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Dopamine Stimulates Propagation of *Toxoplasma gondii* Tachyzoites in Human Fibroblast and Primary Neonatal Rat Astrocyte Cell Cultures

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ABSTRACT: *Toxoplasma gondii* is an obligate intracellular parasite often found in the brain of humans. Research has shown a correlation between prevalence of antibody titers to *T. gondii* and psychological illness in humans. Recent studies indicate that individuals seropositive for *T. gondii* antibodies are more likely to develop psychotic disorders including schizophrenia, which is associated with changes in the dopamine neurotransmitter system. Dopamine in the brain may play a role in proliferation, chemoattraction, infection efficiency, or stage conversion of *T. gondii*. Because tachyzoites are the first developmental stage to reach the brain, the present study was conducted to determine the effects of dopamine on their development in vitro. In human fibroblast host cells, dopamine was added at either 100 nM or 250 nM to cell culture media, and the numbers of tachyzoites produced at 48 hr were determined and compared to vehicle-treated controls. An increase of tachyzoite numbers and increased destruction in cell monolayer were observed at both concentrations of dopamine. Dopamine used at 250 nM caused a significant ($P < 0.05$) increase in tachyzoites counts compared to controls. Dopamine antagonists (10 μM) did not significantly alter dopamine-stimulated tachyzoite production in human fibroblasts. In primary neonatal rat astrocyte cell cultures, dopamine (200 μM) significantly ($P < 0.05$) increased numbers of intracellular tachyzoites after 24 hr. The role that this increase plays in tachyzoite production under the stimulus of dopamine in the modulation of neural infection in humans awaits further studies.

Toxoplasma gondii is an intracellular parasite often found in the central nervous system of humans as latent tissue cysts. Tissue cysts are essential for the maintenance of the *T. gondii* life cycle, and they have historically been considered as simply a latent stage awaiting ingestion by a warm-blooded intermediate host or a feline definitive host. Because of their location in the brain, there has been increasing interest in the association of chronic *T. gondii* infection and alterations in intermediate host behavior. Hutchinson et al. (1980) and Hay et al. (1984) determined that mice chronically infected with *T. gondii* demonstrated increased open field activity, decreased coordination, and diminished capacity to learn a maze. Goodwin et al. (2012) recently reported that minimal behavioral changes, consisting of altered short-term memory, were observed in mice congenitally infected with *T. gondii*. Others have demonstrated altered behavior in chronically *T. gondii*-infected mice and rats (reviewed by Webster, 2007). It is possible that these alterations in behavior are due to *T. gondii*-induced alteration in brain neurochemistry in infected rodents and humans (reviewed by Fekadu et al., 2010).

Schizophrenia is an important neurological disorder affecting approximately 1% of the American population (Regier et al., 1993). Several research groups from different countries have shown that there is an increase in antibodies against *T. gondii* in individuals with schizophrenia compared to individuals without the disease in the same populations (reviewed by Yolken et al., 2009). It has also been demonstrated that children born to mothers with antibodies to *T. gondii* are at an increased risk of developing schizophrenia at the onset of maturity (Yolken et al., 2009).

Humans with schizophrenia have increased dopamine levels or dopamine function, leading to the dopamine hypothesis of schizophrenia which has dominated thinking about the pathophysiology of schizophrenia since the early 1960s (Van Rossum, 1967). Stibbs (1985) found that both acute and chronic *T. gondii* infections may cause an increase in dopamine levels or dopamine turnover in the brains of experimentally infected mice. However, no changes in dopamine levels were found in congenitally infected mice examined at 8 wk of age (Goodwin et al., 2012). Henriquez et al. (2009) discovered that *T. gondii* has encoded in its genome 2 aromatic amino acid hydroxylase enzymes capable of synthesizing dopamine and serotonin precursors to within 1 final step. Gaskell et al. (2009) demonstrated that these genes are turned on when the *T. gondii* stage converts from tachyzoites to bradyzoites. Host genes are also probably involved in the complex interaction between parasite and neurochemical functioning (Carter, 2009).

In order to begin elucidating the parasite–neurochemical interactions, we examined the effect of 100 nM and 250 nM dopamine on the propagation of tachyzoites of *T. gondii* growing in human fibroblast cells. Fibroblast cells have been reported to express the dopamine transporter, DAT, but do not express dopamine receptors (Tang et al., 1994; Manakova et al., 2004). As such, Hs68 cells provide a model system for dopamine receptor-independent actions of dopamine. Results obtained with Hs68 cells were confirmed in a primary neonatal rat brain cell culture model.

Human fibroblast cells (Hs68, American Type Culture Collection, Manassas, Virginia) were maintained in RPMI 1640 medium (Lonza Walkersville, Inc., Walkersville, Maryland) supplemented with 10% (v/v) fetal bovine serum (FBS, Atlanta Biologicals, Inc., Atlanta, Georgia), 100 U/ml penicillin, 100 μg/ml streptomycin (Lonza Walkersville, Inc.), and 1 mM sodium pyruvate (Lonza Walkersville, Inc.). Bovine monocytes (BM) cells were maintained in this same culture medium. Both cell lines were cultured in a humidified incubator set at 37 C with a 5% CO_2 atmosphere. The RH strain of *T. gondii* was propagated in BM cells. For experiments, tachyzoites were harvested from infected cell cultures as previously described (Strobl et al., 2007) and used immediately to infect monolayers of Hs68 cells in 48-well dishes. Each well of Hs68 cells was inoculated with $1–2 \times 10^5$ tachyzoites (MOI = 4) and they were allowed to penetrate for 2–3 hr. Non-infecting tachyzoites were removed by a single medium exchange and 1 ml of fresh medium composed of RPMI 1640 plus 2% (v/v) FBS with freshly prepared dopamine (Sigma, St. Louis, Missouri) at 100 or 250 nM or with sterile water as the solvent control. The infected Hs68 cells were cultured for an additional 48 hr, then tachyzoites in the medium were collected by centrifugation (3,000 *g*, 15 min) and counted using a hemocytometer.

Primary neonatal rat astrocyte cultures were prepared from 1- to 2-day-old Sprague-Dawley rat pup cortices as described previously (Rzigalinski et al., 1999). Cells were plated in multi-well dishes and grown to confluence before infecting with 2×10^5 tachyzoites. Infections were performed for 2 hr, then non-infecting tachyzoites were removed, the monolayer rinsed with medium, and fresh medium added. Freshly prepared dopamine was added to culture medium containing 10% FBS

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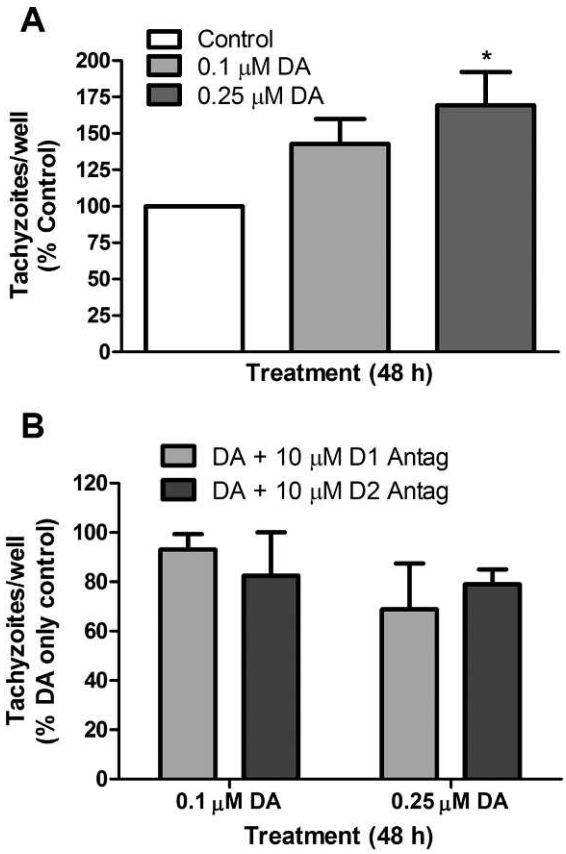

FIGURE 1. Effects of dopamine alone or in combination with dopamine antagonists on the production of *Toxoplasma gondii* tachyzoites in human fibroblast cells. (**A**) Increase in numbers of tachyzoites in human fibroblast cells 48 hr after treatment with 100 nM or 250 nM* dopamine (DA) compared to infected cells treated with vehicle only. Data shown are the mean and SEM of triplicate determinations in four independent experiments. (**B**) Dopamine antagonists (D1/D5 Antag = SCH-23350 and D2 Antag = S- Sulpiride) had no significant effect ($P > 0.05$) on the numbers of tachyzoites in human fibroblast cells 48 hr after treatment with 100 nM or 250 nM dopamine. Data shown are mean and SEM of sextuplicates of DA only and triplicate determinations of each dopamine antagonist in 3 independent experiments. *Indicates significant difference ($P < 0.05$) from control.

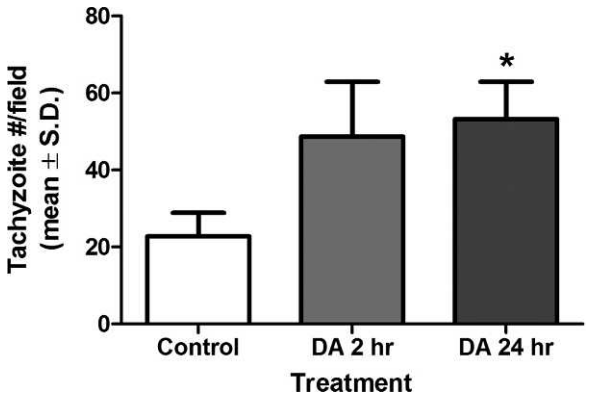

FIGURE 2. Mean number (± standard deviation [SD]) of *Toxoplasma gondii* tachyzoites in triplicate wells containing primary neonatal rat brain cells; for each well, the average number of tachyzoites in 5 microscope fields (×40) was determined 24 hr after treatment with 200 μM dopamine (DA) or control medium. Cells were infected; then cells were either not treated with DA, were treated with DA for 2 hr then placed in maintenance medium (DA 2 hr), or were continuously exposed to DA for 24 hr (DA 24 hr). *Indicates significant difference ($P < 0.05$) from control. Data are representative of n = 4 experiments performed in triplicate.

at a single concentration (200 μM) for either the 2-hr time of infection or for a total of 24 hr. Triplicate determinations were performed in each experiment. The cultures were stained and fixed with crystal violet (Strobl et al., 2007) 24 hr post-infection and images were captured for analysis. Tachyzoites inside the cells were counted in 5 fields for each replicate using the ×40 objective; data were expressed as the mean number of tachyzoites/microscope field.

The dopamine concentrations we tested in fibroblast cells reflect the range (5–1,000 nM) used by others to study dopamine responses in astrocyte cell cultures (Vaarmann et al., 2010; Requardt et al., 2012). Microdialysis analyses conducted in rodent brain (frontal cortex) and spinal cord indicate even higher dopamine concentrations of 1 μM and 133 μM, respectively, occur in vivo (Murata et al., 2009; Gerin et al., 2011). A single dopamine concentration of 200 μM was tested in the primary brain cultures to more closely simulate the level of dopamine identified in the spinal cord. The selective D1/D5 dopamine receptor antagonist, R(+)-SCH23390 HCl was prepared fresh and diluted into the culture medium to a final concentration of 10 μM. The selective D2 dopamine receptor antagonist, S(-)-Sulpiride (Sigma), was prepared fresh as a stock solution in acetic acid and diluted in the culture medium to a final concentration of 10 μM in 0.2% acetic acid. Water and acetic acid vehicle controls were performed.

Experiments were replicated 4 times with triplicate determinations. Statistical analyses were performed using PrismV5.02 (GraphPad, Inc., LaJolla, California). The effect of dopamine in 4 independent experiments was compared using a 1-way analysis of variance and Dunnett's *t*-test.

Dopamine stimulated tachyzoite production in Hs68 cells at both concentrations used (Fig. 1A). Tachyzoite numbers released from infected Hs68 cells treated with 100 nM dopamine were greater than with controls, but the numbers were not significantly different ($P < 0.05$). Treatment of *T. gondii*-infected Hs68 cells with 250 nM dopamine produced a statistically significant ($P < 0.05$) increase in tachyzoite numbers compared to controls. Neither of the 2 dopamine receptor antagonists showed any significant inhibition of the ability of dopamine to augment tachyzoite propagation in Hs68 cells (Fig. 1B), substantiating the idea that dopamine can increase *T. gondii* propagation independently of the involvement of dopamine receptors.

In primary neonatal rat astrocyte cultures, exposure to dopamine during only the 2-hr period of infection resulted in some increase in numbers of tachyzoites in the cells after 24 hr; however, this increase was not statistically significant. When dopamine was continuously present during infection, and during the subsequent 22 hr, there was a significant increase ($P < 0.05$) in the number of intracellular tachyzoites (Fig. 2).

Astrocytes provide a robust model for studying dopamine's influence on cerebral *T. gondii* infections (Rzigalinski et al., 1999). The fibroblast cells constitute a cell culture model to study the influence of dopamine independently of dopamine receptors. Monoamine oxidase (MAO) enzymes in human fibroblast cells (Groshong et al., 1977) and dopamine metabolism by fibroblast cells have been described previously (Crooks et al., 1978), though the absence of dopamine receptors in fibroblast cells has been documented (Tang et al., 1994). We report here that dopamine increased tachyzoite propagation in both models and suggest that dopamine plays a supportive role in *T. gondii* infections. The idea that dopamine fosters *T. gondii* infections provides an explanation for the expression of dopamine biosynthetic enzymes in *T. gondii* (Gaskell et al., 2009) as well as for the report that encysted *T. gondii* in PC12 neuronal cell cultures synthesize dopamine (Prandovszky et al., 2011).

Dopamine is a redox active neurotransmitter that exists in oxidized semi-quinone and quinone forms as well as in the reduced catechol. Redox cycling has been implicated in dopamine signaling and cytotoxicity

(Smythies, 2000). Redox actions of dopamine are involved in intracellular calcium signaling in astrocytes exposed to dopamine (Vaarmann et al., 2010; Requardt et al., 2012). Vaarmann et al. (2010) showed that dopamine-stimulated intracellular calcium levels occurred independently of receptor activation using the D1/D5 and D2 dopamine receptor antagonists, SCH23390 and Sulpiride, respectively. Rather, membrane active anti-oxidants as well as the MAO inhibitor, selegiline, blocked the intracellular rise in calcium. The dopamine redox signaling cascade that occurs in astrocytes involves metabolism of dopamine by mitochondrial MAO and subsequent production of reactive oxygen species. The ensuing lipid peroxidation triggers inositol trisphosphate-mediated release of calcium from the endoplasmic reticulum and a rise in intracellular calcium levels. Based on the critical role intracellular calcium plays in the egress of tachyzoites from infected cells (Nagamune et al., 2008; Chandramohanadas et al., 2009; Lourido et al., 2010), we suggest that this receptor-independent redox signaling pathway may explain the ability of dopamine to increase *T. gondii* tachyzoite propagation in the primary astrocyte cultures as well as in fibroblasts.

Altered dopamine levels are a characteristic of schizophrenia; altered dopamine levels were once thought to be the primary cause of schizophrenia. This has been disproven, in part, by demonstration of alternate neurotransmitters and neurochemical pathways associated with schizophrenia. Altered dopamine levels and dopamine receptor dysfunctions are still believed to be a major contributor to schizophrenia. Hermes et al. (2008) determined that tissue cysts are more frequently found in the diencephalon and cortex than in other regions of the brain. This discovery supports the idea that tissue cysts are in close proximity of regions of the brain with higher than average dopamine levels. The striatum, the region of the brain with the highest dopamine concentration, is located in very close proximity to the diencephalon. The frontal cortex has the second highest concentration of dopamine in the cortex.

Increased dopamine levels were suggested to be a potential explanation of why more males than females seropositive for *T. gondii* had an altered personality profile according to Cattell's questionnaire (Flegr and Hrdy, 1994). The personality changes observed included a 16-fold increase in rule-breaking and in jealousy–suspicious behaviors for seropositive males. It was suggested these behavior changes in men are associated with chronic *T. gondii* infection (Flegr and Hrdy, 1994). However, actual dopamine concentrations were not evaluated in their subjects. The behavior profiles observed are similar to known behavior changes associated with altered dopamine concentrations. Novotná et al. (2005) examined novelty-seeking in humans and attempted to correlate dopamine levels with this activity in *T. gondii* seropositive individuals because decreased novelty-seeking is correlated with increased dopamine levels. They found that *T. gondii* seropositive individuals had a decrease in novelty-seeking, which suggested that increased dopamine levels were present. Flegr et al. (2003) expanded upon this observation by evaluating the size of an individual's residence as a possible factor for novelty-seeking in recent male military conscripts. When the size of residence was factored in and corrected, males seropositive for *T. gondii* demonstrated decreased novelty-seeking compared to seronegative uninfected males. In another study, age-matched male and female blood donors were examined using the same test. *Toxoplasma gondii* seropositive males and females both exhibited decreased novelty-seeking compared to age-matched seronegative males and females (Skallová et al., 2005).

Personality changes discovered in these human behavioral tests are not as drastic as those found in people living with schizophrenia. The personality changes associated with *T. gondii* infection suggests chronic infection is capable of causing behavior alterations. Because dopamine levels were not measured in these human behavioral studies, the amount of dopamine change, if any, is unknown and cannot be compared to fluctuations in dopamine found in people with schizophrenia. However, evidence supports changes in dopamine levels as a cause of behavioral changes.

Certain behavior profiles are associated with increased dopamine levels and chronic *T. gondii* infections. It is argued the behavior alterations associated with chronic *T. gondii* infections help increase transmission of *T. gondii* to its definitive host (Webster, 2007). Further, *T. gondii* infection has been shown to alter astrocyte function; and alterations in astrocyte function, particularly with respect to IP3-mediated signaling, have been associated with schizophrenia (Steiner et al., 2012; Tanahashi et al., 2012). The subtle, and not so subtle, schizophrenic behavior profiles in humans could be a side effect of *T. gondii* cysts in the neural tissue of warm-blooded intermediate hosts. Not all individuals infected with *T. gondii* have mental illnesses, but infection with *T. gondii* may be a factor in initiating the expression of mental illness in susceptible individuals.

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