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Abnormal rat brain monoamine metabolism in iron deficiency anemia

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Iron deficiency in rats produces numerous alterations in brain metabolism as assessed by in vitro *techniques. We used a new method of* in vivo *microdialysis to study the effect of acute iron deficiency anemia on rat brain monoamine metabolism. This method was used to sample extracellular fluid from an implanted microdialysis probe in the caudate putamen from freely moving animals. Method validation experiments showed that steady-state levels of dopamine, norepinephrine, and their metabolites were obtained only after 5 to 7 days of surgical recovery and with prior perfusion of the brain region. Caudate putamen dopamine was significantly increased 30% and 40% in fasted light-exposed and 2-hr-fed dark-exposed iron deficient anemic rats (hemoglobin <6 g/dL), respectively, relative to control rats. Dihydroxyphenyl acetic acid and homovanillic acid concentrations were unaffected by iron deficiency in the fasted state. These metabolites, along with dopamine, increased significantly (50 to 82%) in iron deficient rats with exposure to darkness and food while control rats' metabolites did not change. The present study documents that iron deficiency is associated with altered* in vivo *brain monoamine metabolism in the steady state and in response to the environmental stimuli of food and darkness. While these data are supportive of the previous* in vitro *demonstrations of down-regulation of dopamine D_2 receptors, they also suggest that uptake and processing of monoamines is significantly perturbed by iron deficiency.* (J. Nutr. Biochem. 6:486–493, 1995.)

Keywords: brain; iron deficiency anemia; dopamine; norepinephrine; microdialysis; monoamines

Introduction

Iron deficiency anemia is associated with profound hematological changes, stunted growth, poor immune function, decreased physical performance, altered thermoregulatory performance, and decreased cognitive function.[1,2] In rats made nutritionally iron deficient there is a significant reduction of brain iron,[3] decreased responsiveness to environmental stimuli, and decreased learned task performances.[4–6] Furthermore, behavioral responses to presynaptic and postsynaptic dopaminergic drugs are significantly diminished in these animals.[7,8] Although all the brain biochemical changes that result from iron deficiency are not known, an abnormality of dopaminergic neurotransmission has been demonstrated.[7–12] Iron deficiency had no effect on caudate dopamine (DA) D_1 receptors (dopamine-sensitive adenylate cyclase)[8] but did significantly diminish dopamine D_2 receptors, as evidenced by diminished [^{3}H] spiperone binding sites.[13]

Further indirect evidence of iron having a role in monoaminergic neurotransmission is the colocalization of iron with dopaminergic neurons in the brain, which resembles a pattern similar to that of dopamine and neuropeptides.[13,14] Iron is also reportedly important for monoamine oxidase activity and is a cofactor for tyrosine hydroxylase and tryptophan hydroxylase[2,8,15] though some studies find no influence of nutritional iron deficiency on these brain enzyme activities or on brain norepinephrine (NE), DA, or serotonin (5HT, 5-hydroxy tryptamine) turnover despite a 40% reduction in brain iron.[16]

Given the fact that nearly all of the information regarding brain monoamine metabolism is gathered by in vitro methodology, and therefore suffers from immediate translation into in vivo effects; we sought to investigate the in vivo metabolism of monoamines by using a relatively new meth-

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odology called in vivo microdialysis. This method allows us to measure directly brain monoamines in the extracellular compartment of particular brain regions, in this case the caudate putamen, as a function of iron status in freely moving animals and to determine possible differential responses with exposure to environmental stimuli such as food and darkness.

Methods and materials

Animals and design

This research was developmental and descriptive in nature. Each experiment was based on a simple factorial design with dietary treatment for 6 weeks as the main factor. Male Sprague–Dawley rats were obtained at 21 days of age from a commercial source (Harlan-Sprague Dawley, Indianapolis, IN USA) and were randomly assigned to one of the two dietary treatment groups: iron deficient (ID) diet or control (CN) diet. The two dietary treatment groups received the same nutritionally complete purified diet (AIN76A formulation) ad libitum for 5 to 6 weeks with the only difference being the iron content of the diet (2 ppm versus 50 ppm Fe added as $FeSO_4$). The diets were further modified by replacing sucrose with cornstarch. In addition, cellulose as a fiber source was omitted and replaced with cornstarch due to the variable iron content of cellulose. The dietary iron contents were analyzed by atomic absorption spectrometry to determine their iron concentration.[17] The animals were housed individually in stainless steel cages and maintained at 24 to 25°C on a 12-hr light/dark cycle (6:00 a.m. to 6:00 p.m. as light cycle). The use of laboratory rats as the animal model in this study was approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Microdialysis probe

The CMA/12 probe membrane was obtained from a commercial source (Bioanalytic System, West Lafayette, IN USA) and consisted of a 2 mm length of polycarbonate/polyether copolymer with an outer cannula of 640 μm OD and an inner cannula of 380 μm OD. The membrane has a molecular weight cutoff of 20,000 D and extends 2 mm below the guide cannula. Each probe was tested in vitro to determine its ability to recover known concentrations of the monoamines DA, dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindole acetic acid (5-HIAA), NE, methoxyhydroxyphenyl glycol (MHPG), and dihydroxyphenylalanine (DOPA). The in vitro recoveries of standards for monoamines and their metabolites were measured at room temperature 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%; DA, 33%; DOPAC, 28%; 5-HIAA, 34%; HVA, 34%. The range was not more than 3%. There was no correction for total recovery of samples since in vitro recoveries may not reflect recoveries in vivo where tissue diffusion may differ from that found in a bath. Serotonin was not detectable under the HPLC column conditions necessary to quantitate the other metabolites.

HPLC analysis of perfusates

Concentrations of monoamines in the dialysate were measured with microbore reverse phase HPLC with dual electrochemical detection (Bioanalytical Systems, West Lafayette, IN USA). The concentration of each compound in the 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 V relative to Ag-AgCl. The mobile phase consisted of 50 mM Na_2PO_4, 0.1 mM EDTA, 0.86 mM sodium octyl sulfate, and 5% CH_3OH at a pH of 3.0.

Surgical procedures and probe implantation

After 5 to 6 weeks of dietary treatment, rats were anesthetized with Ketamine hydrochloride (100 mg/mL, 0.00075 mL/g of body weight [BW]) and xylazine (20 mg/mL, 0.00038 mL/g of BW) injected intramuscularly in the hindlimb and an intracerebral guide cannula inserted according to commonly used methods.[18,19] Then rats were placed in a stereotaxic frame with the incisor bar 5 mm below the interaural line. A sterile microdialysis CMA/12 intracerebral guide (Bioanalytical Systems) was placed in the caudate putamen and fixed with dental cement with the following coordinates with respect to bregma: anterior, 0.4 mm; lateral, 3.0 mm; vertical, 4.0 mm. After surgery, except in Experiment 1, animals were allowed to recover for at least 5 to 7 days before dialysis began. During this time, animals were housed singly in hanging stainless steel wire cages in a light-controlled room with free access to food and water. Body weight and temperature were monitored daily to ensure adequate recovery.

Before a dialysis probe was lowered into the caudate putamen, it was connected to a high precision microdialysis pump (CMA/100, Bioanalytic Systems), and sterile degassed perfusion solution was pumped through the probe at 0.5 μL/min. The perfusion solution consisted of 128 mM NaCl, 2.7 mM KCl, 1 mM $CaCl_2$, and 2 mM $MgCl_2$, pH 7.3.[18]

Experiment 1A: Effects of surgery and anesthesia on the basal concentrations of monoamines in dialysate

Both ID anemic (n = 5) and control (n = 5) animals were used in this experiment. At 9:00 a.m. and on the day after surgery the probe was inserted into the guide cannula in the caudate, perfused at 0.5 μL/min, and eight 30-min samples were collected. Samples were collected into 400 μL microfuge tubes that contained 3 μL of 100 μM acetic acid and immediately frozen at −80°C. After the last sample was obtained, the probe was removed, the stylet was placed back into the guide cannula, and the animals were returned to their home cage.

One to 3 days later the animals were killed by decapitation. Trunk blood was collected into heparinized tubes for measurement of hematological parameters (hematocrit, hemoglobin, plasma iron concentration, and total iron binding capacity) and livers were analyzed for nonheme iron content.[17] The brains were removed and stored in 4% formalin fixative solution. The positions of the probes were verified later by histological examination of the fixed brain tissue. All dialysates were analyzed within 1 month.

Experiment 1B: Monoamine levels after at least 5 days recovery, with probe left in position overnight in ID and CN rats

ID rats (n = 6) and control rats (n = 6) were allowed to recover for 5 to 7 days after implantation of the probe guide and when their body weight returned to normal. At 2:00 p.m. the microdialysis probe was lowered into the caudate via the guide cannula and fixed in place. Nine consecutive 20-min samples of dialysate were collected, with the perfusion solution pumped at a rate of 0.5 μL/min. Following collection of the last sample, the pump was turned down to 0.2 μL/min, and the animals were left in the cage overnight with their probe in place and with free access to food and water.

At approximately 9:00 a.m. the next day, the pump was again

set at 0.5 μL/min, and after 1 hr another nine 20-min samples of dialysate were collected. After the last sample was obtained, the probe was removed, the stylet was placed back into the guide cannula, and the animals were returned to their home cages. One to 3 days later, the animals were killed by decapitation. Trunk blood was collected, and the livers were saved for iron status assessment. The brains were removed and stored in 4% formalin fixative solution. The positions of the probes were verified later by histological examination.

Experiment 2: Effect of food deprivation and dark exposure on changes in monoamines and their metabolite concentrations in ID and CN rats

In response to the results of Experiments 1A and 1B, and the demonstration of the conditions necessary to achieve a steady state, we allowed 5 to 7 days of recovery from surgery before we began collecting data on 6 ID rats and 6 CN rats with the purpose of obtaining fasting steady-state data and the response to darkness and food. The probe was placed into the guide cannula and perfused at 0.5 μL/min overnight. Animals were food deprived but with free access to water beginning in the late afternoon. At 6:00 p.m. the following day, six 20-min consecutive samples were collected. At 8:00 p.m. the animals were given access to their regular food and the lights were turned off. This corresponded to the normal time for the lights to be turned off. Another six 20-min samples were collected as previously described, and animals were terminated in the aforementioned fashion.

Statistical analysis

Data from Experiment 1 showed great variability in all neurotransmitter concentrations because individual animals had a highly variable response to anesthesia and neuronal damage during surgery. Data from one rat of each diet treatment group are included in the figures for illustration purposes. Statistical analyses were performed using MINITAB (Version 7.1, Minitab Inc., State College, PA USA) and Crunch (Version 4, Crunch Software Corporation, NE USA). Comparisons of iron status parameters were made using Student's *t* tests. Comparisons of responses between two diet treatment groups to food deprivation and darkness were analyzed using analyses of variance (ANOVA) within Crunch. The data were treated as one between treatment factor, one within subjects factor, and one repeated measures factor. Type III sums of squares were used to calculate the significance. Post hoc tests performed in the last experiment were Tukey's comparisons. Data are expressed as means ± SD in the tables and as the mean ± SEM in the figures. Differences between means were considered significant when $P < 0.05$.

Results

Experiment 1

Following 5 to 6 weeks of dietary treatment, animals from the iron deficient dietary treatment group weighed significantly less than those from the iron sufficient treatment group and had other signs of severe iron deficiency, such as low Hb, Hct, serum iron level, transferrin saturation, and liver iron concentration (*Table 1*). There was no effect of a low iron diet on absolute rat brain weight, although ID rats had significantly higher brain weight to body weight ratios than did control rats, 10.1 ± 0.5 mg/g of BW compared with 6.1 ± 0.6 mg/g of BW.

Substantial variation in monoamines and their metabolites existed between and within each of the dietary treatment groups when data were collected either immediately

Table 1 Iron and hematologic status of rats exposed to low iron (2 ppm Fe) or normal iron diets (50 ppm Fe) for 5 to 6 weeks

	Experiment 1a	Experiment 1b	Experiment 2
Body weight (g)			
ID	178 ± 5	209 ± 22	224 ± 9
CN	305 ± 9*	322 ± 17*	335 ± 9*
Hb (g/dL)			
ID	4.8 ± 0.2	5.9 ± 0.6	5.8 ± 1.0
CN	14.2 ± 0.3*	15.5 ± 0.9*	15.3 ± 2.5*
Hct (% PCV)			
ID	21 ± 1	23 ± 3	24 ± 3
CN	46 ± 2*	46 ± 4*	44 ± 2*
Plasma iron (μmol/L)			
ID	4.3 ± 0.4	6.55 ± 0.7	3.5 ± 0.3
CN	35.6 ± 3.0*	24.0 ± 2.5*	21.9 ± 2.3*
TIBC (μmol/L)			
ID	98.6 ± 5.2	118 ± 9.1	93.2 ± 3.6
CN	51.0 ± 4.5*	65.6 ± 2.8*	54.4 ± 4.6*
Transferrin saturation (%)			
ID	5.2 ± 0.4	4.9 ± 0.4	4.1 ± 0.4
CN	39 ± 6.4*	37 ± 4.9*	40 ± 2.7*
Non-heme Liver iron (μmol/g tissue)			
ID	0.50 ± 0.01	0.51 ± 0.01	1.17 ± 0.02
CN	2.78 ± 0.17*	2.61 ± 0.17*	6.0 ± 0.44*
Brain weight (g)			
ID	1.81 ± 0.05	1.82 ± 0.02	1.86 ± 0.05
CN	1.88 ± 0.06	1.92 ± 0.06*	1.90 ± 0.22

Values represent means ± SE. ID denotes iron deficient rats and CN denotes iron sufficient rats. There were 5 rats per group in Experiment 1a and 6 rats per group in other experiments. A superscript (*) denotes a significant difference between ID and CN rats within an experiment, $P < 0.05$ using unpaired student's *t*-test

after surgery or even the following day. The mean percentage variation of the monoamines measured (MHPG, NE, DOPAC, DA, HVA, 5-HIAA) was 281 ± 82% for CN animals and 258 ± 31% for ID animals during the period immediately after surgery. When metabolites and monoamines were measured 24 hr later, there was little improvement in variability. The mean coefficient of variation for all of these metabolites was now 229 ± 84% in CN rats and 280 ± 93% in ID animals. This large variability showed this model to be unsatisfactory for determinations of steady-state monoamine levels in the caudate putamen of the rat.

Experiment 1B

Six weeks of an iron deficient diet produced changes in iron and growth indices consistent with iron deficiency and similar to animals in Experiment 1A (*Table 1*). *Figures 1a* and *2a* show the mean percentage of initial concentrations of NE, DA, and their metabolites in dialysate measured within 3 hr after probe implantation in CN rats (*Figure 1a*) and in ID rats (*Figure 2a*) after allowing for 5 to 7 days of recovery from surgery. With few exceptions, monoamine concentrations still showed a considerable variation of greater than 50% over this 3 hr interval and meaningful estimates of steady-state levels not attained. Fifteen to 18 hr after the probe was inserted into the guide cannula and perfused in position overnight, the concentrations of most monoamines were much more stable (*Figures 1b* and *2b*). In these ID animals, caudate putamen extracellular DA, DOPAC, HVA, and 5-HIAA were elevated by >50% for DA and DOPAC and by 30% for HVA and 5-HIAA, respectively, compared with control animals (see legends for absolute concentrations). MHPG and NE concentrations in ID rats were significantly lower and significantly higher, respectively, than found in CN rats.

Experiment 2

Following the same dietary treatment as the prior experiments, significant differences existed between ID rats and CN rats with regard to growth and hematologic status (*Table 1*). DA, DOPAC, and HVA concentrations measured over 2 hr in the light and fasting state, followed by a 2 hr dark period along with access to food were significantly altered by iron deficiency (*Figures 3a–3c*). Dopamine was 31% higher in ID animals in the fasted steady state than controls, 30.8 ± 3.2 fmol/μL versus 23.4 ± 1.7 fmol/μL ($P < 0.05$), while DOPAC was 25% lower in the anemic animals, 3,147 ± 1,036 fmol/μl versus 5,557 ± 470 fmol/μL ($P = 0.06$). When 24-hr fasted ID rats were given access to food and simultaneously exposed to darkness, DA and its metabolites, DOPAC and HVA, increased significantly ($P < 0.05$) by the end of the ensuing 2 hr data collection period. This phenomenon, however, did not occur in CN rats. Thus at the end of 2 hr of feeding and dark exposure, iD rats had a 43% higher DA concentration in dialysate from the caudate putamen than did CN animals, 39.7 ± 4.4 fmol/μL versus 27.6 ± 3.3 fmol/μL. There was no difference in DOPAC concentrations between ID and CN animals after this period because of the significant elevation, 82%, in DOPAC in anemic animals. HVA also showed a significant increase of 51% by the end of the data collection period in the dark. The greater percentage increase in these monoamine concentrations in ID rats in response to these environmental stimuli is illustrated in *Figure 3d*.

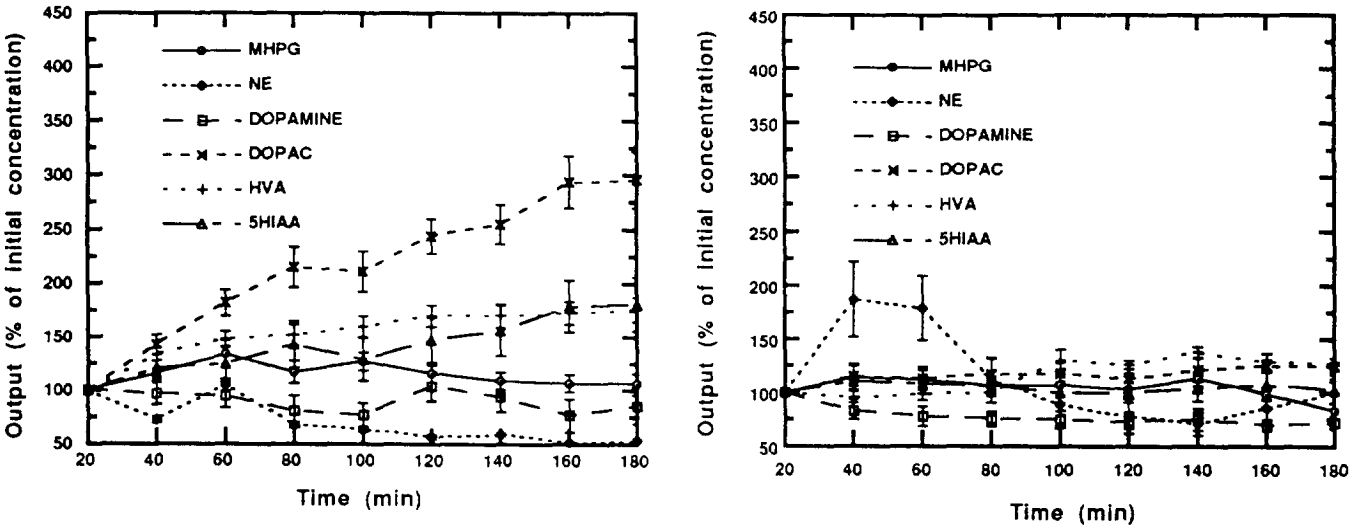

Figure 1 (a) NE, DA, and their metabolite concentrations in dialysate measured after first probe implantation after 1 week of postsurgical recovery in a group of 6 CN rats. Data are expressed as a mean percentage of the first sample. The mean (±SD) absolute initial concentrations as follows: MHPG, 40.13 ± 6.2 fmol/μL; NE, 8.82 ± 0.45 fmol/μL; DA, 23 ± 7.3 fmol/μL; DOPAC, 2,664 ± 622 pmol/μL; 5-HIAA, 998 ± 231 pmol/μL; HVA, 2470 ± 462 pmol/μL. (b) NE, MHPG, DA, DOPAC, HVA, and 5-HIAA concentrations in dialysate measured 24 hr after first probe implantation after 1 week of recovery in a group of 6 CN rats. Data are expressed as a mean percentage of the first sample. The mean (±SD) absolute initial concentrations are as follows: MHPG, 31.25 ± 2.2 fmol/μL; NE, 4.06 ± 0.24 fmol/μL; DA, 41.07 ± 8.2 fmol/μL; DOPAC, 5,952 ± 1,467 pmol/μL; 5-HIAA, 1,498 ± 321 pmol/μL; HVA, 3,479 ± 534 pmol/μL.

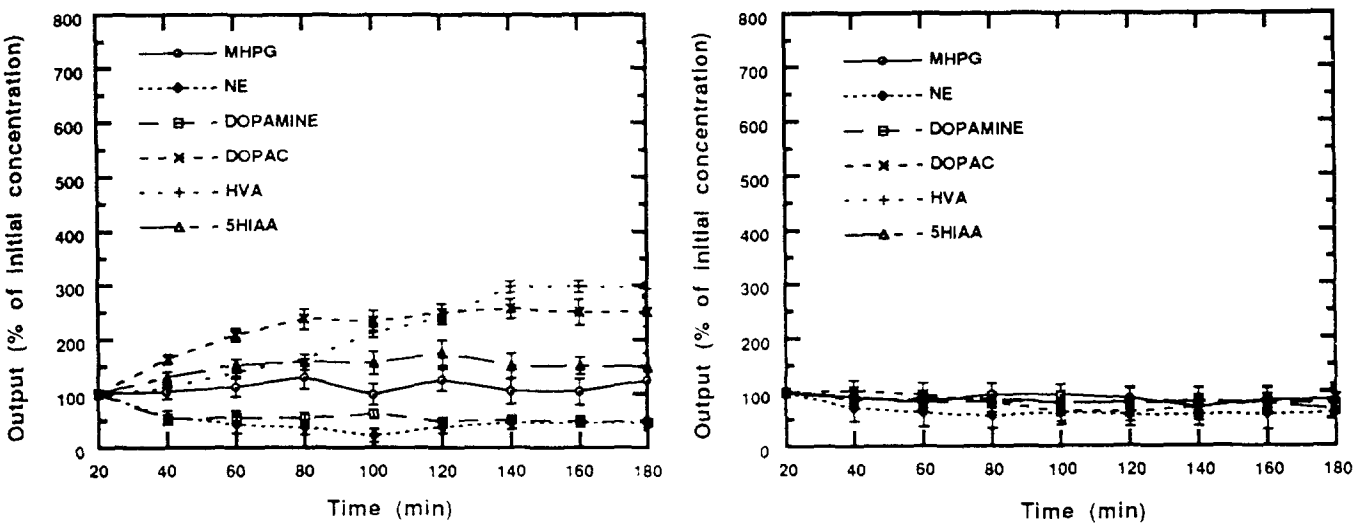

Figure 2 (a) NE, MHPG, DA, DOPAC, 5-HIAA, and HVA concentrations in dialysate measured immediately after probe implantation after 1 week of recovery in a group of 6 ID rats. Data are expressed as a mean percentage of the first sample. The mean (±SE) absolute initial concentrations as follows: MHPG, 15.56 ± 1.02 fmol/μL; NE, 19.79 ± 2.11 fmol/μL; DA, 320.85 ± 48 fmol/μL; DOPAC, 3,351 ± 363 pmol/μL; 5-HIAA, 1,112 ± 59 pmol/μL; HVA, 1,254 ± 162 pmol/μL. (b) NE, DA and their metabolite concentrations measured 15 to 18 hr after perfusion of the probe after 1 week of postsurgical recovery in a group of 6 ID rats. Data are expressed as a mean percentage of the first sample. The mean (±SE) absolute initial concentration is as follows: MHPG, 29.43 ± 3.91 fmol/μL; NE, 9.95 ± .49 fmol/μL; DA, 49.06 ± 3.32 fmol/μL; DOPAC, 6,149 ± 702 pmol/μL; 5-HIAA, 2,553 ± 231 pmol/μL; HVA, 3,703 ± 493 pmol/μL.

Ratios of HVA/DA or DOPAC/DA are often used as indices of the rate of catabolism of DA to its primary metabolites. There was no effect of iron status in either the fasting steady state or at the end of a 2 hr exposure to darkness and access to food on these ratios. The DOPAC/DA ratio averaged 180 ± 40 pmol DOPAC/fmol DA in controls and 140 ± 44 pmol/fmol in anemic animals in the fasted state. These ratios increased to 220 ± 47 pmol/fmol in controls and 170 ± 28 pmol/fmol in anemic rats, again with no statistical significance of dietary treatment. The HVA/DA ratios were also not different in ID animals than in CN rats, 135 ± 15 versus 172 ± 42 pmol/fmol, in the fasted or the refed state, 142 ± 12 versus 175 ± 45 pmol/fmol.

Discussion

This study reports the application of intracerebral microdialysis to monitor the effects of ID on extracellular levels of DA and its main free acid metabolites, DOPAC and HVA, as well as NE and its metabolite MHPG in caudate putamen under different experimental conditions. Microdialysis has been used to study dopamine and its metabolites for more than a decade.[18,19] To date more that 1,500 papers have been published in the scientific literature using this method; we felt it important to carefully establish the criteria and appropriate methodology for steady-state measurements beyond the simple measurements we made previously.[11] Many investigations using the microdialysis method have been performed in anesthetized animals, which raises concerns that the anesthetic may compromise neuronal function, that introduction of the probe into the brain on the day of testing alters results, and that dynamics of exchange of neurotransmitters and metabolites are perturbed through the use of this probe.[19,20] Experiment 1 demonstrates clearly that acute implantation of a probe in the caudate putamen produces wide changes in levels of monoamines immediately after and 1 day after surgery. In addition, it is clearly necessary to perfuse the probe for a significant period of time (in our case 12 to 15 hr), in place, prior to collected data which represent the steady state.[21] From these experiments, it appears that an adequate period for recovery prior to dialysis is between 5 and 7 days after surgery if steady-state concentrations are desired. Reiriz et al.[20] found a decrease in levels of metabolites in dialysate after 4 days of perfusion with the probe consistently in position. Our data suggested that the microdialysis probe be perfused for 1 day before experimentation to avoid recovery problems since recovery of in vitro standards before and after in vivo data collection were nearly identical.

Data collected during the steady-state fasted condition show that iron deficiency leads to a 30% elevation in extracellular DA concentration with an even greater difference, 40%, when the environmental stimuli of darkness and access to food are introduced. This is in contrast to Yehuda's results[16] which showed no elevation in DA or its metabolites, DOPAC and HVA, in iron deficiency, though there was clearly a repeatable decrease in the dopamine D_2 receptor. This decreased receptor density is thought to be associated with decreased functioning of the opiod and dopaminergic system.[22] Explanations for the elevations in monoamines in the caudate putamen observed in our study may include altered intraneuronal DA metabolism, uptake, and release. After release from presynaptic neurons, more than 80% of the dopamine is taken up again in the presynaptic terminals by Na^+-dependent membrane transporter, more than 10% by postsynaptic processes, and the remaining amounts are converted to 3-methoxytyramine (3-MT) or

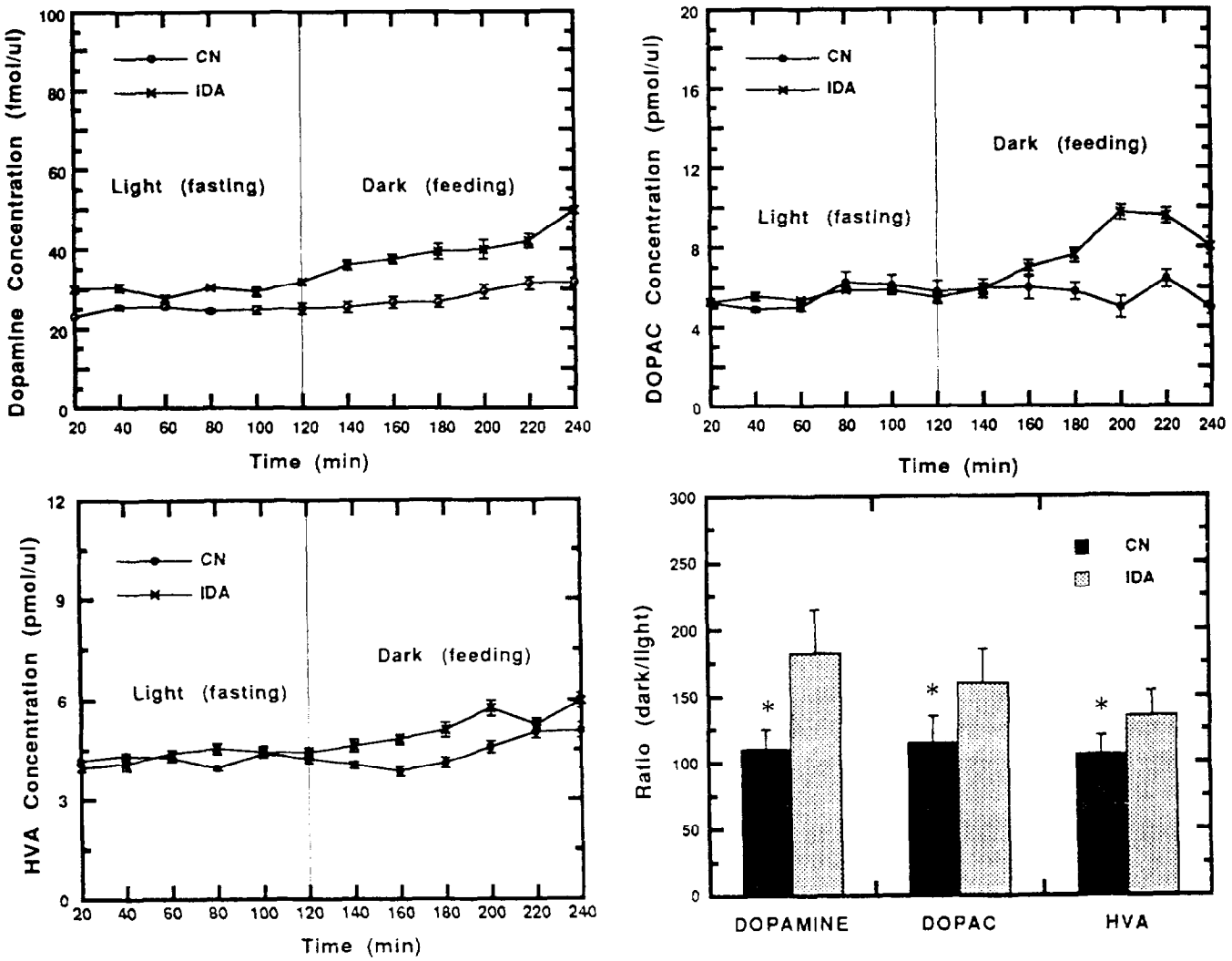

Figure 3 (a) DA concentrations measured during a 2-hr fasting state (lights on), followed by a 2-hr period with darkness and access to food. Groups are iron deficient (ID) and control (CN) rats with $n = 6$ per group. Data are expressed as group means ± SEM. (b) DOPAC concentration measured during a 2-hr fasting state (lights on), followed by a 2-hr period with darkness and access to food. Groups are iron deficient (ID) and control (CN) rats with $n = 6$ per group. Data are expressed as group means ± SEM. (c) HVA concentration measured during a 2-hr fasting state (lights on), followed by a 2-hr period with darkness and access to food. Groups are iron deficient (ID) and control (CN) rats with $n = 6$ per group. Data are expressed as group means ± SEM. (d) Percentage changes in ratios of DA and its metabolites (HVA and DOPAC) during a 2-hr fasting state (lights on), followed by a 2-hr period with darkness and access to food. Groups are iron deficient (ID) and control (CN) rats with $n = 6$ per group. Data are expressed as group means ± SEM during dark (fed) and light (fasting) period. Ratios from IDA rats are significantly higher than ratios from CN rats by t-test ($P < 0.05$). The percentage change is calculated from the difference between the average of the last three time points of individual animal and its baseline average. These changes are then averaged over dietary treatment.

lost to the CSF as DA.[23–25] Thus, a decreased dopamine transporter activity may explain both increased HVA and DA but not an increased DOPAC. In our experiments, the concentration of 3-MT was below the limit of detection, which is approximately 3 fmol/μL. Catechol O-methyl transferase is the extraneuronal enzyme responsible for the metabolism of DA to 3-MT and its further rapid conversion to HVA.[21,26] The extent of its activity changes with iron deficiency are unclear and open to speculation.

Brain monoamines are clearly involved in feeding behavior and response to feeding.[27,28] Experiments show that increased amounts of DA are observed in brain with food consumption due to either increased release of DA caused by central processes of hunger or by the motor movements made during food consumption.[29,30] Twenty hours of food deprivation increased the concentration of HVA with no change in DA or DOPAC levels in the striatum of mice. Dopaminergic responses to feeding vary with brain region and perhaps with strain of rat.[31,32] Increased levels of DOPAC in the nucleus accumbens, the posterior hypothalamus, and the amygdala were observed 4 hr after introduction of food; but changes were not observed in other DA-rich brain areas such as the caudate putamen, septum, frontal cortex, or olfactory tubercle.[31] Zucker rats, however, show an altered route of metabolism of DA as indicated by increased DOPAC/DA and a decreased HVA/DA ratio in the striatum with food deprivation.[32] Inoue et al. investigated the temporal nature of these changes in DA metabolism with in vivo microdialysis of the ventrolateral striatum of freely moving rats and noted that 7 days of a restricted

access to food protocol brought about a rise in HVA and DOPAC with feeding while DA remained unresponsive to access to food.[33] Thus, definitive interpretations of ratios of HVA/DA and DOPAC/DA and absolute elevations in brain DA concentrations in iron-deficient animals are impossible at this time though they may well include altered D_2 receptor function, increased release, and altered uptake via the Na-cotransport system, and altered degradative enzymes. Other studies in our laboratory (in press) note that iron-deficient animals consume more food per gram of body weight or per gram of body weight gain but this is still insufficient to maintain normal growth velocities. The relationship of food intake behavior to iron status thus needs much greater clarification and attention. Other brain regions may not show these effects of iron deficiency on DA metabolism though this particular region was carefully chosen to compare with the studies of Youdim and Yehuda.[12,13]

Iron is heterogeneously distributed in the brain, and this period of immediately after weaning is a period of high rates of accumulation of iron in the rat brain and one in which dietary iron deficiency can modify to brain iron content.[1] The colocalization of iron with dopaminergic neurons demonstrated by others[14] suggests, but does not prove, a functional link of any consequence between these two important elements of brain function. Nonetheless, in vivo microdialysis has been used to demonstrate a significant alteration in brain monoamine metabolism in controlled conditions. Alterations in DA metabolism have been implicated in the decreased cognitive function and behavioral abnormalities of iron-deficient rats and put forward as a possible explanation for decreased cognition in iron deficient humans.[1,16] This experiment provides strong in vivo data to support the argument for altered dopaminergic activity in iron deficiency and describes a new set of methodologic tools to investigate that alteration. A high extracellular DA concentration in iron deficiency may be causally related to the documented decrease in D_2 receptor density; new studies will provide insights into the causal role of iron nutriture in this defect.

Acknowledgments

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