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EFFECT OF PARASITIC INFECTION ON DOPAMINE BIOSYNTHESIS IN DOPAMINERGIC CELLS

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Abstract—Infection by the neurotropic agent *Toxoplasma gondii* alters rodent behavior and can result in neuropsychiatric symptoms in humans. Little is understood regarding the effects of infection on host neural processes but alterations to dopaminergic neurotransmission are implicated. We have previously reported elevated levels of dopamine (DA) in infected dopaminergic cells however the involvement of the host enzymes and fate of the produced DA were not defined. In order to clarify the effects of infection on host DA biosynthetic enzymes and DA packaging we examined enzyme levels and activity and DA accumulation and release in *T. gondii*-infected neurosecretory cells. Although the levels of the host tyrosine hydroxylase (TH) and DOPA decarboxylase and AADC (DDC) did not change significantly in infected cultures, DDC was found within the parasitophorous vacuole (PV), the vacuolar compartment where the parasites reside, as well as in the host cytosol in infected dopaminergic cells. Strikingly, DDC was found within the intracellular parasite cysts in infected brain tissue. This finding could provide some explanation for observations of DA within tissue cysts in infected brain as a parasite-encoded enzyme with TH activity was also localized within tissue cysts. In contrast, cellular DA packaging appeared unchanged in single-cell microamperometry experiments and only a fraction of the increased DA was accessible to high potassium-induced release. This study provides some understanding of how this parasite produces elevated DA within dopaminergic cells without the toxic ramifications of free cytosolic DA. The mechanism for synthesis and packaging of DA by *T. gondii*-infected dopaminergic cells may have important implications for the effects of chronic *T. gondii* infection on humans and animals.

Key words: apicomplexa, neurotransmitter, tyrosine hydroxylase, DOPA decarboxylase, manipulation.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a common parasite that has been found to infect all warm-blooded animals tested, including humans (Hill et al., 2005; Darde et al., 2007). During infection, the parasite forms tissue cysts predominantly in the brain and muscles generating a chronic infection (Hutchinson, 1966). Chronic infection with the encysted form of *T. gondii* has been associated with behavioral changes in rodents, with infected rodents showing higher activity levels and decreases in predator vigilance (Webster et al., 1994; Webster, 1994a,b; Flegr et al., 1996, 2000, 2002; Berdoy et al., 2000; Yereli et al., 2006; Vyas et al., 2007; Kocazeybek et al., 2009). These behavioral traits could facilitate parasite transmission from the rodent to its definitive feline host (Hutchinson, 1966) as rodents lose their strong innate aversion to cat odor upon infection and exhibit, to some degree, an attraction to cat urine-treated areas (Berdoy et al., 2000; Vyas et al., 2007).

It has been postulated that alterations to dopaminergic signaling mediate the behavioral changes seen in rodents following *T. gondii* infection, as high concentrations of dopamine (DA) were found to be localized in tissue cysts within infected mouse brains (Prandovszky et al., 2011). Further support is gained from the administration of DA receptor antagonists, such as DA D2 receptor antagonists haloperidol and GBR 12909, which block establishment of the behavioral changes in rats, furthermore haloperidol also has some antiparasitic activity (Jones-Brando et al., 2003; Skallova et al., 2006; Webster et al., 2006). Further supporting fact of behavior changes involvement in the facilitation of parasite transmission the activation of sexual

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Abbreviations: BSA, bovine serum albumin; BAG1, bradyzoite antigen 1; DA, dopamine; DDC, DOPA decarboxylase and AADC; EDTA, ethylenediaminetetraacetic acid; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; FRET, fluorescence resonance energy transfer; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-performance liquid chromatography; L-DOPA, L-3,4-dihydroxyphenylalanine; PBS, phosphate-buffered saline; PV, parasitophorous vacuole; *T. gondii*, *Toxoplasma gondii*; TH, tyrosine hydroxylase; VMAT1, vesicular monoamine transporter 1.

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arousal pathways in infected male rats when exposed to cat urine has been observed (House et al., 2011); interestingly the three regions identified with increased activity (medial amygdala, ventromedial hypothalamus, and basolateral amygdala) are DA responsive. In addition, *T. gondii* infection has been shown to increase the amount of DA in the striatum, another region of dense DA neurotransmission, of mice with a 38% greater level in infected mice relative to uninfected mice (Xiao et al., 2014).

The molecular mechanisms by which *T. gondii* infection could induce alterations to dopaminergic signaling are unclear, but based on our previous findings, infection of dopaminergic cells *in vitro* increased their DA content by threefold (Prandovszky et al., 2011), suggesting altered DA synthesis. DA synthesis involves two enzymatic steps from L-tyrosine to DA with coincident packaging. Firstly, L-3,4-dihydroxyphenylalanine (L-DOPA) is synthesized from L-tyrosine by tyrosine hydroxylase (TH) which is subsequently metabolized to DA by DOPA decarboxylase and AADC (DDC; also named aromatic-L-amino-acid decarboxylase). As DA is synthesized, it is rapidly imported into vesicles via vesicular monoamine transporters (Cartier et al., 2010) to prevent cellular damage via free-radical generation (Hald and Lotharius, 2005; Chuenkova and Pereiraperrin, 2006). *T. gondii* contains two genes that encode enzymes with TH activity (Gaskell et al., 2009) but has not been reported to possess a version of DDC enzyme and no sequences homologous to this enzyme are detectable in the *T. gondii* genome based on bioinformatic searches in Toxodb (version 24) (www.toxodb.org), NCBI (www.ncbi.nlm.nih.gov), and using a sensitive algorithm based on enzyme profiles for parasitic protozoa (http://www.bioinformatics.leeds.ac.uk/metatiger/). This raises several questions on the mechanism by which *T. gondii* is invoking its changes on dopaminergic cells. This study addresses the contribution of host proteins in DA biosynthesis and packaging and how the process is orchestrated for containment of DA to avoid cellular damage. We hypothesized that infection alters levels and properties of the host DA biosynthetic machinery. To assess the stimulatory activity of infection on amount of DA, changes in the amounts of the enzymes at the RNA and protein level were measured, enzyme activity, phosphorylation, and location were analyzed, and vesicular DA accumulation and release were quantitated.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were of analytical grade or higher and from Fisher Scientific (Loughborough, UK) unless otherwise stated.

Cell and parasite culture

Rat pheochromocytoma (PC12 cells) cells from the European Collection of Cell Cultures (Salisbury, UK) were maintained as previously described (Prandovszky et al., 2011). For all experiments PC12 cells were plated in poly-D-lysine-coated flasks and plates. The *T. gondii* strain Prugniaud was used for all experiments except the fluorescence-activated cell sorting (FACS)/amperometry experiment that used Prugniaud KU80- kindly provided by Louis Weiss (Fox et al., 2011). Parasites were maintained in human foreskin fibroblasts as previously described (Gaskell et al., 2009).

Bradyzoite induction and PC12 infection

Bradyzoite induction was as previously described (Prandovszky et al., 2011). Briefly, liberated tachyzoites were incubated at 37 °C in RPMI supplemented with 1% fetal bovine serum (FBS) at pH 8 for 16–18 h in ambient CO_2, then diluted with DMEM, isolated by centrifugation, and suspended in RPMI (pH 7.4) containing horse serum, FBS and penicillin/streptomycin. PC12 cells were plated at a density of 2.5×10^5 cells/well in 6-well plates (unless otherwise stated), the ‘‘induced tachyzoites” were added to PC12 cell cultures, and cultures were changed 3 days after infection with assays performed on the fifth day. Bradyzoite differentiation was monitored by detection of Bradyzoite antigen 1 (BAG1) expression as described below.

Measurement of DA levels

DA was measured by high-performance liquid chromatography (HPLC) with electrochemical detection using the method described by Prandovszky et al. (2011) with the cells detached from the wells by trypsin treatment, pelleted and an aliquot taken for measurement of cell number using the CyQuant kit (Invitrogen Gaithersburg, MD, USA) as per the manufacturer’s instructions (Prandovszky et al., 2011). All samples were run with three technical replicates and standards. Technical replicates were reproducible within 5% of each other and were within the linear range of detection.

RNA extraction, RT-PCR and qPCR

Infected PC12 cells were detached by trypsin treatment and rinsed in phosphate-buffered saline (PBS). Cell pellets were then resuspended in Tri-reagent and total RNA extracted as per the manufacturer’s instructions (SigmaAldrich, St. Louis, MO, USA). RNA was quantified by Nanodrop (ThermoScientific, Waltham, MA, USA) and 500 ng of RNA was subjected to DNase treatment (Invitrogen, Gaithersburg, MD, USA) as per the manufacturer’s instructions. First strand cDNA was synthesized using random primers (Promega, Southampton, UK) and Superscript II (Invitrogen) as described by the manufacturer. For qualitative PCR 100-ng cDNA was amplified using GoTaq (Promega) as per the manufacturer’s instructions. For quantitative PCR amplification of 100-ng cDNA was undertaken using the Bio-Rad CFX96 (Hemel Hempstead, UK) and SYBR® Green PCR Master Mix (Applied Biosystems, Paisley, UK), as described by the manufacturers. Primer pairs were as follows: BAG1 Sense 5′ ATTCTTCTCAGGGCGGTGCT 3′, Anti-sense 5′ CCTTCTTTGTTTCATCGTCGTCC 3′; Rat GAPDH Sense 5′ GTGGACCTCATGGCCTACAT 3′, Anti-sense 5′ TGTGAGGGAGATGCTCAGTG 3′; Rat DDC Sense