THE EFFECTS OF PROSTAGLANDIN INHIBITION ON THE SYMPATHETIC AND PRESSOR RESPONSES TO MUSCULAR CONTRACTION AND POSTCONTRACTION MUSCLE ISCHEMIA

by

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THE EFFECTS OF PROSTAGLANDIN INHIBITION ON THE SYMPATHETIC AND PRESSOR RESPONSES TO MUSCULAR CONTRACTION AND POSTCONTRACTION MUSCLE ISCHEMIA IN HUMANS

by

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(ABSTRACT)

The purpose of this study was to determine the effect of prostaglandin (PG) inhibition on the sympathetic and pressor responses to isometric handgrip (HG) at 40% of maximal voluntary contraction (MVC) to exhaustion and postcontraction muscle ischemia (PC). To accomplish this heart rate (HR), arterial pressure (n=10) and plasma norepinephrine (NE) levels (n=8) were measured in 10 healthy male subjects during HG at 40% of MVC to exhaustion and during PC. The subjects were given a double-blind administration of either placebo (PLAC) or a single 100 mg dose of indomethacin (IND). The order of administration was counterbalanced and a one week drug washout period was provided between conditions. Mean arterial pressure increased 25±5 vs. 22±4 mmHg during the second minute of HG and 26±2 vs. 21±5 during the last minute of PC in PLAC vs. IND (P>.05), respectively. Heart rate was increased 21±4 vs. 17±3 bpm during the second minute of HG in PLAC vs. IND (P>.05), respectively and returned to control values during PC in both trials. Plasma NE increased 343±89 vs. 289±89 pg/ml after HG and 675±132 vs. 632±132 pg/ml after PC (P>.05) in

PLAC vs. IND, respectively. Therefore, PG inhibition does not alter sympathetic or arterial pressure responses during sustained isometric exercise in humans. This may suggest that 1) PGs not important in metaboreceptor stimulation of sympathetic or pressor responses to sustained isometric contractions in humans or 2) PGs may play only a small role in the regulation of these variables which may be masked by the effects of other stimuli.

Index terms: prostaglandins, pressor reflex, muscle sympathetic nerve activity, static exercise

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Chapter I

INTRODUCTION

The circulatory responses that occur during exercise have been attributed to neural impulses arising from the central nervous system (Krogh & Lindhard, 1913; Eldridge et al., 1985) and from reflexes arising in skeletal muscle (Rowell et al., 1981; Mitchell et al., 1983). The neural mechanisms that are responsible for the changes in efferent autonomic activity to the heart and blood vessels during exercise are incompletely understood.

Static and rhythmic exercise are accompanied by increases in heart rate (HR), arterial blood pressure (BP) and sympathetic (SNS) activity (Rowell, 1986). Alam and Smirk (1937) were the first to demonstrate the reflex nature of the pressor response to static exercise when they observed an elevated arterial pressure during a period of postcontraction circulatory arrest. It was postulated that accumulation of ischemic metabolites stimulated sensory nerves in skeletal muscle and triggered a metaboreflex. This would appear to be the an efficient way of alerting the central nervous system of a mismatch between blood flow and the rate of metabolic activity.

A second mechanism for the autonomic adjustments to exercise is central command, which refers to neural impulses arising from the central activity for the recruitment of motor

units. These neural impulses are thought to excite both medullary and spinal neural circuits (Krogh and Lindhard, 1937; Freychuss, 1970; Mitchell et al., 1983). Freychuss (1970) has shown that blood pressure still increases during intended isometric handgrip when subjects muscles have been paralyzed by curarization. In addition, the pressor response parallels electromyographic activity, reflecting increasing motor unit recruitment, during force constant experiments (Schibye et al., 1981).

During exercise, the increase in BP is partially mediated by a skeletal muscle reflex (Coote et al. 1971; Fisher et al., 1974 Mitchell et al., 1983). The afferent arm of this reflex is composed of group III and IV neural fibers. Mechanical or chemical stimulation of these fibers can influence efferent SNS activity, HR and BP.

Recently, attention has been paid to the metabolic products that may be involved in the pressor reflex from contracting skeletal muscle. Substantial evidence suggests that the metabolic stimuli results from a mismatch between blood flow and the rate of metabolic activity (Wyss et al., 1983; Sheriff et al., 1987). Rybicki et al. (1985) has shown that infusion of potassium into the arterial supply of the gracilis muscle of anesthetized cats increases the activity of group III and IV afferent fibers. However, the activity of these afferents were rapidly adapting suggesting that

potassium may not be the only stimulus. Rotto et al. (1988) has found that other metabolic substances, such as lactic acid and arachidonic acid, increased the discharge rate of group III and IV afferent fibers in anesthetized cats. More importantly, infusion of these substances resulted in reflex increases in BP. Muscle ischemia has also been shown to exaggerate the pressor reflex, presumably a result of accelerated anaerobic metabolism (Stebbins et al., 1989). The rise in BP would serve to restore blood flow to the underperfused tissues.

Recent evidence has suggested that prostaglandins (PGs) may contribute to the pressor reflex in anesthetized cats (Stebbins et al., 1986). Prostaglandins have been shown to mediate the reflex through an action on afferent nerve endings rather than through a regional vascular effect. Blockade of PG synthesis, with either indomethacin or sodium meclofenamate, attenuates the cardiovascular response to electrically induced contraction in anesthetized cats (Stebbins et al., 1986).

Cyclooxgenase products have been shown to accumulate in skeletal muscle during contraction (Symons et al., 1991; Rotto et al.,1989). Rotto et al. (1990) has shown that infusion of arachidonic acid increases the activity of group III afferents to induced contraction. Likewise, indomethacin attenuated responsiveness of the these afferent fibers to contraction.

Cyclooxygenase products have also been shown to be necessary for the full expression of group IV afferents to static contraction (Rotto et al., 1990).

In humans, metaboreceptor stimulation seems more important than mechanical stimulation in eliciting sympathoexcitation (Rowell and O'Leary, 1990). At least three pieces of evidence support this contention. First, tendon vibration in human fails to increase muscle sympathetic nerve activity (MSNA) (Mark et al., 1985). Second, MSNA shows a rather slow pattern of activation during isometric handgrip exercise suggestive of metabolite mediation (Mark et al., 1985 Victor et al., 1988; Seals et al., 1988). And third, MSNA remains elevated during postcontraction circulatory arrest.

There is increasing evidence that the pressor reflex is due to stimulation of chemosensitive afferents by ischemic metabolites. This metaboreflex is thought to be important in the sympathoexcitation during static exercise and at moderate levels of rhythmic exercise (Victor et al., 1987). For example, Victor et al. (1988) has shown that sympathetic nerve discharge during static handgrip in humans is coupled to the cellular accumulation of hydrogen ions. This has been supported by Sinoway et al. (1989) who demonstrated an association between muscle acidosis and calf vasoconstriction during static handgrip. This group has also shown that infusion of dichloroacetate, which inhibits lactic acid

formation, attenuates the rise in MSNA associated with static handgrip and postcontraction muscle ischemia (Ettinger et al., 1991).

Evaluation of the role of metabolic products in stimulating reflex pressor responses has been largely limited to animal studies. However, recently it has been suggested that in humans metaboreceptor stimulation seems more important than mechanoreceptor stimulation in eliciting sympatho-excitation (Rowell and O'Leary, 1990). For example, Victor et al. (1988) has shown that muscle cell pH is correlated to MSNA during exercise. The acidosis that occurs with muscular activity has also been shown to be related to calf vasoconstriction (Sinoway et al. 1989). In addition, Ettinger et al., (1991) found in a recent study that dichloroacetate (DCA) attenuates the rise in MSNA associated with fatiguing handgrip exercise (Ettinger et al. 1991). In addition, the change in mean arterial pressure was significantly less after DCA infusion. This suggests that, as in anesthetized animals, lactic acid is important in stimulating chemosensitive afferent fibers.

Metaboreceptor stimulation has been shown to be important in the pressor response to static contraction in both animal models and human studies. There is evidence that PGs are important, in anesthetized cats, in mediating the pressor response to induced contractions (Rotto et al, 1990; Stebbins

et al.,1986). However, it is not known if this mechanism is important is autonomic-circulatory regulation in humans performing isometric exercise. Therefore, the purpose of this study is to determine whether PGs are important in metaboreceptor stimulation of sympathetic and arterial pressure responses to sustained isometric exercise. In attempt to ensure that any differences observed were due to the effects of PGs on muscle chemoreflexes *per se*, the subjects responses to hand immersion in ice water also were recorded.

Research Problem

The major problem addressed in this investigation was to determine the effects of PG inhibition on the sympathetic and pressor responses to isometric handgrip and postcontraction muscle ischemia in humans.

Research Hypotheses

The following null hypotheses were tested:

Ho_1: There is no difference in the change in arterial pressure responses to isometric handgrip or postcontraction circulatory arrest between the indomethacin and placebo trials.

Ho_2: There is no difference in the change in the plasma norepinephrine response to isometric handgrip or

postcontraction circulatory arrest between the indomethacin and placebo trials.

Ho_3: There is no difference in the change in the arterial pressure response to a cold pressor testing between the indomethacin and placebo trials.

Ho_4: There is no difference in the change in plasma norepinephrine response to a cold pressor challenge between the indomethacin and placebo trials.

Significance of the Study

The results of this study could help to provide more information regarding autonomic-circulatory regulation during static exercise in humans. Specifically, this study may provide insight as to the importance of endogenous prostaglandins in mediating metaboreceptor stimulation of sympathetic nervous system activity and arterial pressure during sustained isometric exercise in humans. In addition, because prostaglandin release is thought to be attenuated in certain pathophysiological states, such as heart failure, results of this study may be important in understanding the impaired metaboreceptor responsiveness in these patients.

Delimitations

The following delimitations were imposed by the investigator due to the nature of the study:

1. The selection of subjects was limited to 10 males, age 18-35, who have not been previously engaged in upper body aerobic or resistance training programs for the prior six months.
2. The exercise mode was limited to isometric handgrip exercise.
3. The subjects were limited to a single dose of 100 mg of indomethacin 2.5 hours before the start of the study.
4. Plasma norepinephrine was the only measure of sympathetic nervous system activity.

Limitations

The following limitations of the study are recognized:

1. Sampling was non-random because subjects were male volunteers, age 18-35, who were not currently engaged in a upper body aerobic or resistance training program.
2. Indomethacin was administered orally; serum levels were not measured and it is uncertain how each subject metabolized the drug.
3. The extent of inhibition of prostaglandin synthesis was not measured and therefore can only be inferred.

Definition and Symbols

1. Prostaglandins - a group of biologically active 20 carbon fatty acids derived from the essential fatty

acids.

2. Indomethacin - a non-steroidal anti-inflammatory drug, typically prescribed for arthritic conditions. Indomethacin is thought to exert its action by inhibition of prostaglandin synthesis.
3. Isometric Handgrip - a sustained contraction of the finger and forearm flexor muscles.
4. Afferent fibers - nerve fibers which conduct impulses from the sensory organs (chemoreceptors and mechanoreceptors) to the central nervous system.
5. Efferent fibers - a nerve fibers which conduct impulses from the central nervous system to effector organs (e.g. skeletal muscle arterioles).
6. Central Nervous System - part of the nervous system consisting of the brain and spinal cord.
7. Sympathetic Nervous System - part of the autonomic nervous systems which utilizes norepinephrine as its primary neurotransmitter. Generally, the end organ effects are excitatory in nature.
8. Norepinephrine - the primary neurotransmitter of the sympathetic nervous system. It is derived from the amino acid tyrosine.

Basic Assumptions

The following assumptions were made:

1. It was assumed that double-blind assignment and counterbalancing of the treatments prevented biasing, despite non-random sampling.
2. It was assumed that all subjects self-administered the indomethacin as instructed.
3. It was assumed that all subjects followed the dietary instructions for the avoidance food and beverages containing caffeine.
4. It was assumed that plasma norepinephrine provided a good index of sympathetic activity during isometric handgrip and the cold stimulation.

Summary

The major objective of this study was to determine if prostaglandins are important in autonomic-circulatory regulation during sustained isometric handgrip in humans. Double-blind and counterbalanced order of administration of either placebo or indomethacin was used to address this issue. The role of prostaglandins in the reflex sympathetic and pressor response to induced muscular contractions in the anesthetized cats has been demonstrated. However, it is not known whether this is an important mechanism in reflex autonomic-circulatory regulation during sustained isometric handgrip in humans. This study was undertaken to address this issue.

Chapter II

REVIEW OF LITERATURE

Introduction

Static and rhythmic exercise are accompanied by increases in heart rate (HR), arterial blood pressure (BP) and sympathetic nervous system (SNS) activity (Rowell, 1986; Christiansen & Galbo, 1983). Two mechanisms have been identified that are responsible for these changes. The increases HR and BP been attributed to neural impulses arising from the central nervous system (Krogh & Lindhard, 1913; Eldridge et al., 1985) and from reflexes arising in skeletal muscle (Rowell et al., 1981; Mitchell et al., 1983). However, the neural mechanisms that are responsible for the changes in SNS activity to the heart and blood vessels during exercise, are incompletely understood.

Alam and Smirk (1937) were the first to demonstrate the reflex nature of the pressor response to static exercise when they observed an elevated BP during a period of post-contraction circulatory arrest. It was postulated that accumulation of ischemic metabolites stimulated sensory nerves in skeletal muscle and triggered a metaboreflex. Functionally, this would appear to be the an efficient way of alerting the central nervous system of a mismatch between blood flow and the rate of metabolic activity. The afferent

arm of the pressor reflex has been shown to reside in the arterially occluded limb of anesthetized cats (Kaufman et al., 1988; Mitchell, 1985; Rowell & Sheriff, 1988).

A second mechanism for the autonomic adjustments to exercise is central command, which refers to neural impulses arising from the central activity for the recruitment of motor units. These neural impulses are thought to excite both medullary and spinal neural circuits (Krogh and Lindhard, 1937; Freychuss, 1970; Mitchell et al., 1983). Freychuss (1970), in particular, has shown that BP still increases during intended isometric handgrip when subjects muscles have been paralyzed by curarization. In addition, the magnitude of the pressor response parallels electromyographic activity, reflecting increasing motor unit recruitment, during force constant experiments (Schibye et al., 1981).

During exercise, the increase in BP is partially mediated by a skeletal muscle reflex (Coote et al. 1971; Fisher et al., 1974 Mitchell et al., 1983). The afferent arm of this reflex is composed of group III and IV neural fibers. Mechanical or chemical stimulation of these fibers can influence efferent sympathetic nerve activity, HR and BP .

Factors Determining the Cardiovascular Response to Exercise

Most forms of exercise are combinations of static and rhythmic contractions (Lind, 1983). Contractions that are

rhythmic in nature involve shortening of muscles and joint rotation. On the other hand, static effort or isometric contractions involve little muscular shortening.

The rise in BP during static exercise is disproportionately high relative to the minimal increase in oxygen consumption. Muscle mass and tension development have been thought to be important determinants of the BP response to static exercise (Shepherd et al., 1981). Mitchell et al. (1981) found that the pressor response to 40% maximal voluntary contraction (MVC) was greater during an extension of the foreleg compared to a scissor-like contraction of the finger muscles. In addition, during postcontraction muscle ischemia the pressor response is dependent on the amount of muscle mass involved; a larger BP response is evoke whenever a larger amount of contracting muscle is involved. Seals (1989) has shown that during static exercise with both arms SNS activity and the BP responses are larger than occurs with the single arm alone, with relative intensity constant. Seals et al. (1988) has also shown that the sympathetic nerve response to static handgrip exercise is dependent on the percentage of MVC. The graded increases in SNS activity appeared to result in proportional BP responses. Thus, the cardiovascular response to static handgrip is dependent on the amount of muscle mass activated and the percentage of MVC. The responsible mechanism has not been identified but it may

involve the relation between receptor distribution and the muscle fiber type and number of motor units in the different muscles activated (Shepherd, 1981).

Arterial and cardiopulmonary baroreceptor have also been thought to modulate the SNS activity during exercise. However, Seals et al. (1988) found that muscle sympathetic nerve activity (MSNA) and BP responses to static exercise when performed during lower body negative pressure were not different from the responses when either were performed alone. The results did not support the hypothesis that cardiopulmonary receptors modulate sympathoexcitation or the pressor response to static exercise. On the other hand, Scherrer et al. (1990) found that arterial baroreceptors buffer the increase in BP and directly measured MSNA that occur during static exercise. Thus, arterial baroreceptors, but not the cardiopulmonary baroreceptor, respond to changes in BP during static exercise and appear to be necessary for normal cardiovascular adjustments to static exercise.

Autonomic Control of the Pressor Response to Static Exercise

In humans, metaboreceptor stimulation seems more important than mechanical stimulation in eliciting sympathoexcitation (Rowell and O'Leary, 1990). At least three sources of evidence support this contention. First, tendon vibration in humans fails to increase muscle sympathetic nerve

activity (MSNA) (Mark et al., 1985). Second, MSNA shows a rather slow pattern of activation during isometric handgrip exercise suggestive of metabolite mediation (Mark et al., 1985 Victor et al., 1988; Seals et al., 1988). And third, MSNA remains elevated during postcontraction circulatory arrest.

Muscle sympathetic nerve activity rises in both the radial and peroneal nerve during static handgrip exercise in humans (Wallin et al., 1989). This pattern suggests homogeneous sympathetic outflow to non-active skeletal muscle which supports the pressor response during static handgrip. Unlike dynamic exercise, static exercise is associated with only a small increase in HR and large increase in BP.

The time course and magnitude of the pressor response to static exercise is strongly influenced by the intensity of contraction. Seals et al. (1988) found that MSNA did not increase during non-fatiguing levels of static handgrip (i.e. 15% MVC). Therefore, tachycardia mediated the elevation of BP during this initial period of contraction. Tachycardia was responsible for the pressor response during the first minute of fatiguing levels of static handgrip (i.e. 25% & 35% MVC). Heart rate plateaued after the first minute indicating that BP during the second minute was mediated primarily by the rise in MSNA. The rise in MSNA would result in increased norepinephrine release from post-ganglionic, sympathetic nerve fibers. Vasoconstriction in non-active skeletal muscle and

possibly other vascular beds serves to support the pressor response. In a such a response heart rate returns to baseline during periods of postcontraction muscle ischemia which suggests that the maintained elevation in MSNA mediates the BP response (Mark et al., 1985).

Plasma Norepinephrine as an Index of Sympathetic Activity

Directly measured microneurographic recordings in man show that muscle sympathetic activity is dominated by pulse synchronous bursts of vasoconstrictor impulses involved in arterial pressure control (Wallin et al., 1987). Plasma levels of norepinephrine in blood samples from an antecubital vein is often used as an index of this sympathetic activity. During various forms of physical stress, plasma NE concentrations correlate with directly measured MSNA. For example, Victor et al. (1987) showed that NE determined from blood samples drawn from an antecubital vein correlated with directly measured MSNA during 2 minutes of cold stimulation. However, the venous NE levels appeared to lag behind, with peak concentrations occurring one minute post immersion. Presumably, increased sympathetic neural outflow, as measured by microneurography, results in increased neurotransmitter spillover where the systemic and end-organ effects are observed. Similar relationships have been demonstrated for various forms of physical exercise. For example, Seals et al.

(1988) found that plasma NE and MSNA increased in an intensity dependent fashion and were qualitatively similar during rhythmic arm cycling exercise. Although, the measurements were positively correlated (r=.80), the magnitude of the response were somewhat different; the MSNA response being much greater than the plasma NE response. These findings are consistent with those of Wallin et al., (1987) who recently reported a positive correlation between MSNA and plasma NE during static handgrip exercise. Thus, it appears that increases in plasma NE during various physical challenges can accurately depict changes in directly measured MSNA.

Plasma Norepinephrine Response to Stress

Isometric handgrip and cold pressor testing has been used extensively to evaluate sympathetic neural control of the peripheral circulation. The cold pressor test has been used in experimental studies of neurocirculatory regulation in humans as a non-specific stimulus to sympathetic neural outflow (Ferguson et al; 1983; Victor &Mark, 1985; Nies et al., 1978). The cold pressor test evokes a generalized sympathetic activation (Victor et al., 1987). Plasma norepinephrine levels rise +23% to +240% (Cummings et al., 1983; Stratton et al., 1983; Robertson et al., 1979; Lake et al., 1976; Winer & Carter, 1977). The differences in the magnitude of the responses are likely due to differences in

the time of sampling, analysis techniques and the duration of the cold pressor test. Other investigators (Cuddy et al., 1966), using a flourometric assay failed to detect any significant change in the plasma NE level from baseline. Generally, although the magnitude of change is smaller than observed with directly measured MSNA, plasma NE amy provided a reasonable representation of the degree of sympathetic activation during cold pressor stimulation.

Isometric handgrip has also been used as a means of evoking large increases in SNS activity (Seals & Victor, 1991). Wallin et al. (1987) found that plasma NE correlated with directly measured MSNA during isometric handgrip exercise (r=.80). The magnitude of change in plasma NE was smaller than observed during directly measured MSNA. Plasma NE rose 21% during isometric handgrip of 30% MVC while directly measured MSNA rose 67%. The plasma NE also lagged behind the MSNA measurements by one to 2 minutes. The peak plasma NE rose from 1.82 $nmol/l^{-1}$ to 2.17 $nmol/l^{-1}$. These are similar to values reported by other investigators (Robertson et al., 1979) using the same percentage of MVC. The differences in the time course of the sympathetic changes were probably related to the slow wash-out from the neuroeffector junctions.

Influence of Exercise Training on Sympathetic Activity

The plasma catecholamine response to large muscle,

dynamic exercise is attenuated in aerobically trained individuals (Galbo, 1983). This is likely due to the fact that the same workload represents a lesser physiological stress when compared with the untrained state. Plasma NE levels are similar at the same relative intensity in the trained and untrained states (Galbo, 1983). However, plasma catecholamine levels or MSNA does not appear to be different in the resting state between trained and untrained subjects (Svedenhag et al., 1984)

Forearm endurance training results in an attenuated sympathetic activation during rhythmic handgrip exercise (Sinoway et al., 1989; Somers et al., 1992). However, during exercise utilizing the untrained limb or during non-specific forms of stress there is no attenuation of the sympathetic response to exercise. For example, Seals (1991) found that there was no difference in the MSNA or the BP response to static handgrip, cold stimulation or orthostatic stress between aerobically trained and untrained individuals. Therefore, during exercise utilizing the untrained musculature or during non-specific laboratory stress sympathetic activation appears unchanged.

Insight from Animal Studies on the Pressor Reflex

The most common animal model for studying the neural mechanisms for eliciting elevations in HR and AP is the

anesthetized cat (Mitchell & Schmidt, 1983). Typically, the ventral roots of L_5 and S_1 are cut and electrodes are placed over them. Stimulation of these cut ends results in contraction of the hindlimb of the cat. The dorsal root is initially left intact. Later, the dorsal roots could be cut to determine if responses were reflexive or central mediated.

Much information regarding cardiovascular control has been gained by studying the receptive properties of group III and IV afferent fibers in anesthetized animals (Mitchell & Schmidt, 1983; McCloskey & Mitchell; 1972). Recording are made from fine filaments either from the sciatic nerve or from fine strands of dorsal rootlets in response to applying a series of stimuli to the tendon or muscle belly. Most of the data regarding the receptive properties of group III and IV afferent fibers have been obtained from anesthetized cat soleus or gastrocnemius muscles (Kniffki et al., 1981).

Classification of Afferent Fibers

Anatomical and physiological measurements have provided for the identification of four afferent fiber types (Mitchell and Schmidt, 1983). Group I fibers have diameters of 12-20 μm and conduction velocities of 71-120 $m \cdot sec^{-1}$. Group II fibers have diameters of 2-16 μm and conduction velocities of 31-71 $m \cdot sec^{-1}$. Group III fibers have diameters of 1-6 μm and conduction velocities of 2.5 and 3.0 $m \cdot sec^{-1}$. Finally, group

IV fibers have diameters of less that 1 μm and conduction velocities of less than 2.5 $m \cdot sec^{-1}$. The groups I-III are myelinated fibers while group IV fibers are unmyelinated. All of the group IV and some of the group III fibers terminate in free nerve endings in the musculature. Some group II fibers stem from secondary endings of muscle spindles and others from pacinian corpuscles.

Group III and IV afferent fibers have also been classified by responses to various mechanical and chemical stimuli (Mitchell and Schmidt, 1983; Kaufman et al., 1984a; 1984b; 1984c). Direct recordings of afferent nerve activity has shown responsiveness to electrically induced contraction, blunt probing, stretching, and to stimulation by various algesic substances. It is important to note that a high degree of polymodality exists among group III and IV fibers. However, group III fibers are generally referred to as mechanosensitive fibers and group IV as chemosensitive fibers.

Mechanical Stimulation of Group III and IV Afferent Fibers

Several investigators have shown that electrically induced contractions via the ventral root stimulation in the anesthetized cats results in increases in HR, BP and afferent nerve activity (Mitchell et al., 1983; Mitchell & Schmidt, 1983; McCloskey et al., 1972; Kaufman et al., 1983). The response are always abolished when the dorsal root is cut.

Coote et al. (1971) showed that static contractions elicited by electrically stimulating the ventral roots of anesthetized cats reflexly increased HR, B and myocardial contractility. These responses could be abolished by severing the dorsal roots at the same level of the spinal cord. McCloskey and Mitchell (1972) found that anodal blockade of group I and II afferent activity did not prevent the contraction induced increase in heart rate and arterial pressure. The conclusion was that the reflex cardiovascular response were due to stimulation of group III and IV afferent fibers.

Metabolic Stimulation Of Group III and IV Afferent Fibers

Recently (Stebbins et al., 1988) and in the past (Waldrop et al., 1982; Mitchell & Schmidt, 1983; Kaufman et al., 1984), attention has been paid to the metabolic products from contracting skeletal muscle that may be involved in the pressor reflex. Substantial evidence suggests that the metabolic stimuli results from a mismatch between blood flow and the rate of metabolic activity (Wyss et al., 1983; Sheriff et al., 1987). Rybicki et al. (1985) has shown that infusion of potassium into the arterial supply of the gracilis muscle of anesthetized cats increases the activity of group III and IV afferent fibers. However, the activity of these afferents were rapidly adapting suggesting that potassium may not be the only stimulus. Rotto et al. (1988) has found that other

metabolic substances, such as lactic acid and arachidonic acid and its' metabolites, increased the discharge rate of group III and IV afferent fibers in anesthetized cats. More importantly, infusion of these substances resulted in reflex increases in BP. Muscle ischemia has also been shown to exaggerate the pressor reflex, presumably a result of accelerated anaerobic metabolism (Kaufman et al., 1984; Stebbins et al., 1989). The rise in BP would presumably serve to restore blood flow to the underperfused tissues.

Role Of Prostaglandins In The Pressor Reflex

Some investigators have shown that certain prostaglandins, such as PGE_2, enhance the bradykinin sensitivity of most muscle afferent fibers (Mense, 1981). These observations have led researchers to investigate the role of PGs in mediating the increase in afferent nerve activity during induced contractions in the anesthetized cat. Recent evidence has suggested that PGs may contribute to the pressor reflex (Stebbins et al., 1986). Prostaglandins have been shown to mediate the reflex through an action on afferent nerve endings rather than through a regional vascular effect. Blockade of PG synthesis, with either indomethacin or sodium meclofenamate, attenuates the cardiovascular response to electrically induced contraction in anesthetized cats (Stebbins et al., 1986).

Cyclooxygenase products have been shown to accumulate in skeletal muscle during contraction (Symons et al., 1991; Rotto et al.,1989). Rotto et al. (1990) has shown that infusion of arachidonic acid increases the activity of group III afferents to induced contraction. Likewise, indomethacin attenuated responsiveness of the these afferent fibers to contraction. Cyclooxygenase products have also been shown to be necessary for the full expression of group IV afferents to static contraction (Rotto et al., 1990).

Nature of the Chemical Stimuli-Human Studies

There is increasing evidence that the pressor reflex is due to stimulation of chemosensitive afferents by ischemic metabolites. This metaboreflex is thought to be important in the sympathoexcitation during static exercise and at moderate levels of rhythmic exercise (Victor et al., 1987). For example, Victor et al. (1988) has shown that sympathetic nerve discharge during static handgrip in humans is coupled to the cellular accumulation of hydrogen ions. This has been supported by Sinoway et al. (1989) who demonstrated an association between muscle acidosis and calf vasoconstriction during static handgrip. This group has also shown that infusion of dichloroacetate, which inhibits lactic acid formation, attenuates the rise in MSNA associated with static handgrip and postcontraction muscle ischemia (Ettinger et al.,

1991). This study corroborates previous finding by Pryor et al. (1990) who has shown an impaired sympathetic activation during static handgrip exercise in patients with muscle phosphorylase disease. Muscle sympathetic nerve activity measured directly from microneurography increased substantially in a normal subjects but not in the patients. In addition, the change in mean BP was significantly less during the static handgrip exercise in the patients as compared to the normal subjects. These findings are consistent with findings in experimental animals. In anesthetized cats, lactic acid stimulates metaboreceptor muscle afferent fibers (Rotto et al., 1988). In conscious dogs, the pressor response to muscle ischemia during treadmill exercise is strongly correlated with venous effluent pH (Sheriff et al., 1987). In humans, it appears that activation of SNS activity is linked to specific metabolic events in skeletal muscle (Seals, D.R. & R.G. Victor, 1991; Rowell, L.B. & O'Leary, 1990). However, it appears that stimulation of chemosensitive fibers may occur only during intensities of exercise that result in the accumulation of metabolic by-products.

Possible Role Of Prostaglandins In The Human Pressor Reflex

At least three lines of evidence suggest that prostaglandins are important in mediating the pressor response

to exercise in humans. First, PGs have been shown to accumulate in muscle (Symons et al.,1991) and plasma of animals (Young et al., 1979; Herbaczynska-Cedro et al., 1976) and in the plasma of humans during exercise (Kilbom et al., 1976; Staessen et al., 1987). Second, infusion of arachidonic acid (the precursor to stable PG metabolites) into the arterial supply of the triceps surae muscle in anesthetized cats increases the impulse activity in group III and group IV afferent fibers (Rotto et al., 1988). And third, inhibition of PG synthesis attenuates the impulse activity of group III and IV fibers and the associated cardiovascular response (Stebbins et al., 1986; Rotto et al. 1990). Infusion of PGE_2 into the arterial supply of the stimulated limb restores these responses.

Prostaglandins Biosynthesis and Its Inhibition

The enzymatic conversion of essential fatty acids to PGs was demonstrated by van Dorp (1964) and Bergstrom (1964). Prostaglandins are the most prevalent autocoids and have been detected in almost every tissue and body fluid (Moncada et al., 1985). The inhibition of their action is now recognized as the mechanism of some of the most widely used therapeutic agents. These include the non-steroidal anti-inflammatory drugs (NSAIDS) such as aspirin, ibuprofen, and the more popular indomethacin.

Prostaglandins, leukotrienes, and thromboxanes are all related compounds known as eicosanoids. They are all derived from 20-carbon essential fatty acids (Shen et al., 1972). Prostaglandin synthesis is accomplished in a stepwise manner beginning with fatty acid cyclooxygenase (Moncada, 1985). The precursor acids are oxygenated and cyclized to form the cyclic endoperoxide derivatives prostaglandin G (PGG) and prostaglandin H (PGH) (see figure 1). The unstable endoperoxides have half lives of approximately 5 minutes, and then are isomerized into different products. PGH_2 is metabolized to thromboxane A_2 thromboxane synthetase and prostacyclin (PGI_2), by prostacyclin synthetase. Both are known to have very short chemical half lives of 30 seconds and 3 minutes, respectively. Prostacyclin can be hydrolyzed to the more stable 6-keto-PGF_α PGE_2, PGD_2 and $PGF_{2\alpha}$ are other products of the intermediate endoperoxides. All tissues have the capability of synthesizing intermediate prostaglandin endoperoxides, however, the fate of which may depend on numerous factors not clearly defined (Moncada et al., 1985).

Non-steroidal anti-inflammatory drugs have the common property of inhibiting PGs. Indomethacin has been used in the treatment of rheumatic disorders, gout, osteoarthritis and acute musculoskeletal problem for over 20 years. Most NSAIDS inhibit the cyclooxygenase-mediated conversion of arachidonic acid to PGG_2 (Hall et al., 1982; Ferriera, 1974). However,

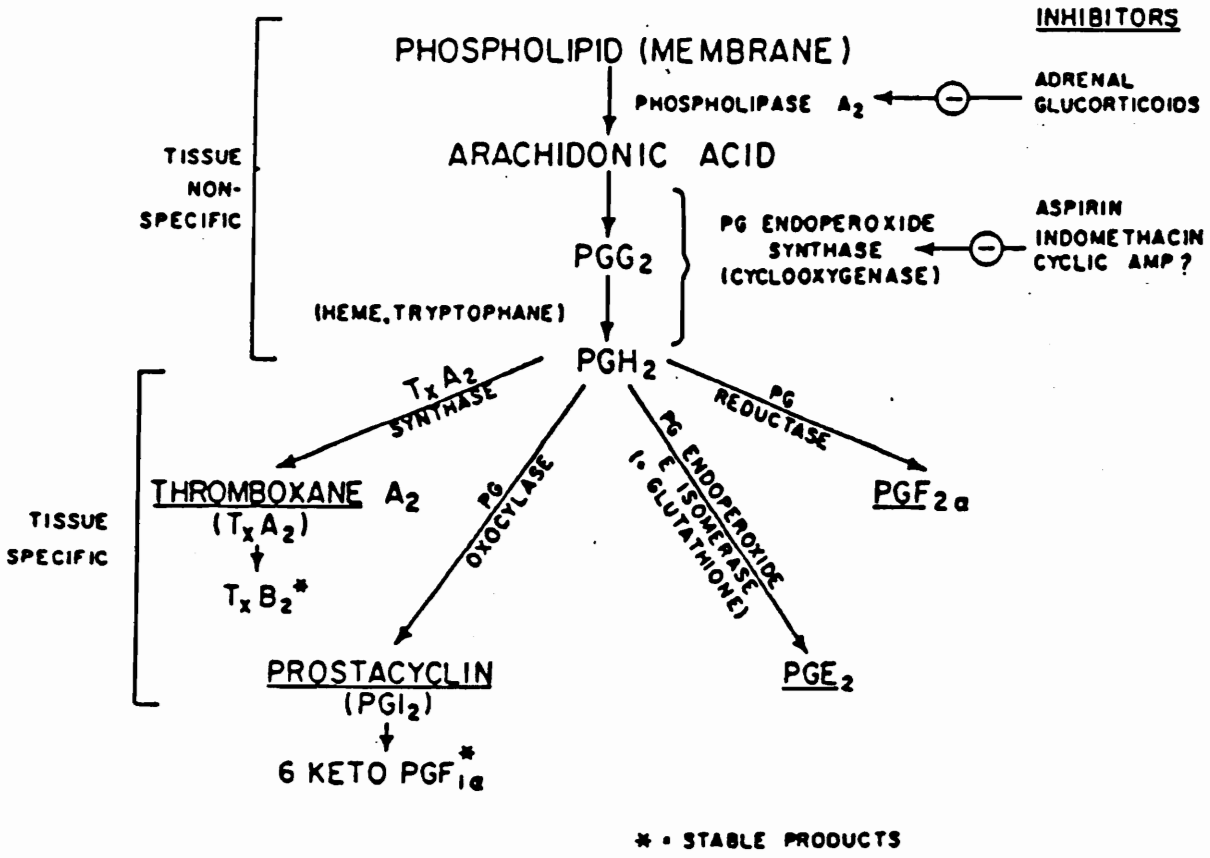

Figure 1. Biosynthesis of Prostaglandins (Shen et al., 1972)

for indomethacin the mode of inhibition is complex and probably involves a site on the enzyme which is different from aspirin (Flower et al., 1985).

Administration of indomethacin orally results in rapid absorption and peak plasma level occurring between 60 and 120 minutes post-ingestion (Hippius & Reinicke, 1982; Alvan et al., 1976). Peak plasma levels after a single indomethacin dose of 100 mg range between 3.0 and 8.0 μg/ml. The plasma level seems to be more dependent on individual differences in absorption, elimination and enterohepatic recycling than differences in bodyweight (Kwan et al., 1976). Exercise does not significantly change plasma concentration indomethacin (Henry et al., 1981).

Evidence of PG synthesis inhibition can be observed as early as one hour after a single indomethacin dose of 50 mg (Rumpf et al., 1975). Clinical effectiveness can be demonstrated when plasma levels are between .3 and 3.0 μg/ml (Moncada et al., 1985). In addition, oral doses of between 75 and 150 mg results in maximal suppression of total body PG production. Rane et al. (1978) showed that urinary excretion of the major PG metabolite (PGE_2) was suppressed by greater than ninety percent with these dosages. Indomethacin is a potent inhibitor of PG production at therapeutic dosages.

Pain Sensitization by Prostaglandins

Studies employing the pharmacological manipulation of PG synthesis should be aware of the role PGs may serve in pain sensitization. Vane et al. (1971) suggested that inhibition of PG synthesis was responsible for the analgesic, antipyretic and anti-inflammatory actions of the aspirin-like drugs. Other investigators (Flower, 1972; Ferriera et al., 1974) have shown that the PGs of the E series sensitize afferent fibers to chemical stimuli such bradykinin, which is an algesic substance often used to produce pain. In humans, aspirin attenuates the pain response to intraarterial injection of bradykinin. Ferriera et al. (1973) found that intra-arterial injection of bradykinin in lightly anesthetized dogs resulted in pain, as inferred from vocalization by the animal, and was accompanied by a reflex increase in BP. Both the BP and pain responses could be attenuated with the administration of indomethacin. Infusion of PGE_1 causes pain sensitization and restores the BP responses. Although these studies suggest a role for PGs in pain sensitization, PGs are probably not important unless trauma, injury or inflammation has occurred (Ferriera et al., 1972). However, if studies are manipulating PG synthesis caution should be taken to understand possible confounding factors.

Summary

Recently, it has been demonstrated that metabolic products are important in mediating the pressor response to induced contractions in anesthetized cats (Stebbins et al., 1986; Rotto et al., 1990; Rotto et al., 1991). It has been postulated that the accumulated ischemic metabolites stimulate the group III and IV afferent fibers. Further evidence suggests that PGs may be important in sensitizing these afferent fibers (Stebbins et al., 1986; Rotto et al., 1988 and 1990).

Evaluation of the role of metabolic products in stimulating group III and IV fibers has been largely limited to animal studies. However, recently it has been suggested that in humans metaboreceptor stimulation seems more important than mechanoreceptor stimulation in eliciting sympatho-excitation (Rowell and O'Leary, 1990). For example, Victor et al. (1988) has shown that muscle cell pH is correlated to MSNA during exercise. Sinoway et al. (1989) has reported similar findings. In addition, Ettinger et al., (1991) found in a later study that dichloroacetate (DCA) attenuates the rise in MSNA associated with fatiguing handgrip exercise (Ettinger et al. 1991). In addition, the change in mean BP was significantly less after DCA infusion. This suggests that, as in anesthetized animals, lactic acid is important in stimulating chemosensitive afferent fibers. Sympathetic

responses are also impaired in phosphorylase deficient patients (Pryor et al., 1991).

Metaboreceptor stimulation has been shown to be important in the pressor response to static contraction in both animal models and human studies. There is evidence that PGs are important, in anesthetized cats, in mediating the pressor response to induced contractions (Rotto et al, 1990; Stebbins et al.,1986). However, it is not known whether this is an important mechanism in arterial pressure or neurohumoral regulation during isometric handgrip exercise in humans.

Chapter III

Journal Manuscript

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EFFECT OF PROSTAGLANDIN INHIBITION ON SYMPATHETIC AND PRESSOR RESPONSES TO MUSCULAR CONTRACTION AND POSTCONTRACTION MUSCLE ISCHEMIA IN HUMANS

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ABSTRACT

The purpose of this study was to test the hypothesis that prostaglandins (PGs) participate in metaboreceptor stimulation of sympathetic and pressor responses to sustained isometric handgrip (HG) in humans. To accomplish this heart rate (HR), arterial pressure (n=10) and plasma norepinephrine (NE) levels (n=8) were measured in healthy male subjects during HG at 40% of MVC to exhaustion and during a period of postcontraction muscle ischemia (PC). The subjects were given a double-blind administration of either placebo (PLAC) or a single 100 mg dose of indomethacin (IND). The order of administration was counterbalanced and a one week period was given for systemic clearance of the drug. Mean arterial pressure increased 25±5 vs. 22±4 mmHg during the second minute of HG and 26±2 vs. 21±5 during the last minute of PC in PLAC vs. IND (P>.05), respectively. Heart rate was increased 21±4 vs. 17±3 bpm during the second minute of HG in PLAC vs. IND (P>.05), respectively and returned to control values during PC. Plasma NE increased 343±89 vs. 289±89 pg/ml after HG and 675±132 vs. 632±132 pg/ml after PC (P>.05) in PLAC vs. IND, respectively. Therefore, PG inhibition does not alter the magnitude of change in plasma NE or MAP. This suggests that 1) PGs may not play an important role in metaboreceptor

stimulation of sympathetic and arterial pressure responses during sustained isometric exercise in humans or 2) may only play a small role in the regulation of these variables, however compensatory mechanisms may be operative.

Index terms: prostaglandins, pressor reflex, sympathetic nervous system activity, isometric exercise

Introduction

Sustained isometric muscle contraction evokes sympathetic nervous system (SNS) activation, increased heart rate (HR) and a marked rise in arterial blood pressure (BP) (7,8,14,15). During exercise, part of these responses are thought to be mediated by active muscle reflexes mediated by mechanical and/or chemical sensitive group III and IV afferent fibers (6,7,14,15).

Several metabolic products have been shown to stimulate chemosensitive afferents in contracting skeletal muscle (6,10-14,19). One such group of metabolites are the prostaglandins (PGs). In anesthetized cats inhibition of PG synthesis attenuates and PGE_2 restores pressor responses to induced muscular contractions (19). Prostaglandin inhibition also has been shown to attenuate afferent nerve activity during induced contractions in the anesthetized cat hindlimb (12,13). It is not known, however, whether PGs play a similar role in the human. Therefore, the purpose of this study was to test the hypothesis that PGs participate in the metaboreceptor stimulation of sympathetic and arterial pressure responses to sustained isometric contraction in humans.

To address this HR, BP and plasma norepinephrine were measured during sustained isometric handgrip exercise. To

gain insight into the specific influence of PGs on muscle chemoreflexes per se, responses to a period of post-contraction circulatory arrest were also performed. During the latter, other potential excitatory CNS inputs such as central command and muscle mechanoreflexes are not engaged. In attempt to ensure that any differences observed were due to the effects of PGs on muscle chemoreflexes and not an effect mediated by central influences or other non-specific effects, the subjects responses to hand immersion in ice water also were recorded. Local cold produces SNS activation and increases in BP presumably via stimulation of cold-pain afferents (22).

Methods

Subjects. Ten male subjects volunteered to participate in this investigation. All subjects were normotensive (arterial pressure <140/90 mmHg), not taking medications or hypersensitive to aspirin products, and apparently healthy as assessed by medical history. None of the subjects were involved in resistance exercise training or upper body aerobic activities for the previous 6 months, as it is known that exercise with trained musculature may modify SNS activity (20). The protocol was explained to subjects and informed consent was obtained. The studies were approved by the Institutional Review Board at Virginia Tech.

Drug Administration. Subjects were given a double-blind administration of either placebo (PLAC) or 100 mg of indomethacin (IND) 2.5 hr prior to the experimental session. The dosage was chosen because it has been shown to maximally suppress prostaglandin production in humans (9). One week was given between trials to allow for systemic clearance of the drug and the order of the administration was counterbalanced.

Hemodynamic measurements. During the experiments, HR was measured from a standard electrocardiographic tracing (lead II). Mean arterial pressure (MAP) was measured in the non-exercising arm by an automated device using the oscillometric technique (Dynamap, Critikon. Tampa, Fla). This device has been validated and shown to correlate highly with direct radial artery measurements during exercise (r=0.98) (17).

Plasma norepinephrine. Six-milliliter blood samples were obtained from an antecubital vein in the non-dominant forearm and immediately place in a chilled heparinized tube containing EGTA and reduced glutathione. The plasma was separated and stored at -70° C. Analysis (in duplicate) for plasma norepinephrine (NE) was made by a single isotope radioenzymatic technique of Henry and Bowsher (5). All

analysis were performed in the Endocrinology Division of the University of Arizona Health Science Center. Individuals performing assays were completely blinded to the nature and conditions of the study. The sensitivity of the assay is ±20 pg/ml, with a coefficient of variation of less than 10%.

Perceived effort and pain ratings. Because differences in effort or pain during the experiments might influence cardiovascular and/or sympathetic activity, subjects were asked to rate their voluntary effort during handgrip and their perceived pain responses during postcontraction muscle ischemia and cold pressor testing. Ratings of effort were made on a Borg scale (6-20) (2). Ratings of pain were made on a validated scale from Gracely (4). Reports of pain were 0 (no pain) to 10 (almost unbearable).

Isometric handgrip exercise. Subjects performed isometric handgrip exercise (HG) with their non-dominant hand, while in the supine posture, at 40% maximal voluntary contraction (MVC) until this force could no longer be maintained. The spring mechanism from a handgrip dynamometer was removed and replaced with an electronic load cell (Genisco, Simi Valley, CA) The signals were amplified (Kube Electronics Inc., Moorepark, CA) and stored on a two channel digital oscilloscope (Tektronics 2201, Beaverton, OR) where the

actual and target forces were displayed for the subject. Subjects were instructed to maintain the target force for as long as possible. The point of exhaustion, was defined as 1) the inability to match actual force with target force (>10% variation for 2 s) despite continued encouragement and 2) the attainment of a maximal rating of perceived effort (23).

Postcontraction muscle ischemia. Approximately 5 s prior to exhaustion, a standard blood pressure cuff was inflated around non-dominant arm to 250 mmHg to occlude circulation to the exercised arm. The subject terminated exercise and postcontraction muscle ischemia (PC) continued for 3 min. This procedure has been shown to isolate metaboreceptor stimulation of sympathetic outflow and BP in humans (7,25).

Cold Pressor Test. Cold pressor testing (CPT) was used as a non-exercising internal control. Subjects immersed their non-dominant hand in a container containing ice water (1° C) for 90 s.

Orientation sessions. All subjects performed the protocol on at least two separate visits to the laboratory before data collection sessions were performed because prior performance

of this task is necessary to attain reliable endurance time (21).

Data collection sessions. Thirty min before the beginning of the exercise protocol subjects were placed in supine position and an indwelling catheter (21G) was inserted into an antecubital vein in the dominant non-exercising arm. Standard blood pressure cuffs were placed approximately 10 cm proximal to antecubital space of both the dominant and non-dominant arms. The subjects MVC was measured in the non-dominant forearm. Muscle contractions of other body regions and Valsalva strain maneuvers were strictly discouraged because they can produce their own cardiovascular effects. The subjects were allowed to rest quietly for approximately 20 min before measurements commenced at which time BP and HR were stable. The submaximal isometric handgrip bout was preceded by a 6 min resting control period. Heart rate and BP were measured 2 min during the control, and each min of HG and PC. Ratings of perceived effort were obtained each 15 s during HG. Pain ratings were obtained each 30 s during PC. Blood samples for plasma NE measurement were drawn during the control period and 1 min after the end of HG and PC. Plasma NE is known to peak approximately 1 min post exercise (16,24). Heart rate and BP measurements were obtained at 2 min

intervals during the 6 minute recovery period.

Twenty-five min after cessation of the HG and PC protocol a second 6 min control period was obtained. Subjects then immersed their non-dominant hand in a container containing ice water (1° C) for 90 s. Heart rate and BP were obtained at two min intervals during the control and at 45 and 90 s of the CPT. Blood samples for plasma NE determination were drawn during control and 1 min post immersion when peak plasma NE levels are observed (22). Ratings of pain were obtained each 30 s during the immersion. Recovery HR and BP were determined at 2 min intervals during 6 min of recovery.

Data analysis. Control and recovery values for hemodynamic variables were calculated as an average of the data obtained over the respective 6 min intervals. The absolute time to exhaustion (in s) was determined for each subject for both PLAC and IND conditions. Blood samples from plasma NE measurement were obtained in only 8 subjects due to unwillingness of one subject to undergo venipuncture and technical difficulties related to the transport of one other subjects' blood samples.

Statistics. Differences in dependent variables were assessed by repeated measured analysis of variance. To determine if

PG inhibition altered the ability to increase HR, BP or plasma NE the magnitude of change from control was also assessed by this procedure. Fishers protected LSD was used when appropriate to locate differences between or across conditions. Paired t-tests were used to assess differences in maximal voluntary contraction and time to exhaustion as well as changes in plasma NE after CPT. Differences were considered significant at the $P<.05$ level. All group data are presented as means ± SE.

Results

Subject characteristics. Age, weight and height for the subjects were 21.8±1.0 yr, 69.3±4.2 kg, and 176.1±2.4 cm, respectively.

Maximal voluntary contraction, time to exhaustion, perceived effort and pain responses. Maximal voluntary contraction during PLAC and IND trials were similar (PLAC: 428±16 N; IND: 439±15 N; $P>.05$). Time to exhaustion was not different between the conditions (PLAC: 147±8 s; IND: 153±6 s; $P>.05$). All subjects achieved a perceived effort rating of 20 units at exhaustion for both trials. Reports of pain increased from 3±1 to 7±1 after PLAC and from 3±1 to 8±1 after IND ($P>.05$). During CPT, reports of pain increased from 3±1 to 6±1 after PLAC and from 4±1 to 7±1 after IND ($P>.05$).

Hemodynamic responses to isometric handgrip and post-contraction muscle ischemia. The absolute level of MAP increased significantly during HG and remained elevated during PC. Mean arterial pressure was significantly elevated after IND (Table 1). However, the magnitude of change in MAP was similar between the trials (P>.05) (Fig. 1). The absolute level of HR increased significantly during HG but returned to control values during PC. Heart rate was attenuated after IND (Table 1). The magnitude of change in HR was similar between trials (Fig. 1).

Hemodynamic responses to cold stimulation. The absolute level of MAP increased significantly during immersion and was significantly elevated after IND (Table 2). Heart rate also increased significantly during immersion but there was no significant difference between trials. The magnitude of change in both heart rate and MAP were not significantly different between trials (P>.05) (Fig. 2).

Plasma norepinephrine responses. The absolute level of plasma NE increased significantly 1 min post HG and PC and was significantly attenuated after IND (Table 3). The magnitude of change in plasma norepinephrine was not different between trials (P>.05) (Fig. 3). During CPT, the

absolute level of plasma NE was similar between trials after IND (Table 3). The magnitude of change after CPT was 200±69 vs. 162±39 (P>.05) in PLAC vs, IND, respectively.

Discussion

In the present investigation PG inhibition failed to alter the magnitude of change in plasma NE and MAP during sustained isometric exercise. Therefore, these results suggest that prostaglandins do not play a significant role in mediating metaboreceptor stimulation of sympathetic or arterial pressure responses to sustained isometric exercise in humans. Furthermore, PG inhibition did not appear to modify these responses to cold stimulation.

Several investigators have found that PGs accumulate in contracting skeletal muscle and plasma of animals (11,27) and the plasma of humans during exercise (18). Inhibition of PG synthesis by infusion of indomethacin attenuates afferent nerve discharge to induced contraction in the anesthetized cat (12,13). Prostaglandin inhibition also has been shown to attenuate the increase in mean arterial pressure during induced contractions (19). In these experiments, stimulation of the cut peripheral ends of the ventral roots supplying motor nerves isolated the reflex nature of these responses, suggesting that PGs act to sensitize group III and IV afferent fibers in the

metabolically active muscle. In contrast, isolation of metaboreceptor stimulation of sympathetic and BP responses was achieved in the present study by inflating cuffs around the previously exercised limb. The observation that plasma NE and arterial pressure remained elevated during the period of postcontraction muscle ischemia while heart rate returned to control values suggests effective isolation of these responses had occurred. It is important to note, however, that experimental and species differences may explain the different results in the study reported here, as compared to studies in the anesthetized cat.

Sympathetic nervous system activation during isometric exercise in humans, particularly muscle sympathetic nerve activity, is considered to be mediated by chemosensitive afferents (7,25). Plasma NE is largely a function of spillover from sympathetic nerve terminals innervating skeletal muscle resistance vessels and provides a reasonable index of MSNA during exercise (r=0.80) (16,24). Thus, blockade or reduced stimulation of these afferents should blunt the increase in sympathetic nervous system activity (i.e., plasma NE) and arterial pressure. Although a blunting of the absolute level of plasma NE and an elevation of arterial pressure was observed in the present study during sustained isometric exercise after PG inhibition, the magnitude of change in either variable was not different

between trials. The significantly higher MAP after PG inhibition may have activated arterial baroreflexes, therefore, reducing plasma NE. That these observations were not evident during cold pressor testing is not clear, but may be related to the relatively smaller magnitude of change in the measured variables. Thus, our results suggest that although the absolute level of plasma NE was lower and MAP was higher, PG inhibition did not alter the ability to increase plasma NE or arterial pressure during sustained isometric exercise in humans. These findings are qualitatively similar to observed arterial pressure responses to sustained isometric handgrip after ingestion of other prostaglandin synthesis inhibitors (acetaminophen, 1200 mg) (Davy, K.P., unpublished observations).

The absolute level of heart rate was also attenuated in this study after PG inhibition. This also may have been due to the activation of arterial baroreceptors. The magnitude of change in heart rate during HG, however, was not different between trials. These findings are consistent with animal studies in that the absolute heart rate is attenuated after PG inhibition but the magnitude of change after PG inhibition is not different (19).

There are some potential limitations that should be considered in this study. First, because the cardiovascular responses to static and dynamic effort are different (1), it

is important not to generalize the present results to more conventional forms of activity such running or cycling. In the present study plasma NE as an index of sympathetic nervous system activity and although well correlated to MSNA during exercise, the magnitude and time course of changes are different (16,24). Thus, plasma NE may not be sensitive to small changes in SNS activity. Therefore, investigators studying this topic in the future may wish to include microneurography in their experimental protocols.

Alterations in muscle blood flow or in the concentration of other metabolites after PG inhibition may have confounded the result of the present study. However, two observation suggest this is improbable. First, muscle blood flow is not altered after PG inhibition in exercising humans (26) or animals (19). Second, because isometric handgrip exercise of this intensity leads to exhaustion in less that 3 minutes, lactate production would appear to be maximal. This suggests that additional changes in the milieu of chemosensitive fibers after PG inhibition is unlikely. In addition, indomethacin has only been shown to inhibit oxidative phosphorylation <u>in vitro</u> at supra-pharmacologic doses (3). In contrast, a maximal therapeutic dose of 100 mg of indomethacin was used in the present study. Finally, plasma prostaglandin levels were not determined in this study. However, studies in humans have

shown maximal suppression of whole body PG production to occur with single dosages of between 75 and 150 mg of IND (9). Thus, although PG levels were not measured in muscle or even plasma the above observation suggests successful inhibition of PG synthesis was achieved.

Of particular interest in this study was the finding that arterial pressure was not lower after PG inhibition despite that attenuation of heart rate and plasma NE. The observations in present study are consistent with the contention that redundant mechanisms may operate to raise or maintain arterial pressure during exercise (8). In the present study the absolute level of MAP was higher after PG inhibition, however, at least part of this elevation may be attributed to the inhibition of certain PGs with local vasodilatory actions on vascular smooth muscle (18,26).

Conclusions

In conclusion, prostaglandin inhibition produces an elevation in arterial pressure and an attenuation of the absolute heart rate and plasma NE at rest and in response to sustained isometric contractions in humans. However, the regulation of sympathetic activity (i.e., plasma NE) and arterial pressure appear unchanged. Therefore, the results of this study suggest that 1) prostaglandins may not play an important role in metaboreceptor stimulation of sympathetic

and arterial pressure responses during sustained isometric exercise in humans or 2) may play only a small role in the regulation of these variables, however compensatory mechanisms may have been operative.

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Table 1. Hemodynamic responses at rest and during isometric handgrip protocol.

	Control	Grip (min)		PC (min)			Recovery	P
		1	2	1	2	3		
Heart rate (bpm)								
PLAC	64±2	83±4	85±4	69±5	69±3	67±3	64±1	.0001
IND	59±2	72±3	76±4	65±4	64±5	62±3	58±2	
Mean arterial pressure (mmHg)								
PLAC	85+2	94±2	110±5	112±3	114±4	111±3	89±2	.0066
IND	91+3	101±2	113+3	114±4	115±3	112±4	94±2	

Values are means±SE; n=10 subjects. P value refers to effect of Indomethacin.

Table 2. Hemodynamic responses during cold stimulation.

	Control	45s	90s	Recovery	P value
Heart rate (bpm)					
PLAC	61±2	70±2	69±4	64±2	NS
IND	57±1	66±3	70±6	61±3	
Mean arterial pressure (mmHg)					
PLAC	88±2	95±2	108±2	94±3	.0422
IND	92±3	99±2	111±3	95±3	

Values are means±SE; n=10 subjects. P value refers to effect of Indomethacin.

Table 3. Plasma norepinephrine concentrations (pg/ml) after isometric handgrip and postcontraction muscle ischemia (top) and after cold pressor testing (bottom).

Control	Post HG	Post PC	P
PLAC			
315.5±65.3	658.5±87.5	990.6±129.4	.0336
IND			
231.3±30.9	520.8±92.0	863.3±143.7	

Control	Post Immersion	P
PLAC		
408.3±88.7	608.3±96.2	NS
IND		
404.5±61.7	566.1±74.6	

Values are means±SE, n=8 subjects. P refers to effect of Indomethacin.

Figure Captions

Figure 1. Top panel; Changes from control in mean arterial (MAP) during isometric handgrip protocol. Bottom panel; changes from control in heart rate during isometric handgrip protocol. Values are means ± SE, n=10 subjects.

Figure 2. Top panel; changes from control in mean arterial pressure (MAP) during 90 s of cold pressor testing. Bottom panel; changes from control in heart rate during 90 s of cold pressor testing. Values are means ± SE, n=10 subjects.

Figure 4. Changes in plasma norepinephrine during isometric handgrip and postcontraction muscle ischemia. Value are means ± SE, n=8 subjects.

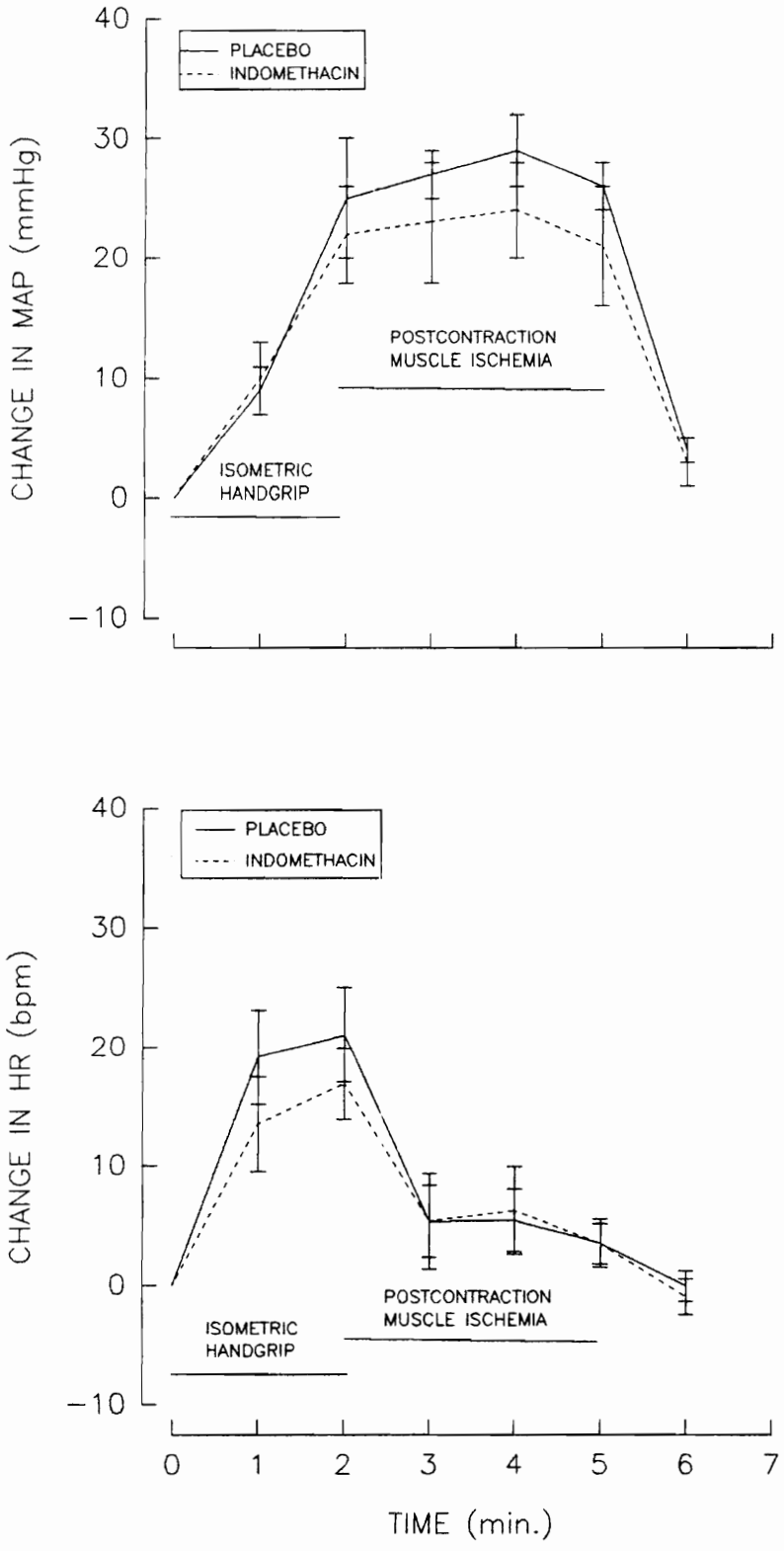
CHANGE IN MAP (mmHg)
40
30
20
10
0
−10
PLACEBO
INDOMETHACIN
POSTCONTRACTION
MUSCLE ISCHEMIA
ISOMETRIC
HANDGRIP
CHANGE IN HR (bpm)
40
30
20
10
0
−10
PLACEBO
INDOMETHACIN
POSTCONTRACTION
MUSCLE ISCHEMIA
ISOMETRIC
HANDGRIP
0
1
2
3
4
5
6
7
TIME (min.)

FIGURE 1

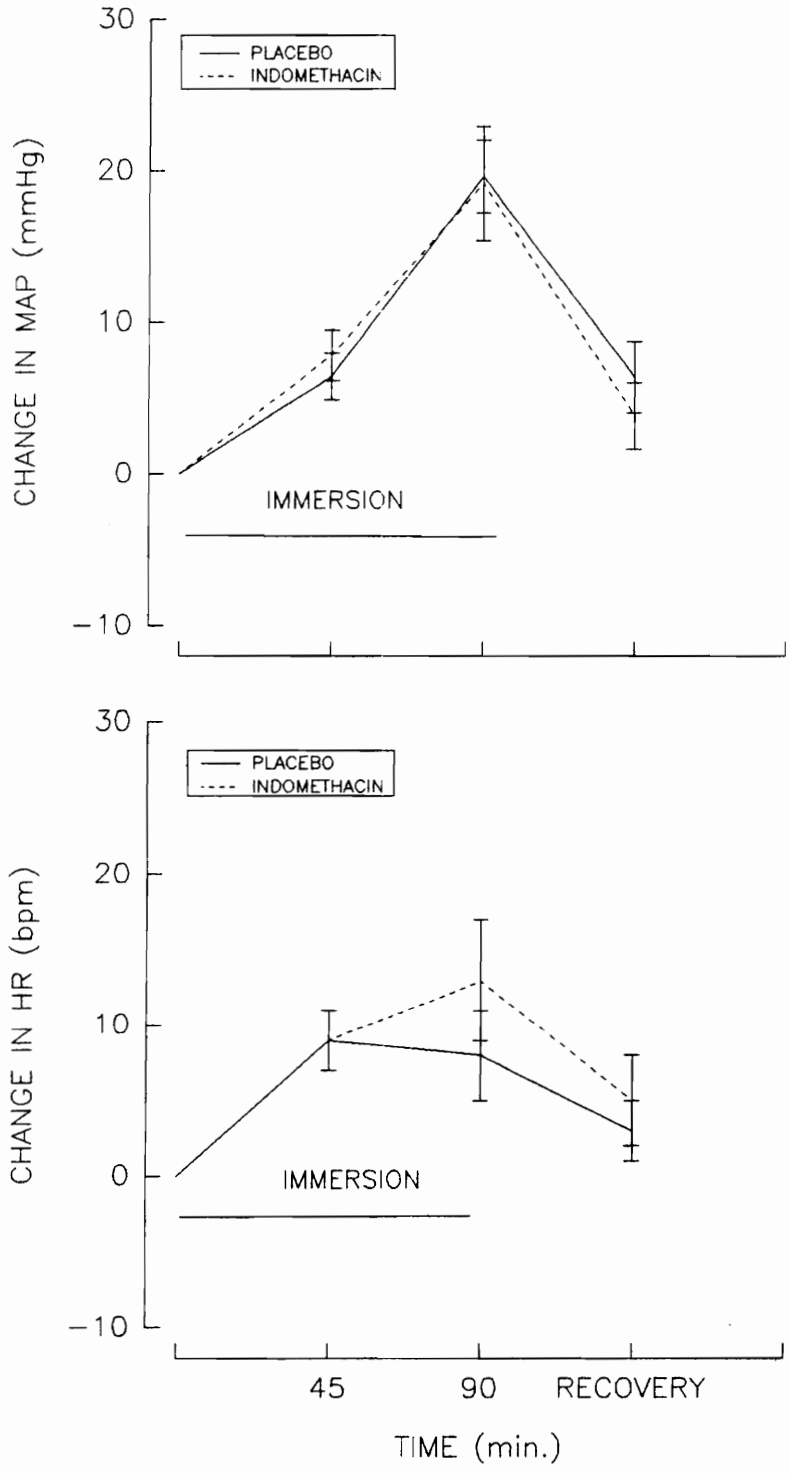
30
20
10
0
−10
PLACEBO
INDOMETHACIN
CHANGE IN MAP (mmHg)
IMMERSION
30
20
10
0
−10
PLACEBO
INDOMETHACIN
CHANGE IN HR (bpm)
IMMERSION
45
90
RECOVERY
TIME (min.)

FIGURE 2

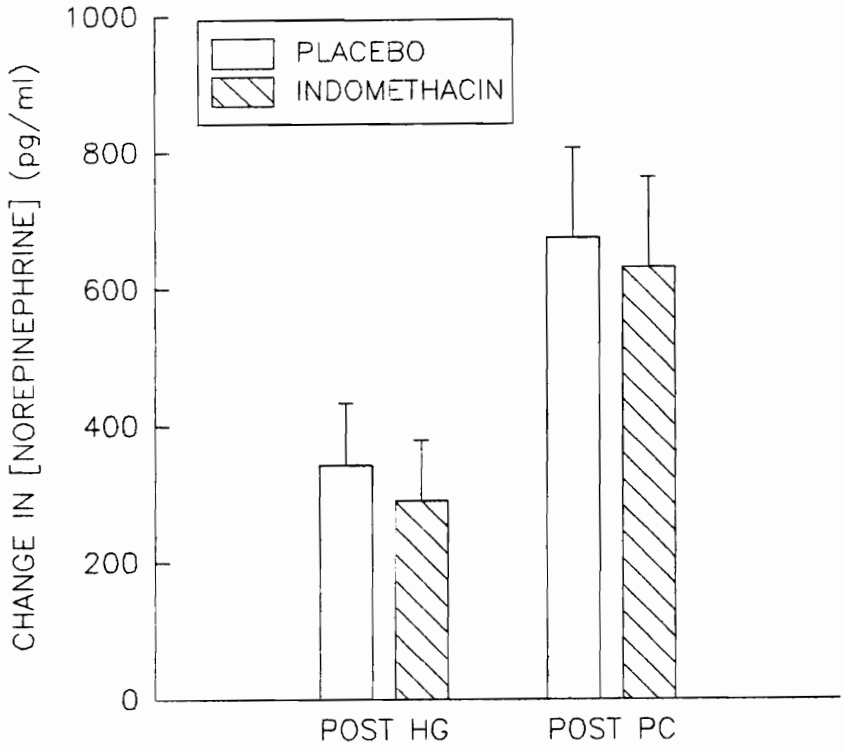
1000
800
600
400
200
0
CHANGE IN [NOREPINEPHRINE] (pg/ml)
PLACEBO
INDOMETHACIN
POST HG
POST PC

FIGURE 3

Chapter IV

SUMMARY AND CONCLUSIONS

Recent studies indicate that certain metabolic products are important in eliciting sympothoexcitation during muscular contraction in both animals and humans. Prostaglandins have been shown to be important in the mediating the reflex cardiovascular and autonomic adjustments that occur during hindlimb stimulation in animals, however, it is not known whether prostaglandins are important in the autonomic-circulatory regulation in humans performing isometric exercise.

The purpose of this study was to determine the effect of prostaglandin inhibition on the sympathetic and pressor responses to sustained isometric handgrip and postcontraction muscle ischemia.

10 males were administered a double-blind administration of either placebo or 100 mg of indomethacin. Subjects perform isometric handgrip exercise at 40% of their previously determined maximal voluntary contraction to exhaustion. At exhaustion, a standard blood pressure cuff was inflated around the previously exercised arm to a suprasystolic level (250 mmHg). The cuff remained inflated around the arm for three minutes. Heart rate and arterial pressures were determined throughout this entire period. In addition, blood samples were obtained in 8 subjects for plasma norepinephrine

determination during baseline, 1 minute post-handgrip and 1 minute post-occlusion. A 90 s cold pressor test was also administered 25 minutes after the isometric handgrip protocol as a non-exercise internal control.

The absolute level of arterial pressure was elevated after IND in this study. This was particularly interesting since both the absolute level of heart rate and plasma norepinephrine were attenuated after IND. Arterial baroreceptors appeared to reduce vasoconstrictor outflow but was unopposed by muscle chemoreflexes. This finding suggests that the regulation of arterial pressure is multifactorial and redundant systems exist to raise of maintain arterial pressure during exercise.

The magnitude of change in heart rate, arterial pressures and plasma norepinephrine increased significantly during both the isometric handgrip protocol and during the cold pressor test. However, administration of 100 mg of indomethacin had no effect on the change in plasma norepinephrine and arterial pressure responses during sustained isometric handgrip exercise. Therefore, the results of this study suggest that prostaglandins do not play a significant role in mediating metaboreceptor stimulation of sympathetic and pressor responses to sustained isometric handgrip exercise in humans.

Recommendations for Future Research

This investigation leaves many unanswered questions that appear worthy of further study. There is little, if any, research investigating the role of prostaglandins in mediating autonomic-circulatory regulation in exercising humans. Further studies are also necessary to determine optimal dosing for inhibiting prostaglandin synthesis in exercising humans.

The following are recommendations for further study:

1. Repeat the same study with direct measurement of sympathetic nerve activity by microneurography. It is possible that microneurography would provide information regarding sympathetic activity that would be undetectable by norepinephrine measurements. In addition, blood samples would be drawn for determining the level of prostaglandin inhibition.

2. Repeat #1, but use aspirin to inhibit prostaglandin synthesis.

3. Repeat #1, but use a thromboxane synthetase inhibitor and/or receptor blocker. Note: these drugs are not Food and Drug Administration approved.

3. Determine the if prostaglandins are important in sensitizing arterial baroreceptors in humans, both at rest and during static exercise. This could be achieved by administering phenylephrine and nitroprusside to raise and

lower arterial pressure, respectively. The changes in the R-R interval and limb vascular resistance would be measured in response to these interventions. Microneurographic studies would also be performed. In addition, responses to graded levels of lower body negative pressure could be performed.

4. Determine the relationship between the metaboreceptor responses and prostaglandin production in heart failure patients versus control subjects. Since metaboreceptor responses may be impaired in heart failure patients (Sterns et al., 1991), it is possible that impaired prostaglandin release may be responsible. Venous blood samples for prostaglandin determination would be obtained during sustained isometric handgrip in heart failure patients versus matched controls. Microneurgraphic recordings would also be obtained.

Conclusions

The results of this study suggest that inhibition of prostaglandin synthesis does not alter metaboreceptor stimulation of sympathetic and arterial pressure responses in humans performing isometric handgrip exercise. Since the autonomic and cardiovascular responses to static and dynamic effort are different, it is important not to generalize the results of this study to more conventional forms of physical activity. Future investigations should be conducted to

clarify the role of prostaglandins in modifying metaboreceptor responses to exercise.

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APPENDIX A

INFORMED CONSENT

Division of Health and Physical Education

Virginia Polytechnic Institute and State University

Informed Consent

I, _____, do hereby voluntarily agree and consent to participate in a research project conducted by Kevin P. Davy of the Division of Health and Physical Education of Virginia Polytechnic Institute and State University.

Title of Study:

The effects of indomethacin on the pressor responses to static handgrip and postcontraction occlusion.

1. The purposes of this study are to determine whether indomethacin, an aspirin-like drug
   - -alters the blood pressure response to static handgrip exercise or postcontraction occlusion
   - -alters the catecholamine (sympathetic hormone) response to static handgrip exercise or postcontraction occlusion
   - -alters the blood pressure response to a cold pressor challenge
   - -alters the catecholamine response to a cold pressor challenge
   - -alters sensations of discomfort associated with the cold pressor challenge

2. I voluntarily agree to participate in this testing program. It is my understanding that my participation will include handgrip exercise (non-dominant hand) with a handgrip

dynamometer (measuring device); the test will continue until I can no longer maintain the required exercise level (approximately 2-3 minutes) and will stress my cardiovascular system to a mild-moderate degree. During this exercise period my heart rate, blood pressure, and rating of perceived exertion from a sensory word scale will be recorded. In addition, a 6 milliliter blood sample will be drawn prior to exercise and again immediately post-exercise (see section 4 on catheterization)

Immediately post-exercise a standard blood pressure cuff will be inflated on my previously exercised arm to stop blood flow to my forearm for a 2 minute period. Another 6 milliliter blood sample will be drawn 30 seconds after this period. I will also be asked to rate the discomfort felt during this period of time on a sensory word scale.

Heart rate and blood pressure will continue to be recorded for twenty minutes after the exercise protocol. At this time I will be asked to immerse my hand in a bucket of ice water (temp 1-4 degrees C) and keep my hand immersed for a two minute time period. Heart rate, blood pressure, and discomfort level (sensory word scale) will be recorded each minute. A 6 milliliter blood sample will also be drawn just before the cold water immersion and just after cold water immersion.

3. Indomethacin, an aspirin-like drug, and/or placebo will

be administered in a randomized trial design. This means that neither Kevin Davy or I will know which one (indomethacin or placebo) I have received until the end of the study. However, I will receive each of these to self-administer during the study. I understand that on the day prior to the testing I will self-administer either placebo or indomethacin (100 mg, maximum dose) 3 hours prior to testing. It is important that I take any of the capsules I receive with milk or food to reduce the possibility of stomach upset.

Indomethacin is only available by prescription. It has been used for the past twenty years for diseases such as arthritis (long term medical management). It has also been used by clinicians to treat acute inflammation. However, research has indicated that this drug may also alter blood pressure during exercise.

Indomethacin is not recommended in certain circumstances. I realize that I should not receive this agent if I ever had any of the following health problems: peptic ulcer, asthma, kidney disorders, problems with fluid retention, Parkinsonism, epilepsy, psychiatric disorders or any adverse reactions to aspirin-like drugs.

I understand that serious side-effects may occur in some individuals who take this agent. This especially related to high doses, ie., greater than 200 mg/day and long term use; these side-effects are more likely to occur in the elderly.

Although infrequent, I understand that the main side-effects of this drug are stomach upset, headache, dizziness, drowsiness, and itching. It may mask the usual signs of infection. Instances have been reported of diarrhea, constipation, nausea, and dyspepsia. In the event that I experience any of these symptoms described, and suspect it is related to the medication, I am obligated to immediately contact the principal investigator or the appointed nurse and to withdraw from the study. I also understand that my response to this agent will be monitored throughout the study by the appointed nurse. It is important to remember that problems associated with this drug are more frequent with higher doses and longer durations than this study requires.

I understand that if any symptoms do develop, the termination of indomethacin will almost always result in relief within 24 hours.

4. A registered nurse will place a catheter in a vein on my non- exercising arm and withdraw approximately 6 ml on 5 different occasions, ie., one sample prior to exercise, one sample just after exercise, one sample after the period of occlusion, one sample prior to cold water immersion, and one sample just after cold water immersion. This will occur during both the placebo and indomethacin conditions and the catheter will remain in place during that testing period. A

new catheter will be placed on the other visit. The total amount of blood drawn during the course of this study will be 60 milliliters. The conditions will be separated by a one week period.

Karen Dorn, R.N., M.S.N. will be operating under standing orders from Michael Payne, M.D.. Mrs. Dorn will be placing all the catheters and monitoring responses to indomethacin.

The time commitment for this project will be approximately two (2) - one and one-half hour sessions spaced one week apart. The nurse will discuss possible side-effects at this time and those subjects with questionable symptoms will be removed from the experiment.

5. I understand that participation in this experiment may produce certain discomforts and risks. These discomforts include forearm fatigue accompanying handgrip exercise and possible sore arm muscles in the few hours to days following the experiment. In addition, discomfort may be expected during the period of forearm circulatory occlusion and during the period of cold water immersion of the non-dominant hand. As this experiment is of low-moderate intensity, the risk of developing cardiovascular problems is minimal. There is a risk of thrombophlebitis (inflammation of vein) resulting from catheter placement. This possibility is often minimized by having experienced persons place the catheter and maintaining aseptic conditions. Thrombophlebitis can be easily treated

with antibiotics.

6. Certain personal benefits may be expected from participation in this experiment. After conclusion of the study results from the maximal voluntary forearm strength and endurance times will be provided.

7. Appropriate alternative procedures which might be advantageous to me include terminating participation in the experiment at any point where discomfort exceeds a level to which you are willing to tolerate.

8. I am required not to engage in any strenuous physical activity during the two week period of participation. I have also been requested not to take aspirin or any other medication during the two weeks of the experiments, unless I first inform the principal investigator or the registered nurse. I have also been asked to refrain from caffeine containing foods or beverages during the days of testing. In general, I have been asked not to significantly change my activity or dietary patterns during the course of this study. I acknowledge that I have read this document in its entirety or that it has been read to me if I request so or I am unable to read it myself.

I consent to all of the procedures as explained herein. I will not hold any of the investigators, the registered nurse, medical supervisor, or Virginia Tech. responsible for any medical consequences foreseen or unforeseen which might

arise as a result of participation in this study.

I understand that all data pertaining to my participation will be held confidential and will be used for research purposes only. I also understand that these data may only be used when not identifiable with me.

I understand that I may abstain from participation in any part of the experiment or withdraw from the experiment should I feel the activities might be injurious to my health. The experimenter may also terminate my participation should he feel participation might be injurious to my health.

I understand that it is my personal responsibility to advise the researchers of any preexisting medical condition that may affect my participation or of any medical problems that might arise in the course of this experiment and that no such medical treatment or compensation is available if injury is suffered as a result of this research. A telephone is available which would be used to call the local hospital for emergency service.

I have read the above statements and have had the opportunity to ask questions. I understand that the researchers will, at any time, answer my inquiries concerning the procedures used in this experiment.

Date_____ Time_____a.m/p.m

Participant Name_____

Participant Signature_____

Witness_____

HPL Personnel

Project Director Kevin Davy

(H) 961-2793 (W) 231-5006

Registered Nurse Karen Dorn, R.N., M.S.N.

(H) 639-4792 (W) 231-5006

Exercise Laboratory Director William G. Herbert, Ph.D.

(W) 231-6565

HPE Human Subjects Chairperson Elizabeth Holford, J.D.

(W) 231-7543

Dr. Ernie Stout, Chairmen, Institutional Review Board for Reseach Involving Human Subjects. 301 Buruss Hall, (W) 231-5281

To receive the results of this investigation, please indicate this choice by marking in the appropriate space

provided below. A copy will then be distributed to you as soon as the results are made available by the investigator. Thank you for making this important contribution.

_____ I request a copy of the results of this study

APPENDIX B

MEDICAL HISTORY

PHYSICAL ACTIVITY HISTORY

Medical History

1. Have you ever experienced any of the following (please check):

__chest pain __ at rest __exertion __ cold weather

__chest pressure __ at rest __exertion __ cold weather

__discomfort/pain in jaw __ at rest __exertion __ cold weather

__in the teeth __ at rest __exertion __ cold weather

__in the throat __ at rest __exertion __ cold weather

__discomfort /pain in arm__ at rest __exertion __ cold weather

__skipped beats __ at rest __exertion __ cold weather

2. Do you smoke or use tobacco products?

If yes, please describe: no./day_____

hours since last _____

3. Are you taking any medications on a regular basis?

__yes __no

If yes, please list any and all medications you are taking (both prescription and non-prescription drugs)

Name of Medication	Dosage	Doses per day
_____	_____	_____
_____	_____	_____
_____	_____	_____

4. Have you ever had any reaction to aspirin or aspirin-like products (Bufferin, Anacin, Advil, etc.)?

__yes ___no

5. Please check if you have ever had any of the following health problems:

Peptic ulcer?__yes __no. If yes, please describe

_____

Bronchospasm?__yes __no. If yes, please describe

_____

Renal Impairment?__yes __no. If yes, please describe _____

_____

Nasal Polyps?__yes __no. If yes, please describe _____

_____

Bleeding problems?__yes __no. If yes, please describe _____

_____

Fluid retention?__yes __no. If yes, please describe _____

_____

Parkonsonism?__yes __no. If yes, please describe _____

_____

Epilepsy?__yes __no. If yes, please describe _____

_____

Psychiatric disorders? __yes __no. If yes, please describe

_____

Diabetes? __yes __no. If yes, please describe _____

_____

6. Do you have any reason to believe that participating in this project would be injurious to your health? If yes, please describe:

_____

_____

Exercise/Activity History

1. Are you currently involved in a regular exercise program (2-3 days/week)? ___yes ___no.
2. Please check the following sports/activities in which you have been involved over the past 3 months.

			Intensity		
Activity	Days/Week	Min/Day	Light	Moderate	Vigorous
Basketball					
Bicycling					
calisthenics					
dancing					
aerobics					
jogging					
swimming					
racquet sports					
weight training					
hiking/climbing					
golf					
handball					
other					

Please include any other physical activities in which you partake which include use of your upper and lower arms.

Appendix C

Detailed Methodology

Detailed Methodology

Introduction

Subjects were given a double blind administration of either placebo (PLAC) or indomethacin (IND) after two initial orientation sessions. Arterial pressures (BP), heart rate (HR), and norepinephrine (NE) were measured during isometric handgrip (HG) and postcontraction muscle ischemia (PC).

Subject Screening and Selection

Subjects were recruited by the use of printed announcement and by word of mouth. Potential subjects were screened and 10 males were selected to participate in the investigation. Selection criteria included: 1) age between 18-35 years of age, 2) males without any health related problems, 3) lack of participation in regular upper body aerobic or resistance training program for the prior 6 months, 4) willingness to adhere to the study protocol.

Subjects. Ten male subjects volunteered to participate in this investigation. All subjects were normotensive (arterial pressure <140/90), not taking medications, and apparently healthy as assessed by medical history. The protocol was explained to subjects and informed consent was obtained. The studies were approved by the Institutional Review Board at Virginia Tech.

Drug Administration. Subjects were give a double-blind random administration of either placebo (PLAC) or 100 mg of indomethacin (IND) 2.5 hours prior to static handgrip session.

A one week time period elapsed between administration of placebo and drug to avoid carry-over effects.

Hemodynamic measurements. During the experiments, heart rate (HR) was measured each two minutes for the last 15 minutes of this period from a electrocardiographic tracing produced by a Cambridge single channel recorder (Cambridge VS-550, Ossining, N.Y.). Systolic, diastolic and mean arterial pressure were determined by a Dynamap™ 1846 blood pressure monitor using the oscillometric technique (Critikon Inc., Tampa, FL.). The blood pressure monitor was calibrated before each session. A mercury manometer was placed in line with the automated device by the use of a "Y" connector. The blood pressure cuff was placed around a non-deformable cylinder and the device was activated during which time the device was compared to the mercury manometer measurement. Comparisons were made at 25 millimeter intervals from 200 mmHg to 0 mmHg.

Plasma norepinephrine. Six-millimeter blood samples were obtained from an antecubital vein in the non-dominant forearm of 9 of the 10 subjects for both conditions. Samples were drawn and immediately place in a chilled heparinized tube containing EGTA and reduced glutathione. The plasma was seperated by centrifugation (6000 xg at 4° c) and stored at -70 C. All samples were packed on dry ice and sent next day air to University of Arizona Health Science Center in Tucson. Analysis for norepinephrine (NE) was made by a single isotope

radioenzymatic technique of Henry and Bowsher (1979). All NE assays were performed in duplicate at the University of Arizona Health Science Center in the Endocrinology Division of the Department of Internal Medicine. This procedure consists of a enzymatic radiolabelling reaction phenylethanolamine N-methyltransferase. Triplicate 25 µl aliquots of plasma were used for each determination. Two tubes received plasma and 25 µl of 1 mM HCl. One tube received 25 µl of 1 mM of HCl which contained 500 pg of NE which provide the internal standard. Duplicate blank tubes received 50 µl of 1 mM HCl. Each methlytransferase reaction was initiated by a 25 µl addition of freshly prepared reaction reagent. A reaction reagent for the assays contained 250 µl of Tris hydrochloride-2 g/100 ml EDTA pH 8.6, 2.5 mg ascorbic acid, 150 µl of distilled water, 50 µl of purified PMNT and 100 µl of (^{3}H) S-adenosyl-L-(methyl-^{3}H)methionine (SAM) (50 µCi).

After incubation of 60 minutes at 37° C, each reaction was terminated by the addition of 100 µl of 2 mM potassium phosphate - 2g/100 ml EDTA pH 10 which contained 2 mM dithiothreitol. The tubes were vortexed and 50 mg of alumina was added. The tubes were vortexed and centrifuged at 2,500 xg for 5 minutes at room temperature. The supernatants were aspirated with a vacuum and the alumina was washed three times by vortexing after the addition of 1-2 ml of distilled water. After each wash, the alumina was allowed to settle by gravity

and the water was removed by aspiration. After the final wash and aspiration the tritiated epinephrine product was eluted by dissolving 2.5 mg of unlabelled SAM in 20 ml of cold 0.1 M perchloric acid which contained 25 μg/ml of unlabelled epinephrine. Each tube was vortexed 1-3 sec after receiving 1 ml of this solution. Next, 200 μl of a phosphotungstic acid solution (125mg/ml distilled water) was added to each tube. After vortexing the tubes, the alumina and SAM precipitate were sedimented by centrifugation at 2500 xg for 10 minutes at room temperature. A 1-ml aliqout of each acidic supernatant was then transfered directly to a scintillation vial which contained 1mM of 1 M potassium phosphate pH 7.1. Next, 10 ml of counting solution was added to each vial. This counting solution was prepared by adding 80 ml of technical grade bis(2-ethylhexyl)hydrogen phosphate to 1 gallon of Econofluor. The vials were capped, shaken and quantified by liguid scintillation spectrometry. The content of NE in each tube was calculated using the following formula:

$$\frac{\text{Sample cpm - blank cpm}}{\text{internal standard cpm - sample cpm}} \times 500 \text{ pg}$$

Ratings of Perceived Exertion and Pain. Subjects ratings of voluntary effort during handgrip were made a scale 6 (very, very light) to 20 (very, very hard) (Borg et al., 1974). Subjects were asked to use peripheral cues for the ratings on both scales. Ratings of discomfort were made on a pain scale

modified from a validated ratio scale. Reports of discomfort were 0 (no pain). 2 (some pain), 4 (mild pain), 6 (moderate pain), 8 (severe pain) and 10 (almost unbearable). No verbal discriptors were attached to the odd numbers.

Maximal voluntary contraction. Maximal voluntary contraction (MVC) of the non-dominant forearm was determined from three trials. Subjects were instructed to squeeze the handgrip as hard as they possible could without involving other muscle groups or performing Valsalva maneuvers. The highest of the three trials was taken as the subjects MVC. Force was recorded on a strip chart recorder (Beckman Cardiopulmonary Instruments, Los Angeles, CA.). The measurement in pounds was converted to Newtons. The force transducer was calibrated before and after each experimental session by hanging known weights from an "S" hook.

Isometric handgrip exercise. Subjects performed isometric handgrip exercise (HG), while in the supine posture, at 40% MVC until this force could no longer be maintained. The spring mechanism from a handgrip dynamometer was removed and replaced with a force transducer. The signals were amplified (Kube Electronics, Simi Valley, California) and routed a digital storage oscilloscope (Tektronics 2201, Beaverton, OR.). The target and actual force dynamometer forces were displayed on the oscilloscope and subjects were instructed to match the actual with the target force for as long as

possible. The point of exhaustion, for the purposes of this study, were defined as 1) the inability to match actual force with target force despite continued encouragement (approx. 10% for 2 sec.) and 2) the attainment of a maximal rating of perceived exertion.

Postcontraction muscle ischemia. Approximately 5 seconds prior to exhaustion, a standard blood pressure cuff was inflated, around the non-dominant arm, to 250 mmHg. Inflation of the cuff was maintained for 3 minutes. This procedure has been shown to isolate metaboreceptor stimulation of sympathetic activity and arterial pressure (Mark et al., 1985).

Experimental procedures

Orientation sessions. All subjects performed the protocol on at least two occasions before the data collection session because prior performance is necessary to attain reliable endurance times. At the end to these sessions, all subjects were able to perform HG to the perceptual and performance endpoints.

Data collection sessions. Thirty minutes before the beginning of the exercise protocol subjects were placed in supine position and an indwelling catheter (21G) was inserted into an antecubital vein in the dominant non-exercising arm. Standard blood pressure cuffs were placed approximately 10 cm proximal to antecubital space of both the dominant and non-dominant

arms. The maximal voluntary contraction (MVC) was measured in the non-dominant forearm. Muscle contractions of other limbs and Valsalva maneuvers were strictly avoided. The subjects were allowed to rest quietly for approximately 20 minutes before measurements commenced. The submaximal isometric handgrip bout was preceded by 10 minute resting control period. Heart rate and arterial pressure were measured each minute during the control, HG, and PC. Ratings of perceived exertion were obtained each 15s during HG. Pain ratings were obtained each 30 seconds during PC. Blood samples for NE measurement were drawn during the control period and 1 min after the end of HG and PC. Subjects were continually reminded to relax all non-exercising body parts and no Valsalva maneuvers were observed. Heart rate and BP measurements were obtained each minute during the 5 minute recovery period.

Cold stimulation. We also used a cold pressor testing (CPT) as a non-exercising internal control. Twenty-five minutes after cessation of the HG and PC protocol another 10 minute control period was obtained. Heart rate and BP were obtained each minute during the control and during the first and last 45s of a 90s of CPT. Blood samples were drawn during control and 1 min post immersion. Ratings of pain were obtained each 30s during the immersion. Recovery HR and BP were determined each minute of recovery for 6 minutes.

Data analysis. Control and recovery values were calculated as an average of the data obtained over an entire 6 minute period. The absolute time to exhaustion (in s) was determined for each subjects under both PLAC and IND conditions. *Statistics.* For all hemodynamic measurements, plasma NE and pain responses, differences between groups for each variable and in the magnitude of change in that variable were assessed by a repeated measured analysis of variance with two within subject factors (drug and time). Fishers protected LSD was used when appropriate to located differences between or across conditions. In attempt to adjust for violation of compound symmetry, the Huynh-Feldt Epsilon correction was applied to the degrees of freedom of the appropriate error term. Paired T-tests were used to assess differences in maximal voluntary contraction and time to exhaustion. Differences were considered significant at the $P<.05$ level. All group data are presented as means ± SE.

APPENDIX D

Raw Data

Raw Data

	Placebo	Indomethacin
Maximal handgrip force: (Newtons)		
Subject 1	505.0	465.8
Subject 2	450.1	436.4
Subject 3	422.7	465.8
Subject 4	436.4	463.9
Subject 5	367.7	367.7
Subject 6	327.5	367.7
Subject 7	450.1	479.5
Subject 8	450.1	465.8
Subject 9	479.5	491.3
Subject 10	395.2	382.5
Endurance time: (seconds)		
Subject 1	155	149
Subject 2	118	135
Subject 3	116	143
Subject 4	152	147
Subject 5	154	170
Subject 6	160	164
Subject 7	140	145
Subject 8	180	157
Subject 9	120	125
Subject 10	184	195

Raw Data

Systolic pressure during static handgrip protocol

	Placebo	Indomethacin
Baseline-Minute 1: (mmHg)		
Subject 1	134	123
Subject 2	108	107
Subject 3	106	105
Subject 4	117	131
Subject 5	133	126
Subject 6	102	111
Subject 7	112	113
Subject 8	115	135
Subject 9	116	133
Subject 10	115	144
Baseline-Minute 3:		
Subject 1	128	123
Subject 2	114	115
Subject 3	110	103
Subject 4	117	127
Subject 5	117	126
Subject 6	100	108
Subject 7	115	114
Subject 8	113	127
Subject 9	114	129
Subject 10	108	135
Baseline-Minute 5:		
Subject 1	127	131
Subject 2	116	111
Subject 3	117	112
Subject 4	112	132
Subject 5	115	122
Subject 6	105	109
Subject 7	110	112
Subject 8	114	120
Subject 9	110	131
Subject 10	110	138

Raw Data

Systolic pressure during static handgrip protocol

	Placebo	Indomethacin
Static handgrip-Minute 1:		
Subject 1	143	136
Subject 2	120	123
Subject 3	121	121
Subject 4	133	133
Subject 5	138	137
Subject 6	115	121
Subject 7	120	128
Subject 8	117	139
Subject 9	133	156
Subject 10	111	131
Static handgrip-Minute 2:		
Subject 1	157	146
Subject 2	133	132
Subject 3	148	135
Subject 4	153	145
Subject 5	152	153
Subject 6	129	134
Subject 7	146	136
Subject 8	127	149
Subject 9	191	179
Subject 10	121	131
Posthandgrip circulatory arrest-Minute 1:		
Subject 1	158	157
Subject 2	142	142
Subject 3	146	154
Subject 4	149	153
Subject 5	177	192
Subject 6	129	136
Subject 7	134	133
Subject 8	154	157
Subject 9	152	163
Subject 10	129	123

Raw Data

Systolic pressure during static handgrip protocol

	Placebo	Indomethacin
Posthandgrip circulatory arrest-Minute 2:		
Subject 1	165	155
Subject 2	147	143
Subject 3	137	153
Subject 4	156	146
Subject 5	186	182
Subject 6	134	137
Subject 7	131	128
Subject 8	146	164
Subject 9	153	163
Subject 10	126	132
Posthandgrip circulatory arrest-Minute 3:		
Subject 1	163	156
Subject 2	147	138
Subject 3	144	147
Subject 4	147	145
Subject 5	187	187
Subject 6	129	135
Subject 7	133	133
Subject 8	151	157
Subject 9	154	168
Subject 10	132	131
Recovery for static handgrip protocol-Minute 1:		
Subject 1	146	138
Subject 2	152	120
Subject 3	115	111
Subject 4	127	131
Subject 5	126	137
Subject 6	116	117
Subject 7	117	120
Subject 8	126	135
Subject 9	133	133
Subject 10	105	115

Raw Data

Systolic pressure during static handgrip protocol

	Placebo	Indomethacin
Recovery for static handgrip protocol-Minute 3:		
Subject 1	136	131
Subject 2	116	114
Subject 3	115	117
Subject 4	126	123
Subject 5	122	136
Subject 6	111	109
Subject 7	110	116
Subject 8	122	133
Subject 9	128	136
Subject 10	112	128
Recovery for static handgrip protocol-Minute 5:		
Subject 1	133	126
Subject 2	123	114
Subject 3	113	111
Subject 4	123	123
Subject 5	127	135
Subject 6	112	114
Subject 7	114	117
Subject 8	116	133
Subject 9	122	133
Subject 10	108	136

Raw Data

Diastolic pressure during static handgrip protocol

	Placebo	Indomethacin
Baseline for static handgrip protocol-Minute 1: (mmHg)		
Subject 1	77	75
Subject 2	69	73
Subject 3	59	65
Subject 4	67	76
Subject 5	68	77
Subject 6	57	61
Subject 7	57	71
Subject 6	71	75
Subject 9	65	70
Subject 10	73	97
Baseline for static handgrip protocol-Minute 3:		
Subject 1	75	77
Subject 2	67	67
Subject 3	60	60
Subject 4	70	70
Subject 5	64	64
Subject 6	59	59
Subject 7	61	61
Subject 6	73	73
Subject 9	62	62
Subject 10	67	67
Baseline for static handgrip protocol-Minute 5:		
Subject 1	74	78
Subject 2	69	73
Subject 3	67	58
Subject 4	67	71
Subject 5	70	76
Subject 6	64	65
Subject 7	60	64
Subject 6	76	84
Subject 9	62	64
Subject 10	70	93

Raw Data

Diastolic pressure during static handgrip protocol

	Placebo	Indomethacin
Static handgrip-Minute 1:		
Subject 1	82	83
Subject 2	76	81
Subject 3	73	81
Subject 4	78	78
Subject 5	81	88
Subject 6	70	74
Subject 7	69	78
Subject 8	81	87
Subject 9	74	87
Subject 10	78	86
Static handgrip-Minute 2:		
Subject 1	92	100
Subject 2	95	86
Subject 3	89	91
Subject 4	100	93
Subject 5	96	99
Subject 6	74	82
Subject 7	85	87
Subject 8	85	94
Subject 9	104	101
Subject 10	84	83
Posthandgrip circulatory arrest-Minute 1:		
Subject 1	95	100
Subject 2	90	96
Subject 3	91	82
Subject 4	92	89
Subject 5	104	115
Subject 6	80	96
Subject 7	76	81
Subject 8	93	102
Subject 9	76	87
Subject 10	83	74

Raw Data

Diastolic pressure during static handgrip protocol

	Placebo	Indomethacin
Posthandgrip circulatory arrest-Minute 2:		
Subject 1	103	102
Subject 2	83	85
Subject 3	95	85
Subject 4	95	90
Subject 5	107	112
Subject 6	78	80
Subject 7	76	79
Subject 8	99	98
Subject 9	86	93
Subject 10	92	87
Posthandgrip circulatory arrest-Minute 3:		
Subject 1	100	100
Subject 2	91	76
Subject 3	95	85
Subject 4	90	85
Subject 5	104	118
Subject 6	82	82
Subject 7	75	81
Subject 8	99	98
Subject 9	84	92
Subject 10	86	83

Raw Data

Diastolic pressure during static handgrip protocol

	Placebo	Indomethacin
Recovery for static handgrip protocol-Minute 1:		
Subject 1	79	80
Subject 2	64	70
Subject 3	71	70
Subject 4	66	57
Subject 5	75	80
Subject 6	65	70
Subject 7	62	70
Subject 8	74	75
Subject 9	59	70
Subject 10	70	75
Recovery for static handgrip protocol-Minute 4:		
Subject 1	78	80
Subject 2	74	66
Subject 3	64	68
Subject 4	69	63
Subject 5	73	83
Subject 6	67	61
Subject 7	59	71
Subject 8	86	81
Subject 9	59	71
Subject 10	73	83

Raw Data

Diastolic pressure during static handgrip protocol

	Placebo	Indomethacin
Recovery for static handgrip protocol-Minute 5:		
Subject 1	80	80
Subject 2	71	66
Subject 3	63	74
Subject 4	66	68
Subject 5	69	82
Subject 6	64	61
Subject 7	59	63
Subject 8	82	72
Subject 9	64	71
Subject 10	76	79

Mean arterial pressure during static handgrip protocol

Baseline for static handgrip protocol-Minute 1: (mmHg)		
Subject 1	97	97
Subject 2	84	88
Subject 3	78	88
Subject 4	87	94
Subject 5	87	88
Subject 6	78	84
Subject 7	82	87
Subject 8	87	101
Subject 9	82	87
Subject 10	93	116
Baseline for static handgrip protocol-Minute 3:		
Subject 1	95	94
Subject 2	80	90
Subject 3	86	78
Subject 4	82	90
Subject 5	84	90
Subject 6	76	85
Subject 7	80	85
Subject 8	88	91
Subject 9	82	85
Subject 10	83	112

Raw Data

Mean arterial pressure during static handgrip protocol

	Placebo	Indomethacin
Baseline for static handgrip protocol-Minute 5:		
Subject 1	91	96
Subject 2	81	88
Subject 3	85	74
Subject 4	85	88
Subject 5	90	93
Subject 6	78	81
Subject 7	83	84
Subject 8	96	106
Subject 9	82	91
Subject 10	87	109
Static handgrip protocol-Minute 1:		
Subject 1	101	103
Subject 2	81	95
Subject 3	91	101
Subject 4	101	99
Subject 5	99	103
Subject 6	91	93
Subject 7	90	95
Subject 8	95	106
Subject 9	98	114
Subject 10	93	103
Static handgrip protocol-Minute 2:		
Subject 1	116	119
Subject 2	93	106
Subject 3	101	106
Subject 4	118	113
Subject 5	115	118
Subject 6	97	103
Subject 7	107	109
Subject 8	98	116
Subject 9	151	133
Subject 10	104	105

Raw Data

Mean arterial pressure during static handgrip protocol

	Placebo	Indomethacin
Posthandgrip circulatory arrest-Minute 1:		
Subject 1	118	122
Subject 2	111	112
Subject 3	116	109
Subject 4	117	115
Subject 5	126	137
Subject 6	106	112
Subject 7	98	99
Subject 8	115	124
Subject 9	108	115
Subject 10	103	95
Posthandgrip circulatory arrest-Minute 2:		
Subject 1	122	121
Subject 2	117	117
Subject 3	118	107
Subject 4	113	113
Subject 5	137	137
Subject 6	105	104
Subject 7	94	104
Subject 8	118	119
Subject 9	108	117
Subject 10	106	108
Posthandgrip circulatory arrest-Minute 3:		
Subject 1	117	117
Subject 2	112	92
Subject 3	115	114
Subject 4	113	106
Subject 5	124	135
Subject 6	99	104
Subject 7	95	104
Subject 8	118	123
Subject 9	109	117
Subject 10	104	107

Raw Data

Mean arterial pressure during static handgrip protocol

	Placebo	Indomethacin
Recovery for static handgrip protocol-Minute 1:		
Subject 1	102	99
Subject 2	95	94
Subject 3	88	81
Subject 4	93	93
Subject 5	93	97
Subject 6	84	90
Subject 7	82	90
Subject 8	84	97
Subject 9	83	92
Subject 10	83	93
Recovery for static handgrip protocol-Minute 3:		
Subject 1	98	101
Subject 2	90	93
Subject 3	90	90
Subject 4	90	88
Subject 5	92	99
Subject 6	84	90
Subject 7	77	91
Subject 8	99	98
Subject 9	86	94
Subject 10	90	101
Recovery for static handgrip protocol-Minute 5:		
Subject 1	97	98
Subject 2	94	90
Subject 3	92	93
Subject 4	86	91
Subject 5	91	98
Subject 6	84	87
Subject 7	83	87
Subject 8	95	96
Subject 9	85	94
Subject 10	88	113

Raw Data

Heart rate during static handgrip protocol

	Placebo	Indomethacin
Baseline for static handgrip protocol-Minute 1: (bpm)		
Subject 1	60	68
Subject 2	60	49
Subject 3	70	61
Subject 4	70	57
Subject 5	69	67
Subject 6	70	58
Subject 7	58	49
Subject 8	60	57
Subject 9	80	66
Subject 10	54	54
Baseline for static handgrip protocol-Minute 3:		
Subject 1	62	58
Subject 2	56	47
Subject 3	65	56
Subject 4	64	60
Subject 5	63	60
Subject 6	72	59
Subject 7	56	50
Subject 8	61	55
Subject 9	61	69
Subject 10	54	54
Baseline for static handgrip protocol-Minute 5:		
Subject 1	69	60
Subject 2	69	47
Subject 3	63	68
Subject 4	65	59
Subject 5	74	61
Subject 6	71	60
Subject 7	53	54
Subject 8	65	58
Subject 9	69	80
Subject 10	56	60

Raw Data

Heart rate during static handgrip protocol

	Placebo	Indomethacin
Static handgrip protocol-Minute 1:		
Subject 1	90	77
Subject 2	70	58
Subject 3	83	73
Subject 4	90	70
Subject 5	94	72
Subject 6	81	74
Subject 7	78	69
Subject 8	106	99
Subject 9	64	70
Subject 10	75	62
Static handgrip protocol-Minute 2:		
Subject 1	97	78
Subject 2	78	62
Subject 3	80	70
Subject 4	72	79
Subject 5	103	82
Subject 6	82	79
Subject 7	82	65
Subject 8	106	99
Subject 9	72	82
Subject 10	78	62
Posthandgrip circulatory arrest-Minute 1:		
Subject 1	69	66
Subject 2	59	56
Subject 3	66	57
Subject 4	75	65
Subject 5	98	90
Subject 6	69	69
Subject 7	42	46
Subject 8	65	56
Subject 9	80	82
Subject 10	70	57

Raw Data

Heart rate during static handgrip protocol

	Placebo	Indomethacin
Posthandgrip circulatory arrest-Minute 2:		
Subject 1	75	68
Subject 2	66	47
Subject 3	57	55
Subject 4	74	65
Subject 5	74	99
Subject 6	71	67
Subject 7	52	52
Subject 8	67	58
Subject 9	88	74
Subject 10	70	57
Posthandgrip circulatory arrest-Minute 3:		
Subject 1	72	64
Subject 2	55	58
Subject 3	59	56
Subject 4	72	62
Subject 5	73	75
Subject 6	79	63
Subject 7	54	49
Subject 8	72	61
Subject 9	74	78
Subject 10	65	58
Recovery for static handgrip protocol-Minute 1:		
Subject 1	67	64
Subject 2	79	48
Subject 3	65	61
Subject 4	65	61
Subject 5	65	55
Subject 6	77	59
Subject 7	59	48
Subject 8	71	68
Subject 9	65	65
Subject 10	61	58

Raw Data

Heart rate during static handgrip protocol

	Placebo	Indomethacin
Recovery for static handgrip protocol-Minute 3:		
Subject 1	63	61
Subject 2	63	50
Subject 3	69	59
Subject 4	65	65
Subject 5	68	57
Subject 6	73	59
Subject 7	56	47
Subject 8	59	61
Subject 9	60	63
Subject 10	59	58
Recovery for static handgrip protocol-Minute 5:		
Subject 1	60	62
Subject 2	53	48
Subject 3	65	65
Subject 4	63	65
Subject 5	73	53
Subject 6	66	59
Subject 7	53	46
Subject 8	55	57
Subject 9	57	60
Subject 10	60	56

Raw Data

Systolic pressure during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 1: (mmHg)		
Subject 1	131	122
Subject 2	112	115
Subject 3	110	113
Subject 4	123	126
Subject 5	120	131
Subject 6	111	112
Subject 7	110	113
Subject 8	121	144
Subject 9	133	116
Subject 10	112	144
Baseline for cold stimulation-Minute 3:		
Subject 1	137	116
Subject 2	110	115
Subject 3	114	109
Subject 4	129	120
Subject 5	120	131
Subject 6	109	110
Subject 7	111	113
Subject 8	127	138
Subject 9	127	123
Subject 10	110	138
Baseline for cold stimulation-Minute 5:		
Subject 1	132	127
Subject 2	111	114
Subject 3	111	115
Subject 4	126	111
Subject 5	120	131
Subject 6	107	106
Subject 7	113	112
Subject 8	112	137
Subject 9	128	115
Subject 10	116	136

Raw Data

Systolic pressure during cold stimulation

	Placebo	Indomethacin
Cold stimulation-45 sec.:		
Subject 1	142	135
Subject 2	117	114
Subject 3	121	116
Subject 4	129	132
Subject 5	129	137
Subject 6	117	116
Subject 7	116	123
Subject 8	114	135
Subject 9	129	146
Subject 10	123	136
Cold stimulation-90 sec.:		
Subject 1	145	139
Subject 2	146	149
Subject 3	129	127
Subject 4	146	151
Subject 5	139	155
Subject 6	122	127
Subject 7	133	123
Subject 8	136	157
Subject 9	135	152
Subject 10	134	132
Recovery for cold stimulation-Minute 1:		
Subject 1	145	131
Subject 2	134	134
Subject 3	129	127
Subject 4	115	116
Subject 5	133	136
Subject 6	111	116
Subject 7	116	122
Subject 8	145	136
Subject 9	137	144
Subject 10	117	146

Raw Data

Systolic pressure during cold stimulation

	Placebo	Indomethacin
Recovery for cold stimulation-Minute 3:		
Subject 1	135	132
Subject 2	120	115
Subject 3	120	120
Subject 4	112	116
Subject 5	126	133
Subject 6	115	109
Subject 7	129	100
Subject 8	133	152
Subject 9	120	138
Subject 10	111	136

Diastolic pressure during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 1: (mmHg)		
Subject 1	83	79
Subject 2	66	75
Subject 3	58	68
Subject 4	67	62
Subject 5	70	77
Subject 6	60	70
Subject 7	69	70
Subject 8	60	74
Subject 9	84	77
Subject 10	73	92

Raw Data

Diastolic pressure during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 3:		
Subject 1	82	78
Subject 2	69	77
Subject 3	60	69
Subject 4	70	67
Subject 5	72	74
Subject 6	63	66
Subject 7	63	71
Subject 8	67	80
Subject 9	73	74
Subject 10	74	88
Baseline for cold stimulation-Minute 5:		
Subject 1	80	74
Subject 2	67	74
Subject 3	62	72
Subject 4	67	70
Subject 5	71	76
Subject 6	62	62
Subject 7	69	71
Subject 8	62	80
Subject 9	81	72
Subject 10	77	86

Raw Data

Diastolic pressure during cold stimulation

	Placebo	Indomethacin
Cold stimulation-45sec:		
Subject 1	81	79
Subject 2	76	83
Subject 3	75	76
Subject 4	7	76
Subject 5	79	76
Subject 6	73	69
Subject 7	70	78
Subject 8	66	80
Subject 9	83	85
Subject 10	85	93
Cold stimulation-90sec:		
Subject 1	89	93
Subject 2	96	99
Subject 3	83	83
Subject 4	99	93
Subject 5	91	87
Subject 6	73	79
Subject 7	77	78
Subject 8	75	94
Subject 9	97	87
Subject 10	91	82
Recovery for cold stimulation-Minute 1:		
Subject 1	81	85
Subject 2	88	85
Subject 3	70	80
Subject 4	65	78
Subject 5	70	71
Subject 6	77	77
Subject 7	70	65
Subject 8	69	69
Subject 9	86	82
Subject 10	78	78

Raw Data

Diastolic pressure during cold stimulation

	Placebo	Indomethacin
Recovery for cold stimulation-Minute 3:		
Subject 1	73	93
Subject 2	80	80
Subject 3	71	92
Subject 4	59	66
Subject 5	60	64
Subject 6	80	77
Subject 7	65	59
Subject 8	65	67
Subject 9	70	72
Subject 10	76	79
Recovery for cold stimulation-Minute 5:		
Subject 1	81	-
Subject 2	78	87
Subject 3	67	94
Subject 4	63	65
Subject 5	58	66
Subject 6	73	80
Subject 7	68	60
Subject 8	65	71
Subject 9	73	79
Subject 10	81	80
Recovery for cold stimulation-Minute 5:		
Subject 1	81	-
Subject 2	78	87
Subject 3	67	94
Subject 4	63	65
Subject 5	58	66
Subject 6	73	80
Subject 7	68	60
Subject 8	65	71
Subject 9	73	79
Subject 10	81	80

Raw Data

Mean arterial pressure during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 1: (mmHg)		
Subject 1	97	97
Subject 2	84	88
Subject 3	78	88
Subject 4	87	94
Subject 5	87	88
Subject 6	78	84
Subject 7	82	87
Subject 8	87	101
Subject 9	82	87
Subject 10	93	116
Baseline for cold stimulation-Minute 3:		
Subject 1	95	94
Subject 2	80	90
Subject 3	86	78
Subject 4	82	90
Subject 5	84	90
Subject 6	76	85
Subject 7	80	85
Subject 8	88	91
Subject 9	82	85
Subject 10	83	112
Baseline for cold stimulation-Minute 5:		
Subject 1	91	96
Subject 2	81	88
Subject 3	85	74
Subject 4	85	88
Subject 5	90	93
Subject 6	78	81
Subject 7	83	84
Subject 8	96	98
Subject 9	82	91
Subject 10	87	109

Raw Data

Mean arterial pressure during cold stimulation

	Placebo	Indomethacin
Cold stimulation-45 sec:		
Subject 1	102	98
Subject 2	92	96
Subject 3	101	97
Subject 4	93	96
Subject 5	97	103
Subject 6	88	88
Subject 7	88	96
Subject 8	84	101
Subject 9	101	107
Subject 10	99	112
Cold stimulation-90 sec:		
Subject 1	112	112
Subject 2	122	119
Subject 3	114	116
Subject 4	102	102
Subject 5	107	123
Subject 6	98	98
Subject 7	98	99
Subject 8	102	119
Subject 9	111	112
Subject 10	111	107
Recovery for cold stimulation-Minute 1:		
Subject 1	102	99
Subject 2	95	94
Subject 3	88	81
Subject 4	93	93
Subject 5	93	97
Subject 6	84	90
Subject 7	82	90
Subject 8	84	97
Subject 9	83	92
Subject 10	83	93

Raw Data

Mean arterial pressure during cold stimulation

	Placebo	Indomethacin
Recovery for cold stimulation-Minute 3:		
Subject 1	98	101
Subject 2	90	93
Subject 3	90	90
Subject 4	90	88
Subject 5	92	99
Subject 6	84	90
Subject 7	77	91
Subject 8	99	98
Subject 9	86	94
Subject 10	90	101
Recovery for cold stimulation-Minute 5:		
Subject 1	97	98
Subject 2	94	90
Subject 3	92	93
Subject 4	86	91
Subject 5	91	98
Subject 6	84	87
Subject 7	83	87
Subject 8	95	96
Subject 9	85	94
Subject 10	88	113

Raw Data

Heart rate during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 1: (bpm)		
Subject 1	69	53
Subject 2	53	57
Subject 3	59	65
Subject 4	70	62
Subject 5	69	56
Subject 6	65	56
Subject 7	56	46
Subject 8	65	61
Subject 9	62	58
Subject 10	57	58
Baseline for cold stimulation-Minute 3:		
Subject 1	63	51
Subject 2	52	48
Subject 3	62	59
Subject 4	66	62
Subject 5	75	54
Subject 6	70	52
Subject 7	58	46
Subject 8	53	64
Subject 9	61	58
Subject 10	58	58

Raw Data

Heart rate during cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation-Minute 5:		
Subject 1	62	50
Subject 2	54	49
Subject 3	65	66
Subject 4	69	62
Subject 5	69	56
Subject 6	66	60
Subject 7	58	50
Subject 8	68	68
Subject 9	72	57
Subject 10	59	58
Cold stimulation-45sec:		
Subject 1	80	72
Subject 2	67	57
Subject 3	67	61
Subject 4	76	77
Subject 5	71	58
Subject 6	71	59
Subject 7	66	54
Subject 8	74	84
Subject 9	62	75
Subject 10	66	66

Raw Data

Heart rate during cold stimulation

	Placebo	Indomethacin
Cold stimulation-90sec:		
Subject 1	73	68
Subject 2	60	56
Subject 3	55	56
Subject 4	71	78
Subject 5	69	58
Subject 6	70	63
Subject 7	62	59
Subject 8	88	110
Subject 9	85	88
Subject 10	55	65
Recovery for cold stimulation-Minute 1:		
Subject 1	69	64
Subject 2	52	47
Subject 3	69	62
Subject 4	57	57
Subject 5	75	69
Subject 6	60	59
Subject 7	52	48
Subject 8	57	57
Subject 9	66	74
Subject 10	56	55
Recovery for cold stimulation-Minute 3:		
Subject 1	66	64
Subject 2	51	57
Subject 3	64	62
Subject 4	65	60
Subject 5	68	73
Subject 6	62	57
Subject 7	63	49
Subject 8	55	53
Subject 9	64	90
Subject 10	76	55

Raw Data

Heart rate during cold stimulation

	Placebo	Indomethacin
Recovery for cold stimulation-Minute 5:		
Subject 1	69	58
Subject 2	61	53
Subject 3	64	62
Subject 4	69	59
Subject 5	73	56
Subject 6	79	57
Subject 7	58	51
Subject 8	61	53
Subject 9	65	78
Subject 10	72	56

Pain responses during posthandgrip circulatory arrest

	Placebo	Indomethacin
Pain responses during posthandgrip circulatory arrest-30 sec:		
Subject 1	2	3
Subject 2	1	1
Subject 3	6	5
Subject 4	2	4
Subject 5	2	1
Subject 6	2	3
Subject 7	4	4
Subject 8	2	4
Subject 9	5	4
Subject 10	2	3

Raw Data

Pain responses during posthandgrip circulatory arrest

	Placebo	Indomethacin
Pain responses during posthandgrip circulatory arrest-60 sec:		
Subject 1	2	3
Subject 2	2	3
Subject 3	7	7
Subject 4	2	4
Subject 5	3	2
Subject 6	4	5
Subject 7	5	5
Subject 8	3	6
Subject 9	7	5
Subject 10	4	4

Raw Data

Pain responses during posthandgrip circulatory arrest

	Placebo	Indomethacin
Pain responses during posthandgrip circulatory arrest-90 sec:		
Subject 1	5	6
Subject 2	3	4
Subject 3	8	8
Subject 4	4	5
Subject 5	4	3
Subject 6	5	6
Subject 7	6	6
Subject 8	5	7
Subject 9	8	6
Subject 10	6	6

Raw Data

Pain responses during posthandgrip circulatory arrest

	Placebo	Indomethacin
Pain responses during posthandgrip circulatory arrest-120 sec:		
Subject 1	5	6
Subject 2	4	5
Subject 3	9	9
Subject 4	4	5
Subject 5	4	4
Subject 6	6	6
Subject 7	6	6
Subject 8	7	7
Subject 9	8	8
Subject 10	7	7
Pain responses during posthandgrip circulatory arrest-150 sec:		
Subject 1	6	9
Subject 2	5	6
Subject 3	9	9
Subject 4	4	5
Subject 5	5	5
Subject 6	6	7
Subject 7	7	7
Subject 8	8	8
Subject 9	9	9
Subject 10	8	7

Raw Data

Pain responses during posthandgrip circulatory arrest

	Placebo	Indomethacin
Pain responses during posthandgrip circulatory arrest-180 sec:		
Subject 1	6	9
Subject 2	5	6
Subject 3	10	10
Subject 4	4	5
Subject 5	5	6
Subject 6	7	7
Subject 7	8	10
Subject 8	9	10
Subject 9	9	8
Subject 10	8	7

Pain responses during cold stimulation

	Placebo	Indomethacin
Pain responses during cold stimulation-30 sec:		
Subject 1	1	1
Subject 2	3	4
Subject 3	6	6
Subject 4	5	6
Subject 5	2	2
Subject 6	3	3
Subject 7	5	5
Subject 8	3	5
Subject 9	2	1
Subject 10	4	4

Raw Data

Pain responses during cold stimulation

	Placebo	Indomethacin
Pain responses during cold stimulation-60 sec:		
Subject 1	3	3
Subject 2	5	6
Subject 3	7	8
Subject 4	7	7
Subject 5	3	3
Subject 6	4	5
Subject 7	6	7
Subject 8	4	6
Subject 9	3	3
Subject 10	6	6

Raw Data

Pain responses during cold stimulation

	Placebo	Indomethacin
Pain responses during cold stimulation-90 sec:		
Subject 1	4	4
Subject 2	6	7
Subject 3	8	9
Subject 4	7	8
Subject 5	4	4
Subject 6	6	6
Subject 7	7	7
Subject 8	5	7
Subject 9	5	4
Subject 10	8	8

Raw Data

Norepinephrine during handgrip protocol

	Placebo	Indomethacin
Baseline for handgrip protocol: (pg/ml)		
Subject 1	305	113
Subject 2	225	271
Subject 3	109	212
Subject 4	315	393
Subject 5	364	315
Subject 6	179	152
Subject 7	633	348
Subject 8	532	291
Subject 10	177	148
Static handgrip-1 minute post:		
Subject 1	455	275
Subject 2	606	332
Subject 3	899	987
Subject 4	1551	472
Subject 5	1017	730
Subject 6	502	628
Subject 7	825	477
Subject 8	687	537
Subject 10	277	200

Raw Data

Norepinephrine during handgrip protocol

	Placebo	Indomethacin
Postcontraction Muscle Ischemia-1 minute post:		
Subject 1	465	624
Subject 2	923	793
Subject 3	1194	1015
Subject 4	1965	730
Subject 5	1501	1772
Subject 6	1192	678
Subject 7	1137	617
Subject 8	1059	927
Subject 10	454	480

Norepinephrine for cold stimulation

	Placebo	Indomethacin
Baseline for cold stimulation: (mmHg)		
Subject 1	231	228
Subject 2	312	386
Subject 3	139	272
Subject 4	1599	490
Subject 5	223	612
Subject 6	894	311
Subject 7	644	647
Subject 8	401	227
Subject 10	454	480
Cold stimulation-1 minute post:		
Subject 1	345	263
Subject 2	312	646
Subject 3	513	641
Subject 4	1427	613
Subject 5	642	765
Subject 6	1189	466
Subject 7	421	811
Subject 8	767	667
Subject 10	580	270

Appendix E

Statistical Analyses

Repeated measures Analysis of Variance
Summary ANOVA

Dependent variable: Systolic pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	359.13	359.13	7.67	.0218
ERROR(TRIAL)	9	421.23	46.80		
TIME	3	3634.99	1211.67	8.77	.0016
ERROR TIME)	27	3730.48	138.17		
DRUG*TIME	3	17.08	5.69	0.10	.9509
ERROR(DRUG* TIME)	27	1573.47	58.27		
	HUYNH-FELDT EPSILON= .9118				

Repeated measures Analysis of Variance
Summary ANOVA

Dependent variable: Diastolic pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	196.25	196.25	2.81	.1283
ERROR(DRUG)	9	629.58	69.95		
TIME	3	2813.31	937.77	26.60	.0001
ERROR(TIME)	27	951.79	35.25		
DRUG*TIME	3	59.76	19.92	1.72	.1913
ERROR (DRUG* TIME)	27	312.21	11.56		
	HUYNH-FELDT EPSILON= .9128				

Repeated measures Analysis of Variance
Summary ANOVA

Dependent variable: Mean arterial pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	191.27	191.27	5.60	.0422
ERROR (DRUG)	9	307.64	34.18		
TIME	3	4055.37	1351.79	39.15	.0001
ERROR (TIME)	27	932.24	34.53		
DRUG*TIME	3	39.48	13.16	0.54	.6312
ERROR (DRUG* TIME)	27	655.96	24.29		
		HUYNH-FELDT EPSILON= .8519			

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Heart rate during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	120.78	120.78	1.34	.2773
ERROR (DRUG)	9	812.78	90.31		
TIME	3	1428.19	476.06	6.36	.0089
ERROR (TIME)	27	2020.24	74.82		
DRUG*TIME	3	105.26	35.09	2.00	.1464
ERROR (DRUG* TIME)	27	474.00	17.56		
		HUYNH-FELDT EPSILON= .8881			

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Systolic pressure during isometric protocol

Source	df	SS	MS	F	PR>F
DRUG	1	396.48	396.48	2.62	.1401
ERROR (DRUG)	9	1363.11	151.46		
TIME	6	22392.43	3732.07	27.48	.0001
ERROR (TIME)	54	7334.40	135.82		
DRUG*TIME	6	332.58	55.43	1.99	.0827
ERROR (DRUG* TIME)	54	1502.31	27.82		

HUYNH-FELDT= 1.037

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Diastolic pressure during isometric protocol

Source	df	SS	MS	F	PR>F
DRUG	1	276.93	276.93	5.44	.0446
ERROR (DRUG)	9	458.30	50.92		
TIME	6	11535.47	1922.58	34.09	.0001
ERROR (TIME)	54	3045.56	56.40		
DRUG*TIME	6	248.50	41.42	2.44	.0366
ERROR (DRUG* TIME)	54	915.48	16.95		

HUYNH-FELDT EPSILON = .8382

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Mean arterial pressure during isometric protocol

Source	df	SS	MS	F	PR>F
DRUG	1	455.04	455.04	12.33	.0066
ERROR (DRUG)	9	332.08	36.90		
TIME	6	14806.29	2467.71	27.18	.0001
ERROR (TIME)	54	4901.86	90.77		
DRUG*TIME	6	178.45	29.74	1.22	.3153
ERROR (DRUG* TIME)	54	1318.98	24.43		

HUNYH-FELDT EPSILON = .7952

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Heart rate during isometric handgrip protocol

Source	df	SS	MS	F	PR>F
DRUG	1	1505.52	1505.52	40.33	.0001
ERROR (DRUG)	9	355.99	37.33		
TIME	6	7072.29	1178.72	11.81	.0003
ERROR (TIME)	54	5388.38	99.78		
DRUG*TIME	6	169.79	28.30	0.86	.5004
ERROR (DRUG* TIME)	54	1784.22	33.04		

HUYN-FELDT EPSILON = .6763

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Pain response during postcontraction muscle ischemia period

Source	df	SS	MS	F	PR<F
DRUG	1	5.63	5.63	2.92	.1217
ERROR (DRUG)	9	17.37	1.92		
TIME	5	284.07	56.81	83.55	.0001
ERROR (TIME)	45	30.60	0.68		
DRUG*TIME	5	0.37	0.73	0.21	.7947
ERROR (DRUG* TIME)	45	15.63	0.35		

HUYNH-FELDT EPSILON= .3688

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Pain responses during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	3.75	3.75	4.55	.0617
ERROR (DRUG)	9	7.42	0.82		
TIME	5	7.637	35.32	135.26	.0001
ERROR (TIME)	45	4.70	0.26		
DRUG*TIME	5	0.10	0.05	0.73	.4681
ERROR (DRUG* TIME)	45	1.23	0.06		

HUYNH-FELDT EPSILON = .7882

Repeated Meausures Analysis of Variance
Summary ANOVA

Dependent variable: Plasma norepinephrine during isometric hangrip protocol

Source	df	SS	MS	F	PR>F
DRUG	1	162750.5	162750.2	6.95	.0336
ERROR (DRUG)	7	163826.9	23403.9		
TIME	2	3418334.5	1709167.3	24.22	.0001
ERROR (TIME)	14	987997.5	70571.2		
DRUG*TIME	2	6439.5	3219.8	0.15	.8598
ERROR (DRUG* TIME)	14	295094.4	21078.2		

HUYN-FELDT EPSILON=0.6918

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Plasma norepinephrine during cold stimulation

Sournce	df	SS	MS	F	PR<F
DRUG	1	4186.1	4186.2	0.05	.8355
ERROR (DRUG)	7	631182.9	90168.9		
TIME	1	261364.5	261364.5	18.79	.0034
ERROR (TIME	7	97359.5	13908.5		
DRUG*TIME	1	2926.1	2926.1	0.26	.6284
ERROR (DRUG* TIME)	14	79999.9	11428.6		

HUYN-FELDT EPSILON=1.012

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Change in mean arterial pressure during isometric handgrip protocol

Source	df	SS	MS	F	PR<F
DRUG	1	217.8	217.8	1.61	.2368
ERROR (TIME)	9	1219.9	135.5		
TIME	6	14806.3	2467.7	27.18	.0001
ERROR (TIME)	54	4901.9	90.8		
DRUG*TIME	6	178.4	29.7	1.22	.3153
ERROR (TIME*	54	1318.9	24.4		

HUYNH-FELDT EPSILON=0.8764

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Change in systolic pressure during isometric handgrip protocol

Source	df	SS	MS	F	PR>F
DRUG	1	616.1	616.1	3.31	.1022
ERROR (DRUG)	9	1674.9	186.11		
TIME	6	22227.5	3704.6	27.00	.0001
ERROR (TIME)	54	7409.9	137.2		
DRUG*TIME	6	333.7	55.6	2.04	.0759
ERROR (DRUG* TIME)	54	1471.9	27.3		

HUYNH-FELDT EPSILON=1.024

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent varible: Changes in diastolic pressure during isometric handgrip

Source	df	SS	MS	F	PR>F
DRUG	1	490.7	490.7	2.8	.1287
ERROR (TIME)	9	1578.3	175.4		
TIME	6	11391.1	1898.5	35.47	.0001
ERROR (TIME)	54	2890.4	53.5		
DRUG*TIME	6	285.7	47.6	2.98	.0137

HUYNH-FELDT EPSILON=0.7752

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Change in mean arterial pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	3.3	3.3	0.03	.8729
ERROR (DRUG)	9	1103.4	122.6		
TIME	3	4057.6	1352.5	39.24	.0001
ERROR (TIME)	27	930.7	34.5		
DRUG*TIME	3	40.3	13.4	0.55	.6517
ERROR(DRUG* TIME)	27	658.6	24.39		

HUYNH-FELDT EPSILON=0.8606

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Changes in systolic pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	41.3	41.3	0.22	.6536
ERROR (DRUG)	9	1727.3	191.9		
TIME	3	3634.99	1211.7	8.77	.0003
ERROR (TIME)	27	3730.5	138.2		
DRUG*TIME	3	17.1	5.69	0.10	.9606
ERROR (DRUG* TIME)	27	1573.5	58.3		

HUYNH-FELDT EPSILON=0.9118

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Changes in diastolic pressure during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	69.8	69.8	2.36	.1587
ERROR (DRUG)	9	265.8	29.5		
TIME	3	2627.0	875.7	23.17	.0001
ERROR (TIME)	27	1020.6	37.8		
DRUG*TIME	3	90.8	30.3	2.75	.0621
ERROR (DRUG* TIME)	27	297.1	11.0		

HUYNH-FELDT EPSILON=0.9789

Repeated Measures Analysis of Variance
Summary ANOVA

Dependent variable: Changes in heart rate during isometric handgrip protocol

Source	df	SS	MS	F	PR>F
DRUG	1	69.4	69.4	0.88	.3730
ERROR (DRUG)	9	711.0	79.0		
TIME	6	7035.2	1172.5	11.72	.0001
ERROR (TIME)	54	54.01.4	100.0		
DRUG*TIME	6	184.1	30.7	0.96	.4605
ERROR (DRUG* TIME)	54	1724.2	31.9		

HUYNH-FELDT EPSILON=0.6496

Repeated Measure Analysis of Variance
Summary ANOVA

Dependent variable: Changes in heart rate during cold stimulation

Source	df	SS	MS	F	PR>F
DRUG	1	73.7	73.7	1.04	.3335
ERROR (DRUG)	9	635.4	70.6		
TIME	3	1385.2	461.7	5.70	.0037
ERROR (TIME)	27	2187.3	81.0		
DRUG*TIME	3	123.0	40.7	2.43	.0865
ERROR (DRUG* TIME)	27	450.9	16.7		

HUYNH-FELDT EPSILON=0.8878

Repeated Measures of Analysis of Variance
Summary ANOVA

Dependent variable: Change in plasma norepinephrine during isomotric handgrip protocol

Source	df	SS	MS	F	PR>F
DRUG	1	12448.5	12448.5	0.82	.3956
ERROR (DRUG)	7	106405.9	15200.9		
TIME	2	3418334.5	1709167.3	24.22	.0001
ERROR (TIME)	14	987997.5	70571.2		
DRUG*TIME	2	6439.5	3219.8	0.15	.8598
ERROR (DRUG* TIME)	14	295094.5	21078.2		

HUYNH-FELDT EPSILON=0.6918

Paired T-test Results

Dependent variable: Change in plasma norepinephrine after cold stimulation

	Means±SE
Placebo	199.9±69.4
Indomethacin	161.6±39.00
Mean difference	38.25
t value	0.5060
P value	0.6284

Dependent variable: Endurance time (seconds)

	Means±SE
Placebo	147.9±7.7
Indomethacin	153.0±6.2
Mean difference	5.1
t value	1.14
P value	0.2824

Dependent variable: Maximal voluntary contraction (Newtons)

	Means±SE
Placebo	428.45+16.65
Indomethacin	438.65+15.10
Mean difference	10.20
t value	1.22
P value	.2550

Results of Fishers Protected LSD

Hemodynamic measurements at rest during static handgrip protocol

	Control	Grip		PHG-CA			Recovery	
		Min 1	Min 2	Min 1	Min 2	Min 3		
Heart rate (bpm)								
PLAC	63.9±1.8	83.1±3.9	85.0±3.9	69.3±4.5	69.4±3.2	67.5±2.8	63.8±1.4	
IND	68.9±2.1 a	72.4±3.4 a	75.8±3.6	64.5±4.2	64.2±4.7	62.4±2.7	57.9±1.9	b
Systolic blood pressure (mmHg)								
PLAC	114.3±2.3	125.1±3.4	145.7±6.4	147.0±4.6	148.1±5.7	148.7±5.4	121.6±3.0	
IND	121.8±3.4	132.5±3.3 a	144.0±4.6 a	151.0±6.1 a	150.3±5.2 a	149.7±5.6 a	124.7±2.9	b
Diastolic blood pressure (mmHg)								
PLAC	66.6±1.7	76.2±1.5	90.4±2.8	88.0±2.9	91.4±3.3	90.6±2.9	69.3±2.2	
IND	73.0±2.9	82.3±1.5 ab	91.6±2.2 a	92.2±3.8 ab	91.1±3.3 a	90.0±3.9 a	72.0±2.1	
Mean arterial pressure (mmHg)								
PLAC	84.9±1.6	94.0±2.0	110.0±5.3	111.8±2.6	113.8±3.7	110.6±2.8	89.3±1.7	
IND	91.1±2.9	101.2±2.0 a	112.8±2.9 a	114.0±3.8 a	114.7±3.2 a	111.9±3.8 a	94.0±1.6	

Values are means±SE; n=10 subjects. a indicates column mean significantly different from control. b indicates significant drug effect. ab significantly different from placebo.

Results of Fishers Protected LSD

Hemodynamic measurements during cold stimulation.

	Base	45s	90s	Recovery
Heart rate (bpm)				
PLAC	61.3±1.8	70.0±1.7	68.8±3.6	64.1±1.9
IND	56.6±1.8	66.3±3.2 a	70.1±5.5 a	61.4±2.8 b
Systolic blood pressure (mmHg)				
PLAC	119.0±2.8	123.7±2.7	136.5±2.5	124.2±2.8
IND	121.8±3.5	129.0±3.5 a	141.2±4.1 a	128.4±3.8 b
Diastolic blood pressure (mmHg)				
PLAC	69.4±2.3	76.6±1.9	87.1±3.0	71.9±2.2
IND	74.1±2.1	79.5±2.0 a	87.5±2.2 a	76.3±3.0 b
Mean arterial pressure (mmHg)				
PLAC	86.1±2.4	92.5±1.9	103.6±2.7	89.0±1.9
IND	90.0±2.4	96.8±2.3 a	106.3±3.0 a	93.5±3.3 b

Values are means±SE; n=10 subjects. a indicates column mean significantly different from control. b indicates significant drug effect.

Results of Fishers Protected LSD

Pain responses during posthandgrip circulatory arrest and cold stimulation.

30s	60s	90s	120s	150s	180s
Posthandgrip circultory arrest:					
PLAC					
2.8±1.6	3.9±1.9	5.4±1.6	6.0±1.8	6.7±1.8	7.1±2.1
IND					
3.2±1.3	4.4±1.5 a	5.7±1.4 a	6.3±1.5 a	7.2±1.5 a	7.7±1.8 a
Cold stimulation:					
PLAC					
3.4±1.6	4.8±1.6	6.0±1.5	-	-	-
IND					
3.8±1.9	5.4±1.8 a	6.5±1.9 a	-	-	-

Values are means±SE, n=10 subjects. a indicates column mean significantly different from 30s value.

Results of Fishers Protected LSD

Norepinephrine responses to static handgrip and postcontraction muscle ischemia

Baseline	Post HC	Post PC	
Norepinephrine (pg/ml):			
PLAC			
315.5±65.3	658.5±87.5	990.6±129.4	
IND			
231.3±30.9	520.8±92.0 a	863.3±143.7 a	b

Values are means±SE, n=9 subjects. a indicates column mean significantly different from control. b indicates significant drug effect.

Norepinephrine responses to cold stimulation

Control	Post immersion
Norepinephrine (pg/ml):	
PLAC	
408.3±88.6	608.1±96.2
IND	
404.5±61.7	566.1±74.6 a

Values are means±SE, n=9 subjects. a indicates column mean signifcantly different from control.

VITA

Kevin Patrick Davy was born in Flushing, New York on April 4, 1964. He comes from a large family with 4 brothers and 2 sisters. Kevin spent the first twenty years of his life in Baldwin, New York. Consequently, while at SUNY Cortland, he majored in Physical Education, graduating with a B.S. degree with an emphasis in Adult Fitness. After attending Adelphi University in Garden City, New York part time he was offered a graduate assistantship in the Human Performance Laboratory and left that school with an M.A. in Physical Education with a certificated in Exercise Physiology.

He spent one year working on Long Island at a local hospital and at a free standing cardiac rehabilitation center. Kevin came to Virginia Tech. in 1989, completing the requirements for the Ph.D. degree in June 1992. He will begin post-doctoral training at the University of Colorado in Boulder. Kevin will marry Brenda M. Mueller in November of 1992.