpeduncular nu
IPF interpeduncular fossa
IPit intermediate lobe pituitary
LDTg laterodorsal tegmental nu
lfp longitudinal fasciculus pons
LHb lat habenular nu
LSD lat septal nu, dors
LSI lat septal nu, intermediate
MD mediodorsal th nu
Med med cerebellar nu
MHb med habenular nu
ML med mammillary nu, lat
ml med lemniscus
mlf med longitudinal fasciculus
MM med mammillary nu, med
MP med mammillary nu, post
mp mammillary peduncle
MPO med preoptic area
MS med septal nu
mt mammillothalamic tr
mtg mammillotegmental tr
MVe med vestibular nu
Op optic nerve layer sup colliculus
ox optic chiasm
PaMC paraventricular hy nu, magnocellular
PaPC paraventricular hy nu, parvocellular
pc post commissure
PCg post cingulate cortex
PF parafascicular th nu
PH post hy nu
Pi pineal gland
PMD premammillary nu, dors
PMn paramedian reticular nu
PN paranigral nu
Pn pontine nuclei
PnC pontine reticular nu, caudal
PnO pontine reticular nu, oral
PPit post lobe pituitary
PrF primary fissure
PrH prepositus hypoglossal nu
PT paratenial th nu
PVP paraventricular th nu, post
py pyramidal tr
pyx pyramidal decussation
RCh retrochiasmatic area
Re reuniens th nu
RF rhinal fissure
Rh rhomboid th nu
RSpl retrosplenial cortex
RtTg reticulotegmental nu pons
scc splenium corpus callosum
SCh suprachiasmatic nu
SFi septofimbrial nu
SFO subfornical organ
SGe suprageniculate nu pons
SHy septohypothalamic nu
sm stria medullaris thalamus
smv sup medullary velum
Sol nu solitary tr.
sox supraoptic decussation
SPF subparafascicular th nu
SuG superficial grey layer sup colliculus
SuM supramammillary nu
tfp transverse fibers pons
TS triangular septal nu
TT taenia tecta
Tu olf tubercle
tz trapezoid body
VDB nu vertical limb diagonal band
vhc vent hip commissure
VMH ventromedial hy nu
VTA vent tegmental area
VTg vent tegmental nu
xscp decussation sup cerebellar peduncle
Zo zonal layer sup colliculus
The ten lobules of the cerebellum are indicated by the numerals 1-10.

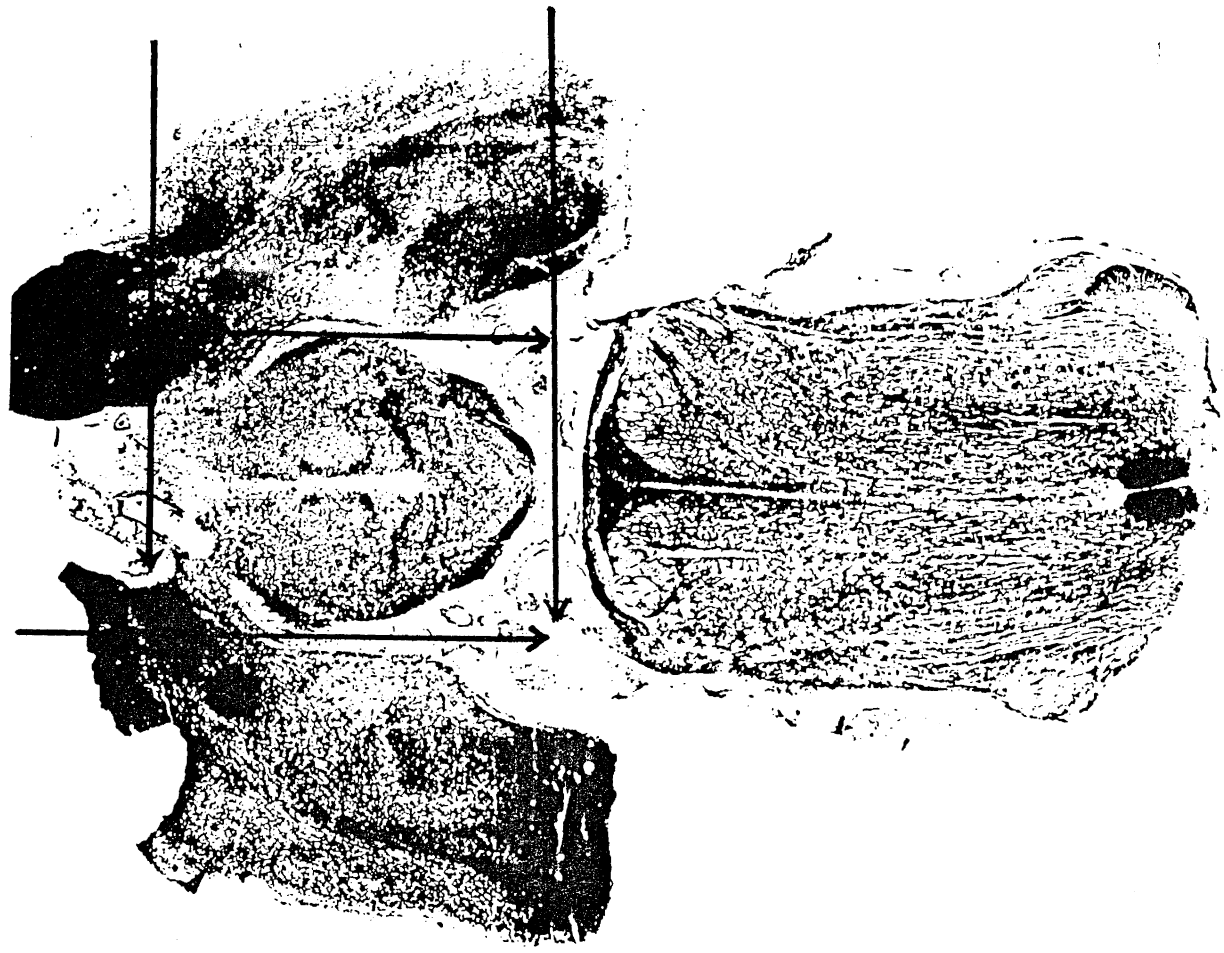
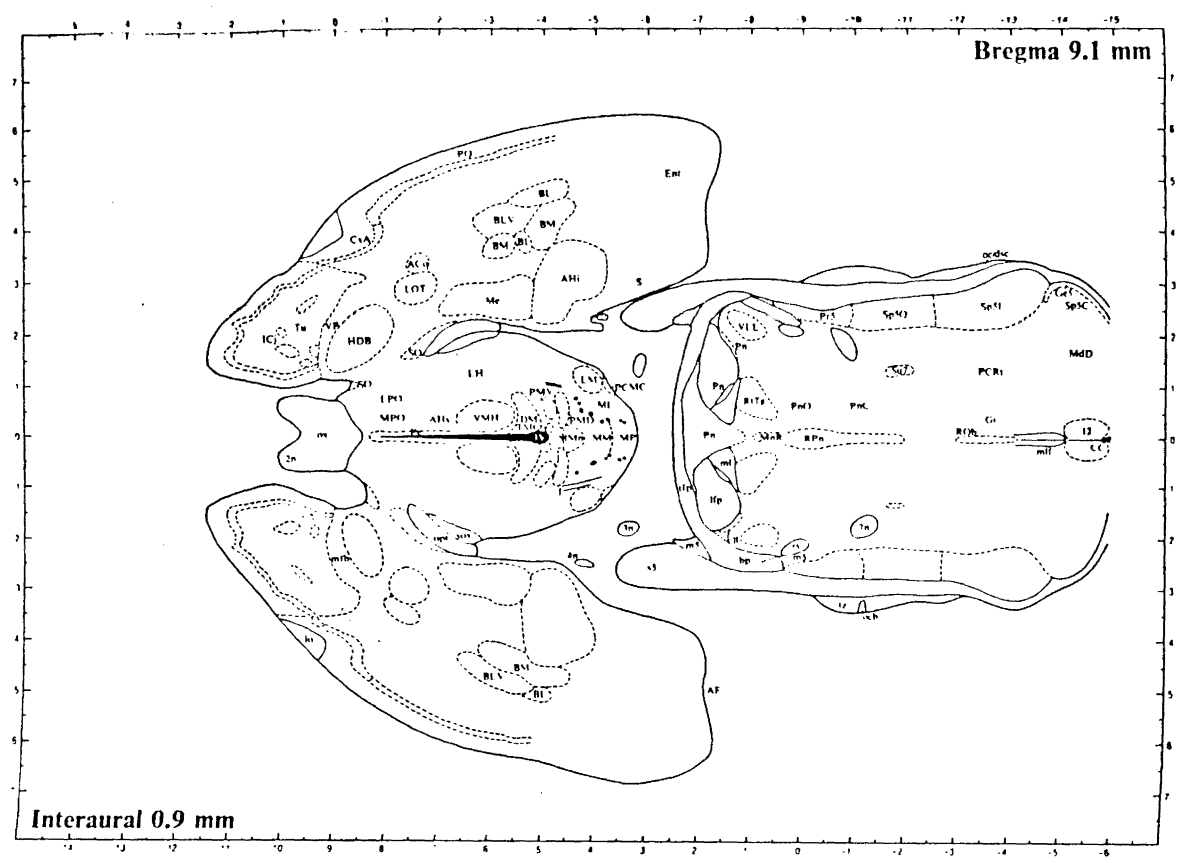


Figure 11. Location of the hypothalamus in a photographic ventral view of the rat brain matched against a diagram of the structures with stereotaxic reference points. (204).



2n optic nerve
3n oculomotor nerve
3V third ventricle
4n trochlear nerve
7n facial nerve
12 hypoglossal nu
ACo ant cortical amygdaloid nu
AF amygdaloid fissure
AHi amygdalohippocampal area
AHy ant hy area
BL basolateral amygdaloid nu
BLV basolateral amygdaloid nu, vent
BM basomedial amygdaloid nu
bp brachium pontis
CC central canal
CxA cortex-amygdala transition zone
DM dorsomedial hy nu
dsc dors spinocerebellar tr
Ent entorhinal cortex
f fornix
Ge5 gelatinosus subnu caudal nu
spinal tract trigeminal nerve
Gi gigantocellular reticular nu
HDB nu horizontal limb diagonal band
ICj islands of Calleja
lfp longitudinal fasciculus pons
LH lat hy area
ll lat lemniscus
LM lat mammillary nu
lo lat olf tr
LOT nu lat olf tr
LPO lat preoptic area
m5 motor root trigeminal nerve
MdD reticular nu medulla, dors
Me med amygdaloid nu
mfb med forebrain bundle
ML med mammillary nu, lat
ml med lemniscus
mlf med longitudinal fasciculus
MM med mammillary nu, med
MMn med mammillary nu, median
MnR median raphe nu
MP med mammillary nu, post
MPO med preoptic area
oc olivocerebellar tr
ocb olivocochlear bundle
opt optic tr
ox optic chiasm
PCMC postmamillary caudal magnocellular nu hypothalamus
PCRt parvocellular reticular nu
Pe periventricular hy nu
PMD premammillary nu, dors
PMV premammillary nu, vent
Pn pontine nuclei
PnC pontine reticular nu, caudal
PnO pontine reticular nu, oral
PO primary olf cortex
Pr5 principal sensory trigeminal nu
RObs raphe obscurus nu
RPn raphe pontis nu
rs rubrospinal tr
RtTg reticulotegmental nu pons
S subiculum
s5 sensory root trigeminal nerve
SO supraoptic hy nu
sox supraoptic decussation
Sp5C nu spinal tr trigeminal nerve, caudal
Sp5I nu spinal tr trigeminal nerve, interpolar
Sp5O nu spinal tr trigeminal nerve, oral
Su7 suprafacial nu
tfp transverse fibers pons
TMC tuberal magnocellular hy nu
Tu olf tubercle
tz trapezoid body
VLL vent nu lat lemniscus
VMH ventromedial hy nu
VP vent pallidum

The hypothalamus was quickly weighed, placed in 1.0 ml of 0.2 N HCl, homogenized by sonication, and frozen. Trunk blood was also collected in chilled heparinized tubes and centrifuged at 4 degrees C to obtain plasma which was then frozen.

Laboratory Assays

Preparation for the analysis of hypothalamic β-endorphin is described by Wardlaw et al. (291). Frozen hypothalamus samples that had been sonicated in 0.2 N HCl were thawed and centrifuged at 4000g for 30 minutes. β-endorphin is soluble in low acid concentrations. Fifty µl of the supernatant was added to 950 µl of 0.1 M phosphate buffer at pH 7.0. Two hundred µl of this 1:20 dilution was assayed for beta-endorphin using a radioimmunoassay kit.[5] Procedures for the β-endorphin assay are described in Appendix A. A regression analysis was used to calculate β-endorphin values from counts per minute (CPM). Dorsa et al. (59) reported a parallel displacement of the Immuno Nuclear β-endorphin with camel β-endorphin which is equivalent to rat β-endorphin. Results reported by the Immuno Nuclear Corporation indicate that the β-endorphin antibody cross-reactivity (molar basis) was 100 percent with [Lue5] camel β-endorphin, N-acetyl-β-endorphin, human β-endorphin, and less than 0.01 percent with α-endorphin, leucine enkephalin, methionine enkephalin, ACTH (1-39), and ACTH (1-24) (Appendix A).

The protein content of the supernatant from the centrifuged sonicated hypothalamus was determined by a Coomassie Brilliant Blue G-250[6] assay (25). Several trial samples were run to determine if any difference existed between the hypothalamic protein content in the homogenate and

---

[5] Immuno Nuclear Corporation, (Catalog No. 1600), Stillwater, MN.

[6] Sigma Chemical Co., St. Louis, MO.

supernatant. Supernatant values were slightly higher and more consistent than those obtained from the homogenate. This may have been due to the lack of interference from the larger particles found in the homogenate which would have influenced absorbance, and therefore, protein concentrations. The low acid concentration of the 0.2 N HCl did not precipitate the protein. The details of the protein assay and standard curve are described in Appendix B. Blood plasma was analyzed for corticosterone using a radio immunoassay kit.[7] The cross-reactivity of the corticosterone antiserum was reported by the company to occur 100 percent with corticosterone, and less than 0.01 percent for estrone, estradiol-17 α, estradiol-17 β, estriol, and 17-α-hydroxyprogesterone (Appendix C). Procedures for the corticosterone radioimmunoassay are detailed in Appendix C. Determination of plasma estrogen and progesterone was also to be done, but the assay was disrupted by a fire alarm in the Veterinary Medicine building.

Statistical Analysis

A One-Way Factorial Analysis of Variance was used to calculate significant differences between the four treatment groups for the following variables: total weight gain, daily food intake, total food intake/100 g body weight, hypothalamic β-endorphin content (ng/mg protein, ng/hypothalamus, & ng/g hypothalamus), hypothalamic weight, protein mg/hypothalamus, protein mg/g hypothalamus, adrenal weight, adrenal weight/100 g body weight, and plasma corticosterone levels. A Duncan's multiple range test determined which groups were significantly different. Relationships between values for parameters were analyzed using Pearson r correlation coefficients. Group means and standard error of the means (SEMs) were also determined. Comparisons at $p < 0.05$ or less were considered statistically significant.

---

[7] Radioassay Systems Laboratories, Inc., Carson, CA.

chapter IV

RESULTS AND DISCUSSION

Weight Gain and Food Intake

The direct effects of ovarian steroid treatment on body weight and hypothalamic β-endorphin were determined, and the results support the hypothesis that they are related.

Estrogen-treated rats had the lowest weight gain and food intake (Table 3). Weight gain in the estrogen-treated group averaged 9.2 ± 1.2 g over 14 days and was significantly less than the controls that gained 45.1 ± 2.4 g, the estrogen/progesterone group that gained 24.3 ± 2.9 g, and the progesterone group that gained 48.8 ± 2.4 g. Daily food intake in the estrogen-treated rats averaged 24.8 ± 0.4 g and was significantly less than the control's average of 27.0 ± 0.6 g and the progesterone group's average of 27.1 ± 0.5 g; however, not the estrogen/progesterone group's average of 25.4 ± 0.4 g.

TABLE 3

TOTAL BODY WEIGHT GAIN AND DAILY FOOD INTAKE AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	WEIGHT GAIN (g)	FOOD INTAKE (g)
OIL	20	45.1 ± 2.4^{a}	27.0 ± 0.6^{d}
EST	20	9.2 ± 1.2^{b}	24.8 ± 0.4^{e}
EST/PROG	20	24.3 ± 2.9^{c}	25.4 ± 0.4^{e}
PROG	20	48.8 ± 2.4^{a}	27.1 ± 0.5^{d}

Values represent means $\pm$ SEM.

$^{a-c}$ Means with matching superscripts are not statistically different, $p < 0.01$.

$^{d-e}$ Means with matching superscripts are not statistically different, $p < 0.05$.

Progesterone and control groups gained the most weight, but were not significantly different from each other. Weight gain was significantly lower than the control or progesterone groups in both the estrogen- and estrogen/progesterone-treated group, which were also significantly different from each other. Figure 12 graphically depicts the weight gain of the four treatment groups.

The weight gain of 48.8 ± 2.4 g in the ovariectomized progesterone treated rats corresponds to results reported by Ashby et al. (10), Morley et al. (184), Shirling et al. (250), and Wade (282). Morley et al. (184) administered 2 mg of progesterone to Sprague-Dawley rats weighing an average of 183.0 ± 2.0 g and reported a 60.7 ± 1.6 g increase in body weight after 6 days of treatment (Table 4). The estrogen-treated group had significantly less weight gain than the progesterone group; however, unlike the results reported in this study, the estrogen-treated group was not significantly different from the combined estrogen/progesterone-treated group. The difference in weight gain between the two studies may be attributed to the 2 mg progesterone dose used by Morley and associates which was less than the 5 mg used in this experiment.

Wade (282) observed a 26 g weight gain in ovariectomized Sprague-Dawley rats (200-250 g) after administrating progesterone for a two week period. Shirling et al. (250) implanted 200 mg progesterone disks in female Wister Albino rats (200 g) and after three weeks reported a 25.8 g weight gain over sham ovariectomized controls. The same progesterone treatment given to rats in the study by Ashby and associates (10) resulted in an increase of 37.1 g after 28 days. Ovariectomized progesterone-treated rats in this experiment gained 48.8 g, which is higher than most of the above studies. However, the starting weights of rats in this study ranged 278.0-345.9 g ($\bar{x}$ = 313.0), which is heavier than the average 200 g starting weight observed in other studies. Rats used in this study were 18-20 weeks of age at the start of the treatments and had higher weights than what Poiley (214) extrapolated should be the 260-299 g range for Sprague-Dawley females.

Daily food intake for female rats of this weight according to the NAS/NRC (191) average 10-15 g, which is low compared to the 24-27 g range observed in this study. However, the results are comparable to the 25-26 g average intake of ovarian steroid treated rats in the study by Morley and

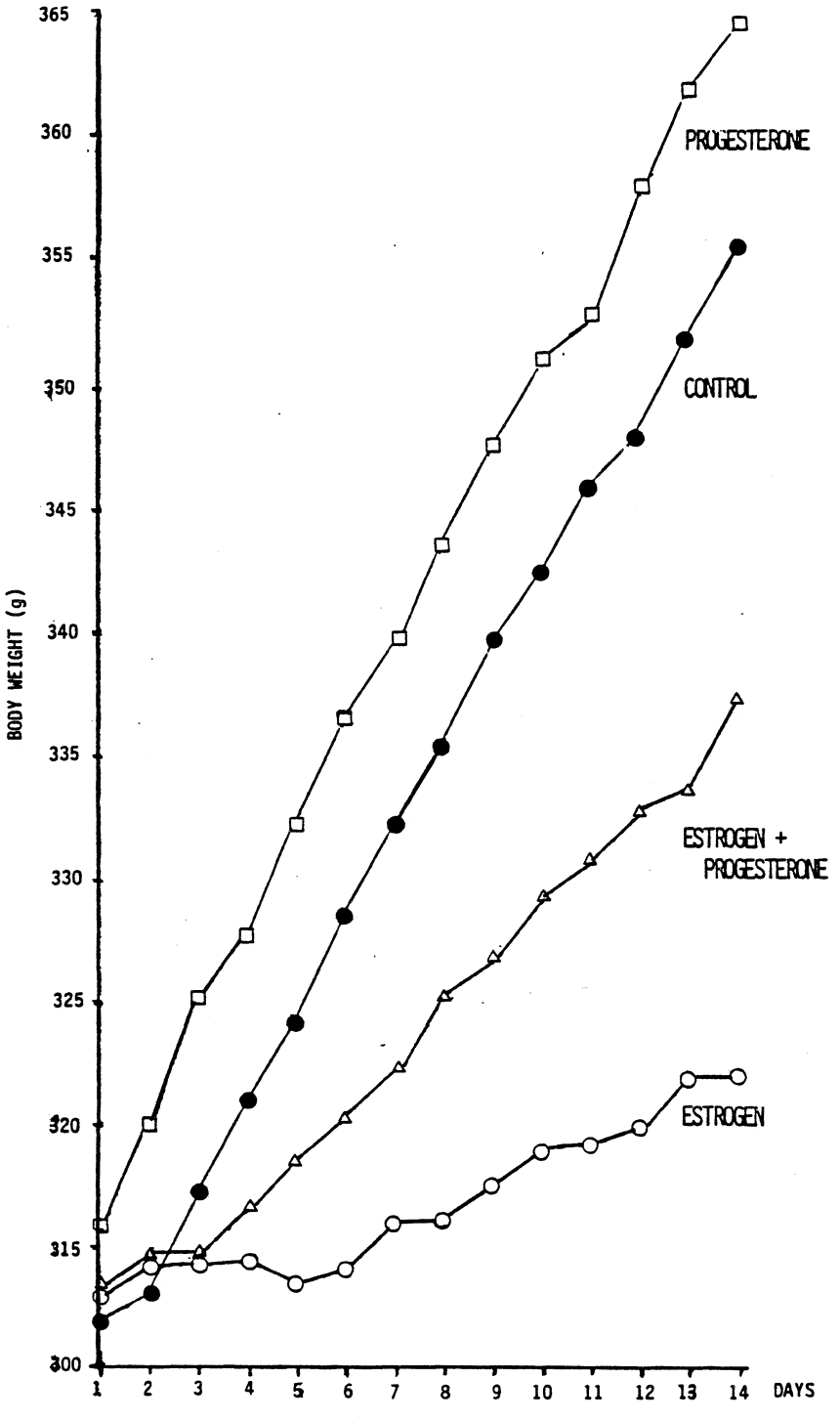


Figure 12. Body weight in rats after treatment with oil, estrogen, estrogen + progesterone, and progesterone for 14 days.

TABLE 4

BODY WEIGHTS, WEIGHT GAIN, AND 24 HOUR FOOD INTAKE AFTER ADMINISTRATION OF OIL, ESTRADIOL BENZOATE (E_2), E_2 AND PROGESTERONE (P), AND P ALONE TO OVARIECTOMIZED (OVX) ANIMALS AND OF OIL ADMINISTRATION TO SHAM OPERATED FEMALES

GROUP	N	BW (g)	WT. GAIN (g)	FOOD INTAKE (g)
OVX + OIL	30	177.8 ± 2.6	57.1 ± 2.4*	25.16 ± 0.56**
OVX + E_2	30	176.4 ± 2.3	50.3 ± 1.5	23.70 ± 0.43
OVX + E_2 + P	30	177.1 ± 2.4	52.3 ± 1.8	25.38 ± 0.44**
OVX ± P	30	180.3 ± 2.0	60.7 ± 1.6*	26.70 ± 0.53**
SHAM + OIL	30	168.8 ± 1.6*	50.3 ± 1.2	25.77 ± 0.55**

*Different from groups without asterisks, $p < 0.05$.

**Different from OVX + E_2.

Source: Morley et al. (184).

associates (184) (Table 4). A difference of 3.0 g of food intake was reported by Morley et al. between the progesterone and estrogen groups, which is close to the 2.3 g difference observed in the equivalent groups of this study. Ashby et al. (10) and Shirling et al. (250) both reported that progesterone-treated rats ate 4 g more than the control group.

Pearson correlation coefficients were run on all 12 variables and are shown on Table 5. A Pearson correlation coefficient of 0.53 ($p < 0.0001$) was observed between body weight and food intake. Food intake was determined per 100 g body weight. Although there was a slight tendency for the estrogen rats to ingest more chow and gain less weight, there was no significant difference between any of the groups (Table 6). This supports the influence of food intake, rather than steroid treatment alone regardless of diet, on the rats' weight gain.

The direct effects of ovarian steroid treatment on body weight and hypothalamic β-endorphin were observed in this study. The results support research indicating that estrogen acts as an appetite suppressant and is antagonized by progesterone (nicknamed the "hungry hormone") (10,22,61,101,226,247). Stern et al. (259), Oomura (198), and Blaustein and Wade (22) suggested that estrogen withdrawal is responsible for the characteristic hyperphagia and rapid weight gain observed with ovariectomy (179,259). Progesterone secretion from the adrenal glands occurs with ovariectomy (225) and may also contribute to the weight gain.

Since there was no sham ovariectomized group in the study, conclusions regarding the effect of ovariectomy on weight gain are not possible. However, a weight difference between estrogen- and progesterone-treated groups was demonstrated by Morley et al. (184). The additional group of rats would have been ideal but impratical due to the available lab animal facilities. Sham ovariectomized rats would theoretically also have varying hypothalamic β-endorphin levels due to different estrus stages.

Despite reported results suggesting an influence of ovarian hormones on body weight, certain questions remain. Rats subjected to estrogen, progesterone, or ovariectomy treatments can gain

TABLE 5

CORRELATION COEFFICIENTS

	WT	FOOD	FOOD/BW	βNG/PRO	βNG/HYPO	βNG/gHYPO	HYPO	PRO/HYPO	PRO/gHYPO	AD	AD/BW	CORT
WT	1.00	0.53	-0.05	-0.32	-0.41	-0.04	-0.09	0.12	0.20	-0.29	-0.50	-0.10
	0.00	0.0001*	0.65	0.01*	0.001*	0.75	0.42	0.30	0.08	0.01*	0.0001*	0.37
FOOD		1.00	0.67	-0.21	-0.20	-0.06	0.03	0.12	0.08	-0.09	-0.28	-0.02
		0.00	0.0001*	0.06	0.08	0.59	0.82	0.30	0.48	0.41	0.01*	0.85
FOOD/BW			1.00	0.04	0.02	-0.06	0.08	-0.04	-0.11	0.16	0.20	0.11
			0.00	0.70	0.87	0.58	0.48	0.73	0.33	0.16	0.07	0.33
βNG/PRO				1.00	0.17	0.43	0.17	-0.86	-0.18	0.01	0.14	0.03
				0.00	0.14	0.0001*	0.14	0.0001*	0.11	0.96	0.21	0.79
βNG/HYPO					1.00	0.24	0.45	0.31	0.14	0.24	0.29	-0.20
					0.00	0.03*	0.69	0.005*	0.22	0.03	0.01*	0.08
βNG/gHYPO						1.00	-0.88	-0.33	0.80	-0.05	-0.01	-0.04
						0.00	0.0001*	0.01*	0.0001*	0.64	0.93	0.73
HYPO							1.00	0.48	-0.71	0.11	0.09	-0.00
							0.00	0.0001*	0.0001*	0.32	0.42	0.99
PRO/HYPO								1.00	0.20	0.06	-0.04	-0.14
								0.00	0.07	0.57	0.76	0.21
PRO/gHYPO									1.00	-0.09	-0.14	-0.06
									0.00	0.43	0.22	0.59
AD										1.00	0.92	-0.07
										0.00	0.0001*	0.55
AD/BW											1.00	-0.04
											0.00	0.74
CORT												1.00
												0.00

LEGEND

WT - Total Weight Gain
FOOD - Daily Food Intake
FOOD/BW - Food Intake/100g Body weight
βNG/PRO - β-endorphin/ng/mg protein
βNG/HYPO - β-endorphin/hypothalamus
βNG/gHYPO - β-endorphin/g hypothalamus
HYPO - hypothalamus
PRO/HYPO - Protein mg/g hypothalamus
PRO/gHYPO - Protein mg/g hypothalamus
AD - Adrenal
AD/BW - Adrenal mg/100 g body weight
CORT - Plasma certicosterone

TABLE 6

TOTAL FOOD INTAKE G/100 G BODY WEIGHT AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	TOTAL FOOD INTAKE g/100 g BODY WEIGHT
OIL	20	106.1 ± 1.7[a]
EST	20	107.7 ± 1.6[a]
EST + PROG	20	105.2 ± 1.6[a]
PROG	20	104.4 ± 1.9[a]

Values represent means ± SEM.

[a] Means with matching superscripts are not statistically different, $p < 0.01$.

or lose weight without hyperphagia or hypophagia (112,232,250). Ovarian steroids also act differently in certain species (158). For instance, adult female hamsters have androgens rather than estrogens as their major hormones (262,307). Female gerbils were reported to experience a decrease in body weight after ovariectomy and increased, rather than decreased, food intakes with estrogen treatments (219).

Hypothalamic β-Endorphin

The mechanism(s) of weight alterations involving steroid hormones are not understood; however, the ovarian hormones may be related to changing hypothalamic β-endorphin levels. The body weight of ovarian steroid-treated rats in this study was significantly related to β-endorphin when expressed as ng β-endorphin/mg protein (Table 7). Both the estrogen- and estrogen/progesterone-treated groups had the highest hypothalamic β-endorphin (ng/mg protein) levels, but were not significantly different from each other. The estrogen- and estrogen/progesterone-treated groups had 21.62 ± 0.56 and 20.80 ± 0.72 ng/mg protein in the hypothalamus, respectively, which were significantly higher than the control group's concentration of 18.59 ± 0.47 ng/mg. The hypothalamic β-endorphin concentrations of the estrogen-, but not estrogen/progesterone-treated group, was significantly different from the progesterone group's average of 19.35 ± 0.83 ng/mg. The ng β-endorphin/mg protein levels of the progesterone-treated group were not significantly different from the control group.

A Pearson correlation coefficient of -0.32 ($p < 0.01$) was observed between ng β-endorphin/mg protein and body weight; this tends to support the hypothesis that the weight gain observed with different steroid treatments is related to hypothalamic β-endorphin. As body weight decreased with estrogen- and estrogen/progesterone-treatments, hypothalamic ng β-endorphin/mg protein increased. The elevated body weight observed in the control and progesterone groups corresponded

TABLE 7

HYPOTHALAMIC BETA-ENDORPHIN CONTENT (NG/MG PROTEIN) AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	BETA-ENDORPHIN (NG/MG PROTEIN)
OIL	20	18.59 ± 0.47^{a}
EST	20	21.62 ± 0.56^{b}
EST/PROG	20	20.80 ± 0.72^{bc}
PROG	20	19.35 ± 0.83^{ac}

Values represent means $\pm$ SEM.

$^{a-c}$ Means with matching superscripts are not statistically different, $p < 0.05$.

to decreased hypothalamic ng β-endorphin/mg protein. A Pearson correlation coefficient of -0.21 ($p < 0.06$) existed for food intake, and was not significantly correlated to to ng β-endorphin/mg protein, which weakens this study's hypothesis.

Rats treated with ovarian steroids in this study had significantly different hypothalamic weights, which did not differ in protein content (Table 8). This is important because expressing β-endorphin as ng/hypothalamus or ng/g hypothalamus would yield different results (Table 9). The majority of studies report hypothalamic β-endorphin as ng/mg protein, which can be expressed in a number of different units which further complicate comparisons - ng/mg protein, ng/tissue, pmole, pmole/10 g tissue, and various combinations.

Hypothalamic β-endorphin concentrations in this study ranged between 18-22 ng/mg protein. This is comparable to the results of Wardlaw et al. (291), whose methodology was partially adapted in determining β-endorphin and protein levels in this study. Tables 10 and 11 depict the brain β-endorphin results of several different studies. Certain values (291,63,231,16,124) are comparable to the ones reported in this study, while others tend to be lower (58,82,74,108,147). Values become lower if higher protein concentrations are used in the calculations. Ten percent of the brain's wet weight normally is protein. The rats' average hypothalamic weight in this study was 53 mg which would result in a protein content of 5.3 mg if the 10 percent value is assigned. Dividing the average 8.41 ± 0.08 ng of β-endorphin/hypothalamus by 5.3 would result in 1.59 ± 0.02 ng β-endorphin/mg protein; this approximates values reported by Gambert et al. (82) and Forman et al. (74). A Pearson r correlation coefficient of -0.86 ($p < 0.0001$) was observed between ng β-endorphin/mg protein and hypothalamic protein, indicating that as one increased, the other tended to decrease. The correlation of just protein to hypothalamic weight was 0.48 ($p < 0.0001$), which tends to support the possibility that β-endorphin in the hypothalamus is related to protein content.

Another reason that β-endorphin is reported as ng/mg protein is because of the variability that exists when detecting hypothalamic landmarks for dissection. Jenkins (131) reported that the ana-

TABLE 8

HYPOTHALMIC WEIGHT, PROTEIN/HYPOTHALAMUS, AND PROTEIN/G HYPOTHALAMUS AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	HYPOTHALAMUS MG	PROTEIN HYPOTHALAMUS	PROTEIN G HYPOTHALAMUS
OIL	20	46 ± 2^{a}	0.462 ± 0.011^{d}	10.42 ± 0.43^{d}
EST	20	49 ± 2^{ab}	0.420 ± 0.014^{d}	8.66 ± 0.24^{e}
EST/PROG	20	60 ± 3^{c}	0.408 ± 0.015^{d}	6.96 ± 0.25^{f}
PROG	20	55 ± 4^{bc}	0.422 ± 0.018^{d}	7.85 ± 0.29^{ef}

Values represent means $\pm$ SEM.

$^{a-c}$ Means with matching superscripts are not statistically different, $p < 0.05$.

$^{d-f}$ Means with matching superscripts are not statistically different, $p < 0.01$.

TABLE 9

HYPOTHALMIC BETA-ENDORPHIN CONTENT, EXPRESSED AS NG/HYPOTHALAMUS AND NG/G HYPOTHALAMUS AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

		BETA-ENDORPHIN	
GROUP	N	NG/HYPOTHALAMUS	NG/G HYPOTHALAMUS
OIL	20	8.50 ± 0.14^{a}	193.97 ± 10.11^{d}
EST	20	8.95 ± 0.14^{b}	187.14 ± 7.01^{d}
EST/PROG	20	8.30 ± 0.14^{a}	144.22 ± 6.30^{e}
PROG	20	7.90 ± 0.11^{c}	151.59 ± 7.37^{e}

Values represent means $\pm$ SEM.

$^{a-c}$ Means with matching superscripts are not statistically different, $p < 0.05$.

$^{d-e}$ Means with matching superscripts are not statistically different, $p < 0.01$.

TABLE 10

VARIOUS VALUES OF BRAIN BETA-ENDORPHIN CONTENT

TISSUE/CONDITION	BETA-ENDORPHIN NG/MG PROTEIN	REFERENCE
Hypothalamus/estrus	0.525 ± 0.036	
constant estrus	0.262 ± 0.028	(74)
Hypothalamus/control	3.45 ± 1.7	
fasted	1.22 ± 0.3	(82)
Hypothalamus	4.10 (estimated)	(108)
	6.10 ± 1.04	(147)
Arcuate nucleus (beli)	10	
Medium eminence	7 (estimated)	
Periventricular nucleus	4 (estimated)	(58)
N. arcuatus v/proestrus	13 ± 2	(16)
Hupothalamus/control	27.1 ± 1.5	
est	20.7 ± 1.9	
est/prog	23.1 ± 0.86	
controls	31.8 ± 1.4	
early pregnancy	41.4 ± 1.8	
late pregnancy	39.2 ± 1.9	(291)
Hypothalamus/proestrus	6.2 ± 0.4 ng/tissue	
oestrus	5.4 ± 0.4	
metoestrus	8.0 ± 0.1	
diestrus	9.4 ± 0.6	(124)
Hypothalamus	217 ± 32 ng/g tissue	(231)

TABLE 11

REGIONAL DISTRIBUTION OF BETA-ENDORPHIN IN THE BRAIN

Brain area	β-endorphin ng/mg protein
Frontal cortex	ND
Septum	0.77 ± 0.08
Tractus diagonalis	1.06 ± 0.33
Medial preoptic nucleus	21.93 ± 0.96
Lateral preoptic nucleus	2.90 ± 0.13
N. interstitialis striae terminalis	15.46 ± 1.57
N. accumbens	ND
Tubercle olfactorium	ND
Striatum A 9000	ND
Striatum A 7000	—
Globus pallidus	—
N. amygdaloideus medialis A 4250	4.28 ± 1.14
N. amygdaloideus medialis A 3500	3.47 ± 0.29
N. amygdaloideus centralis A 4250	5.76 ± 1.25
N. amygdaloideus lateralis, pars post	3.09 ± 0.91
N. amygdaloideus basalis, pars lat	4.43 ± 1.73
N. amygdaloideus corticalis	ND
N. anterior hypothalami	21.17 ± 1.80
N. lateralis hypothalami	5.81 ± 1.32
N. suprachiasmaticus	25.76 ± 2.83
N. periventricularis	30.79 ± 3.16
Paraventricular nucleus	13.18 ± 0.22
Median eminence	41.78 ± 0.77
Arcuate nucleus I-II-III	29.67 ± 0.37
N. ventromedialis hypothalami	21.84 ± 1.80
N. dorsomedialis hypothalami	25.68 ± 4.92
N. arcuate IV-V	26.39 ± 8.01
N. premamillaris ventralis	9.60 ± 0.40
N. premamillaris dorsalis	4.59 ± 0.95
N. mamillaris medialis, pars medialis	6.32 ± 0.94
N. lateralis thalami	1.84 ± 0.21
N. medialis thalami	6.16 ± 1.41
N. ventralis thalami	1.32 ± 0.41
Hippocampus	1.47 ± 0.20
N. habenulae lateralis	6.32 ± 0.94
Substantia nigra	ND
Area ventralis tegmenti	ND
Formatio reticular + lemniscus medialis	ND
Interpeduncular nucleus	1.33 ± 0.15
Periaqueductal gray	8.19 ± 1.77
N. medianus raphes	ND

The brains were frozen and specific areas were punched as described under Materials and Methods. Means ± SEM of 3 groups of 4 rats (pool) are presented. ND = not detectable. Values in ng/mg of protein.

Source: Dupont et al. (63).

tomical boundaries of the hypothalamus are not well defined. Different methods of removing this section for analysis can influence β-endorphin levels. Even when the hypothalamus is dissected, some researchers argue that the tissue removed from each rat may not be 100 percent hypothalamus. Rats in this study were sacrificed by group instead of random sequence to eliminate bias in technique. The average hypothalamic weight for the four groups was 53 mg, which is slightly larger than the 40 mg which Dorsa (57) suggested would occur in 18 week old rats. Rossier et al. (231) reported that the hypothalamus averaged 31 mg in male rats weighing only 150-200 g. The possibility also exists that ovarian steroid treatments influence hypothalamic weight. Chowers and McCann (37) and Lisk (152) reported that estrogen increased pituitary wet weight. In the presence of estrogen, the uterus also gains weight by imbibing water. Increased myelin formation which can influence fat content also occurs with estrogen treatment. It is difficult to determine whether ovarian steroids influence the protein content of the hypothalamus (171). Anterior hypothalamic protein content has been reported to differ in rats according to gender (241) and gonadectomy (178); however, protein content did not significantly change in female rats during different stages of the estrous cycle (241). Dorsa (57) recommended that β-endorphin be expressed as ng/mg protein because of the large variability that exists when dealing with the hypothalamus. In light of these considerations, β-endorphin was reported as ng/mg protein. When β-endorphin is reported as ng/hypothalamus or g hypothalamus, the control and estrogen groups tend to have the highest β-endorphin levels compared to the estrogen/progesterone and progesterone groups (Table 9). This is reflected from the significantly lighter hypothalamic weights of the control and estrogen groups compared to the estrogen/progesterone group. The progesterone group also had significantly heavier hypothalamic weights than the controls, but was heavier that the estrogen-treated rats (Table 8).

Ovarian Steroids Related to Weight Gain, Food Intake, and β-Endorphin

It appears from the results of this study that elevated hypothalamic β-endorphin is related to the decreased weight gain observed with estrogen treatment. Progesterone treatment seemed to antagonize the suppressed food intake of estrogen. Progesterone also corresponded to lowered hypothalamic β-endorphin concentrations, while estrogen was related to higher hypothalamic β-endorphin levels.

Wardlaw et al. (291) and Wehrenberg et al. (295) suggested that ovarian steroids might be important regulators of brain β-endorphin content. If this is true, then perhaps the natural weight gains occurring with ovariectomy, pseudopregnancy, pregnancy, the reproductive cycle, and oral contraceptive use are related to hypothalamic β-endorphin. The results of the present research and several other studies both support and conflict with this hypothesis.

The increased hypothalamic β-endorphin levels observed in the estrogen treated rats of this experiment support Petraglia et al. (209), who reported increased β-endorphin in the mediobasal hypothalamus and median eminence of estrogen-treated rats. Barden (16) also reported that estrogen-treated ovariectomized rats had increased mediobasal hypothalamic β-endorphin concentrations.

Knuth et al. (139) expected to find higher levels in high dose estrogen treated rats, but he observed no significant difference. Perhaps the five-day treatment too short. Hulse and Coleman (126) also reported no change in hypothalamic β-endorphin, however; they used conjugated estrogen and treatment spanned only 3 days. Wardlaw et al. (289,291) reported data that conflicted with the results of this study. In both of the studies by Wardlaw and associates, hypothalamic β-endorphin decreased with estrogen treatment. In the later study, hypothalamic β-endorphin levels were re-

ported in pmoles instead of beta-endorphin units/weight protein. The results by Wardlaw and associates are supported by Forman et al. (74), who reported decreased hypothalamic β-endorphin in estrogen treated rats when compared to controls in constant estrus. Such controls have significantly lower hypothalamic β-endorphin concentrations than rats on the day of estrus. Petraglia et al. (108) reported increased β-endorphin concentrations in the mediobasal hypothalamus of pregnant rats which have naturally high progesterone levels during pregnancy.

Forman et al. (74) stated that discrepancies in hypothalamic β-endorphin levels associated with ovarian-steroid treatment may depend on whether estrogen treatments are acute or chronic, and how long the animals are ovariectomized. Other possible factors include dose amounts, steroid form, stress, and method or time of sacrifice. Ogawa et al. (195) reported that different sacrifice methods and extracting from the brain can yeild β-endorphin concentrations which vary by tenfold. There are many radioimmunoassay techniques for determining β-endorphin; in some data, especially from early studies, β-endorphin like immunoreactivity (BELI) is reported. As detailed above, the various methods of expressing β-endorphin can influence the results.

Despite discrepencies, the reproductive cycle provides indirect evidence linking ovarian steroids and β-endorphin to body weight. The four-day estrus cycle in the rat is split into four phases: estrus, diestrus I, diestrus II, and proestrus. Estrogen peaks at proestrus (8 a.m. to 4 p.m.), while progesterone peaks twice: proestrus (4 p.m. to 8 p.m.), diestrus I & II (180). Rats lose weight at proestrus, just after estrogen's peak. Knuth et al. (139) reported increased β-endorphin in the arcuate nucleus during proestrus, which supports the results of this study. Opposite data in which hypothalamic β-endorphin decreased during estrus was reported by Hulse et al. (124). Rats regain weight at diestrus, and Hulse et al. reported increased hypothalamic β-endorphin levels during this stage. Such data conflicts with this study's results; however, the four-day rat cycle is quite short, and brain β-endorphin levels have been reported to change from morning to afternoon and even increase/decrease simultaneously in different parts of the brain. Barden et al. (16) reported that on the afternoon of proestrus, β-endorphin decreased by 50 percent in the arcuate nucleus when compared to the mean content of all other days in the cycle. In contrast, during the same time,

β-endorphin increased in the suprachiasmatic nucleus by 100 percent and in the median eminence by 65 percent.

Measuring hypothalamic β-endorphin during different phases of the human reproductive cycle is not practical; however, food intake fluctuations occurring with varying ovarian steroid concentrations has been documented. Dalvit-McPhilips (52) reported increased food intake and weight gain after ovulation when progesterone levels naturally rise. Weizenbaum et al. (296) also reported that females ate more M&M's and peanuts during the luteul phase of the menstrual cycle. Increased food consumption during the luteal phase has also been reported in female rhesus monkeys (227).

Casper et al. (36) reported that women on oral contraceptives, an exogenous source of ovarian steroids, have a tendency to eat sweets, gain weight, and have increased opioid activity. Berger and Talwar (19) also reported increased weight with progestin-based oral contraceptives. Tejawani et al. (267) administered ovarian hormones (in the form of oral contraceptives) to female rats. Their results both support and conflict with the hypothalamic-β-endorphin-adrenal hypothesis of this study. Acute (five days) or chronic (7 weeks) administration of progesterone (norethindrone) to rats resulted in no significant difference in pituitary or hypothalamic β-endorphin when compared to controls. When given acutely, higher doses of progesterone and estrogen (ethinyl estradiol) decreased both pituitary and hypothalamic β-endorphin levels. Chronic doses of the combined higher doses resulted in no significant difference in the pituitary, but decreased hypothalamic β-endorphin levels.

The above studies indicate that hypothalamic β-endorphin does fluctuate with the changing ovarian steroid concentrations observed with pregnancy, the reproductive cycle, and oral contraceptive use. It appears from the results of this study that estrogen treatment is related to increased hypothalamic β-endorphin and suppressed food intake and weight gain. More data needs to be generated to determine if increased hypothalamic β-endorphin is related to decreased weight gain.

The findings of this study challenge the bulk of the evidence suggesting that opioids act to stimulate food intake. Numerous studies report that β-endorphin injected into hypothalamic nuclei stimulates feeding (55,98,150,173,175,235,273). Increased food intake also occurs with morphine injected into the rat hypothalamus, but chronic morphine and heroin are both known to decrease body weight in rats (269,270). Reduced brain β-endorphin levels also occur in rats chronically treated with morphine (184). β-endorphin was named after morphine; yet, Schneider et al. (244) reported that narcotic addicts lost weight while addicted and gained weight after drug abstinance.

The influence of other drugs on hypothalamic β-endorphin levels supports that elevated hypothalamic β-endorphin levels are related to satiety. Amphetamine was administered for 3 weeks by Schultz et al. (245), who observed hypothalamic β-endorphin levels increase by 36 percent. Harsing et al. (108) injected rats with D-fenfluramine, an anorexic drug that releases serotonin, and reported increased hypothalamic β-endorphin content. Chronic treatment with haloperidol, a drug that decreases food intake, increased levels of hypothalamic β-endorphin (118).

Caution is necessary when interpreting data, because acute or chronic doses of drugs often have different results. Nohtomi et al. (194) reported that a single injection of sulpiride, a dopamine receptor antagonist, decreased β-endorphin in the arcuate nucleus, paraventricularis, and median eminence. Repeated sulpiride injections increased β-endorphin concentrations in these same areas. Other conditions support a satiety role for β-endorphin in the hypothalamus. Fasting rats were reported by Gambert et al. (82) to have decreased hypothalamic β-endorphin. Dum et al. (62) observed lower hypothalamic β-endorphin concentrations in rats fed palatable diets.

Proponents of a stimulative role for β-endorphin in food intake have often based their hypothesis on the assumption that the naloxone response is a criterion of an opioid effect. Naloxone, an opioid antagonist, has been demonstrated to depress food intake which theoretically implies that opioids stimulate intake. However, most of the studies demonstrating a decreased food intake with naloxone treatment have measured intake for only several hours and often in food deprived animals. What is frequently not addressed in the results obtained with naloxone is that acute naloxone doses

decrease feeding, but chronic doses increase intake (249). Shimomura et al. (249) reported that lean rats increased consumption 140-200 percent above controls while obese rats elevated their intakes 200 percent. Sanger (237) reported that repeated doses increased intake by 60 percent. In addition, naloxone is also not equally specific for different receptor types (95). An opioid antagonist was not used in this study because of its questionable reliability as an indicator of an opioid effect on food intake. Also, increased weight gain was observed in rats treated with naltrexone in a pilot study.

Nevertheless, naloxone and naltrexone appear to affect food intake and may even involve the hypothalamus. Naloxone treatment was reported by Lee et al. (147) to decrease rat hypothalamic β-endorphin levels, and this would support the results of this study if naloxone actually reduces food intake. Conflicting evidence was reported by Stein (258), who found elevated hypothalamic β-endorphin concentrations with naloxone treatment. Either way hypothalamic β-endorphin appears to be affected and whether this is related to food intake remains to be determined.

Another reason why β-endorphin has been associated with appetite was that initial studies reported that elevated plasma β-endorphin levels occurred with increased food intake. Plasma is the most accessible and practical tissue to measure β-endorphin levels, and as a result, researchers may have been misled from the origin and ultimate control of β-endorphin. The pituitary is the source for circulating β-endorphin, but the hypothalamus has long been known to regulate pituitary peptides and hormones. Initial evidence indicates that hypothalamic and pituitary β-endorphin can not only differ from each other, but also from plasma β-endorphin levels. Alterations in synthesis, degradation, release, reuptake, and/or a combination of these processes can change brain β-endorphin levels. β-endorphin research is relatively new and relationships between hypothalamic, pituitary, and plasma concentrations are not quite clear. Increased plasma β-endorphin may reflect increased release, and therefore decreased hypothalamic and/or pituitary β-endorphin content. Increased hypothalamic and/or pituitary β-endorphin synthesis and turnover may increase these brain levels, or have them remain "unchanged" as the excess overflows to the blood to elevate plasma β-endorphin levels. Pituitary β-endorphin synthesis/turnover may elevate hypothalamic β-endorphin. Removal of the pituitary (hypophysectomy) was reported to decrease brain

β-endorphin by 90 percent (195). This was suggested to occur through retrograde transport from the pituitary to the hypothalamus. Even if elevated β-endorphin levels did occur, it may merely reflect storage pool amounts and be unrelated to food intake.

Studies reporting increased plasma β-endorphin with stimulation of food intake include Guilleman et al. (105), who demonstrated elevated plasma β-endorphin in rats subjected to stress. Fasting plasma levels of obese children and adolescents were reported by Genazzani et al. (84) to be twice that of lean controls. Contrary data has been reported by Awoke et al. (15), who reported that plasma β-endorphin in 15 obese humans were not significantly higher than controls. Margules and Inturrisi (163) reported no significant difference in the plasma β-endorphin of 6 patients with Prader-Labhart-Willi syndrome compared to 7 of their normal siblings. Wallace et al. (288) stated that little evidence exists for the role of circulating β-endorphin in the control of food intake in the rat. McLaughlin and Baile (174) hypothesized that β-endorphin plays a role in satiety. Rats were immunized against plasma β-endorphin and reported by McLaughlin and Baile to have elevated food intakes and body weights. The lowered plasma levels in McLaughlin and Baile's study were related to the decreased food intake and body weight, but not hypothalamic β-endorphin. Such data do not support the hypothesis stated in this study that hypothalamic β-endorphin levels are related to food intake and body weight gain. Research weakening plasma β-endorphin's role in stimulating food intake include Petraglia et al. (210), who reported that drugs enhancing brain serotonin neurotransmission, known to decrease food intake, increased plasma β-endorphin. Serotonin antagonists have also been reported to decrease plasma β-endorphin (31).

Several researchers support the satiety role of opioid peptides and suggest several mechanisms. McCloy and McCloy (170) stated that endorphins and enkephalins are as addictive as morphine, and hypothesized that the purpose of enkephalins is to inhibit the enkephalinergic neurons to switch off feeding in the brain. Shimomura et al. (249) also suggested that endogenous opioids and opioid receptors may play a role in signaling satiety. Decreased appetite is common among morphine addicts. Opiate receptors in the brain led McCloy and McCloy (169) to hypothesize that obesity is a form of autoaddiction to endogenous opiates. Increased levels of opioids may result in

tolerance and therefore larger amounts of food would be required to satisfy the opioid level needed to reach the "reward state". Excess opioids after meals, like uniform doses of morphine, soon cease to satisfy.

Studies cited by Gold et al. (93) reveal that endogenous opioid peptides decrease firing in the locus coeruleus, and that naloxone acts to prevent this decrease in firing (94). The locus coerulus neurons are known to increase firing in response to pain and this is blocked by naloxone. Theoretically, exogenous opiates could lead to a decrease in the synthesis/release of β-endorphin. Removing these exogenous opiates may weaken the ability of the endogenous system to inhibit the locus coeruleus, resulting in withdrawal (94). Addiction was assumed by Collier (45) to involve cellular changes (Figure 5). McCloy and McCloy (169) suggested that opiates inhibit cAMP production and therefore the neuron activity. If inhibition continues, the neuron's capacity to make more cAMP increases and more opiates are required to suppress it, resulting in tolerance. Withdrawal occurs when the removal of excess opiate unleashes the increased capacity to produce cAMP. This results in neuron hyperactivity. Gold et al. (93) supports the role of cAMP in tolerance/dependence. Opiates initially decrease cAMP, but chronic opiate treatments increase cAMP and lead to tolerance. Greater opiate amounts become necessary to decrease cAMP levels. It is known that opioid peptides decrease cerebral calcium content. Calcium and cyclic nucleotide concentrations closely parallel tolerance and dependence development. Giugliano (89) cited an article in which the opioid peptide-receptor decreases intracellular cAMP levels. This rise was blocked by naloxone, an opiate-receptor blocker.

Estrogen is related to suppressed food intake and β-endorphin which may involve mechanisms interacting with the hypothalamus. The brain has receptor sites for estrogen, progesterone (172), and β-endorphin. Morrell et al. (188) demonstrated that many estradiol-concentrating neurons are intermingled with β-endorphin-immunoreactive neurons. The local connections between these two types of cells could allow steroids to act on dopamine-producing neurons which then synapse with adjacent opioid-producing neurons. Steroids may also influence the opioid receptor content of neurons. Morrell et al. suggested that estrogen's ability to increase progesterone brain receptors

could also play a role due to progesterone's influence on opioid mediation of pituitary secretion. The effect of estrogen and progesterone on gonadotropin release has been suggested to be through the catecholamine system (299). Opiates are known to interact with the catecholamine system (203) which is involved in self-stimulation reward (300). β-endorphin levels are high in the hypothalamus and have been suggested to be directlly or indirectly linked to food intake by interacting with brain monoamines (203). Opiate receptors have been reported to be localized on dopamine terminals (69).

Morley et al. (182) suggested that food intake is via a tonic dopaminergic-opioid mechanism in the lateral hypothalamus. Estrogen has potent anti-dopamine action (64,96,128). Dopamine receptor sensitivity in the rat striatum is altered by estrogen treatment (64,96). Ferland et al. (71) presented data in which 17-β-estradiol increases hypothalamic dopamine release. The results of the present study which reported increased hypothalamic β-endorphin levels with estrogen treatment are supported by Hollt and Bergmann (118), who demonstrated that dopamine receptor antagonists increase hypothalamic β-endorphin. Locatteli et al. (153) reported decreased β-endorphin in the mediobasal hypothalamus and median eminence in rats treated with dopamine agonists. The lowered food intake and weight gain in the estrogen-treated group is supported by Harsing et al. (108), who stated that the anorexia produced by D-fenfluramine might be partially due to inhibition of β-endorphin release from the hypothalamus.

Estrogen is capable of lowering brain β-dopamine hydroxylase, the enzyme responsible for converting dopamine to norepinephrine. Wise et al. (299) suggested that falling norepinephrine levels are related to satiety and this would further support the results of this study. Progesterone, which antagonizes estrogen's effect, is known to increase norepinephrine levels. Progesterone was also suggested by Morley et al. (184) to decrease opioid receptor sensitivity. It has been suggested that the dopamine mediated components of feeding could be partially inhibited by estrogen and β-endorphin, in addition to GABA, acetylcholine, serotonin, and norepinephrine systems (203).

Adrenal Weights

Other evidence supporting a role for β-endorphin in food intake stems from research investigating the hypothalamic-pituitary-adrenal axis. The weight gain observed in ovariectomized rats (103,147,179,259) and genetically obese rats is abolished by adrenalectomy (303,234). Obesity was reported by Dallman (50) to be prevented by adrenalectomy in all animal models tested. Lee et al. (147) reported elevated hypothalamic β-endorphin in adrenalectomized, rats. Stein (258) reported opposite results.

The adrenals appear to play a crucial role in weight regulation. Estrogen and estrogen/progesterone groups had significantly heavier adrenals than the progesterone or control groups. There was no significant difference between the estrogen and estrogen/progesterone groups, or between the controls and progesterone. The same pattern of significance was observed when adrenal weight was reported per 100 g body weight (Table 12). Rats with the heavier adrenals ingested and weighed the least. The results of this study support Muellar (189), who reported that estrogen treatment consistently resulted in adrenal hyperplasia, although no data was provided for comparison. A Pearson correlation coefficient of -0.29 ($p < 0.01$) was observed between adrenal weight and body weight which tends to support the existance of a small relationship between the two in regard to estrogen treatment. Perhaps the adrenal hyperplasia is partially due to estrogen's ability to stimulate cortisol secretion and increase corticosteroid-binding globulin levels (41).

Plasma Corticosterone

Weight regulation may involve a relationship between brain β-endorphin and ovarian steroids through the hypothalamic-pituitary-adrenal axis. Yuckimura (304) reported that food intake was

TABLE 12

ADRENAL WEIGHT AND ADRENAL WEIGHT MG/100 G BODY WEIGHT AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	ADRENAL WT. MG.	ADRENAL WT. MG./100G BW
OIL	20	66 ± 3^{a}	18.7 ± 0.9^{c}
EST	20	77 ± 3^{b}	24.4 ± 1.0^{d}
EST/PROG	20	75 ± 3^{b}	22.4 ± 0.8^{d}
PROG	20	62 ± 2^{a}	17.2 ± 0.6^{c}

Values represent means $\pm$ SEM.

$^{a-b}$ Means with matching superscripts are not statistically different, $p < 0.05$.

$^{c-d}$ Means with matching superscripts are not statistically different, $p < 0.01$.

restored with corticosterone treatment in fatty rats that had lowered food intake due to adrenalectomy. Saito and Bray (234) also reported that the food intake/weight gain of adrenalectomized ob/ob mice receiving cortisone returned to the levels observed in intact ob/ob mice. Adrenal glucocorticoids were suggested by Saito and Bray (234) to play a role in the phenotypic expression of genetic obesity. Estrogen stimulates increased adrenal glucocorticoids which are potent inhibitors of β-endorphin secretion (189). The adrenal gland is stimulated by estrogen to secrete cortisol (41) (corticosterone in the rat) which then inhibits the release of CRF from the hypothalamus (Figure 6). Normally, CRF would then signal the concomitant release of ACTH and β-endorphin from the pituitary. ACTH, often stimulated by stress, signals cortisol secretion from the adrenal cortex.

No significant difference was found in the plasma corticosterone levels of the four treatment groups in this study which may have been due to the very large SEMs (Table 13). The baseline range for rat corticosterone levels, according to the RIA kit, is 100-250 ng/ml which is quite large. The results of this study conflict with those of Muellar (189), who reported that estrogen treatment resulted in a dose-related increase in plasma corticosterone levels. Muellar detected a difference using five estradiol benzoate doses, while one dose was used in this study. Corticosterone levels also vary throughout the day and values can jump 2-3 fold by the mere perception of a stressful situation.

Ovarian steroids influence the hypothalamic-pituitary-adrenal axis and therefore β-endorphin, which may explain why β-endorphin levels change with ovarian hormone concentrations as reported in the results of this and other studies. β-endorphin parallels corticosterone's rhythm during estrus, and levels of corticosterone and β-endorphin are altered concomitantly with ACTH (125).

Although there appears to be some connection between the hypothalamic-pituitary-adrenal axis, β-endorphin, and ovarian steroids, the exact link(s) remains a mystery. It remains to be determined whether the β-endorphin involved is of a hypothalamic origin. To determine if corticosterone is related to hypothalamic β-endorphin, several doses could be administered to rats followed by hypothalamic β-endorphin radioimmunoassays. Another study could administer corticosterone

TABLE 13

PLASMA CORTICOSTERONE LEVELS AFTER TREATMENT WITH OIL, ESTROGEN, ESTROGEN + PROGESTERONE, AND PROGESTERONE TO OVARIECTOMIZED RATS FOR 14 DAYS.

GROUP	N	PLASMA CORTICOSTERONE NG/ML
OIL	20	91.9 ± 21.0[a]
EST	20	124.3 ± 23.9[a]
EST/PROG	20	165.4 ± 37.2[a]
PROG	20	128.2 ± 15.5[a]

Values represent means ± SEM.

a Means with matching superscripts are not statistically different, $p < 0.01$.

replacements to adrenalectomized rats to observe if the weight gain before and after adrenalectomy was related to hypothalamic, pituitary, or plasma β-endorphin.

Data Summary

The data are summarized in Table 14. Estrogen- and estrogen/progesterone-treated groups were significantly different from control and progesterone groups in four ways; 1) lower body weight gains (estrogen > estrogen/progesterone), 2) lower food intakes, 3) higher adrenal weights, and 4) higher ng β-endorphin/mg protein in the hypothalamus. No significant difference occurred with total food intake/100 g body weight which indicates that weight gain was related to food intake. Hypothalamic β-endorphin, when expressed as ng/mg protein, appeared in this study to be affected by ovarian steroid hormone treatment and to be related to the weight gain.

A follow-up to the present study would pair-feed the animals administered with different steroid treatments to determine if hypothalamic β-endorphin was related to food intake and weight gain or the steroid treatment. A problem would occur if body weight were influenced by the ovarian steroids regardless of food intake. Ideally, pituitary and plasma β-endorphin concentrations would be determined in addition to hypothalamic β-endorphin.

Regardless of which tissues are analyzed for β-endorphin concentrations, caution is necessary when relating it to food intake. Data does exist, but more often than not, it conflicts. The research controversy of Margules et al. (166) and Rossier et al. (230) should not be forgotten. Margules et al. (166) reported that genetically obese mice contained double the pituitary β-endorphin levels of non-obese controls. Weight gain was abolished following pituitary removal. Margules et al. (166)

TABLE 14

TOTAL BODY WEIGHT GAIN, DAILY FOOD INTAKE, TOTAL FOOD INTAKE/100 G BODY WEIGHT, HYPOTHALAMIC BETA-ENDORPHIN (NG/MG PROTEIN, NG/HYPOTHALAMUS, NG/G HYPOTHALAMUS), HYPOTHALAMIC WEIGHT, PROTEIN/HYPOTHALAMUS, PROTEIN/G HYPOTHALAMUS, ADRENAL WEIGHT, ADRENAL WEIGHT/100 G BODY WEIGHT, AND PLASMA CORTICOSTERONE.

	TOTAL WT GAIN	DAILY FOOD INTAKE	FOOD INTAKE G / 100 G BW	BETA-ENDORPHINNG / MG PROTEIN	BETA-ENDORPHIN NG / HYPOTHALAMUS
OIL	45.1 ± 2.4[a]	27.0 ± 0.6[a]	106.1 ± 1.7[a]	18.59 ± 0.47[a]	8.50 ± 0.14[a]
EST	9.2 ± 1.2[b]	24.8 ± 0.4[b]	107.7 ± 1.6[a]	21.62 ± 0.56[b]	8.95 ± 0.14[b]
EST/PROG	24.3 ± 2.9[c]	25.4 ± 0.4[b]	105.2 ± 1.6[a]	20.80 ± 0.72[bc]	8.30 ± 0.14[a]
PROG	48.8 ± 2.4[a]	27.1 ± 0.5[a]	104.4 ± 1.9[a]	19.35 ± 0.83[ac]	7.90 ± 0.11[c]
	($p < 0.01$)	($p < 0.05$)	($p < 0.01$)	($p < 0.05$)	($p < 0.05$)

a-c Means with matching superscripts are not statistically different.

TABLE 14 (continued)

TOTAL BODY WEIGHT GAIN, DAILY FOOD INTAKE, TOTAL FOOD INTAKE/100 G BODY WEIGHT, HYPOTHALAMIC BETA-ENDORPHIN (NG/MG PROTEIN, NG/HYPOTHALAMUS, NG/G HYPOTHALAMUS), HYPOTHALAMIC WEIGHT, PROTEIN/HYPOTHALAMUS, PROTEIN/G HYPOTHALAMUS, ADRENAL WEIGHT, ADRENAL WEIGHT/100 G BODY WEIGHT, AND PLASMA CORTICOSTERONE.

GROUP	BETA-ENDORPHIN NG / G HYPOTHALAMUS	HYPOTHALAMUS MG	PROTEIN MG / HYPOTHALAMUS	PROTEIN MG / G HYPOTHALAMUS	ADRENAL MG
OIL	193.97 ± 10.11[a]	46 ± 2[a]	0.462 ± 0.011[a]	10.42 ± 0.43a	66 ± 3a
EST	187.14 ± 7.01[a]	49 ± 2[ab]	0.420 ± 0.014[a]	8.66 ± 0.24[b]	77 ± 3[b]
EST/PROG	144.22 ± 6.30[b]	60 ± 3[c]	0.408 ± 0.015[a]	6.96 ± 0.25[c]	75 ± 3[b]
PROG	151.59 ± 7.37[b]	55 ± 4[bc]	0.422 ± 0.018[a]	7.85 ± 0.29[bc]	62 ± 2[a]
	($p < 0.01$)	($p < 0.05$)	($p < 0.01$)	($p < 0.01$)	($p < 0.05$)

[a-c] Means with matching superscripts are not statistically different.

TABLE 14 (continued)

TOTAL BODY WEIGHT GAIN, DAILY FOOD INTAKE, TOTAL FOOD INTAKE/100 G BODY WEIGHT, HYPOTHALAMIC BETA-ENDORPHIN (NG/MG PROTEIN, NG/HYPOTHALAMUS, NG/G HYPOTHALAMUS), HYPOTHALAMIC WEIGHT, PROTEIN/HYPOTHALAMUS, PROTEIN/G HYPOTHALAMUS, ADRENAL WEIGHT, ADRENAL WEIGHT/100 G BODY WEIGHT, AND PLASMA CORTICOSTERONE.

GROUP	ADRENAL MG 100 G BW	PLASMA CORTICOSTERONE NG/ML
OIL	18.7 ± 0.9^{a}	91.9 ± 21.0^{a}
EST	24.4 ± 1.0^{b}	124.3 ± 23.9^{a}
EST/PROG	22.4 ± 0.8^{b}	165.4 ± 37.2^{a}
PROG	17.2 ± 0.6^{a}	128.2 ± 15.5^{a}

[a-c] Means with matching superscripts are not statistically different.

concluded that the increased pituitary β-endorphin may play a role in the hyperphagia of obesity. Rossier et al. (230) questioned the findings of Margules and associates, when they reported that the elevated pituitary β-endorphin occurred 3 months after birth -- after the greatest percentage of weight gain had occurred in the ob/ob mice.

It appears from the results of this study that increased hypothalamic β-endorphin is related to the suppressed food intake and weight gain observed in estrogen and estrogen/progesterone treated rats. However, enough information exists to question this hypothesis, and a conservative approach would wait for the generation of additional data. Different methods of reporting β-endorphin content can alter results, and must be considered when generating conclusions. Protein was used as a baseline because of hypothalamic variability; and therefore, β-endorphin was reported as ng/mg protein. Also, conflicting evidence supporting a stimulative role for β-endorphin in food intake cannot be ignored. The elevated hypothalamic β-endorphin levels reported in this study do not imply a functional effect. The possibility exists that β-endorphin is being stored, synthesized, and/or even released in the hypothalamus with no effect on food intake. Turnover of hypothalamic β-endorphin was not measured and is a crucial factor in understanding its relationship, if any, to food intake. Reactions at the molecular level within and between cells determine functions on the larger scale. Reported hypothalamic β-endorphin levels and other indirect evidence exist to suggest, but not substantiate, a link between β-endorphin body weight, food intake, and ovarian steroids. Additional indirect support of this study's hypothesis is provided by the following section on implications.

Implications

Studying the link between ovarian steroid weight gain/loss and β-endorphin may not only shed more light on appetite, but help decipher the unknown mechanisms behind certain related medical and psychiatric conditions. The hypothalamus regulates numerous physiological functions such as food intake through several systems involving dopamine, norepinephrine, acetycholine, serotonin, prostaglandin, and GABA. Both β-endorphin and ovarian steroids interact with these systems in the hypothalamus. The possibility that estrogen and progesterone interact with β-endorphin to influence the catecholamine system lends support to the statement by Labrie and associates that the ovarian hormones have clinical implications in neurological as well as psychiatric diseases. Catecholamine imbalance has been associated not only with appetite, but to schizophrenia, depression, and Parkinson's disease. Depression, which is more prevalent in women, has been linked to ovarian steroids (83) and β-endorphin (4,43). Hypothyroidism, and its often accompanying depression, occur more frequently in women than in men (92,305). Tricyclic antidepressants have long been known to affect appetite (116).

Ovarian hormones and β-endorphin have also both been linked to the immune system (72,162,256). Certain autoimmune disorders were stated by Grossman (102) to be significantly influenced by sex steroid hormones. Women are more prone to certain medical conditions such as lupus erythematosus, idiopathic thrombocytompenic purpura, and rheumatoid arthritis. A genetic defect occurs in women with Turner's syndrome who are believed to carry an increased risk of obesity (151), and the disease has been paradoxically linked to anorexia nervosa (142).

Endogenous opioids are capable of inhibiting gonadotropin release and influencing prostaglandin activity. Certain menopause symptoms have been hypothesized by Casper and Alapin-Rubillovitz (35) to be due to endogenous opioid withdrawal. Ried and Yen (222) suggested that opioids are related to premenstrual syndrome. Increased food intake and weight gain have also been reported

to occur after ovulation (51,52,227) and oral contraceptive use (19,36). Ovarian steroids may also influence long term body weight. Hoyenga and Hoyenga (121) stated that almost all weight surveys from almost every country have found women to be more obese than men at all ages. Twenty-four percent of American women are obese, compared to only 14 percent of the men. Among genetically or dietetically obese rodents, females often get fatter than males (121).

The relationship between β-endorphin, ovarian steroid hormones, and various medical conditions which may involve the hypothalamic-pituitary-adrenal axis. Stress increases opioid concentrations which may disrupt the luteinizing hormone surge and block ovulation (127). Jewelewicz (132) and Russell et al. (233) suggested that increased plasma β-endorphin and catechol estrogens occurring with strenuous exercise may help explain the oligomenorrhea observed in some female athletes. Females with oligo-amenorrhea were reported to have elevated plasma β-endorphin levels (90). Women with polycystic ovarian disease were also reported by Aleem and McIntosh (5) to average 124 percent above their ideal body weight which was significantly correlated to plasma β-endorphin. β-endorphin, like morphine (40), acts on the hypothalamic-pituitary axis (223) to decrease luteinizing hormone. Humans addicted to morphine often experience amenorrhea and sterility (139).

β-endorphin and ovarian steroids interacting with the hypothalamic-pituitary-adrenal axis may involve other conditions such as Cushing's disease (119) which is more prevalent in women, and Addison's disease (190,261) which afflicts more men. Anorexia nervosa (56,87,137) and bulemia (80,106) have been related to β-endorphin and the hypothalamic-pituitary-adrenal axis. β-endorphin interacting with the hypothalamic-pituitary-adrenal axis has also been reported in diabetes (74,101), alcoholism (114), Nelson's syndrome (190), and stress-induced eating (105,187).

Medical dysfunctions, which influence β-endorphin, ovarian steroids, and the hypothalamic-pituitary-adrenal axis, provide clues to the master mechanism map governing food intake and perhaps numerous other regulatory systems based in the hypothalamus.

chapter V

SUMMARY AND CONCLUSION

This study was conducted to test the hypothesis that hypothalamic β-endorphin levels were related to body weight and food intake changes occurring with ovarian steroid treatments.

Eighty adult female Sprague-Dawley (Charles River) were ovariectomized and allowed to recover for 7 days. Rats in four treatment groups of 20 each were subjected to daily injections for 14 days with A) oil, B) β-estradiol benzoate (2μg), C) β-estradiol benzoate (2μg) plus progesterone (5mg), or D) progesterone (5mg). Body weight and food intake was measured daily. After sacrifice, the hypothalamus was immediately dissected, weighed, and prepared for radioimmunoassay analysis of β-endorphin. Adrenals were dissected and weighed. Trunk blood was collected for analysis of plasma corticosterone.

In the estrogen-treated group, weight gain was significantly lower ($p < 0.01$) than the control, estrogen/progesterone, or progesterone groups. The estrogen/progesterone group also gained significantly less than the control or progesterone groups. A significant ($p < 0.05$) decrease in food intake was also observed in the estrogen and estrogen/progesterone groups compared to the control

or progesterone groups, but not between each other. The progesterone-treated group was not significantly different from the controls in either weight gain or food intake.

Hypothalamic β-endorphin (ng/mg protein) levels were significantly ($p < 0.05$) higher in the estrogen- and estrogen/progesterone-treated groups, compared to the control and progesterone groups. Again, much like the weight gain, food intake, and hypothalamic β-endorphin (ng/mg protein), the estrogen and estrogen/progesterone groups had significantly ($p < 0.05$) heavier adrenal weights when compared to the control and progesterone groups. There was no significant difference in plasma corticosterone levels between any of the groups.

Ovarian steroid treatment appearded to influence hypothalamic β-endorphin (ng/mg protein). Whether this is related to the weight gain also observed with the treatments remains to be determined. Elevated hypothalamic β-endorphin levels do not necessarily imply a functional effect. The possibility exists that β-endorphin is being stored, synthesized, and/or released in the hypothalamus with no effect on food intake.

Numerous components of appetite have been studied to determine if it is the long awaited key to food intake regulation. β-endorphin and steroids are no exception. They are just two of the many links fitting snugly into an interdependent chain of events. Altering one, more than likely influences others. Both β-endorphin and ovarian steroids are known to interact with the hypothalamus through dopamine, norepinephrine, acetylcholine, serotonin, prostaglandin, and gabaminergic systems. These physiological systems are not only linked to food intake, but numerous other regulatory mechanisms in the brain. Further investigation may not only shed more light on appetite, but help decipher the unknown mechanisms behind certain related medical and psychiatric conditions.

Bibliography

1. Abou Samra AB, Loras B, Pugeat M, Tourmiaire J, Bertrand J. Demonstration of an antiglucocorticoid action of progesterone on the corticosterone inhibition of beta-endorphin release by rat anterior pituitary in primary culture. *Endocrinology* 115:1471-1475, 1984.

2. Akil H, Shio H, Mathews J. Induction of the intermediate pituitary by stress: Synthesis and release of a nonopioid form of beta-endorphin. *Science* 227:424-427, 1985.

3. Akil H, Watwon SJ, Barchas JD, Li CH. Beta-endorphin immunoreactivity in rat and human blood: radioimmunoassay, comparative levels and physiological alterations. *Life Sci* 24:1659, 1979.

4. Albala AA, Carroll BJ, Lohr NE. Opioid regulation of hypothalamic-pituitary-adrenal function in depression. *Arch Gen Psychiatry* 42(4)383-386, 1985.

5. Aleem FA, McIntosh T. Elevated plasma levels of beta-endorphin in a group of women with polycystic ovarian disease. *Fert and Steril* 42(5):686-689, 1984.

6. Amir S, Bernstein M. Endogenous opioids interact in stress-induced hyperglycemia in mice. *Physiol & Behav* 28:575-577, 1982.

7. Amir S, Brown ZW, Amit Z. The role of endorphins in stress: Evidence and speculations. *Neurosci Biobehav Rev* 4:77-86, 1979.

8. Antelman SM, Rowland N. Endogenous opiates and stress-induced eating. *Science* 214:1149-1150, 1981.

9. Antelman SM, Caggiula AR. Norepinephrine-dopamine interactions and behavior. *Science* 195:646-653, 1977.

10. Ashby JP, Shirling D, Baird JD. Differential changes in body composition during growth and progesterone treatment in intact female rats. *J Endocr* 93:391-395, 1982.

11. Ashby JP, Shirling D, Baird JD. Effect of progesterone on the secretion and peripheral acton of insulin and glucagon in the intact rat. *J Endocr* 88:49-55, 1981.

12. Ashby JP, Shirling D, Baird JD. Effect of progesterone on insulin secretion in the rat. *J Endocr* 76:479-486, 1978.

13. Atkinson RL, Berke LK, Drake CR, Bibbs ML, Williams FL, Kaiser DL. Effects of long-term therapy with naltrexone on body weight in obesity. *Clin Pharmacol Ther* 38:419-422, 1985.

14. Atkinson RL. Naloxone decreases food intake in obese humans. *J Clin Endocrin Metab* 55:196-198, 1982.

15. Awoke S, Voyles N, Wade A, Smith S, Bhathena S, Tanenberg R, Recant L. Altered opiate levels in obese human subjects. *Diabetes* 31(2)61A, 1982.

16. Barden N, Merand Y, Rouleau D, Garon M, Dupont A. Changes in the Beta-endorphin content of discrete hypothalamic nuclei during the estrous cycle of the rat. *Brain Res* 204:441-445, 1981.

17. Barden N, Merand Y, Rouleau D. A physiological role of beta-endorphin in the control of prolactin secretion during the estrous cycle in the rat. *Program of the 62nd Annual Meeting of the Endocrine Society*. (Abstract) Washington D.C., p.222.

18. Belluzzi JD, Stein L. Enkephalin may mediate euphoria and drive-reduction reward. *Nature* 266:556-558, 1977.

19. Berger GS, Talwar PP. Oral contraceptive potencies and side effects. *Obstet Gynecol* 51(5)545, 1978.

20. Bernardis LL, Skelton FR. Growth and obesity following ventomedial hypothalamic lesions placed in female rats at four different ages. *Neuroendocrinology* 1:265, 1965-66.

21. Biegon A, Reches A, Snyder L, McEwen BS. Serotonergic and noradrenergic receptors in the rat brain: modulation by chronic exposure to ovarian hormones. *Life Sci* 32:2015-2021, 1983.

22. Blaustein JD, Wade GN. Ovarian influences on the meal patterns of female rats. *Physiol Behav* 17(2)201-208, 1976.

23. Blum K. Psychogenics of drug seeking behavior. In: Muller EE, Genazzani AR (eds). Central and Peripheral Endorphins: Basic and Clinical Aspects. Raven Press, 1984. Pgs.339-356.

24. Bohus B, De Kloet ER. Adrenal steroids and extinction behavior: antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone. *Life Sci* 28:433-440, 1981.

25. Bradford MM. A rapid Sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254, 1976.

26. Brands JP, Thronhill JA, Hirst M, Gowdey CW. Suppression of food intake and body weight gain by naloxone in rats. *Life Sci* 24:1773-1778, 1979.

27. Bray GA. Autonomic and endocrine factors in the regulation of food intake. *Brain Res Bull* 14(6)505-510, 1985.

28. Bray GA. Endocrine factors in the control of food intake. *Federation Proc* 33(5)1140-1145, 1974.

29. Brown DR, Holtzman SG. Suppression of deprivation-induced food and water intake in rats and mice by naloxone. *Pharmacol Biochem Behav* 11:567-573, 1979.

30. Browning AJF, Butt WR, Lynch SS, Shakespear RA. Maternal plasma conctrations of beta-lipotrophin, beta-endorphin and gamma-lipotrophin throughout pregnancy. *Br J Obstet Gynecol* 90:1147-1151, 1983.

31. Bruni JF, Hawkins RL, Yen SSC. Serotonergic mechanism in the control of beta-endorphin and ACTH release in male rats. *Life Sci* 30:1247-1254, 1982.

32. Buckingham JC, Cooper TA. Influence of opioid substances on hypothalamo-pituitary-adrenocortical activity in the rat. In: Delitala G, Motta M, Serio M (eds). Opioid Regulation of Endocrine Function. Raven Press, 1984. Pgs.81-87.

33. Bueno J, Pfaff DW. Single unit recording in hypothalamus and preoptic area of estrogen-treated and untreated ovariectomized female rats. *Brain Res* 101:67-78, 1976.

34. Carr DB, Bullen BA, Skrinar GS, Arnold MA, Rosenblatt M, Beitins IZ, Martin JB, McArthur JW. Physical conditioning facilitates the exercise-induced secretion of beta-endorphin and beta-lipotropin in women. *N Eng J Med* 305: 560-563, 1981.

35. Casper RF, Alapin-Rubillovitz S. Progestins increase endogenous opioid peptide activity in postmenopausal women. *J Clin Endocrinol Metab* 60:34-36,1985.

36. Casper RF, Bhanot R, Wilkinson M. Prolonged elevation of hypothalamic opioid peptide activity in women taking oral contraceptives. *J Clin Endocrinol Metab* 58:582-584, 1984.

37. Chowers I, McCann SM. Comparison of the effect of hypothalamic and pituitary implants of estrogen and testosterone on reproductive system and adrenal of female rats. *Proc Soc Exp Biol Med* 124:260-6, 1967.

38. Chretien M, Seidah NG, Dennis M. Processing of precursor polyproteins in rat brain: regional differences in acetylation of POMC peptides. In: Muller EE, Genazzani AR (eds). Central and Peripheral Endorphins: Basic and Clinical Aspects. Raven Press, 1984. Pgs.27-33.

39. Cicero TJ, Schainker BA, Meyer ER. Endogenous opioids participate in the regulation of the hypothalamic-pituitary-luteinizing hormone axis and testoserones negative feedback control of luteinizing hormone. *Endocrinology* 104:1286-1291, 1979.

40. Cicero TJ, Badger TM, Wilcox CE, Bell RD, Meyer ER. Morphine decreases luteinizing hormone by an action on the hypothalamic-pituitary axis. *J Pharmacol Exp Ther* 203:548, 1977.

41. Coe CL, Murai JT, Wiener SG, Levine S, Siiteri PK. Rapid cortisol and corticosteroid-binding globulin responses during pregnancy and after estrogen administration in the squirrel monkey. *Endocrinology* 118(1)435-440, 1986.

42. Cohen MR, Cohen RM, Pickar D, Murophy DL. Naloxone reduces food intake in humans. *Psychosomat Med* 47:132-138, 1985.

43. Cohen MR, Pickar D, Extein I, Gold MS, Sweeney DR. Plasma cortisol and beta-endorphin immunoreactivity in nonmajor and major depression. *Am J Psychiatry* 141(5)628-632, 1984.

44. Collier HOJ, Francis DL, McDonald-Gibson WJ, Roy AC, Saeed SA. Prostaglandins, cyclic AMP and the mechanism of opiate dependence. *Life Sci* 17:85-90, 1975.

45. Collier HOJ. Consequences of interaction between opioid and receptor. *Biochem Soc Trans* 5(1)70-73, 1977.

46. Colt EWD, Wardlaw SL, Frantz AG. The effect of running on plasma beta-endorphin. *Life Sci* 28:1637-1640, 1981.

47. Copolov DL, Helm RD. Enkephalins and endorphins: clinical, pharmacological and therapeutic implications. *Drugs* 26:503-519, 1983.

48. Costrini NV, Kalkhoff RK. Relative effects of pregnancy, estradiol, and progesterone on plasma insulin and pancreatic islet insulin secretion. *J Clin Invest* 50:992-999, 1971.

49. Csontos K, Rust M, Hollt V, Mahr W, Kromer W, Teschemacher HJ. Elevated beta-endorphin levels in pregnant women and their neonates. *Life Sci* 25:835, 1979.

50. Dallman M, Bray GA. Neural and endocrine regulation of ingestive behavior. *Fed Am Soc Exp Biol* 45(5)1383, 1986.

51. Dalvit SP. The effect of the menstrual cycle on patterns of food intake. *Am J Clin Nutr* 34:1811-1815, 1981.

52. Dalvit-McPhillips SP. The effect of the human menstrual cycle on nutrient intake. *Physiol Behav* 31(2)209-212, 1983.

53. Dark J, Wade GN, Zucker I. Ovarian modulation of lipoprotein lipase activity in white adipose tissue of ground squirrels. *Physiol Behav* 32(1)75-78, 1984.

54. Davis JM, Lowly MT, Yim GKW, Lamb DR, Malven PV. Relationship between plasma concentrations of immunoreactive beta-endorphin and food intake in rats. *Peptides* 4(1)79-83, 1983.

55. Della-Fera MA, Baile CA. Control of feed intake in sheep. *J Animal Sci* 59(5)1362-1368, 1984.

56. Donohoe TP. Stress-induced anorexia: implications for anorexia nervosa. *Life Sci* 34:203-218, 1984.

57. Dorsa DM. Personal communication.

58. Dorsa DM. Localization and identification of gamma-endorphin and beta-endorphin-like peptides in the hypothalamus and ventral forebrain of the rat. *Life Sci* 33:337-345, 1983.

59. Dorsa DM, Majumdar LA, Chapman MB. Regional distribution of gamma & beta endorphin-like peptides in the pituitary and brain of the rat. *Peptides* 1(Suppl. 1):71-77, 1981.

60. Drago F, Facchinetti F, Petraglia F, Panissidi GB, Bellomio F, Genazzani AR, Scapagnini U. Ocular opioids: distribution and function. In: Muller EE, Genazanni AR (eds). Central and Peripheral Endorphins: Basic and Clinical Aspects. Raven Press, 1984. Pgs.151-156.

61. Dubuc PU. Effects of estrogen on food intake, body weight, and temperature of male and female obese mice. *Proc Soc Exp Biol* 180:468-473, 1985.

62. Dum J, Gramsch CH, Herz A. Activation of hypothalamic beta-endorphin pools by reward induced by highly palatable food. *Pharmacol Biochem Behav* 18(3)443-447, 1983.

63. Dupont A, Barden N, Cusan L, Merand Y, Labrie F, Vaudry H. Beta-endorphin and met-enkephalins: their distribution, modulation by estrogens and haloperidol, and role in neuroendocrine control. *Federation Proc* 39:2544-2550, 1980.

64. Euvrard C, Oberlander C, Boissier JR. Antidopaminergic effect of estrogens at the striatal level. *J Pharmacol Exp Ther* 214(1)179-185, 1980.

65. Evans CJ, Erdelyi E, Weber E, Barchas JD. Identification of pro-opiomelanocortin-derived peptides in the human adrenal gland. *Science* 221:957-960, 1983.

66. Facchinetti F, Giovannini C, Barletta C, Petraglia F, Buzzetti R, Burla F, Lazzari R, Genazzani AR, Scavo D. Hyperendorphinemia in obesity and relationships to affective state. *Physiol Behav* 36:937-940, 1986.

67. Facchinetti F, Volpe A, Nappi G, Petraglia F, Genazzani AR. Impairment of adrenergic-induced proopiomelacortin-related peptide release in heroin addicts. *Acta Endocrinol* 108(1)1-5, 1985.

68. Feder HH, Ruf KB. Stimulation of progesterone release and estrous behavior by ACTH in ovariectomized rodents. *Endocrinology* 84:171-174, 1969.

69. Feigenbaum JJ, Yanai Y. The role of dopaminergic mechanisms in mediating the central behavioral effects of morphine in rodents. *Neuropsychobiology* 11:98-105, 1984.

70. Ferin M, Van Vugt D, Wardlaw S. The hypothalamic control of the menstrual cycle and the role of endogenous opioid peptides. *Rec Prog Horm Res* 40:441-477, 1981.

71. Ferland L, Meunier H, Di Paolo T, Giguer V, Veilleux R, Labrie F. The pituitary gland: a model for the study of dopaminergic systems. In: Collu R, Ducharme JR, Barbeau A, Tolis G. Brain Neurotransmitters and Hormones Pgs.55-65. Raven Press, 1982.

72. Fischer EG, Falke NE. Beta-endorphin modulates immune functions. *Psychother Psychosom* 42:195-204, 1984.

73. Foley KM, Kourides IA, Inturrisi CE, Kaiko RF, Zaroulis CG, Posner JB, Houde RW, Hao Li C. Beta-endorphin: analgesic and hormonal effects in humans. *Proc Natl Acad Sci* 76(10)5377-5381, 1979.

74. Forman LJ, Marquis DE, Stevens R. The effect of chronic estrogen treatment on immunoreative beta-endorphin levels in intact female rats. *Proc Soc Exp Biol* 197:365-372, 1985.

75. Forman LJ, Marquis DE, Stevens R, Adler R, Vasilenko P. Diabetes induced by streptozocin results in a decrease in immunoreactive beta-endorphin levels in the pituitary and hypothalamus of female rats. *Diabetes* 34:1104-1107, 1985.

76. Fraioli F, Fabbri A, Moretti C, Santoro C, Isidori A. Endogenous opioid peptides and neuroendocrine correlations in a case of congenital indifference to pain. *Endocrinology* 108:238A, 1981.

77. Fraioli FC, Moretti D, Paolucci E, Alicicco E, Crescenzi F, Fortunio G. Physical exercise stimulates marked concomitant release of beta-endorphin and adrenocorticotropin hormone (ACTH) in peripheral blood in man. *Experientia* 36:987-989, 1980.

78. Frantz AG, Wardlaw SL, Ragavan VV, Thoron L, Wehrenberg WB, Ferin M. Opioid regulation of pituitary secretion. In: Liss AR. Physiopathology of Hypophysial Disturbances and Diseases of Reproduction. Alan R. Liss, Inc., 1982. Pgs.153-177.

79. Frenk H, Rogers GH. The suppressant effect of naloxone on food and water intake in the rat. *Behav Neural Biol* 26:23-40, 1979.

80. Fullerton DT, Getto CJ, Swift WJ, Carlson IH. Sugar, opioids and binge eating. *Brain Res Bull* 14:673-680, 1985.

81. Gambert SR, Garthwaite TL, Pontzer CH, Cook EE, Tristani FE, Duthie EH, Martinson DR, Hagen TC, McCarty DJ. Running elevates plasma beta-endorphin immunoreactivity and ACTH in untrained human subjects. *Proc Soc Exp Biol Med* 168:1-4, 1981.

82. Gambert SR, Garthwaite TL, Pontzer CH, Hagen TC. Fasting associated with decrease in hypothalamic beta-endorphin. *Science* 210:1271-1272, 1980.

83. Ganten D, DW Pfaff (eds). Current Topics in Neuroendocrinolgy. Springer-Verlag, 1982.

84. Genazzani AR, Facchinetti F, Petraglia F, Pintor C, Corda R. Hyperendorphinemia in obese children and adolescents. *J Clin Endorinol Metab* 62:36-40, 1986.

85. Genazzani AR, Facchinetti F, Parrini D. Beta-lipotropin and beta-endorphin plasma levels during pregnancy. *Clinical Endocrinology* 14:409-415, 1981.

86. Gentry RT, Wade GN. Androgenic control of food intake and body weight in male rats. *J Comp Physiol Psychol* 90(1)18-25, 1976.

87. Gillman MA, Lichtigfeld FJ. The opioid system in anorexia nervosa. (letter) *Am J Psychiatry* 140(3)371, 1983.

88. Gintzler AR. Endorphin-mediated increases in pain threshold during pregnancy. *Science* 210:193-195, 1980.

89. Giugliano D. Morphine, opioid peptides, and pancreatic islet function. *Diatbetes Care* 7(1)92-98, 1984.

90. Givens JR, Wiedemann E, Andersen RN, Kitabchi AE. Beta-endorphin and beta-lipotropin plasma levles in hirsute women: correlation with body weight. *J Clin Endocrin Metab* 50:975-976, 1980.

91. Goland RS, Wardlaw SL, Stark RI, Frantz AG. Human plasma beta-endorphin during pregnancy, labor, and delivery. *J Clin Endocrinol Metab* 52:74-78, 1981.

92. Gold MS, Pearsall HR. Hypothyroidism - or is it depression? *Psychosomatics* 24(7)646, 1983.

93. Gold MS, Dackis CA, Pottash ALC, Sternbach HH, Annitto WJ, Martin D, Dackis MP. Naltrexone, opiate addiction, and endorphins. *Medicinal Res Rev* 2(3)211-246, 1982.

94. Gold MS, Pottash AC. Naloxone and naltrexone: endorphin antihormones. In: Agarwal MK (ed). Hormone Antagonists. Walter de Gruyter & Co., 1982. Pgs.671-678.

95. Goldstein A. Opioid peptides: function and significance. In: Hughes J, Collier HOJ, Rance MJ, Tyers MB (eds). Opioids: Past, Present & Future. Taylor & Francis, 1984. Pgs.127-143.

96. Gordon JH. Modulation of apomorphine-induced stereotypy by estrogen: time course and dose response. *Brain Res Bull* 5(6)679-682, 1980.

97. Govoni S, Yang HYT. Sex differences in the content of beta-endorphin and enkephalin-like peptides in the pituitary of obese (ob/ob) mice. *J Neurochem* 36:1829-1833, 1981.

98. Grandison L, Guidotti A. Stimulation of food intake by muscimol and beta endorphin. *Neuropharmacology* 16:533-536, 1977.

99. Gray JM, Greenwood MRC. Time course of effects of ovarian hormones on food intake and metabolism. *Am J Physiol* 243:E407-E412, 1982.

100. Greene J, Wells J Ivy A. Progesterone will maintain adrenalectomized rats. *Proc Soc Exp Biol Med* 40:83-86, 1939.

101. Greenberg J, Ellyin F, Pullen G, Ehrenpreis S, Singh SP, Cheng J. Methionine-enkephalin and beta-endorphin levels in brain, pancreas, and adrenals of db/db mice. *Endocrinology* 116:328-330, 1985.

102. Grossman CJ. Interactions between the gonadal steroids and the immune system. *Science* 227(4684):257-261, 1985.

103. Grunt JA. Effects of adrenalectomy and gonadectomy on growth and development in the rat. *Endocrinology* 75:446-451, 1964.

104. Guillemin R. Endocrine and reproductive diseases. In: Wyngaarden JB, Smith LH. Cecil Textbook of Medicine. WB Saunders Co., 1985. Pgs.1234-1237.

105. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F. Beta-endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 197:1367-1369, 1977.

106. Gwirtsman HE, Roy-Byrne P, Yager J, Gerner RH. Neuroendocrine abnormalities in bulimia. *Am J Psychiatry* 140(5)559-563, 1983.

107. Hahn EF, Fishman J. Changes in rat brain opiate receptor content upon castration and testosterone replacement. *Biochem Biophys Res Com* 90(3):819-823, 1979.

108. Harsing LG,Yang, H YT, Govoni S, Costa, E. Elevation of met-enkephalin and beta-endorphin hypothalamic content in rats receiving anorectic drugs: differences between D-fenfluramine and D-amphetamine. *Neuropharmacology* 21:141-145, 1982.

109. Herberg L, Coleman DL. Laboratory animals exhibiting obesity and diabetes syndromes. *Metabolism* 26:59-99, 1977.

110. Herman BH, Holtzman SG. Repeated administration of naltrexone and diprenorphine decreases food intake and body weight in squirrel monkeys. *Life Sci* 34:1-12, 1983.

111. Herrero SM. Radio-frequency-current and direct current lesions in the ventromedial hypothalamus. *Am J Physiol* 217:403-410, 1969.

112. Hervey E, Hervey GR. Energy storage in female rats treated with progesterone in the absence of increased intake of food. *J Physiol* 200:118P, 1968.

113. Hervey E, Hervey GR. The effects of progesterone on body weight and composition in the rat. *J Endocr* 37:361-384, 1967.

114. Ho AKS, Allen JP. Alcohol and the opiate receptor: Interactions with the endogenous opiates. In: Stimmel B (ed). Advances in Alcohol and Substance Abuse. 1(1)53-76. The Haworth Press, Inc., 1981.

115. Ho WWK, Wen HL, Ling H. Beta-endorphin-like immunoactivity in the plasma of heroin addicts and normal subjects. *Neuropharmacology* 19:117-120, 1980.

116. Hoebel BG. Brain neurotransmitters in food and drug reward. *Am J Clin Nutr* 42:1133-1150, 1985.

117. Hollister LE, Johnson K, Boukhabza D, Gillespie HK. Aversive effects of naltrexone in subjects not dependent on opiates. *Drug Alcohol Depend* 8:37-41, 1981.

118. Hollt V, Bergmann M. Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive beta-endorphin in plasma, pituitary and brain of rats. *Neuropharmocology* 21:147-154, 1982.

119. Hollt V, Muller OA, Fahlbusch R. Beta-endorphin in human plasma: Basal and pathologically elevated levels. *Life Sci* 25:37-44, 1979.

120. Holtzman SG. Behavioral effects of separate and combined administration of naloxone and D-amphetamine. *J Pharmacol Exp Ther* 189:51-60, 1974.

121. Hoyenga KB, Hoyenga KT, Gender and energy balance: Sex differences in adaptations for feast and famine. *Physiol Behav* 28:545-563, 1982.

122. Hughes J. Biogenesis, release and inactivation of enkephalins and dynorphins. *Br Med Bull* 39(1):17-24, 1983.

123. Hughes J, Smith TW, Kosterlitz HW, Fothergill LA, Morgan BA, Morris HR. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258:577-579, 1975.

124. Hulse GK, Coleman GJ, Copolov DL, Clements JA. Relationship between endogenous opioids and the oestrous cycle in the rat. *J Endocr* 100:271-275, 1984.

125. Hulse GK, Coleman GJ. The role of endogenous opioids in the blockade of reproductive function in the rat following exposure to acute stress. *Pharmacol Biochem Behav* 19(5):795-799, 1983.

126. Hulse GK, Coleman GJ. Effect of stress on tissue and plasma levels of immunoreactive beta-endorphin in ovariectomized rats primed with oestrogen and progesterone. *J Endocr* 100:277-280, 1984.

127. Hulse G, Coleman G, Nichaolas J, Greenwood K. Reversal of the anti-ovulatory action of stress in rats by prior administration of naloxone hydrochloride. *J Reprod Fert* 66:451-456, 1982.

128. Ignatkov VY, Babichev VN. Changes in dopamine sensitivity in isolated neurons of the preoptic region during the estrous cycle. *Neurosci Behav Physiol* 14(5)380, 1984.

129. Imura H, Kato Y, Nakai Y, Nakao K, Tanaka I, Jingami H, Koh T, Yoshimasa T, Tsukada T, Suda M, Sakamoto M, Morii N, Takahashi H, Tojo K, Sugawara A. Endogenous opioids and related peptides: From molecular biology to clinical medicine. *J Endocr* 107:147-157, 1985.

130. Jaloweic JE, Panksepp J, Zolovick AJ, Najam N, Herman B. Opioid modulation of ingestive behavior. *Pharmacol Biochem Behav* 15:477-484, 1981.

131. Jenkins JS. The hypothalamus. *Br Med J* 2:99-102, 1972.

132. Jewelewicz R. The role of endogenous opioid peptides in control of the menstrual cycle.*Fert and Steril* 42(5):683-685, 1984.

133. Johnson HM, Smith EM, Torres BA, Blalock JE. Regulation of the in vitro antibody response by neuroendocrine hormones. *Proc Natl Acad Sci* 79:4171-4174, 1982.

134. Kalkhoff RK. Metabolic effects of progesterone. *Am J Obstet Gynecol* 142:735-738, 1982.

135. Kalkhoff RK, Jacobson M, Lemper D. Progesterone, Pregnancy and the Augmented Plasma Insulin Response. *J Clin Endocr* 31:24-28, 1970.

136. Kasson BG, George R. Endocrine influences on the actoins of morphine: IV. Effects of sex and strain. *Life Sci* 34(17)1624-1634, 1984.

137. Kaye WH, Pickar D, Naber D, Ebert MH. Cerebrospinal fluid opioid activity in anorexia nervosa. *Am J Psychiatry* 139:643-645, 1982.

138. Kral JG, Gortz L, Terenins L. Endorphin-like activity in morbidly obese patients, a pilot study. *Intl J Obesity* 5:539, 1981.

139. Knuth UA, Sikand GS, Casanueva FF, Havlicek V, Friesen HG. Changes in beta-endorphin content in discrete areas of the hypothalamus throughout proestrus and diestrus of the rat. *Life Sciences* 33:1443-1450, 1983.

140. Krieger DT, Martin JB. Brain peptides. *The New England Journal of Medicine* 304(15):876, 1981.

141. Kriegar DT, Liotta AS, Brownstein MJ, Zimmerman EA. ACTH, beta-lipotropin, and related peptides in brain, pituitary, and brain. *Rec Progr Horm Res* 36:277-336, 1980.

142. Kron L, Katz JL, Gorzynski G, Weiner H. Anorexia nervosa and gonadal dysgenesis. *Arch Gen Psychiatry* 34:332-335, 1977.

143. Krotkiewski M, Fagerberg B, Bjorntop P, Terenius L. Endorphins in genetic human obesity. *Intl J Obesity* 7:597-598, 1983.

144. Kyriakides M, Silverstone T, Jeffeoate W, Laurance B. Effects of naloxone on hyperphagia in Prader-Willi-Syndrome. *Lancet* I:876-877, 1980.

145. LaBella FS. Opiate-specific displacement of steroid hormones from microsomes. *Life Sciences* 16:1783-1784, 1975.

146. Labrie F, Ferland L, Denizeau F, Beaulieu M. Sex steroids interact with dopamine at the hypothalamic and pituitary levels to modulate prolactin secretion. *J Steroid Biochem* 12:323-330, 1980.

147. Lee S, Panerai AE, Bellabarba D, Friesen HG. Effect of endocrine modifications and pharmacological treatments on brain and pituitary concentrations of beta-endorphin. *Endocrinology* 107:245-248, 1980.

148. Leibowitz SF. Brain monoamines and peptides: Role in the control of eating behavior. *Federation Proc* 45:1396-1403, 1986.

149. Leibowitz SF, Hor L. Endorphinergic and beta-noradrenergic systems in the paraventricular nucleus: Effects on eating behavior. *Peptides* 3:421-428, 1982.

150. Leibowitz SF, Hor L. Behavioral effect of beta-endorphin and norepinephrine in the hypothalamic paraventricular nucleus. *Soc Neurosci Abst* 6:318, 1980.

151. Levine LS. Treatment of Turners syndrome with estrogen. *Pediatrics* 62:1178-1183, 1978.

152. Lisk RD. Estrogen: Direct effects on hypothalamus or pituitary in relation to pituitary weight changes. *Neuroendo* 4:368-373, 1969.

153. Locatelli V, Petraglia F, Penalva A, Panerai AE. Effect of dopaminergic drugs on hypothalamic and pituitary immunoreactive beta-endorphin concentrations in the rat. *Life Sci* 33:1711-1717, 1983.

154. Lowly MT, Yim GKW. Stimulation of food intake following opiate agonists in rats but not hamsters. *Psychopharmacology* 81:28-32, 1983.

155. Lowly MT, Maickel RP, Yim GKW. Naloxone reduction of stress-related feeding. *Life Sciences* 26:2113-2118, 1980.

156. Lowry PJ, Gillies G, Hope J, Jackson S. Structure and biosynthesis of peptides related to corticotrophin and lipotrophin. *Hormone Res* 13:201-210, 1980.

157. Lymangrover JR, Dokas LA, Kong A, Martin R, Saffran M. Naloxone has a direct effect on the adrenal cortex. *Endocrinology* 109:1132, 1981.

158. Maass CA, Wade GN. Effects of gonadal hormones on eating and body weight in mongolian gerbils (Meriones unguiculatus). *Horm Behav* 9:178-187, 1977.

159. Maggio CA, Presta E, Bracco EF Vasselli JR, Kissileff HR, Pfohl DN, Hashim SA. Naltrexone and human eating behavior: A dose-ranging inpatient trial in moderately obese men. *Brain Res Bull* 14(6)657-661, 1985.

160. Malcolm R, ONeil PM, Sexquer JD, Riddle FE, Currey HS, Counts C. A controlled trial of naltrexone in obese humans. *Intl J Obesity* 9:347-353, 1985.

161. Mandenoff A, Fumeron F, Apfelbaum M, Margules DL. Endogenous opiates and energy balance. *Science* 215:1536-1538, 1982.

162. Mandler RN, Biddison WE, Mandler R, Serrate SA. Beta-endorphin augments the cytolytic activity and interferon production of natural killer cells. *J Immunol* 136(3)934, 1986.

163. Margules DL, Inturrisi CE. Beta-endorphin immunoreactivity in the plasma of patients with Prader-Labhart-Willi syndrome and their normal siblings. *Experimentia* 39:766-777, 1982.

164. Margules DL. The obesity of middle age: A common variety of Cushings syndrome due to a chronic increase in adrenocorticotrophin (ACTH) and beta-endorphin activity. *Neurosci Biobehav Rev* 3(2):107-111, 1979.

165. Margules DL. Beta-endorphin and endoloxone: Hormones of the autonomic nervous system for the conservation or expenditure of bodily resources and energy in anticipation of famine or feast. *Neurosci Biobehav Rev* 3(2):155-162, 1979.

166. Margules DL, Moisset B, Lewis MJ, Shibuya H, Pert CB. Beta-endorphin is associated with overeating in genetically obese mice (ob/ob) and rats (fa/fa). *Science* 202:988-991, 1978.

167. Matsumura M, Yamanoi A, Sato K, Tsuda M, Chikamori K, Mori H, Saito S. Alterations in the levels of beta-endorphin-like immunoreactivity in plasma and tissues of obese rats with hypothalamic lesions. *Horm Metabol Res* 16:105-106, 1984.

168. Matsumura M, Saito S, Fujino M. Effects of solution of low pH and taurocholate on release of beta-endorphin-like immunoreactivity from human duodenal mucosa in vitro. *Regulat Peptides* 3:173-181, 1982.

169. McCloy RF, McCloy J. Autoaddiction: A hypothesis on hunger and obesity. *Am Heart J* 100(6)933-934, 1980.

170. McCloy RF, McCloy J. Enkephalins, hunger, and obesity. *Lancet* II(8134)156, 1979.

171. McEwen BS. Personal communication.

172. McEwen BS. The brain as a target organ of endocrine hormones. In: Krieger DT, Hughes JC (eds). Neuroendocrinology. Sinauer Assoc., Inc., 1980. Pgs.33-42.

173. McKay LD, Kenney NJ, Edens NK, Williams RH, Woods SC. Intracerebroventricular beta-endorphin increases food intake of rats. *Life Sciences* 29:1429-1434, 1981.

174. McLaughlin CL, Baile CA. Autoimmunization against beta-endorphin increases food intakes and body weights of obese rats. *Physiol Behav* 35(3)365-370, 1985.

175. McLean S, Hoebel BG. Opiate and norepinephrine-induced feeding from the paraventricular nucleus of the hypothalamus are dissociable. *Life Sci* 31:2379-2382, 1982.

176. McLean S, Hoebel BG. Local injection of morphine or an opiate peptide into the hypothalamic paraventricular nucleus elicits feeding. *Soc Neurosci Abstr* 6:532, 1980.

177. Meites J, Bruni JF, Van Vugt DA, Smith AF. Relation of endogenous opioid peptides and morphine to neuroendocrine functions. *Life Sci* 24:1325-1336, 1979.

178. Moguilevsky JA, Scacchi P. RNA and protein content in different hypothalamic areas: Influence of castration. *J Endocr* 51:219-220, 1971.

179. Mook DG, Kenney NJ, Roberts S, Nussbaum A, Rodier WI. Ovarian adrenal interactions in regulation of body weight by female rats.*J Comp Physiol Psychol* 81(2):198-211, 1972.

180. Morali G, Beyer C. Neuroendocrine control of mammalian estrous behavior. In: Beyer C. Endocrine Control of Sexual Behavior. Raven Press, New York, 1979, pgs.33-75.

181. Morley JE, Parker S, Levine AS. Effect of butorphanol tartrate on food and water consumption in humans. *Am J Clin Nutr* 42:1175-1178, 1985.

182. Morley JE, Levine AS, Gosnell BA, Billington CJ. Neuropeptides and appetite: contribution of neuropharmacological modeling. *Fed Am Soc Exp Biol* 43:2903-2907, 1984.

183. Morley JE, Levine AS, Gosnell BA, Billington CJ. Which opioid receptor mechanism modulates feeding? *Appetite* 61:61-68, 1984.

184. Morley JE, Levine AS, Grace M, Kneip J, Gosnell BA. The effect of ovariectomy, estradiol and progesterone on opioid modulation of feeding. *Physiol Behav* 33(2)237-241, 1984.

185. Morley JE, Levine AS, Yim GK, Lowy MT. Opioid modulation of appetite. *Neurosci Biobehav Rev* 7(2)281-305, 1983.

186. Morley JE. The endocrinology of the opiates and opioid peptides. *Metabolism* 30(2)195-209, 1981.

187. Morley JE, Levine AS. Stress-induced eating is mediated through endogenous opiates. *Science* 209:1259-1260, 1980.

188. Morrell JI, McGinty JF, Pfaff DW. A subset of beta-endorphin- or dynorphin-containing neurons in the medial basal hypothalamus accumulates estradiol. *Neuroendocrinology* 41:417-426, 1985.

189. Mueller GP. Attenuated pituitary beta-endorphin release in estrogen-treated rats. *Proc Soc Exp Biol* 165:75-81, 1980.

190. Nakao K, Nakai Y, Jingami H, Oki S, Fukata J, Imura H. Substantial rise of plasma beta-endorphin levels after insulin-induced hypoglycemia in human subjects. *J Clin Endocrinol Metab* 49:838-841, 1979.

191. NAS/NRC. Nutrient Requirements of Laboratory Animals. No. 10, Washington, D.C., 1978.

192. Newnham JP, Tomlin S, Ratter SJ, Bourne GL, Rees LH. Endogenous opioid peptides in pregnancy. *Br J Obstet Gynecol* 90:535-538, 1983.

193. Nimrod A, Ryan KJ. Aromatization of androgens by human abdominal and breast fat tissue. *J Clin Endocr Metab* 40:367-372, 1975.

194. Nohtomi A, Itoh M, Yufu N. Effects of sulpiride treatment on the levels of immunoreactive beta-endorphin in rat hypothalamic nuclei. *Brain Res* 300:152-156, 1984.

195. Ogawa N, Panerai AE, Lee S, Forsbach G, Havlicek V, Friesen HG. Beta-endorphin concentration in the brain of intact and hypophysectomized rats. *Life Sci* 25:317-326, 1979.

196. Oliver C, Mical RS, Porter JC. Hypothalamic pituiatary vasculature: Evidence for retrograde blood flow in the pituitary stalk. *Endocrinology* 101:598, 1977.

197. Olson GA, Olson RD, Kastin AJ, Coy DH. Endogenous opiates: Through 1978. *Neurosci Biobeh Rev* 3:285-299, 1979.

198. Oomura Y. Input-output organization in the hypothalamus relating to food intake behavior. In: Morgane PJ, Panksepp J (eds). Handbook of the Hypothalamus: Physiology of the Hypothalamus. Vol 2:557-620, 1980.

199. O'Riordan FLH, Molan PG, Gould RP. Essentials of Endocrinolgy. Blackwell Scientific Publications, 1982.

200. Orosco M, Trouvin JH, Cohen Y, Jacquot C. Ontogeny of brain monoamines in lean and obese female Zucker rats. *Physiol Behav* 36:853-856, 1986.

201. Panerai AE, Sawynok J, LaBella FS, Friesen HG. Prolonged hyperprolactinemia influences beta-endorphin and met-enkephalin in the brain. *Endocrinology* 106:1804-1807, 1980.

202. Pang CN, Zimmermann E, Sawyer CH. Morphine inhibition of the preovulatory surges of plasma luteinizing hormone and follicle stimulating hormone in the rat. *Endocrinology* 101:1726, 1977.

203. Panksepp J. Hypothalamic integration of behavior: Rewards, punishments, and related psychological processes. In: Morgane PJ, Panksepp J (eds). Handbook of the Hypthalamus: Behavioral Studies of the Hypothalamus. Vol 3:289-431, 1981.

204. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. Academic Press, 1982. Plates 46 & 53.

205. Pecquery R, Leneveu MC, Giudicelli Y. Estradiol treatment decreases the lipolytic responses of hamster white adipocytes through a reduction in the activity of the adenylate cyclase catalytic subunit. *Endocrinology* 118(6)2210-2216, 1986.

206. Perry MLS, Carrasco MA, Dias RD, Izquierdo I. Beta-endorphin like immunoreactivity of brain, pituitary gland and plasma of rats submitted to postnatal protein malnutrition: Effect of behavioral training. *Peptides* 5:15-20, 1984.

207. Pert CB, Snyder SH. Operate receptor binding antagonists and antagonists affected directly by sodium. *Molec Pharmac* 10:868-899, 1974.

208. Petraglia F, Baraldi M, Giarre G, Facchinetti F, Santi M, Volpe A, Genazzani AR. Opioid peptides of the pituitary and hypothalamus: Changes in pregnant and lactating rats. *J Endocr* 105:239-245, 1985.

209. Petraglia F, Penalva A, Locatelli V, Cocchi D, Panerai AE, Genazzani AR, Muller EE. Effect of gonadectomy and gonadal steroid replacement on pituitary and plasma beta-endorphin levels in the rat. *Endocrinology* 111:1224-1229, 1982.

210. Petraglia F, Penalva A, Genazzani AR, Muller EE. Stimulation of beta-endorphin and beta-lipotropin release from the anterior but not the neurointermediate pituitary lobe in the rat after acute administration of serotonin-acting drugs. *Life Sci* 31:2809-2817, 1982.

211. Pickar D, Cohen MR, Naber D, Cohen RM. Clinical studies of the endogenous opioid system. *Biol Psychiatry* 17(11)1243-1275, 1982.

212. Pilotte NS, Burt DR, Barraclough CA. Ovarian steroids modulate the release of dopamine into hypophysial portal blood and the density of anterior pituitary (3H)spiperone-binding sites in ovariectomized rats. *Endocrinolgoy* 114:2306, 1984.

213. Pinsky C, Koven SJ, LaBella FS. Evidence for role of endogenous sex steroids in morphine antinociception. *Life Sci* 16:1785-1786, 1975.

214. Poiley SM. Growth: Mammals other than man. In: Altman PL, Dittmer DS (eds). Biology data book I (2nd ed.). Federation of American Societies for Experimental Biology, 1972.

215. Quigley ME, Sheehan KL, Casper RF, Yen SSC. Evidence for increased dopaminergic and opioid activity in patients with hypogonadotropic amenorrhea. *J Clin Endocrinol Metab* 50(5)949, 1980.

216. Quigley ME, Yen SSC. The role of endogenous opiates on LH secretion during the menstrual cycle. *J Clin Endocrinol Metab* 51(1)179, 1980.

217. Quigley ME, Sheehan KL, Casper RF, Yen SSC. Evidence for an increased opioid inhibition of luteinizing hormone secretion in hyperprolactemic patients with pituitary microadenoma. *J Clin Endocrinol Metab*50(3)427, 1980.

218. Racz K, Glaz E, Kiss R, Lada G, Varga I, Vida S, Di Gleria K, Medzihradszky K, Lichtwald K, Vecsei P. Adrenal cortex - a newly recognized peripheral site of action of enkephalins. *Biochem Biophys Res Rev* 97(4)1346-1353, 1980.

219. Railble LH, Gorzalka BB. Food intake, body weight and lordosis in male and female mongolian gerbils: Effects of ovarian steroids. *Physiol Behav* 35(5) 767-774, 1985.

220. Raps D, Barth PL, Desaulles PA. Plasma and adrenal corticosterone levels during different phases of the sexual cylce in normal female rats. *Experimentia* 27:339-340, 1971.

221. Recant L, Voyles N, Wade A, Awoke S, Bhathena SJ. Studies on the role of opiate peptides in two forms of genetic obesity: ob/ob mouse and fa/fa rat. *Horm Metabol Res* 15:589-593, 1983.

222. Reid RL, Yen SSC. The premenstrual syndrome. *Clin Obstet Gynecol* 26(3)710-717, 1983.

223. Reid RL, Hoff JD, Yen SSC, Li CH. Effects of exogenous beta-endorphin on pituitary hormone secretion and its disappearance rate in normal human subjects. *J Clin Endocrinol Metab* 52:1179, 1981.

224. Reilly MA, Kline NI, Smith AA. Uptake of 125 by mouse tissue after intravenous injection 125I beta-endorphin. *Fed Proc* 37:237, 1978.

225. Resko JA. Endocrine control of adrenal progesterone secretion in the ovariectomized rat. *Science* 164:70-71, 1969.

226. Roberts S, Kenney NJ, Mook DG. Overeating induced by progesterone in the ovariectomized, adrenalectomized rat. *Horm Behav* 3:267-276, 1972.

227. Rosenblatt H, Dyrenfurth I, Ferin M, vande Wiele RL. Food intake and the menstrual cycle in rhesus monkeys. *Physiol Behav* 24:447-449, 1980.

228. Ross GE, Zucker I. Progesterone and the ovarian-adrenal modulation of energy balance in rats. *Horm Behav* 5:43-62, 1974.

229. Rossier J, French E, Rivier C, Shibasaki T, Guillemin R, Bloom FE. Stress-induced release of prolactin: blockade by dexamethasone and naloxone may indicate beat-endorphin mediation. *Proc Natl Acad Sci* 77:666-669, 1980.

230. Rossier J, Rogers J, Shibasaki T, Guillemin R, Bloom FE. Opioid peptides and alpha-melanocye-stimulating hormone in genetically obese (ob/ob) mice during development. *Proc Natl Acad Sci* 76(4)2077-2080, 1979.

231. Rossier J, Vargo TM, Minick S, Ling N, Bloom FE, Guillemin R. Regional dissociation of beta-endorphin and enkephalin contents in rat brain and pituitary. *Proc Natl Acad Sci* 74(11)5162-5165, 1977.

232. Roy EJ, Wade GN. Role of food intake in estradiol-induced body weight changes in female rats. *Horm Behav* 8:265-274, 1977.

233. Russell JB, Mitchell DE, Musey PI, Collins DC. The role of beta-endorphins and catechol estrogens on the hypothalamic-pituitary axis in female athletes. *Fertil Steril* 42:690-694, 1984.

234. Saito M, Bray GA. Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. *Am J Physiol* 246:R20-R25, 1984.

235. Sanger DJ, McCarthy PS. The anorectic action of naloxone is attenuated by adaption to a food deprivation schedule. *Psychopharmacology* 77:336-338, 1982.

236. Sanger DJ, McCarthy PS, Metcalf G. The effects of opiate antagonists on food intake are stereospecific. *Neuropharmacology* 20:45-47, 1981.

237. Sanger DJ. Endorphinergic mechanisms in the control of food and water intake. *Appetite: J Intake Research* 2:193-208, 1981.

238. Sanger DJ, McCarthy PS. Increased food and water intake produced in rats by opiate receptor agonists. *Psychopharmacology* 74:217-220, 1981.

239. Santen RJ, Worgul TJ, Samojlik E, Interrante A, Boucher AE, Lipton A, Harvey HA, White DS, Smart E, Cox C, Wells SA. A randomized trial comparing surgical adrenalectomy with aminoglutethimide plus hydrocortisone in women with advanced breast cancer. *N Eng J Med* 305(10)545-551, 1981.

240. Sapun-Malcolm D, Farah JM, Mueller GP. Evidence for serotonergic stimulation of pituitary beta-endorphin release: preferential release from the anterior lobe in vivo. *Life Sci* 33:95-102, 1983.

241. Scacchi P, Moguilevsky JA, Libertun C, Christot J. Sexual differences in the protein content of the hypothalamus in rats. *Proc Soc Exp Biol Med* 133:845, 1970.

242. Schally AV, Kastin AJ, Arimura A. Hypothalamic hormones: The link between brain and body. *Am Scientist* 65:712, 1977.

243. Schmauss C, Emrich HM. Dopamine and the action of opiates: a reevaluation of the dopamine hypothesis of schizophrenia. *Biol Psychiatry* 20:1211-1231, 1985.

244. Schneider BS, Freedman IM, Hirsch J. Feeding behavior. In: Krieger DT, Brownstein MI, Martin IB (eds). Brian Peptides. John Wiley & Sons, 1983. Pgs.251-279.

245. Schultz R, Wuster M, Herz A. Interaction of amphetamine and naloxone in feeding behavior in guinea pig. *Eur J Pharmac* 63:313-319, 1980.

246. Schultz R, Wuster M, Herz A. Supersensitivity to opioids following chronic blockade of endorphin action of naloxone. *Naunyn-Schmiedebergs Arch Pharmacol* 306:93-96, 1979.

247. Schwartz SM, Wade GN. Effects of estradiol and progesterone on food intake, body weight, and carcass adiposity in weanling rats. *Am J Physiol* 240:E499-E503, 1981.

248. Shanker G, Shama RK. Beta-endorphin stimulates corticosterone synthesis in isolated rat adrenal cells. *Biochem Res Commun* 86:1-5, 1979.

249. Shimomura Y, Oku J, Glick Z, Bray GA. Opiate receptors, food intake and obesity. *Physiol Behav* 28(3)441-445, 1982.

250. Shirling D, Ashby JP, Baird JD. Effect of progesterone on lipid metabolism in the intact rat. *J Endocr* 90:285-294, 1981.

251. Shoupe D, Montz FJ, Lobo RA. The effects of estrogen and progestin on endogenous opioid activity in oophorectomized women. *J Clin Endocrinol Metab* 60:178-183, 1985.

252. Si ECC, Bryant HU, Yim GKW. Opioid and non-opioid components of insulin-induced feeding. *Pharm Biochem Behav* 24(4)899-903, 1986.

253. Silverstone T (ed). Appetite and Food Intake. *Dahlem Konfernzen*, 1976.

254. Simnatov R. Glucocorticoids inhibit endorphin synthesis by pituitary cells. *Nature* 280:684-685, 1979.

255. Sims EAH. Syndromes of obesity. In: DeGroot LJ, Cahill GF, Odell WD, Martini L, Potts JT, Nelson DH, Steinberger E, Winegrad AI (eds). Endocrinology. Vol 3:1941-1962. Grune & Stratton, 1979.

256. Smith EM, Harbour-McMenamin D, Blalock JE. Lymphocyte production of endorphins and endorphin-mediated immunoregulatory activity. *J Immunology* 135(2)779s-782s, 1985.

257. Smyth DG. Beta-endorphin and related peptides in pituitary, brain, pancreas and antrum. *Br Med Bull* 39(1)25-30, 1983.

258. Stein EA. Effects of intracranial self-stimulation on brain opioid peptides. *Peptides* 6(1)67-73, 1985.

259. Stern JJ, Porterfield AL, Krupa RJ. Endocrine interactions in regulation of body weight by female rats. *J Comp Physiol Psychol* 86(5)926-929, 1974.

260. Sternbach H, Annito W, Pottash ALC, Gold MS. Anorexic effects of naltrexone in man. *Lancet* I:388-389, 1982.

261. Suda T, Liotta AS, Krieger DT. Beta-endorphin is not detectable in plasma from normal human subjects. *Science* 202:221-223, 1978.

262. Swanson HH. Effect of progesterone on the body weight of hamsters. *J Endocr* 41:13, 1968.

263. Takayama S, Makino T, Shinichi Y, Kondo Z, Iizuka R. The study of opioid peptides in normal menstrual cycle, pregnancy, and labor. *Acta Obstet Gynaecol Jpn* 37(9)1813-1819, 1985.

264. Tallman JF, Paul SM, Skolnick P, Gallager DW. Receptors for the age of anxiety: Pharmacology of the benzodiazepines. *Science* 207:271-281, 1980.

265. Tarttelin MF, Gorski RA. The effects of ovarian steroids on food and water intake and body weight in the female rat. *Acta Endocrinol* 72:551-568, 1973.

266. Tarttelin MF, Gorski RA. Variations in food and water intake in the normal and acyclic female rat. *Physiol Behav* 7(6)847-852, 1971.

267. Tejwani GA, Vaswani KK, Barbacci JC. Effect of oral contraceptives on the rat brain and pituitary opioid peptides. *Peptides* 6:555-561, 1985.

268. Tepperman FS, Hirst M, Gowdey CW. Hypothalamic injection of morphine: Feeding and temperature responses. *Life Sci* 28:2459-2467, 1981.

269. Thompson DA, Welle SL, Lilavivat U, Penicaud L, Campbell RG. Opiate receptor blockage in man reduces 2-deoxy-D-glucose induced food intake but not hunger, thrist, and hypothermia. *Life Sci* 31:847-852, 1982.

270. Thornhill JA, Hirst M, Cowdey CW. Disruption of diurnal feeding patterns of rats by heroin. *Pharmac Biochem Behav* 4:129-135, 1976.

271. Thornhill JA, Hirst M, Cowdey CW. Changes in diurnal temperature and feeding patterns of rats during repeated injections of heroin and withdrawal. *Archs Int Pharmacodyn* 223:120-131, 1976.

272. Trenchard E, Silverstone T. Naloxone reduces the food intake of normal human volunteers. *Appetite: J Intake Res* 4:43-50, 1983.

273. Tseng LF, Cheng DS. Acute and chronic administration of beta-endorphin and naloxone on food and water intake in rats. *Fed Proc* 39:606, 1980.

274. Vale W, Rivier C, Yang L, Minick S, Guillemin R. Effects of purified hypothalamic corticotropin-releasing factor and other substances on the secretion of adrenocotropin and beta-endorphin- like immunoactivities in vitro. *Endorcrinology* 103:1910-1915, 1978.

275. Valenstein ES, Cox VC, Kakolewski JW. Sex differences in hyperphagia and body weight following hypothalamic damage. *Am NY Acad Sci* 157:1030-1046, 1969.

276. Van Vugt DA, Sylvester PW, Aylsworth CF, Meites J. Counteraction of gonadal steroid inhibition of luteinizing hormone release by naloxone. *Neuroendocrinology* 34:274-278, 1982.

277. Van Vugt DA, Bruni JF, Sylvester PW, Chen HT, Ieiri T, Meites J. Interaction between opiates and hypothalamic dopamine on prolactin release. *Life Sci* 24:2361-2368, 1979.

278. Veith JL, Anderson J, Slade SA, Thompson P, Laugel GR, Getzlaf S. Plasma beta-endorphin, pain thresholds and anxiety levels across the human menstrual cycle. *Physiol Behav* 32(1)31-34, 1984.

279. Vrbicky KW, Baumstark JS, Wells IC, Hilgers TW, Kable WT, Elias CJ. Evidence for the involvement of beta-endorphin in the human menstural cycle. *Fert Steril* 38(6)701, 1982.

280. Wade GN, Gray JM, Bartness TJ. Goandal influences on adiposity. *Intl J Obesity* 9(Suppl 1)83-92, 1985.

281. Wade GN, Gray JM. Theoretical review - gonadal effects on food intake and adiposity: A metabolic hypothesis. *Physiol Behav* 22:583-593, 1979.

282. Wade GN. Some effects of ovarian hormones on food intake and body weight in female rats. *J Comp and Physiol Psychol* 88(1)183-193, 1975.

283. Wade GN. Theoretical review - gonadal hormones and behavioral regulation of body weight. *Physiol Behav* 8:523-534, 1972.

284. Wade GN. Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants. *J Comp Physiol Psychol* 72(2)328-336, 1970.

285. Wade GN, Zucker I. Development of hormonal control over food intake and body weight in female rats. *J Comp Physiol Psychol* 70(2)213-220, 1970.

286. Wade GN, Zucker I. Hormonal and developmental influences on rat saccharin preferences. *J Comp Physiol Psychol* 69:291-300, 1969.

287. Wade GN, Zucker I. Taste preferences of female rats: Modification by neonatal hormones, food deprivation and prior experience. *Physiol Behav* 4:935-913, 1969.

288. Wallace M, Fraser CD, Clements JA, Funder JW. Naloxone, adrenalectomy, and steroid replacement: Evidence against a role for circulating beta-endorphin in food intake. *Endocrinology* 108:189-192, 1981.

289. Wardlaw SL, Wang PJ, Frantz AG. Regulation of beta-endorphin and ACTH in brain by estradiol. *Life Sci* 37:1941-1947, 1985.

290. Wardlaw SL, Frantz AG. Brain beta-endorphin during pregnancy, parturition and the postpartum period. *Endocrinology* 113:1664-1668, 1983.

291. Wardlaw SL, Thoron L, Frantz AG. Effects of sex steroids on brain beta-endorphin. *Brain Res* 245:327-331, 1982.

292. Wardlaw SL, Wehrenberg WB, Ferin M, Antunes JL, Frantz AG. Effect of sex steroids on beta-endorphin in hypophyseal portal blood. *J Clin Endocrinol Metab* 55(5)877, 1982.

293. Watson SJ, Akil H, Richard CW, Barchas JD. Evidence for two separate opiate peptide neuronal systems. *Nature* 275:226-228, 1978.

294. Way EL. History of opiate use in the orient and the United States. *NY Acad Sci* 398:12, 1982.

295. Wehrenberg WB, Wardlaw SL, Frantz AG, Ferin M. Beta-endorphin in hypophyseal portal blood: Variation throughout the menstrual cycle. *Endocrinology* 111:879-881, 1982.

296. Weizenbaum F, Benson B, Solomon L, Brehony K. Relationship among reproductive variables, sucrose taste reactivity and feeding behavior in humans. *Phsyiol Behav* 24(6)1053-1056, 1980.

297. Wilcox JN, Roberts JL. Estrogen dereaces rat hypothalamic proopiomelanocortin messenger ribonucleic acid levels. *Endocrinology* 117:2392-2396, 1985.

298. Wise J. Endorphins and metabolic control in the obese: A mechanism for food addiction. *Obesity and metabolism* 1(3)165-181, 1981.

299. Wise PM, Rance N, Barraclough CA. Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. *Endocrinology* 108:2186-2193, 1981.

300. Wise RA. Catecholamine theories of reward: A critical review. *Brain Res* 152:215-247, 1978.

301. Wright P, Crow RA. Menstrual cycle: Effect on sweetness preferences in women. *Hormones Behav* 4:387-391, 1973.

302. Yim GKW, Lowy MT. Opioids, feeding, and anorexias. *Federation Proc* 43:2893-2897, 1984.

303. Yukimura Y, Bray GA. Effects of adrenalectomy on body weight and the size and number of fat cells in the Zucker (fatty) rat. *Endocrine Res Com* 5(3)189-198, 1978.

304. Yukimura Y, Bray GA, Wolfsen AR. Some effects of adrenalectomy in the fatty rat. *Endocrinology* 103?:1924-1928, 1978.

305. Zaninovich AA. Direct and indirect effects of estrogens on thyroidal economy in man. In: Physiopathology of Endocrine Diseases and Mechanisms of Hormone Action. Alan R. Liss, Inc., 1981. Pgs.95-102.

306. Zioudrou C, Streaty RA, Klee WA. Opioid peptides derived from food proteins. *J Biol Chem* 254(7)2446-2449, 1979.

307. Zucker I, Wade GN, Ziegler R. Sexual and hormonal influences on eating, taste preferences, and body weight of hamsters. *Physiol Behav* 8(1)101-111, 1972.

308. Zucker I. Hormonal determinants of sex differences in saccharin preference, food intake and body weight. *Physiol Behav* 4:595-602, 1969.

Appendix A

Immuno Nuclear Corporation Procedure for the β-Endorphin Assay

BETA ENDORPHIN

BY

RADIOIMMUNOASSAY

Methods and Reagents of the

IMMUNO NUCLEAR CORPORATION

1951 Northwestern Avenue

Stillwater, Minnesota 55082

612-439-9710

800-328-1482

Revised 8/84

BETA ENDORPHIN

BY

RADIOIMMUNOASSAY

(FOR EXTRACTED SAMPLES)

TABLE OF CONTENTS

Revised 8/84
Catalog No. 16-L2
Part No. XP0307

BETA ENDORPHIN

BY

RADIOIMMUNOASSAY

(FOR EXTRACTED SAMPLES)

I. INTRODUCTION

Intended Use

This kit is intended for research use only and is not for use in diagnostic procedures. It contains materials and instructions for the quantitative measurement of human beta endorphin (β-endorphin) by radioimmunoassay.

Purpose

β-endorphin is one of a new class of neuropeptide substances. It is a 31 amino acid C-terminal segment of a larger molecule called beta lipotropin:

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-
Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-
Tyr-Lys-Lys-Gly-Glu

In vivo animal experiments show that β-endorphin possesses extremely powerful analgesic qualities, and its influences on the mechanisms of pain are currently under active investigation. Other research suggests that β-endorphin affects human "reward" behavior, and that it also influences the release of several gut peptides, including somatostatin and glucagon.

Method Description

The Immuno Nuclear Beta Endorphin RIA can be done in approximately 34 hours. The RIA method is based on an antibody with high sensitivity to β-endorphin. Sample and first antibody are added, followed by a 16 - 24 hour incubation

at 2 - 8° C. Tracer is added, followed by a 16 - 24 hour incubation at 2 - 8° C. Phase separation is done in two hours at 2 - 8° C. with a pre-precipitated complex of second antibody, carrier, and PEG added in a single pipetting step.

Literature Background for Plasma Analysis

Many laboratories report methods for β-endorphin analysis. Suda et al adsorb both β-endorphin and β-LPH onto silicic acid and elute with acid acetone. They could not detect β-endorphin, but did find 400 pg/ml β-LPH in pooled serum using column separation of the two peptides.

Wardlaw and Frantz extracted 20 ml samples of serum with talc, followed by elution with 1:1 acetone: 0.1 N HCl. Samples were dried and chromatographed. β-LPH ranged from 54-160 pg/ml and β-endorphin ranged from 14-32 pg/ml. Both rose after metapirone stimulation.

Hollt et al analyzed rat plasma by adsorption onto silicic acid, finding β-endorphin-like activity to be about 200 pg/ml.

Wiedemann et al have reported a method using a β-endorphin antibody with virtually no cross-reactivity with β-LPH. Their assay does not appear to have any difficulty with nonspecific binding of tracer to plasma proteins. The antibody is peculiar in that the 50% suppression appears to be 170 times the minimum detectible dose.

β-endorphin levels in spinal fluid are reported by Rossier et al to be as high as 200 pg/ml. Their extraction consisted of exposure of spinal fluid to acetic acid for 10 minutes at 100° C., lyophilization, and subsequent radioimmunoassay.

Scientific Detail from Immuno Nuclear Research and Development

Immuno Nuclear has made progress in defining some of the problems of measuring β-endorphin-like peptides in human pituitary and plasma. Details of studies in our laboratory are provided below to aid the investigator using Immuno Nuclear Corporation products.

The major problem affecting the direct assay of β-endorphin and β-LPH in plasma is the nonspecific binding of ^{125}I β-endorphin to high molecular weight plasma components at alkaline pH (Orf et al)(Figure 1). This binding interferes with RIA and can be circumvented by either extraction or column chromatography. Such interference problems do not appear to be present in acid tissue extracts and are lessened at neutral pH. Fortunately, uniodinated β-endorphin does not seem to bind to plasma proteins.

Several methods of extraction of β-endorphin and β-lipotropin have been studied. Neither silicic acid nor SP-Sephadex adequately separate these peptides from nonspecific binding material. Affinity gel extraction has been found to be an excellent method of concentrating β-endorphin and β-LPH from plasma, and we recommend it where measurement of both β-LPH and β-endorphin are necessary.

When plasma is extracted with Sepharose antibody complex and eluted, both β-endorphin and β-LPH are observed (see Figure 2, panel A). There is also a small peak seen in the void volume, which may be a large molecular weight cross-reacting material (as seen in pituitary) or aggregated endorphin of LPH. In panel B of Figure 2, the elution profile of a mixture of pure β-LPH and pure β-endorphin is shown, demonstrating the partition coefficients for these materials on G-75 superfine Sephadex, which are nearly

identical to those observed in extracted plasma. This is the preferred method for analysis, since it allows concentration and separation of these substances.

For plasma β-endorphin measurements, another method is direct chromatography of plasma on G-75 superfine Sephadex in 1% BSA 0.1 M borate, pH 8.4. As much as 2 ml of plasma may be chromatographed on a 11 ml bed volume column. Collection volumes vary with the sample size applied. The analysis can be accomplished by collecting 1 ml samples tube by tube, or by batch elution. β-endorphin-like activity is detectible by the INC assay without concentration, as seen in Figure 3. The area extending from tube 21 through tube 32 is nonspecific interference and β-LPH. In this particular column, a concentration of 7 picomoles/ℓ of β-endorphin-like activity was found, which is within the assay sensitivity and compares well with the antibody extracted β-endorphin-like activity.

II. SPECIMEN COLLECTION

Tissue Extraction

Tissue extraction is less difficult than serum because an acid extract can easily be made. A protocol we have used is as follows:

1. Weigh tissue and grind with 10 volumes of ice cold 0.2 N HCl. Allow to extract for 2 hours at 4° C. (Literature suggests that a hot extraction (95° C.) can also be used in certain applications.)
2. Centrifuge until clarified.
3. Lyophilize extract to concentrate and remove acid.
4. Redissolve in 1% bovine serum albumin in borate buffer 0.1 M pH 8.4 for subsequent analysis.

Plasma Analysis

Affinity gel extraction has found to be an excellent method of concentrating β-endorphin in EDTA plasma samples. If this method is to be utilized, it requires the purchase of Immuno Nuclear Plasma β-endorphin RIA kit (catalog number 4600).

III. PRECAUTIONS

This kit contains radioactive material which should be used by responsible persons in designated areas. Appropriate precautions and good laboratory practices should be used in the storage, handling, and disposal of this material.

This kit is intended for research use only and is not for use in diagnostic procedures. It is not for internal or external use in animals or humans.

THE RADIOACTIVE CONTENT OF THIS KIT DOES NOT EXCEED 2 MICROCURIES OF ^{125}IODINE.

This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and the general license of the U.S. Nuclear Regulatory Commission or of the State with which the Commission has entered into an agreement for the exercise of regulatory authority.

IV. TEST PROCEDURE

Material Provided

1. 1% BSA-borate
2. β-endorphin standard (80 pmol/ℓ)
3. Rabbit anti-β-endorphin (first antibody)
4. ^{125}I β-endorphin
5. Goat anti-rabbit precipitating complex (second antibody)

Materials Required but not Provided

In addition to the reagents supplied with the Plasma Beta Endorphin Kit, the following are suggested or required:

1. Disposable borosilicate glass tubes, 12 x 75 mm (Some manufacturers' tubes yield very elevated nonspecific bindings; therefore, we recommend tubes that, in our experience, have been satisfactory: CMS Dispo, SP Dispo, Kimble.)
2. Centrifuge to accommodate 12 x 75 mm tubes (temperature controlled)
3. Gamma scintillation counter capable of counting ^{125}I
4. Vortex
5. Pipetting devices

   Recommended pipetting devices:

   a. Micropipettors such as MLA or Eppendorf, 100 µl, 200 µl, and 500 µl
   b. Hamilton repeating dispensers, 5 ml to deliver 100 µl
   c. B-D Cornwall syringe, 2 ml

Reagents

The material and reagents supplied with each kit are as follows:

	65 tubes
1. 1% BSA-BORATE lyophilized	1 vial 35 ml
2. β-ENDORPHIN STANDARD 80 pmol/ℓ, lyophilized	1 vial 2.0 ml
3. β-ENDORPHIN ANTIBODY Rabbit anti-β-endorphin serum, lyophilized	1 vial 7 ml
4. ^{125}I β-ENDORPHIN lyophilized	1 vial 7 ml
5. PRECIPITATING COMPLEX Goat anti-rabbit precipitating complex (GAR-PPT) consisting of pre-precipitated normal rabbit serum, goat anti-rabbit serum, and polyethylene glycol, lyophilized	1 vial 35 ml

Reagent Storage Instructions

1. Upon receipt, reagents must be stored at 2 - 8° C. or lower.
2. After reconstitution, reagents must be stored at -15° C. or lower.

Reagent Preparation

1. 1% BSA-borate

   Reconstitute the vial with 35 ml of distilled or deionized water.

   Allow the vial to stand 15 - 20 minutes for complete reconstitution.

   Mix thoroughly before use in the assay.

2. β-endorphin standard

   Reconstitute the vial with 2.0 ml of distilled or deionized water to give 80 pmol/ℓ. Mix carefully without foaming. Allow the vial to stand 15 - 20 minutes for complete reconstitution. In order to obtain the entire standard curve, make serial dilutions by adding 500 µl of standard to 500 µl of 1% BSA-borate to give standards of 40, 20, 10 and 5 pmol/ℓ.

   Add 500 µl of 80 pmol/ℓ standard to 500 µl of 1% BSA-borate and mix to give 40 pmol/ℓ.

   Add 500 µl of 40 pmol/ℓ standard to 500 µl of 1% BSA-borate and mix to give 20 pmol/ℓ.

   Add 500 µl of 20 pmol/ℓ standard to 500 µl of 1% BSA-borate and mix to give 10 pmol/ℓ.

   Add 500 µl of 10 pmol/ℓ standard to 500 µl of 1% BSA-borate and mix to give 5 pmol/ℓ.

3. Rabbit anti-β-endorphin serum

   Reconstitute the vial with 7 ml of distilled or deionized water.

   Allow the vial to stand 15 - 20 minutes for complete reconstitution.

   Mix thoroughly before use in the assay.

4. ^{125}I β-endorphin

   Reconstitute the vial with 7 ml of distilled or deionized water.

   Allow the vial to stand 15 - 20 minutes for complete reconstitution.

5. Goat anti-rabbit precipitating complex (GAR-PPT)

   Reconstitute the vial with 35 ml of distilled or deionized water. Mix THOROUGHLY until the suspension appears homogeneous and then allow the vial to stand a minimum of 30 minutes at room temperature with occasional mixing.

Procedure

NOTE: Put all specimens from one patient into the same assay.

1. Reconstitute lyophilized reagents. Allow any frozen reagents to thaw completely at a temperature not to exceed 25° C. Place reagents on crushed ice after thawing. Do not heat reagents to speed thawing time. Mix gently before use in the assay.

2. Set up labeled 12 X 75 mm glass tubes in duplicate according to the protocol in Table I. All volumes are in microliters. Place rack on crushed ice.

3. Add reagents to the tubes as follows:

   a. Total count

      100 µl of ^{125}I β-endorphin

      Set aside until step number 11.

   b. Nonspecific binding (NSB)

      200 µl of 1% BSA-borate

   c. 0 standard

      200 µl of 1% BSA-borate

      100 µl of rabbit anti-β-endorphin

   d. β-endorphin standard

      200 µl of β-endorphin standard

      100 µl of rabbit anti-β-endorphin serum

4. Vortex gently and incubate for 16 - 24 hours at 2 - 8° C.
5. Add 100 µl of ^{125}I β-endorphin to each tube.
6. Vortex gently and incubate for 16 - 24 hours at 2 - 8° C.
7. Add 500 µl of goat anti-rabbit precipitating reagent (GAR-PPT) to each tube.
8. Vortex gently without foaming and incubate for 2 hours (± 15 minutes) at 2 - 8° C.
9. Centrifuge the tubes at a minimum of 760 x g* for 20 minutes at 20-25° C.
10. Decant or aspirate supernatant.
11. In a gamma scintillation counter, count the precipitate of each tube, and the total count tubes, for at least 60 seconds (see Limitations of the Procedure, page 14).

---

* For your convenience, the formula for computing g's is:

$$g = (1118 \times 10^{-8})(\text{radius in cm})(\text{rpm})^2$$

$$760 = (1118 \times 10^{-8})(\text{radius in cm})(\text{rpm})^2$$

$$\text{RPM} = \sqrt{\frac{760}{(1118 \times 10^{-8})(\text{radius in cm})}}$$

60 ASSAYS, KIT
6 = Std

BETA ENDORPHIN

Table I

SET UP ASSAY ON CRUSHED ICE

	TOTAL COUNT	NSB	STANDARDS pmol/liter						UNKNOWN SAMPLES			
			0	3.75 5	7.5 10	15 20	30 40	60 80	1	2	3	4
Tube number	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24
1% BSA-borate		200	200									
Standards				200	200	200	200	200				
Unknown samples									200	200	200	200
Rabbit anti-β-endorphin serum			100									
	Vortex gently. Incubate for 16 - 24 hours at 2 - 8° C.											
^{125}I β-endorphin	100											
	Vortex gently. Incubate for 16 - 24 hours at 2 - 8° C.											
Goat anti-rabbit precipitating complex (GAR-PPT)		500										
	Vortex gently. Incubate for 2 hours (± 15 minutes) at 2 - 8° C.											
All volumes are in microliters	NOT TC 3030P Centrifuge at 760 x g* for 20 minutes at 20 - 25° C. Decant or aspirate supernatants. Count precipitate of each tube for 60 seconds or longer.											

12 pm

Calculation

1. Calculate the average CPM for each standard, control and sample.
2. Subtract the average CPM of the NSB tubes from all counts.
3. Divide the correct CPM of each standard, control, or sample by the corrected CPM of the 0 standard.

$$B/Bo\ (\%) = \frac{\text{CPM of Standard or Sample - CPM of NSB}}{\text{CPM of 0 Standard - CPM of NSB}} \times 100$$

4. Using 2 cycle semi-log or log-logit graph paper, plot percent B/Bo for the β-endorphin standards (vertical axis) versus the concentration (horizontal axis).
5. Draw a best-fit line through the points.
6. Interpolate the levels of β-endorphin in the samples from the plot.
7. If any sample reads greater than 80 pmol/ℓ, it should be diluted appropriately in 0 standard and assayed again.
8. If a sample has been diluted, correct for appropriate dilution factor.
9. Calculate maximum binding by dividing CPM of 0 standard by the average total counts obtained in the total count tubes.

Sensitivity

When defined as the apparent concentration at three standard deviations from the counts at maximum binding, the minimum detectable amount is 0.6 pmol/tube (3 pmol/ℓ).

Specificity

Comparison of the reactivity of the β-endorphin antibody was made with the following peptides:

	cross-reactivity (molar basis)		cross-reactivity (molar basis)
Human β-endorphin	100 %	$ACTH^{1-39}$	< 0.1 %
Human β-lipotropin	50 %*	$ACTH^{1-24}$	< 0.1 %
[$Des\text{-}Tyr^1$] human β-endorphin	> 100 %	α-MSH	< 0.1 %
[$2\text{-}Me\text{-}Ala^2$] β-endorphin	> 100 %	β-MSH	< 0.1 %
α-endorphin ($\beta\text{-}LPH^{61-76}$)	< 0.1 %	Prolactin	< 0.1 %
α-endorphin ($\beta\text{-}LPH^{61-77}$)	< 0.1 %	Luteinizing hormone	< 0.1 %
[$D\text{-}Ala^2$]-β-endorphin	< 0.1 %	Follicle stimulating hormone	< 0.1 %
Dynorphin	< 0.1 %	Thyroid stimulating hormone	< 0.1 %
α-neo-endorphin	< 0.1 %	Vasopressin	< 0.1 %
α-endorphin	< 0.1 %	Oxytocin	< 0.1 %
Leucine enkephalin	< 0.1 %	N-acetyl-β-endorphin	100 %
Methionine enkephlin	< 0.1 %	[Leu^5] camel β-endorphin	100 %

* Standardization of β-LPH is not yet precise.

Limitations of the Procedure

1. Care must be taken so that all samples are extracted uniformly.
2. Counting times should be sufficient to prevent introduction of statistical error due to counting (for example, accumulation of 2,000 cpm will yield 5% error, 10,000 cpm will yield 1% error).

REFERENCES

1. Akil, H., S.J. Watson, J.D. Barchas, C.H. Li, "β-Endorphin-like Immunoreactivity by RIA in Rat Blood - Normal Levels and Comparison to Human Plasma," Society for Neuroscience 8th Annual Meeting, Nov. 5-9, 1978, Abstract 1273.

2. Chang, W.C., P.A. Jagannadha, and C.H. Li, "Rate of Disappearance of Human β-Lipotropin and β-Endorphin in Adult Male Rats as Estimated by Radioimmunoassay," Int. J. Peptide Protein Res. 11:93, 1978.

3. Guillemin, R., N. Ling, and T.M. Vargo, "Radioimmunoassay for α-Endorphin and β-Endorphin," Biochem. and Biophy. Res. Com. 77:361-6, 1977.

4. Hollt, V., R. Przewlocki, and A. Herz, "Radioimmunoassay of β-Endorphin Basal and Stimulated Levels in Extracted Rat Plasma," Naunyn-Schmiedeberg's Archives of Pharmacology 303 (2):171, 1978.

5. Hosobuchi, Y., J. Rossier, F.E. Bloom, R. Guillemin, "Stimulation of Human Periaqueductal Gray for Pain Relief Increases Immunoreactive β-Endorphin in Ventricular Fluid," Science 203 (4377):279, 1979.

6. Jeffcoate, W.J., L.H. Rees, P.J. Lowry, and G.M. Besser, "A Specific Radioimmunoassay for Human β-Lipotropin," Journal of Clinical Endocrinology and Metabolism 47:160, 1978.

7. Jeffcoate, W.J., P.J. Lowry, L.H. Rees, J. Hope, and G.M. Besser, "β-Lipotropin and β-Endorphin in Human Plasma and CSF," The Endocrine Society 60th Annual Meeting, June 14-16, 1978, Abstract No. 142.

8. Jeffcoate, W.J., L. Rees, L. McLoughlin, S. Ratter, J. Hope, P.J. Lowry, and G.M. Besser, "β-Endorphin in Human Cerebrospinal Fluid," The Lancet II (8081):119, 1978.

9. Marx, J., Review of "Neurobiology: Researchers High on Endogenous Opiates," Science 193:1227-29, 1976.

10. Orf, J.W., J. Ells, N. Johanson, F. Vanderloise, and A.W. Lindall, "Extraction of β-endorphin from Serum Binding Proteins," Clinical Research 27 (4):680A, 1979.

11. Orth, D.N., R. Guillemin, N. Ling, and W.E. Nicholson, "Immunoreactive Endorphins, Lipotropins and Corticotropins in a Human Nonpituitary Tumor: Evidence for a Common Precursor," Journal of Clinical Endocrinology and Metabolism 46:849-852, 1978

12. Rossier, J., A. Bayon, T.M. Vargo, N. Ling, R. Guillemin, and F. Bloom, "Radioimmunoassay of Brain Peptides: Evaluation of a Methodology for the Assay of Beta Endorphin and Enkephalin," Life Sciences 21:847-852, 1977.

13. Shinkmanter, B., N. Aronin, A. Liotta, T. Suda, and D.T. Kreiger, "Presence of α-MSH and Endorphin-like Immunoreactivity in Human Cerebrospinal Fluid (CSF)," The Endocrine Society 60th Annual Meeting, June 14-16, 1978, Abstract No. 387.

14. Suda, T., A.S. Liotta, and D.T. Krieger, "β-Endorphin is Not Detectable in Plasma from Normal Human Subjects," Science 202:221, 1978.

15. Wardlaw, S.L. and A.G. Frantz, "Measurement of β-Endorphin in Human Plasma," Journal of Clinical Endocrinology and Metabolism 48 (1):176, 1979.

16. Wiedermann, E., T. Saito, J.A. Linfoot, and C.H. Li, "Specific Radioimmunoassay of Human β-Endorphin in Unextracted Plasma," Journal of Clinical Endocrinology and Metabolism 49 (3):478, 1979.

17. Yoshimi, H., S. Matsukura, S. Sueoka, M. Fukase, M. Yokota, Y. Hirata, and H. Imura, "Radioimmunoassay for Beta-Endorphin - Presence of Immunoreactive Big-Big Beta-Endorphin (Big Beta-Lipotropin) in Human and Rat Pituitaries," Life Sciences 22(24):2189, 1978.

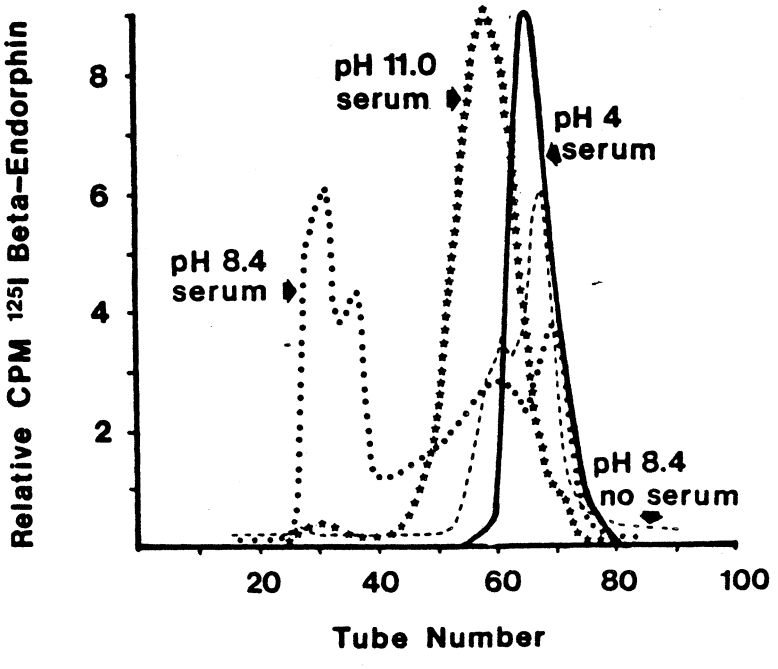


Figure 1. The effect of pH on binding of ^{125}I β-endorphin to serum at pH 8.4 (dotted line); peaks are seen at tubes 30 and 60. In the absence of serum at pH 8.4 (dashed line) only a peak at tubes 60 - 70 is seen. In either acid (solid) or base (stars) in the presence of serum only one peak is seen at tubes 60 and 70.

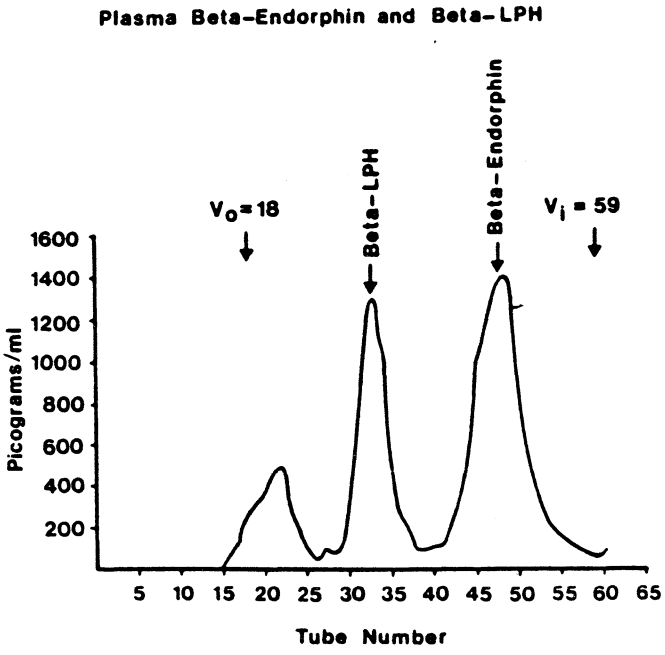


Figure 2A. A chromatogram of the eluted peptides from an affinity gel extraction of plasma. Chromatography was done on a 1 x 30 cm, G-75 superfine column using 0.1 M borate buffer pH 8.4 as the eluant. The affinity gel was made by coupling β-endorphin antibody to Sepharose.

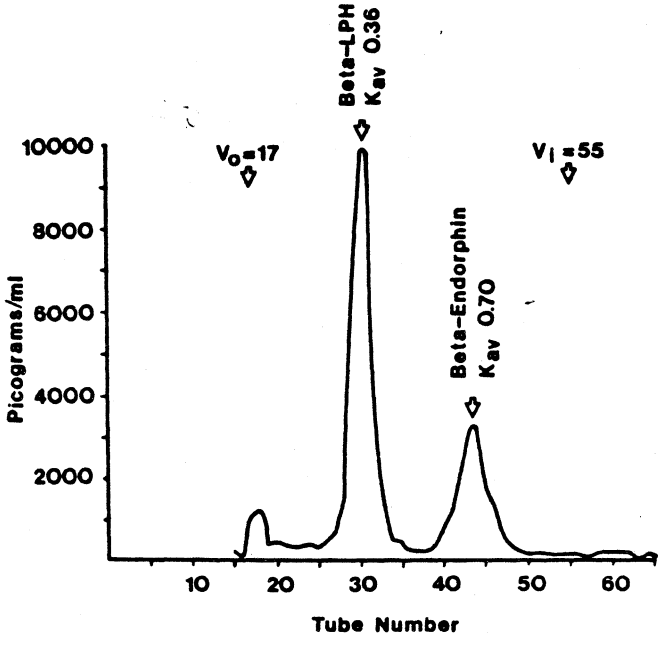


Figure 2B. Column chromatography of a mixture of pure β-LPH and β-endorphin as in figure 2A.

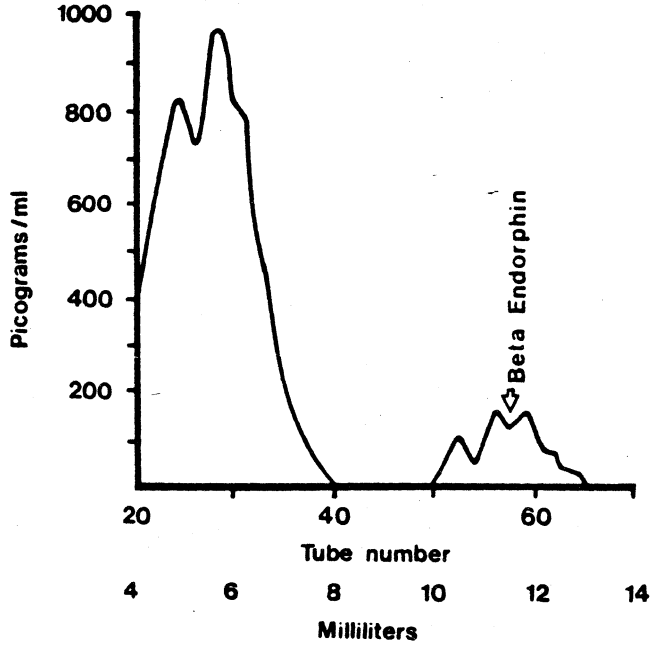
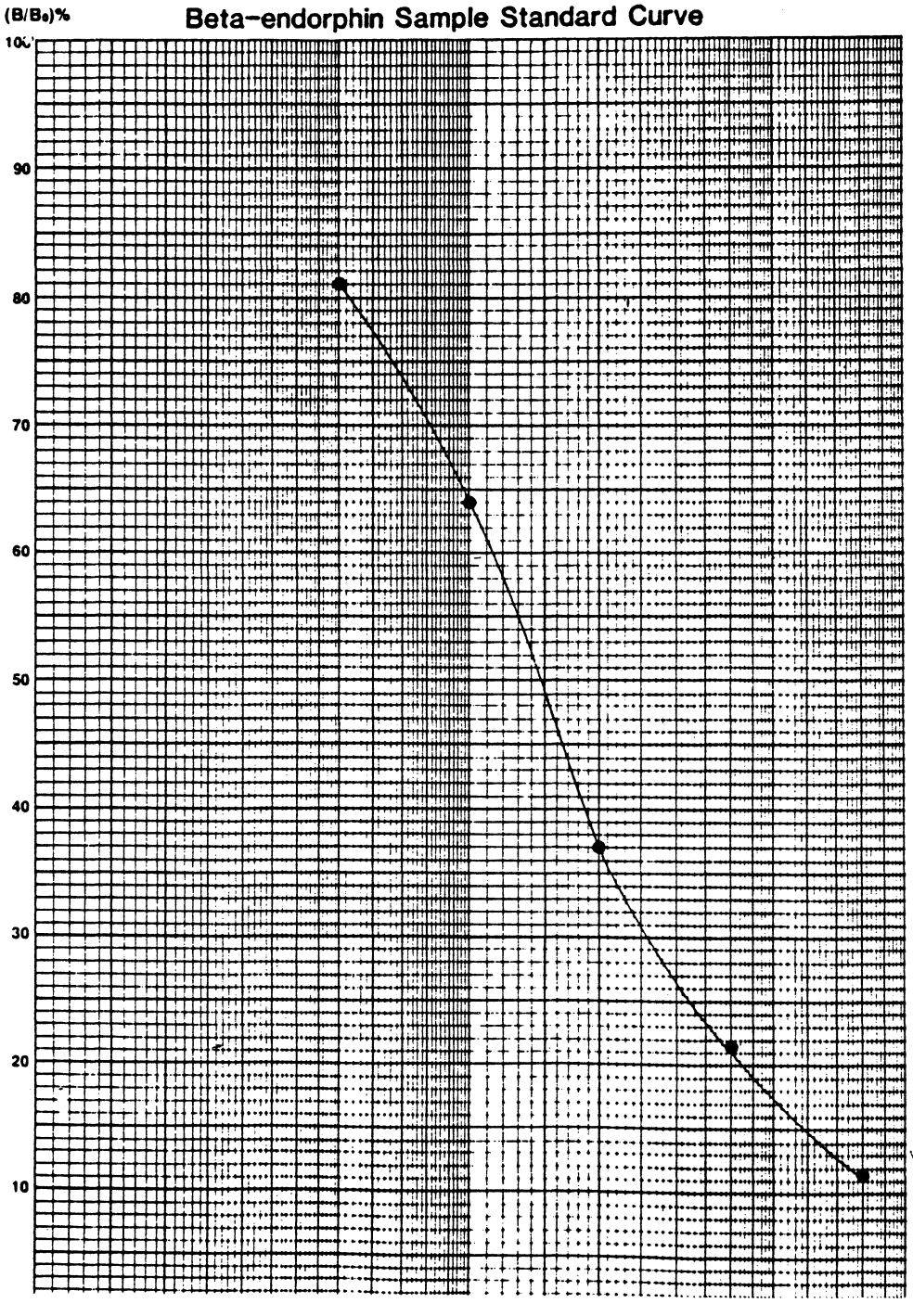


Figure 3. Direct chromatography of 2 ml plasma on 0.5 x 30 cm, G-75 superfine column. 0.2 ml fractions were collected and assayed directly. Recovery of β-endorphin was 115 pg/ml.


(B/B₀)%
Beta-endorphin Sample Standard Curve
100
90
80
70
60
50
40
30
20
10

Appendix B

Protein Assay Procedure

PROTEIN DETERMINATION - DYE BINDING ASSAY

Protein Reagent:

Dissolve 100 mg. Coomassie Brilliant blue G-250 dye (Sigma) in 50 ml 95% methanol. (Be sure not to use the R-250 or electrophoresis type dye).

Add 100 85% phosphoric acid.

Dilute with dH_2O to 1 liter.

Filter with Whatman no. 1 paper.

Standard:

Use a 1 mg/ml (1ug/ul) bovine serum albumin standard made up in dH_2O.

Std. Curve	Vol 1 mg/ml BSA
0	0 ul
10	10 ul
25	25 ul
50	50 ul
75	75 ul
100	100 ul

Procedure:

Place the appropriate volume of BSA into 13 x 100 mm culture tubes. 50 ul of the unknown samples were used for this assay.

Dispense 5.0 ml dye-binding protein reagent into test tubes.

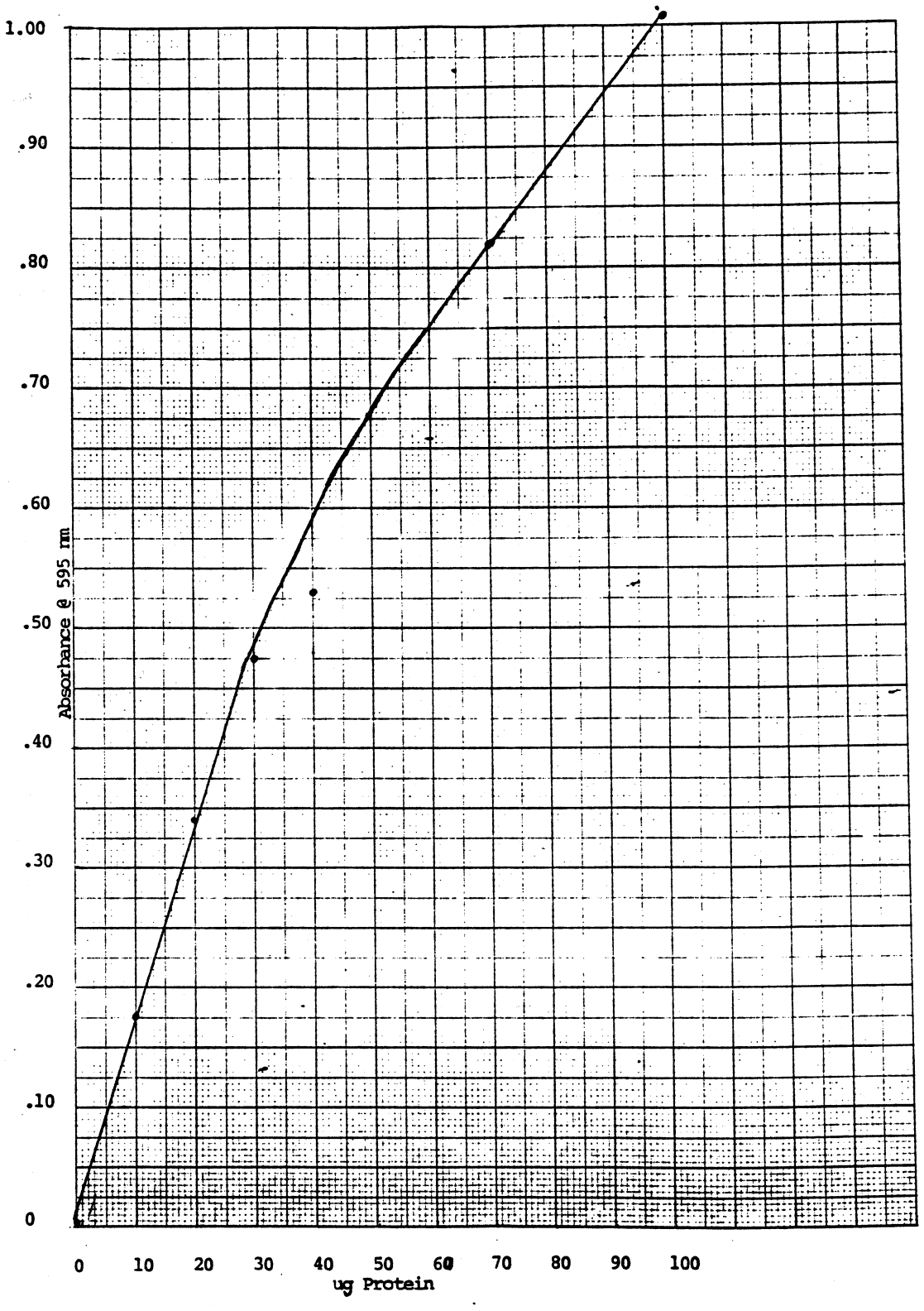
Vortex briefly.

Zero cuvettes against blank.

Read absorbance at 595 nm between 5 minutes and one hour.

Plot absorbance vs ug protein.

Source: Bradford (25).


1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
Absorbance @ 595 nm
0
10
20
30
40
50
60
70
80
90
100
ug Protein

Appendix C

Radioassay Systems Laboratories, Inc. Procedure for Rat Serum

Radioassay Systems Laboratories, Inc.
20770 LEAPWOOD AVE. CARSON, CALIFORNIA 90746
TELEPHONE: (213) 537-2141 (213) 774-0730 TWX: 910-346-7727

RSL CORTICOSTERONE (^{3}H) KIT

PROCEDURE FOR THE RADIOIMMUNOASSAY OF RAT SERUM OR PLASMA CORTICOSTERONE

IMPORTANT NOTICE

DO NOT FREEZE ENTIRE KIT.

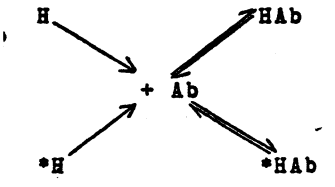
HOWEVER, FOR BEST STABILITY THE TRACER (IN ETOH), ANTI-SERUM AND STANDARD VIALS SHOULD BE STORED AT -15°C IMMEDIATELY UPON RECEIPT OF KIT.

TABLE OF CONTENTS

I. PRINCIPLES OF RIA

Radioimmunoassay (RIA) is the term applied to the measurement of the concentration of antigen molecules using a radioactive label that quantitates the amount of antigen (i.e., hormone) by determination of the extent to which it combines with its antibody.

In the assay, a limited amount of specific antibody (Ab) is reacted with the corresponding hormone (*H) labeled with a radioisotope. Upon addition of an increasing amount of the hormone (H), a correspondingly decreasing fraction of *H added is bound to the antibody. After separation of the bound from the free *H by various means, the amount of radioactivity in one or both of these two fractions is evaluated and used to construct a standard curve against which the unknown samples are measured.



II. PRECAUTIONS IN HANDLING RADIOACTIVE MATERIAL

A. This radioactive material may be received, acquired, possessed, and used only by physicians, clinical labs, or hospitals, and is intended for in-vitro laboratory tests not involving internal or external administration of the materials. Thus, the possession, use and transfer of the radiation herein are subject to the regulations of, and with a general license from, the U.S. NRC or the state with which the NRC has entered into agreement for the exercise of regulatory authority.

B. Immediately upon receipt of this kit, check for breakage and verify contents as per the packing list. Should there be breakage or questions regarding this kit's contents, immediately notify your supplier by telephone.

C. Kit reagents should be stored and used only at clean, designated work stations of the laboratory. Although exposure to radiation from the small amount of radioactive material supplied is negligible, it is good practice to designate a storage area at least 10 feet from any work station. Furthermore, persons under the age of 18 should not be permitted to handle radioactive material or enter an area where it is present.

D. Should there be spillage of any radioactive material, the following clean-up procedure is recommended. While wearing rubber gloves, blot the spillage with a paper towel. This contaminated towel should be disposed as radioactive waste. Wash the affected area with a detergent, then rinse gloves with water, tear to prevent further use and discard as ordinary waste.

E. The pipetting of radioactive material by mouth should not be permitted. Smoking, eating, or drinking while performing tests involving radioactive material should be prohibited. Lastly, persons handling radioactive material should wash their hands immediately after handling and prior to leaving the laboratory.

III. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

A. This kit is intended for investigational purposes only.

B. Care must be taken that unknown samples contain no exogenous radioactivity since its presence may lead to erroneous results.

C. The reagents provided in this kit are intended for the direct, specific measurement of serum or plasma corticosterone in rats only. Quantitating corticosterone levels in human serum or plasma requires extraction/chromatography before assay.

D. Strict adherence to the protocol is recommended. Any changes should be done at the discretion of the user.

E. The reagents provided in the kit are for IN-VITRO DIAGNOSTIC USE ONLY.

IV. EQUIPMENT AND REAGENTS REQUIRED

In addition to the reagents supplied with the kit, the following materials are required:

A. Pipettor and/or pipets that can accurately and precisely deliver the required volumes.

B. Liquid scintillation counter with windows set for counting tritium (^3H).

C. Refrigerated centrifuge.

D. Heat block or water bath at 98°C (large enough to accommodate all assay tubes during denaturization of rat binding proteins).

E. Test tube rack.

F. Disposable glass tubes for RIA (10 x 75 mm).

G. Liquid scintillation cocktail - any formula that will accept 0.9 ml of aqueous material should be acceptable.

V. KIT CONTENTS

A. 1 x 11 ml ANTI-CORTICOSTERONE.

B. 1 x 0.5 ml CORTICOSTERONE-^{3}H in ETOH.

C. 6 x 5 ml CORTICOSTERONE STANDARDS, range 0.025-1.0 ng/0.5 ml.

D. 1 x 25 ml CHARCOAL DEXTRAN SOLUTION.

E. 1 X 250 ml DILUENT BUFFER.

VI. STABILITY OF KIT REAGENTS

All kit reagents will have a minimum stability of 4 months from day of receipt when stored at the following conditions:

A. Store at -15°C:

1. CORTICOSTERONE^{3}H (in ETOH; as received in kit).

2. CORTICOSTERONE STANDARDS.

3. ANTI-CORTICOSTERONE.

B. Store at 2-8°C:

1. CHARCOAL DEXTRAN SOLUTION. DO NOT FREEZE.

2. DILUENT BUFFER.

VII. REAGENT PREPARATION PRIOR TO ASSAY

With the exception of the CORTICOSTERONE-^{3}H tracer (in ETOH), all reagents are shipped ready to use. To prepare "working" CORTICOSTERONE-^{3}H tracer for assay, evaporate the storage solvent (ETOH) under a gentle stream of dry nitrogen gas. Add 12 ml of DILUENT BUFFER and equilibrate at room temperature for at least 15 minutes. Mix well and check the concentration of the solution; 0.1 ml of this "working" CORTICOSTERONE-^{3}H tracer should contain ~ 10,000 cpm.

The stability of "working" CORTICOSTERONE-^{3}H tracer is approximately 2 months from the day of reconstitution when stored at 4°C. DO NOT FREEZE "working" CORTICOSTERONE-^{3}H tracer.

VIII. SAMPLE PREPARATION PRIOR TO ASSAY

Dilute rat serum/plasma 1:500 with DILUENT BUFFER. We suggest diluting 10 µl of serum/plasma to 5.0 ml.

NOTE: The baseline corticosterone levels in rats can vary greatly according to handling techniques and sample collection methods. It is best for each laboratory to determine its own range of values for baseline and stimulated samples. Generally, a range of 100 - 250 ng/ml can be expected for baseline rat samples.

IX. ASSAY PROCEDURE

A. Bring reagents to room temperature prior to use.

B. Using 10 x 75 mm glass tubes, add 0.6 ml of DILUENT BUFFER to tube numbers 1 and 2.

C. Add 0.5 ml DILUENT BUFFER to tube numbers 3 and 4.

D. Add 0.5 ml (in duplicate) of each CORTICOSTERONE STANDARD (0.025 - 1.0 ng/0.5 ml) to tube numbers 5 - 16.

E. Add 0.5 ml (in duplicate) of diluted SAMPLE or CONTROL to tube numbers 17 to end of assay.

F. Incubate ALL TUBES at 98°C for 10 minutes (this step is necessary to denature the CORTICOSTERONE binding proteins in rat serum/plasma).

G. Allow sufficient time for the tubes and samples to cool to room temperature (~30 minutes).

H. WITH THE EXCEPTION OF TUBE NUMBERS 1 AND 2, add 0.1 ml of ANTI-CORTICOSTERONE to all the rest of the assay tubes.

I. Add 0.1 ml of "working" (i.e. diluted) CORTICOSTERONE-^{3}H (containing ~ 10,000 cpm/0.1 ml) to ALL the assay tubes.

J. Mix and incubate for a minimum of 1 hour or a maximum of 24 hours in a 4°C water bath.

K. After incubation add 0.2 ml of COLD (4°C) CHARCOAL DEXTRAN solution (SHAKE WELL initially then mix continuously). Mix for 20 seconds by shaking the whole test tube rack VIGOROUSLY and incubate for 20 minutes at 4°C.

L. Centrifuge at 2500 rpm for 15 minutes then decant entire supernatant into scintillation cocktail. Count for a minimum of 1 minute in a beta counter.

X. CALCULATIONS

A. Percent Bound:

1. Average the counts of tubes 1 and 2 (blank or NSB) and subtract from the average counts of all other duplicate assay tubes.

2. Divide the value obtained from tubes 3 and 4 into the values obtained for duplicate sample tubes. Multiplying by 100 gives percent bound.

Formula:

$$\%B = \frac{\overline{CTS}\ (\text{sample}) - \overline{CTS}\ (\text{Blank NSB})}{\overline{CTS}\ (\text{0 standard}) - \overline{CTS}\ (\text{Blank NSB})} \times 100$$

$\overline{CTS}$ = Average counts of duplicate tubes.

Sample = Standard, control, or serum unknown.

Blank (NSB) = Blank tube (also known as non-specific binding tube; tubes #1 and #2).

0 Standard = 0 tube (also known as 100% binding tube; tubes #3 and #4)

Sample Calculation (from sample assay):

$$\text{Control II} = \frac{\overline{CTS}\ (\text{sample}) - \overline{CTS}\ (\text{Blank NSB})}{\overline{CTS}\ (\text{0 standard}) - \overline{CTS}\ (\text{Blank NSB})} \times 100$$

$$= \frac{2669 - 181}{5710 - 181} \times 100$$

$$= \frac{2488}{5529} \times 100$$

$$= 45\%$$

B. Final Results:

Plot percent bound using 100% as the starting point against the CORTICOSTERONE STANDARDS ranging from 0.025-1.0 ng/0.5 ml.

Then, if sample was diluted 1:500;

ng read off standard curve x 1000 = ng/ml CORTICOSTERONE.

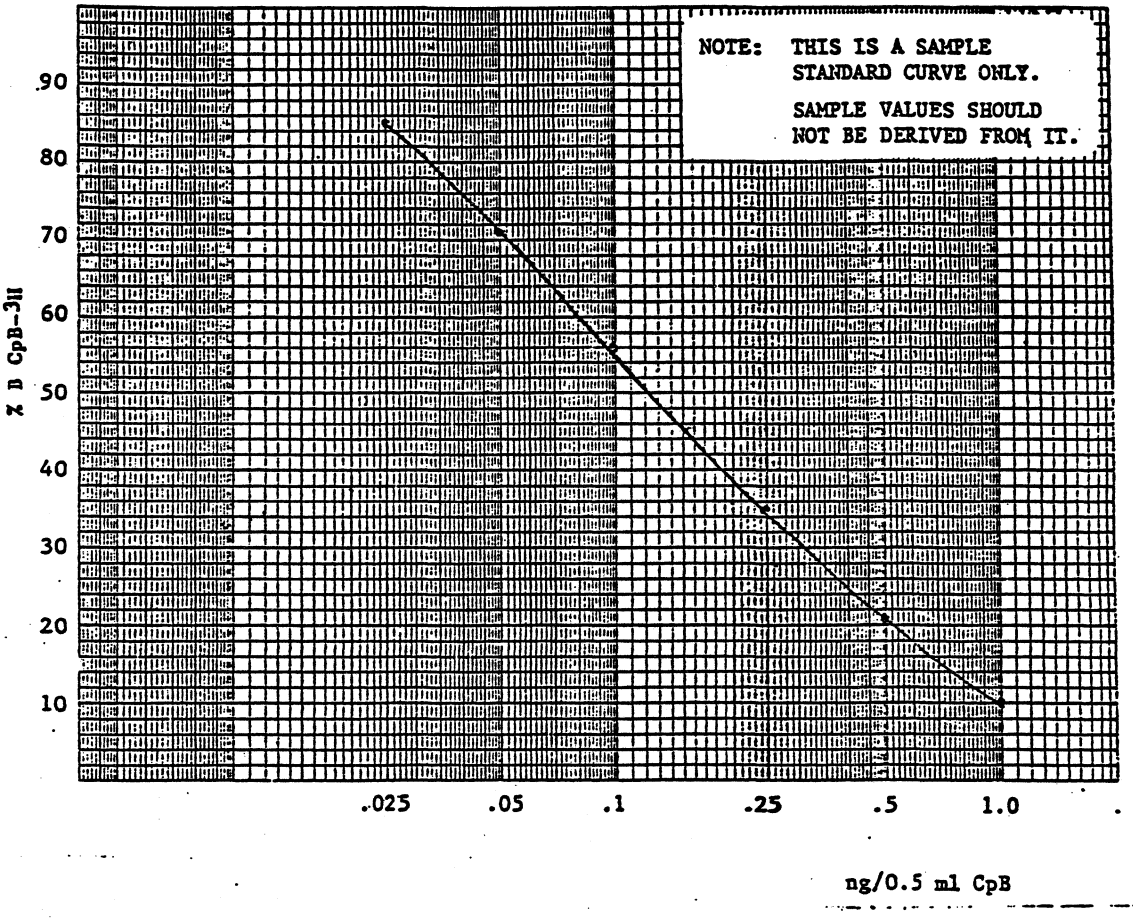
XI. SPECIFICITY OF ANTISERUM

STEROIDS	% CROSS REACTION
Corticosterone	100.00
Desoxycorticosterone	6.10
Progesterone	0.29
Cortisol	0.19
Aldosterone	0.08
20α-Dihydroprogesterone	0.08
Testosterone	0.08
11-Desoxycortisol	0.03
Androstenedione	0.01
Cholesterol	<0.01
Dehydroepiandrosterone	<0.01
Dehydroepiandrosterone-sulfate	<0.01
Dihydrotestosterone	<0.01
Estradiol-17β	<0.01
Estradiol-17α	<0.01
Estrone	<0.01
Estriol	<0.01
Pregnenolone	<0.01
17α-Hydroxypregnenolone	<0.01
17α-Hydroxyprogesterone	<0.01

XII. SAMPLE ASSAY

Sample	CPM	AVG. CPM	Avg.-Blank CPM	%B	CORTICOSTERONE (ng/ml)
Blank (NSB)	166 196	181			
0.0 ng/0.5ml	5696 5724	5710	5529	100	
0.025ng/0.5ml	4795 4931	4863	4682	85	
0.05 ng/0.5ml	4121 4119	4120	3939	71	
0.1 ng/0.5ml	3176 3394	3285	3104	56	
0.25ng/0.5ml	2081 2140	2110	1929	35	
0.5 ng/0.5ml	1350 1388	1369	1188	21	
1.0 ng/0.5ml	748 767	757	576	10	
CONTROL I	4626 4804	4715	4534	82	0.030X1000=30
CONTROL II	2620 2718	2669	2488	45	0.16X1000=160
CONTROL III	1672 1664	1668	1487	27	0.37X1000=370

XIII. STANDARD CURVE



Reagent Addition Sequence

TUBE NO.	(1) DILUENT BUFFER (ml)	(2) STANDARDS SERUMS OR CONTROLS (ml)	(3) ANTI-SERUM (ml)	(4) ^{3}H TRACER (ml)	(5) CHARC. DEXTRAN (ml)	DESCRIPTION	% BOUND	RESULTS
1	0.6	0	0	0.1	0.2	Blank (NSB)		
2	0.6	0	0			Blank (NSB)		
3	0.5	0	0.1			0.0 ng/0.5ml		
4	0.5	0				0.0 ng/0.5ml		
5	0	0.5				0.025ng/0.5ml		
6						0.025ng/0.5ml		
7						0.05 ng/0.5ml		
8						0.05 ng/0.5ml		
9						0.1 ng/0.5ml		
10						0.1 ng/0.5ml		
11						0.25 ng/0.5ml		
12						0.25 ng/0.5ml		
13						0.5 ng/0.5ml		
14						0.5 ng/0.5ml		
15						1.0 ng/0.5ml		
16						1.0 ng/0.5ml		
17						SERUM CONTROL		
18						SERUM CONTROL		
19						SERUM CONTROL		
20						SERUM CONTROL		
21						SERUM UNKNOWN		
22	↓	↓	↓	↓	↓	SERUM UNKNOWN		

The vita has been removed from the scanned document