

Changes in brain concentrations of catecholamines and indoleamines in *Toxoplasma gondii* infected mice

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Brain concentrations of dopamine, homovanillic acid, norepinephrine, serotonin and 5-hydroxy-indoleacetic acid were measured in mice with acute and chronic, adult-acquired toxoplasmosis. Mice with acute infections showed a 40% rise in homovanillic acid levels as compared with controls; dopamine levels, however, remained unchanged. Norepinephrine levels in this group were 28% lower than in controls. Dopamine levels were 14% higher in the mice with chronic infections than controls. Serotonin and 5-HIAA levels were not altered in infected mice. These neurochemical changes may be factors contributing to mental and motor abnormalities that accompany or follow toxoplasmosis in rodents and possibly in man.

Toxoplasmosis, a disease caused by the coccidian protozoan, *Toxoplasma gondii*, is commonly acquired either congenitally or through ingestion of infectious parasites (e.g. in infected, undercooked meats, or in food or drink contaminated by infected cat faeces). Congenital toxoplasmosis can result in meningoencephalitis, which may have serious sequelae such as blindness, hydrocephaly and mental retardation. Learning and motor disabilities and personality changes have been linked to the congenital disease (Witting, 1979; Hutchison and Hay, 1981). Postnatal, ingestion-acquired toxoplasmosis in adults is generally mild or asymptomatic; cysts form in the brain, musculature and elsewhere, but they do not elicit an inflammatory response and meningoencephalitis is unusual. Behavioural changes or mental impairment have not yet been proved to occur in the latter type of infection in man. However, a clear cause and effect relationship between behavioural or mental changes and infection is difficult to prove, since the number of complicating factors is so large and the disease itself is often not recognized. On the other hand, experiments with chronic *Toxoplasma* infections in mice and rats have shown that learning, memory, motor performance and response to novel stimuli are all affected significantly by post-natally acquired infections (Witting, 1979; Hutchison *et al.*, 1980; Hutchison and Hay, 1981; Hay *et al.*, 1983). Congenital infections also have been found to affect motor performance in the mouse (Hutchison and Hay, 1981; Hay *et al.*, 1983).

The possibility that toxoplasmosis may alter the metabolism or balance of neurochemicals in the host brain has never been examined. An imbalance in the metabolism of catecholamine or indoleamine neurotransmitters might explain the appearance of mental and motor changes during infection. These substances mediate locomotor activity, mood, aggression, sleep, learning, memory and cerebral blood flow (Hornykiewicz, 1973; DeFeudis, 1979). Also, defects in the metabolism of these compounds have been associated with various neurological disorders, including Parkinson's disease (Hornykiewicz, 1973) and schizophrenia (Bird *et al.*, 1979). The purpose of the present study was to test the hypothesis that post-natally acquired toxoplasmosis causes changes in indoleamine and catecholamine metabolism in the brain of mice. Levels of the catecholamines, dopamine and noradrenaline, of the indoleamine, serotonin, and of two of their major metabolites, were measured in the brains of mice following acute and chronic infections with *T. gondii*.

MATERIALS AND METHODS

Parasite

The C56 strain of *T. gondii* was used in these experiments. It was obtained from Dr. Jack Remington of the Palo Alto Medical Foundation, Palo Alto, California, and was maintained by serial passage in mice.

Animals

Thirty, one-month-old female Swiss-Webster mice, aged six weeks, were obtained from Tyler Laboratories, Bellevue, Washington. They were maintained at five mice per cage and fed dry rodent chow and water *ad libitum*. An electronically timed lighting schedule was set at 16 hours light : eight hours dark (light from 0600–2200 hours). At seven weeks of age, 20 mice were inoculated intraperitoneally with tachyzoites (1×10^4 per mouse in 0.25 ml of 0.85% saline), obtained by draining the peritoneal cavity of an acutely infected mouse. Ten of these infected animals constituted the 'chronic infection' group and were treated with sodium sulfadiazine in the drinking water (0.5 mg ml^{-1}), beginning at four days post-infection (p.i.) and ending at 19 days p.i. The second group of ten infected mice constituted the 'acute infection' group and were not treated with the sulphonamide. The ten remaining uninfected mice were kept as the control group. Mice with acute, untreated infections showed swollen abdomens and sluggishness by seven days p.i. By 12 days p.i., two of these mice had died from toxoplasmosis and the remaining mice in this group were now killed. Mice in the chronic infection group were killed at five weeks p.i., and did not exhibit any symptoms. The mice in the control group were all killed three days after the 'chronic' group.

Tissue Preparation

Mice were killed by decapitation, using a guillotine designed for use with small rodents (EDCO, Inc., Chapel Hill, North Carolina). Brains were removed within 90 seconds of death and were placed directly onto a $\frac{1}{4}$ inch (6 mm) thick aluminium slab resting on ice. The olfactory lobes and the pineal were not included with the rest of the brain. Approximately 4 mm of spinal cord was left attached to the medulla. Brains were carefully dissected into right and left halves by making a longitudinal incision with a scalpel, and the two halves were rapidly frozen on dry ice and stored at -70°C for up to two weeks. Animals were always killed between 1400 and 1700 hours.

Neurochemical Analysis

The left half of each brain was analyzed for serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and dopamine (DA). The right half of each brain was analyzed for norepinephrine (NE). Measurements were made using high performance liquid chromatography (HPLC). Equipment included a Beckman Model 100A solvent-metering pump (Beckman Instruments, Inc., Fullerton, California), an Altex 210 injector with 20 μl sample loop (Beckman), an ODS-18 guard column (Bioanalytical Systems, Inc., W. Lafayette, Indiana), a 25 cm \times 5 mm Biophase ODS-18 reversed phase column with 5 μm particle size (Bioanalytical Systems, Inc.), a Model LC4A/17 electrochemical detector with glassy carbon electrode (Bioanalytical Systems, Inc.) and a Model 3390A recorder-integrator (Hewlett-Packard, Inc., Palo Alto, California).

For analysis of 5-HT, 5-HIAA, HVA and DA, the tissue was quickly thawed at 37°C , weighed, and homogenized in ten volumes of 0.1 M perchloric acid containing 0.23 mM ascorbate, using a Ten Broeck hand homogenizer resting in ice. The homogenate was centrifuged at 12 000 g for 15 minutes at 4°C , then the supernatant was filtered through an MF-1 centrifugal microfilter (Bioanalytical Systems, Inc.) and 20 μl injected in the HPLC system.

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An isocratic mobile phase of 0.1 M sodium acetate containing 4% methanol and 0.1 mM EDTA was used. Oxidative potential on the EC detector was +0.60 V; flow rate through the column was 2.0 ml minute⁻¹. Quantitation was done by the external standard method, i.e. using standard curves of peak areas obtained using known amounts of pure standards. Standards were always analyzed two to three times immediately prior to analysis of experimental samples. Samples were always analyzed two to three times in succession and the values averaged. Data were not corrected for percentage recovery because the latter was found to be virtually 100% for each compound so analyzed. Linearity of response for each compound was confirmed in the appropriate concentration range.

NE was analyzed after alumina extraction from the perchloric acid homogenate. Briefly, 30 ng of the internal standard, 3,4-dihydroxybenzylamine (DBA), were added to 1.0 ml of the supernatant, which was then mixed with 1.0 ml of 0.5 M Tris-HCl buffer, pH 8.6, after which 10 mg of dry, acid-washed alumina (BAS) were added and the tube shaken by hand for ten minutes. The alumina was rinsed three times with 1.0 ml 5 mM Tris-HCl, pH 8.6, and the NE was extracted from the alumina using 0.2 ml of 0.1 M perchloric acid. The eluate was filtered with a microfilter and then analyzed by HPLC. The mobile phase for NE analysis was a 0.1 M KH₂PO₄ buffer, pH 3.0, containing 4% methanol, 0.2 mM sodium octyl sulfonate (BAS) and 0.1 mM EDTA. The flow rate was 1.5 ml minute⁻¹ and oxidative potential on the detector was +0.72 V. Percentage recovery for NE ranged from 63-75% and the data were adjusted accordingly.

Statistical Analysis

Data were analyzed by a one-tailed Student's *t*-test. Differences were considered significant when $P < 0.05$.

Reagents

Chemicals were purchased from the following sources: purified neurochemical standards, sulfadiazine (2-sulphanilamidopyrimidine) and sodium acetate trihydrate (grade I), Sigma Chemical Co., St. Louis, Missouri; monobasic potassium phosphate, Mallinkrodt, Inc., St. Louis, Missouri; HPLC grade water and 'Baker-analyzed' perchloric acid, J. T. Baker Chemical Co., Phillipsburg, New Jersey; HPLC grade methanol, Burdick and Jackson Laboratories, Muskegon, Michigan; and sodium octyl sulfonate, Bioanalytical Systems, Inc., W. Lafayette, Indiana.

RESULTS AND DISCUSSION

Concentrations of serotonin and 5-HIAA did not change in the brains of animals during infection (see Table). However, three significant changes did occur in infected animals. One of these was a 40% increase in HVA levels in the acutely infected group of mice. Since DA levels did not change, this may be interpreted as indicating an increased turnover rate of DA on the pathway to HVA, and also a compensatory increase in the rate of DA synthesis, i.e., an overall increase in DA turnover. However, a retardation in transport of HVA out of the brain might have yielded the same result and must be considered. Norepinephrine levels were 28% lower in the acute group relative to controls, and DA was 14% higher in the chronic group than controls. The significant decrease in NE in acutely infected animals suggests that activity of the enzyme, dopamine-beta-hydroxylase, may be reduced in noradrenergic neurons in infected animals. This enzyme is located in noradrenergic neurons but not in dopaminergic neurons, therefore the rate of conversion of DA to HVA in the latter may not directly affect NE formation in noradrenergic neurons. The primary rate-limiting step in NE as well as DA formation is at the level of the enzyme tyrosine hydroxylase. This

TABLE
Neurochemical changes in brains of mice with chronic and acute infections with Toxoplasma gondii

	Neurotransmitter				
	DA	HVA	5-HT	5-HIAA	NE
Control (N=10)	1102 ± 33*	181 ± 14	1176 ± 92	430 ± 21	894 ± 90‡
Chronic (N=10)	1257 ± 53	185 ± 83	1157 ± 68	429 ± 17	802 ± 70§
P value†	<0.01	n.s.	n.s.	n.s.	n.s.
% Change	+14%	—	—	—	—
Acute (N=8)	1050 ± 77	254 ± 25	1036 ± 48	448 ± 17	646 ± 51¶
P value†	<0.25(n.s.)	<0.01	<0.1(n.s.)	n.s.	<0.025
% Change	—	+40%	—	—	-28%

*Expressed as nanograms per gram wet weight, mean ± s.e.m.

†Derived from a one-tailed Student's *t*-test; n.s. = not significant.

‡N=4.

§N=9

||Percentage change from controls

¶N=5.

enzyme catalyzes the first step in conversion of tyrosine to the catecholamines. It is possible that the activity of this enzyme is reduced in noradrenergic neurons but unusually active in the dopaminergic neurons of acutely infected animals. Dopaminergic neuron terminals are primarily located in the basal ganglia, while those of noradrenergic neurons are primarily in the brainstem (midbrain, pons and medulla).

The demonstration of these changes suggests that in chronic as well as in acute toxoplasmosis, the balance between synthesis and breakdown of catecholamines is upset. These neurochemical changes may be factors contributing to the psychological and motor changes which have been shown to occur in experimentally infected rodents.

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