Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 3, pp. 621–624, 1994

0091-3057/94 $6.00 + .00

0091-3057(93)E0075-F

Altered Monamine Metabolism in Caudate-Putamen of Iron-Deficient Rats

JOHN L. BEARD,*†[1] QING CHEN,* JAMES CONNOR‡ AND BYRON C. JONES†

*Nutrition Department, †Biobehavioral Health Program, The Pennsylvania State University, University Park, PA 16802
‡Department of Neurosciences, Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033

Received 16 April 1993

BEARD, J. L., Q. CHEN, J. CONNOR AND B. C. JONES. *Altered monamine metabolism in caudate-putamen of iron-deficient rats.* PHARMACOL BIOCHEM BEHAV **48**(3) 621–624, 1994.—The effect of iron deficiency on brain monoamine metabolism using in vivo microdialysis techniques has not been previously reported. We, therefore, examined the monoamines, dopamine and norepinephrine, and their metabolites at steady state by in vivo microdialysis in rat brain caudate-putamen in 11-week-old iron-deficient anemic (hemoglobin < 7 g/dl) and control rats (Hb > 14 g/dl). Caudate-putamen dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA) concentrations were increased by 53%, 57%, and 30% ($p < 0.001$), respectively, in iron-deficient rats in samples collected over a 4-h period. While diminished numbers of D_2 receptors have been previously reported, the present findings suggest an additional defect in monoamine uptake and catabolism.

Dopamine Monoamines Catecholamines Iron deficiency

IRON deficiency (ID) anemia is associated with poor cognitive performance in infants, young children, and adolescents (2). Neurobiologic explanations are tentative, as systematic investigations have not yet been conducted regarding the functional role of iron in different brain regions, though it is clearly involved in the synthesis of monoamines by virtue of the non-heme iron enzymes tyrosine and tryptophan hydroxylase (5,6,17). Iron is distributed in the brain in a heterogeneous fashion that generally parallels that of dopamine and is transported by transferrin to both neuronal and nonneuronal cells (2). The highest concentrations of iron are usually found in the globus pallidus, caudate-putamen, putamen, and substantia nigra, with significant heterogeneity in brain regions (4,6). Dallman demonstrated that a brief period of severe ID in young rats resulted in an irreversible 30–40% deficit of brain iron that was not repaired after 45 days of iron therapy (6). Iron deficiency in the postnatal period in the rat results in significant downregulation of the dopamine D_2 receptors in the caudate-putamen, though Youdim *et al.* (17) found no alteration in turnover or concentrations of DA, NE, or 5-HT. Our previous studies, however, showed significant decreases in NE content and increases in fractional turnover in peripheral tissues during iron deficiency anemia (3). Thus, we believed that, by applying a different methodology, viz, in vivo microdialysis, we would be afforded a different perspective, i.e., events in the extracellular compartment, of the impact of ID on brain monoamines. We, therefore, conducted a study of ID in rats in which we measured steady-state extracellular monoamine neurotransmitters and their metabolites to determine if, using this method, we would observe similar effects of ID in the central nervous system.

METHOD

Male Sprague–Dawley rats, 3 weeks of age, were divided into two dietary treatment groups: iron deficient and control. After 8 weeks of dietary treatment that consisted of nutritionally complete diets except for the amount of iron in low iron diet (<2 μg/kg), rats were anesthetized with Xylazine and placed in a stereotaxic frame, with an incision bar 5 mm below the interaural line. A microdialysis CMA/12 intracerebral guide (BioAnalytical Systems, West Lafayette, IN) was placed in the caudate-putamen with the following coordinates with respect to bregma: anterior: 0.4 mm, lateral: 3.0 mm, vertical: 4.0 mm, and were fixed with dental cement. After surgery, animals were allowed to recover for at least 5 days before dialysis began. Preliminary studies in 16 animals showed steady-state concentrations of brain monoamines in the cau-

[1] To whom requests for reprints should be addressed.

TABLE 1
BODY AND HEMATOLOGICAL CHARACTERISTICS

	Hct	Hb	Body Weight	Brain Weight	Brain Weight/ Body Weight (%)
Controls					
Average	45.56	15.47	344.89	1.90	0.57
SD	3.74	2.23	37.87	0.12	0.02
Iron deficient anemic					
Average	23.25	5.78	199.50	1.80	0.92
SD	5.63	1.28	50.63	0.07	0.20

date-putamen could not be obtained before this time. Data were collected in eight ID anemic and eight control animals in the current report.

Before the CMA/12 microdialysis probe was lowered into the caudate-putamen via the guide cannula, it was connected to a high precision pump (CMA/100) and sterile perfusion fluid was pumped through the probe at 0.5 μl/min. The perfusion fluid, which was always filtered and degassed, consisted of 128 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, and 2 mM $MgCl_2$, pH 7.3.

In the late afternoon the animals were placed in a Plexiglas cage that allowed free movement and were given ad lib access to food and water. The animals were left in the cage overnight with their probe in place and a perfusion rate of 0.2 μl/min. Again, preliminary studies showed very clearly that monoamines and their metabolites were not in a kinetic steady state before that time. At approximately 0900 h the day after probe implantation, the pump was again set at 0.5 μl/min, and nine 20-min samples of dialysate were collected. Each collection vial contained 3 μl 100 mM acetic acid and was placed in ice. The first sample in the data collection period was not included in the data analysis, as it usually differed significantly from the rest. For data analysis, the remaining eight samples were averaged to represent the mean concentration of monoamines and metabolites for each animal.

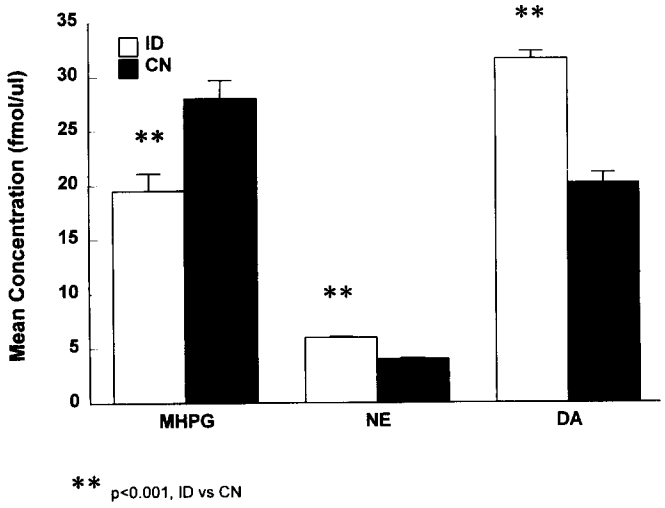

FIG. 1. Extracellular MHPG, NE, DOPA, and dopamine concentration. Student's *t*-test showed significant difference between IDA and CN ($p < 0.05$). Bar height is the mean with bracket 1 SD.

The in vitro recoveries of standards for catecholamines and their metabolites, and for the serotonin metabolite, 5-hydroxyindoleacetic acid, were measured for each probe before and after each experiment. Recoveries for each probe were averaged for each substance over all probes. The average in vitro recoveries at room temperature at a flow rate of 0.5 μl/min. were: MHPG: 37%, NE: 35%, DOPA: 37%, dopamine: 33%, DOPAC: 28%, 5-HIAA: 34%, HVA: 34%. 5-HT was not detected under the assay conditions. There was no correction for total recovery of samples because in vitro recoveries may not reflect recoveries in vivo where tissue diffusion may differ from that found in a bath.

The concentrations of monoamines/metabolites in dialysate were determined with micro-bore reverse phase HPLC with electrochemical detection (Bioanalytical System, West Lafayette, IN). The concentration of each compound in dialysate was determined by comparison with the peak heights of standards run with each experiment. The flow rate was 90 μl/min with dual detector potentials at (+)0.800 volts relative to Ag-AgCl. The mobile phase consisted of 50 mM Na_2PO_4, 0.1 mM EDTA, 0.86 mM sodium octyl sulfate, 5% CH_3OH, and pH 3.0.

At the end of the experiment, the brains were removed, weighed, and stored in 4% formalin fixative solution. The positions of the probes were verified later by histological examination. The hematologic status of each animal was determined by standard methods (8).

Differences between means were determined by Student's *t*-test using MINITAB software. Steady-state conditions were determined by trend-line analysis and outliers identified by boxplot analysis. Less than 1% of the data were identified as statistical outliers and were removed from the analysis. Data were considered significant when $p < 0.05$.

RESULTS

Overall, for each treatment condition, there were no effects for time or time × diet interaction. This indicates that the animals were, indeed, at a steady state for extracellular flux in the monoamines and metabolites.

As concerns the treatment conditions, IDA rats had significantly lower hemoglobin, hematocrit, body weight, serum iron level, transferrin saturation, and higher brain weight to body weight ratio than controls. There was no effect of ID on absolute rat brain weight (Table 1).

The steady-state extracellular fluid monoamine/metabolite concentrations are shown in Figs. 1 and 2. Caudate-putamen

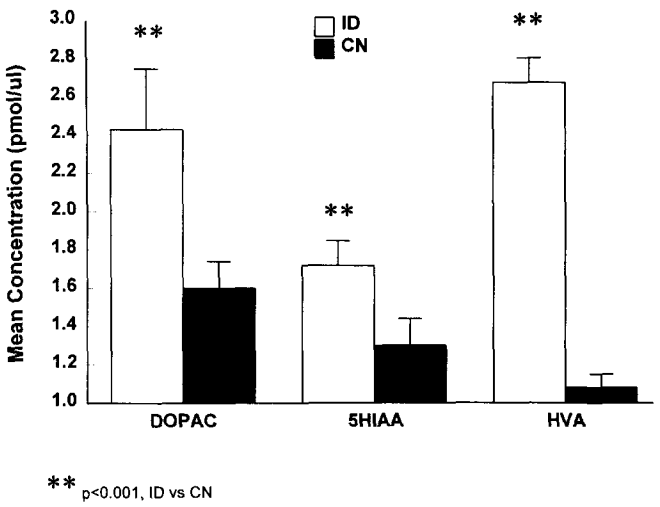

FIG. 2. Extracellular DOPAC, 5-HIAA, and HVA concentration. Student's *t*-test showed significant difference for DOPAC and HVA but not for 5-HIAA ($p < 0.05$). Bar height is the mean with bracket 1 SD.

extracellular dopamine, DOPAC, HVA, and 5-HIAA increased by 53, 57, 30, and 29% ($p < 0.01$), respectively, in IDA. MHPG and NE concentration in IDA rats were 70% and 146% of that in CN rats ($p < 0.05$).

DISCUSSION

ID has been linked repeatedly to abnormalities in CNS function, behavioral abnormalities, and altered cognition (12). Because dopaminergic systems play an important role in these processes (18), it is reasonable to suggest that brain extracellular dopamine levels and, therefore, dopaminergic function have been changed by ID. However, very few studies have been carried out to elucidate the biochemical changes associated with this dopamine function.

Other investigators have reported that the nutritional lack of iron, even though brain nonheme iron content, was significantly lowered by 40%, was without effect on brain iron MAO and tyrosine or tryptophan hydroxylases, succinate dehydrogenase, and aldehyde dehydrogenase activities in vitro or in vivo (1,17,18). Changes in brain content and turnover of NE, DA, and 5-HT were not seen. These findings were partly confirmed by Shukla et al. (11) when they showed activities of MAO and aldehyde dehydrogenase unaltered after 8 weeks of latent ID. They, however, found brain 5-HT reduced by 21%.

In contrast to Youdim's results (17,18), the present study showed rat brain caudate-putamen dopamine and its metabolites, DOPAC and HVA were significantly increased by ID as measured by in vivo microdialysis. If dopamine synthesis is not altered by ID as indicated by the measurements of synthetic monoamine enzyme activities (12), the current results suggest that the dopamine reuptake system, or the rate of utilization or catabolism has been changed by ID. After release from presynaptic neurons, most of the dopamine is to be taken up again in the presynaptic terminals by Na^+-dependent membrane dopamine transporter (7,15,16). Nicolaysen and Justice (10) demonstrated that at steady state in the mathematical model, 87.5% extracellular DA is removed by presynaptic uptake, 10.9% by postsynaptic uptake, and the remaining 1.6% by extraneuronal metabolism to 3-MT. Although 3-MT steady state tissue levels are less than 1% of the DA steady-state levels, this pool possesses extremely rapid dynamics and about 30% of the remaining extracellular DA is metabolized to 3-MT (15). Because COMT, the enzyme responsible for the metabolism of DA to 3-MT, appears to be extraneuronal, investigators have suggested that extracellular DA metabolism is mainly via 3-MT to HVA (13,14). If the dopamine transporter were diminished by ID, accumulation of extracellular DA would occur with an increase in HVA concentration as a secondary metabolite of both 3-MT and DOPAC. The 53% and 30% respective increases in extracellular dopamine and HVA concentration in ID rats support this hypothesis.

The importance of iron in DNA synthesis, as a cofactor for many heme and nonheme enzymes, and its structural role in many membrane- and nonmembrane-bound proteins (2,5) offer the possibility that nutritional ID can decrease dopamine transporter number or cause dopamine transporter dysfunction. The finding by Youdim and his co-workers that iron deficiency is associated with fewer D_2 receptors supports the former hypothesis. They could not rule out, however, either that the DA receptors are modified by some conformational change or that the lack of iron diminishes the rate of receptor synthesis (17,18). Our finding of iron deficiency-related increases in both dopamine and homovanillic acid suggest another possibility, i.e., that D_2 receptor downregulation may be secondary to impairment of dopamine transporters, and subsequent increased synaptic levels of dopamine. Our data also revealed higher DOPAC concentrations in ID rats compared to CN rats. Westerink (14) showed that DOPAC can be formed intraneuronally, both prior to release and after reuptake, as well as extraneuronally, although DOPAC accumulation is considered by some to reflect primarily intraneuronal DA metabolism (15). The increase in DOPAC observed in ID rats probably indicates a multiplicity of effects of iron deficiency, intraneuronally as well as extraneuronally.

In summary, ID not only reportedly diminishes dopamine receptors, but now may also diminish dopamine transporters. As a result, in ID rat brain caudate-putamen, extracellular dopamine level increases as reuptake decreases while dopamine-mediated functions remain altered because of altered dopamine receptors.

REFERENCES

1. Altman, P. L.; Dittmer, D. S., eds. Blood and other body fluids. Washington, DC: Federation of American Societies for Experimental Biology (FASEB); 1961.
2. Beard, J.; Connor, J. D.; Jones, B. C. Iron in the brain. Nutr. Rev. 51:157–170; 1993.
3. Beard, J. L.; Tobin, B. W.; Smith, S. M. Effects of iron repletion and correction of anemia on norepinephrine turnover and thyroid metabolism in iron deficiency. Proc. Soc. Exp. Biol. Med. 193: 306–312; 1990.
4. Connor, J. R. Proteins of iron regulation in the brain in Alzheimer's disease. In: Lauffer, R. B., ed. Iron and human disease. Ann Arbor, MI: CRC Press; 1992:365–393.
5. Dallman, P. R. Biochemical basis for the manifestations of iron deficiency. Annu. Rev. Nutr. 6:13–40; 1986.

6. Dallman, P. R.; Siimes, M. A.; Manies, E. C. Brain iron: Persistent deficiency following short-term iron deprivation in the young rat. Br. J. Haematol. 31:209–215; 1975.
7. Horn, A. S. Dopamine uptake: A review of progress in the last decade. Prog. Neurobiol. 34:387–400; 1990.
8. International nutritional anemia consultative group. Measurements of iron status. Report of the Nutrition Foundation; 1985.
9. Lozoff, B. Behavioural aspects of iron deficiency in infancy. Am. J. Clin. Nutr. 50(Suppl):641–655; 1989.
10. Nicolaysen, L. C.; Justice, J. B. Effects of cocaine on release and uptake of dopamine in vivo: Differentiation by mathematical modeling. Pharmacol. Biochem. Behav. 31:327–335; 1988.
11. Shukla, A.; Agarwal, K. N.; Chansuria, J. P. N.; Taneja, V. Effect of latent iron deficiency on 5-hydroxytryptamine metabolism in rat brain. J. Neurochem. 52:730–735; 1989.
12. Sourkes, T. L. Influence of specific nutrients on catecholamine synthesis and metabolism. Pharmacol. Rev. 24:359–379; 1973.
13. Uretsky, N. J.; Iversen, L. L. Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. J. Neurochem. 17:268–278; 1970.
14. Westrink, B. H.; Tuinite, M. H. Chronic use of intracerebral dialysis for the in vivo measurement of 3,4-dihydrophenylethelamine and its metabolite, 3,4 dihydroxyphenylacetic acid. J. Neurochem. 46:181–185; 1986.
15. Wood, P. L.; Kim, H. S.; Marien, M. R. Intracerebral dialysis: Direct evidence for the utility of 3-MT measurements as an index of dopamine release. Life Sci. 41:1–5; 1987.
16. Wood, P. L. Opioid regulation of CNS dopaminergic pathways: A review of methodology, receptor types, regional variations and species differences. Peptides 4:595–601; 1983.
17. Youdim, M. B. H.; Green, A. R. Iron deficiency and neurotransmitter synthesis and function. Proc. Nutr. Soc. 37:173–179; 1978.
18. Youdim, M. B. H.; Ben-Schachar, D.; Yehuda, S. Putative biological mechanisms on the effects of iron deficiency on brain biochemistry. Am. J. Clin. Nutr. 50:607–617; 1989.