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Effects of African Trypanosomiasis on Brain Levels of Dopamine, Serotonin, 5-Hydroxyindoleacetic Acid, and Homovanillic Acid in the Rabbit

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Abstract: Serotonin (5-HT) levels fell by 21% in the midbrain-thalamus-hypothalamus (MTH) region of the rabbit brain after chronic infection with the protozoan *Trypanosoma brucei gambiense*. 5-HT did not decrease significantly in the caudate/putamen (CP) or the pons/medulla (PM) region. 5-Hydroxyindoleacetic acid (5-HIAA) levels were unchanged in the MTH and caudate/putamen (CP) but increased by 17% in the pons/medulla (PM) after infection. Dopamine (DA) levels rose by 19% and homovanillic acid (HVA) by 33% in the PM during infection. DA and HVA tended to be lower in the CP of infected rabbits, but the apparent decreases were not statistically significant. DA and HVA levels in the MTH were also unchanged by infection. These neurochemical changes may be involved in the behavioral symptoms that frequently accompany this disease in man and cattle. **Key Words:** Trypanosome—*Trypanosoma*—Trypanosomiasis—Sleeping sickness—Dopamine—Serotonin. **Stibbs H. H.** Effects of African trypanosomiasis on brain levels of dopamine, serotonin, 5-hydroxyindoleacetic acid, and homovanillic acid in the rabbit. *J. Neurochem.* **43**, 1253–1256 (1984).

African trypanosomiasis, or sleeping sickness, is a protozoal disease of man and domestic mammals in Africa. *Trypanosoma brucei gambiense* and *T. b. rhodesiense* cause human infections, whereas in cattle *T. b. brucei, T. vivax,* and *T. congolense* are the most important etiologic agents. Emaciation, exhaustion, anemia, meningoencephalitis, and neurologic behavioral symptoms commonly occur during infection in both man and domestic mammals. The behavioral symptoms coincide with the onset of meningoencephalitis, and in man include stupor, ataxia, incoordination, tremors, depression (sometimes interrupted by periods of mania and violence), and irregular, prolonged sleep episodes (Kellersberger, 1933; Duggan and Hutchinson, 1966; Lambo, 1966). Incidence of neurologic behavioral symptoms among patients is about 80–90% (Lambo, 1966). Duggan and Hutchinson (1966) observed that excessive, irregular, and uncontrollable sleep was a symptom in 25 of 66 patients at the time of diagnosis and in virtually all patients in the terminal stage of the disease. The meningoencephalitis is usually most severe in the region of the basal ganglia, midbrain, and brainstem; however, it commonly extends to other brain areas. Histologically, it is characterized by congestion and dilation of cerebral blood vessels, perivascular infiltration by plasmocytes and histiocytes, perivascular proliferation of glial cells (gliosis), and demyelination of neurons (Calwell, 1937; Chalgren and Baker, 1946; Manuelidis et al., 1956; Morrison et al., 1983).

A possible neuropharmacologic basis for the behavioral syndrome accompanying African trypanosomiasis has not been investigated. However, alterations in metabolism of the aromatic acids in infected laboratory animals have been reported. Newport et al. (1977) found that in infected voles free serum tyrosine decreased by 50% and free serum tryptophan was below detection. Newport and Page (1977) also reported that free tyrosine in infected vole brain decreased by 45%. Stibbs and Seed (1975*a*, 1976) found that urinary *p*-hydroxy-

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Abbreviations used: CP, Caudate/putamen; DA, Dopamine; 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HT, T-Hydroxytryptamine, or serotonin; HVA. Homovanillic acid; MTH, Midbrain/thalamus/hypothalamus; PM, Pons/medulla.

phenylpyruvate and the activity of serum and hepatic tyrosine aminotransferase both increased significantly in infected voles, and also that the incorporation of labeled tryptophan into the free amino acid pool of the infected vole brain was reduced. Stibbs and Seed (1975*b*) also reported that in trypanosome-infected rats, intravenously inoculated [^{14}C]tryptophan is rapidly metabolized to indole-3-acetic acid and indole-3-ethanol (tryptophol). The latter is a compound that possesses pharmacologic properties (Seed et al., 1978; Fukumori et al., 1980). This information suggests that central neurochemicals dependent on the aromatic amino acids as precursors might undergo changes in levels or turnover during this disease.

The purpose of the present study was to measure the concentrations of dopamine, serotonin, and some of their important metabolites in the brain of rabbits after chronic infection with *T. b. gambiense*, the etiologic agent of Gambian, or West African, trypanosomiasis in man.

MATERIALS AND METHODS

Parasites

The Wellcome TS strain of *T. b. gambiense* was obtained from the American Type Culture Collection, Rockville, MD (ATCC catalog #30025).

Animals

Female white New Zealand rabbits weighing 8–9 lb were housed individually and fed a diet of rabbit chow and water *ad libitum*. Rabbits were maintained on a lighting schedule of 7 h light, 17 h dark (light from 0800–1500 h). Eight control and 9 infected rabbits were used in these experiments. Rabbits were infected by subcutaneous inoculation of 2.5×10^6 parasites in 0.5 ml of 0.85% saline with 1% glucose. Infection in all inoculated animals was confirmed through microscopic examination of peripheral blood.

Infected rabbits exhibited no obvious signs of disease for several weeks after infection. At 3–4 weeks postinfection inflamed, cracked, and often purulent areas of skin appeared on the nose, ears, genitals, around the eyes, and sometimes on the paws, and the animals began to look exhausted and to lose weight. By 6–8 weeks the animals generally looked emaciated and exhausted but were not comatose. (Infected rabbits usually die at 8–10 weeks postinfection.)

Infected rabbits were sacrificed at 6–8 weeks postinfection using a guillotine designed for use with rabbits (EDCO, Inc., Chapel Hill, NC). Animals were always sacrificed at 1100–1600 h.

Neurochemical analysis

After decapitation, brains were immediately removed from the skull, rinsed for 10 s in ice-cold 1% sodium citrate, and placed directly on dry ice. Frozen brains were stored at −70°C for up to 3 weeks.

At time of analysis, the following three brain regions were removed and weighed: (1) midbrain/thalamus/hypothalamus (MTH), (2) caudate/putamen (CP), and (3) pons/medulla (PM). Each tissue was homogenized in 5 vol of ice-cold 0.1 *M* perchloric acid containing 0.23 m*M* ascorbate using a Ten Broeck hand tissue homogenizer resting in ice. The homogenate was centrifuged at 12,000 *g* at 4°C for 10 min and the supernatant filtered using an MF-1 centrifugal microfilter (Bioanalytical Systems, Inc., W. Lafayette, IN) containing a regenerated cellulose filter.

Dopamine (DA), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the supernatant using HPLC. The HPLC equipment included a Beckman Model 100A solvent-metering pump (Beckman Instruments, Inc., Fullerton, CA), an Altex 210 injector with a 20-μl sample loop (Beckman), on ODS-18 guard column (Bioanalytical Systems, Inc., W. Lafayette, IN), a 25-cm × 5-mm Biophase ODS-18 reversed-phase column with 5-μm particle size (B.A.S.), a Model LC4A/17 electrochemical detector with glassy carbon electrode (B.A.S.), and a Model 3390A recorder-integrator (Hewlett-Packard, Inc., Palo Alto, CA).

Isocratic flow was used with a solvent system of 0.1 *M* sodium acetate containing 4% methanol and 0.1 m*M* EDTA. Flow rate was 2.0 ml/min, and oxidative potential of the electrochemical detector was set at +0.60 V. Quantitation was done using standard curves of peak areas obtained with known amounts of pure standards; linearity of response in the appropriate concentration range was confirmed. Standards were always analyzed immediately prior to analysis of experimental samples, and the latter samples were in turn analyzed at least twice in succession and the values averaged. Data were not corrected for percent recovery, as this was found to be close to 100% for each compound being studied.

Reagents

Reagents were obtained from the following sources: neurochemical standards and sodium acetate trihydrate (grade 1), Sigma Chemical Co. (St. Louis, MO); HPLC grade water and "Baker-analyzed" perchloric acid (70–72%), J. T. Baker Chemical Co. (Phillipsburg, NJ); HPLC grade methanol, Burdick and Jackson Laboratories (Muskegon, MI); and sodium octyl sulfonate, Bioanalytical Systems, Inc. (W. Lafayette, IN).

RESULTS AND DISCUSSION

The 5-HT levels in the MTH showed the most striking change during infection, a 21% decrease in mean levels (see Table 1). 5-HT levels also averaged 14% lower in the CP and 8% lower in the PM of infected rabbits; however these changes were not statistically significant. 5-HIAA levels increased by 17% in the pons/medulla, indicating an increased turnover rate of 5-HT in this region.

The observation that 5-HT levels diminished in the MTH region (and tended to do so in the CP) of infected animals, but did not significantly decrease in the PM, where the cell bodies of the serotonergic neurons are located, is puzzling. One would have expected a significant elevation in 5-HIAA in the MTH to accompany the fall in 5-HT as a reflection of increased turnover; instead, 5-HIAA levels in MTH were unchanged but rose significantly in the PM. Perhaps it is the neuronal transport of 5-HT

TABLE 1. *Levels of DA, 5-HT, 5-HIAA, and HVA in brain regions of rabbits infected with* T. b. gambiense

Brain region	DA	5-HT	5-HIAA	HVA
Caudate/putamen	(C) 8868 ± 937[a] (I) 7719 ± 1406 $p < 0.25$ (NS)[b] −13%[c]	(C) 841 ± 55 (I) 720 ± 99[d] $p < 0.15$ (NS) −14%	(C) 652 ± 61 (I) 627 ± 30 $p > 0.25$ (NS) −4%	(C) 5377 ± 262 (I) 4748 ± 450 $p < 0.2$ (NS) −12%
Midbrain/thalamus/ hypothalamus	(C) 160 ± 8[e] (I) 177 ± 10 $p < 0.25$ (NS) +11%	(C) 1280 ± 30 (I) 1009 ± 94 $p < 0.01$ −21%	(C) 1118 ± 47 (I) 1151 ± 80 $p > 0.25$ (NS) +3%	(C) 827 ± 28 (I) 756 ± 57 $p < 0.25$ −9%
Pons/medulla	(C) 83 ± 5[e] (I) 99 ± 7 $p < 0.05$ +19%	(C) 701 ± 30 (I) 647 ± 63 $p < 0.25$ (NS) −8%	(C) 698 ± 31 (I) 816 ± 51 $p < 0.05$ +17%	(C) 275 ± 16 (I) 366 ± 30 $p < 0.01$ +33%

[a] Expressed as ng/g wet weight of tissue, mean ± SEM. C, Controls (n = 8); I, infected (n = 9).
[b] By Student's *t* test, one-sided. NS, Not significant ($p > 0.05$).
[c] Percent change from controls.
[d] n = 8.
[e] n = 7.

from the PM to terminals in the MTH that is most affected by the disease. Turnover may be increased in the PM, as suggested by the rise in 5-HIAA here, but increased 5-HT synthesis could compensate for this and thus maintain normal 5-HT levels here. Diversion of 5-HT metabolism away from 5-HIAA formation and into the pathway leading to 5-hydroxytryptophol may also occur in the MTH of infected animals and could explain the absence of a significant elevation of 5-HIAA in this region. Levels of 5-hydroxytryptophol were not measured in this study. Because of the pathologic changes in cerebral microcirculation occurring in this disease, brain tissue probably becomes relatively anoxic. This condition would favor reduction of the 5-hydroxyindoleacetaldehyde intermediate (formed by the action of monoamine oxidase) to yield 5-hydroxytryptophol. Parasite metabolism of tryptophan to tryptophol and indole-3-acetic acid, and of 5-hydroxytryptophan to 5-hydroxytryptophol and 5-HIAA, has been shown to occur *in vitro* (Stibbs and Seed, 1973, 1975*c*), and may also contribute to the decline in 5-HT levels by diverting these amino acids away from 5-HT synthesis. Trypanosomes are often abundant in extravascular as well as vascular sites in the brain of chronically infected animals and their metabolism of glucose and amino acids may occur at such a rate that neuronal metabolism is affected.

A decrease in serum tryptophan levels, or in the ratio of serum tryptophan to other neutral amino acids, may also contribute to the lowered brain 5-HT. A nearly total disappearance of free serum tryptophan was reported to occur in trypanosome-infected voles (Newport et al., 1977), and Stibbs and Seed (1975*a*) found that incorporation of [^{14}C]tryptophan into the free amino acid pool of the brain of voles was reduced by 62% during trypanosome infection. Also, rapid conversion of serum tryptophan to indole acetate and tryptophol was reported in acutely infected rats (Stibbs and Seed, 1975*b*) and may have occurred in the infected rabbits, thus tending to lower their serum and brain tryptophan levels. The rate at which tryptophan is metabolized along the pathway leading to kynurenine, a pathway occurring primarily in the liver, also influences serum tryptophan levels and thus the availability of tryptophan to the brain. The activity of this metabolic pathway has not been studied in this disease.

Lowered brain 5-HT levels have also been found in trypanosome-infected rats, mice, and voles (Stibbs, submitted). In rats it is the PM region that exhibits the decrease (21%) in 5-HT, whereas in mice 5-HT decreases in both the PM (17%) and MTH (23%).

A 13% decrease in DA and a 12% decrease in HVA in the CP were observed in infected animals (see Table 1), but did not prove to be statistically significant. This tendency toward a decline in DA and HVA in the CP, however, suggests that the synthesis rate of dopamine may decrease in the nigrostriatal pathway of the brain. A decline in free brain tyrosine, as observed by Newport and Page (1977) in voles, may have occurred in the infected rabbits and would have tended to depress DA levels. However DA levels in the MTH region, which contained the substantia nigra, did not significantly decrease in infection; in fact they averaged 11% higher than controls, although this difference was not significant. HVA levels in the MTH also did not change significantly. In contrast a significant 19% increase in DA and 33% increase in HVA occurred in the PM region, suggesting that DA synthesis and metabolism were increased here. The tendency toward a DA decrease in the CP region is similar to the

decrease in 5-HT in the MTH, discussed above, in that the level of each amine is apparently unchanged in the region where most synthesis occurs. Possibly either reduced transport of these amines to the region containing the neuron terminals or degradation of the amines during transport may occur in infected animals. Interestingly in rats chronically infected with a different strain of *T. b. gambiense* (the Gemena strain) DA levels in the CP region have been found to increase by 34% whereas the levels of 3,4-dihydroxyphenylacetic acid were found to fall by 30% (Stibbs, submitted).

In conclusion, significant changes in brain neurochemical concentrations have been shown to occur in experimental African trypanosomiasis. Given the established relationship between these neurochemicals and various facets of animal behavior (Vogt, 1973; Seiden et al., 1975; DeFeudis, 1979), it seems reasonable to conclude that the observed neurochemical changes, together with others yet to be discovered, should be considered as possible causes of the behavioral symptoms that accompany this disease. Future experiments involving pharmacologic intervention in infected animals will prove whether correction of the neurochemical imbalances can simultaneously eliminate disease-related behavioral aberrations.

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