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Changes in Extracellular PVN Monoamines and Macronutrient Intake After Idazoxan or Fluoxetine Injection

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PAEZ, X. AND S. F. LEIBOWITZ. *Changes in extracellular PVN monoamines and macronutrient intake after idazoxan or fluoxetine injection.* PHARMACOL BIOCHEM BEHAV 46(4) 933-941, 1993.—Norepinephrine (NE) and serotonin (5-HT) in the paraventricular nucleus (PVN) have opposite effects on feeding, with NE stimulating carbohydrate intake through α_2 noradrenergic receptors and 5-HT inhibiting carbohydrate intake. This study examined the action of drugs that affect brain monoaminergic systems, in terms of their impact on nutrient intake and on PVN monoamines measured using microdialysis. The drugs studied were idazoxan, a blocker of α_2 receptors, or fluoxetine, a 5-HT reuptake blocker. In rats maintained on pure macronutrient diets, idazoxan (1 mg/kg) and fluoxetine (10 mg/kg), 120 min after injection both reduced total food intake, and specifically carbohydrate intake. In dialysis experiments, successive 20-min dialysate samples were taken, three samples before and seven samples after intraperitoneal injection of idazoxan (5 and 20 mg/kg), fluoxetine (10 mg/kg), or vehicle. Idazoxan increased NE, homovanillic acid, and dihydroxyphenylacetic acid in the PVN. Fluoxetine induced a significant increment of 5-HT in PVN, while producing a smaller increase in NE, dopamine, and homovanillic acid. These results support the conclusion that the impact of these drugs on macronutrient intake may be a consequence of their action on endogenous monoamine systems in the PVN. Thus, in this nucleus, the blockade of α_2-noradrenergic receptors, like stimulation of 5-HT receptors, attenuates normal ingestion of carbohydrate.

Idazoxan Fluoxetine Paraventricular nucleus Monoamines Food intake

IN the brain, the paraventricular nucleus (PVN) of the hypothalamus is believed to be an important area in the control of ingestive behavior (24-27). Two neurochemicals in the PVN possibly involved in this process are the monoamines, norepinephrine (NE) and serotonin (5-HT), which appear to function antagonistically in modulating nutrient intake. Whereas PVN NE through postsynaptic α_2-noradrenergic receptors acts to stimulate specifically carbohydrate intake (29), 5-HT in this nucleus is an inhibitor of this behavior (31). Drugs that activate these monoaminergic systems, such as the α_2-receptor agonist clonidine (44,23) and the 5-HT-releasing agent *d*-norfenfluramine (48), produce similar effects to the monoamines themselves. Moreover, these drugs act similarly whether administered peripherally or directly into the PVN, supporting the suggestion that changes in feeding behavior after peripheral drug administration may be attributed, in part, to changes in the activity of the PVN monoaminergic systems. This hypothesis is strengthened by evidence that lesions in the PVN attenuate the feeding effects produced by peripherally injected monoaminergic drugs (26,36).

The present study tested this hypothesis further by examining the feeding effects, as well as the neurochemical changes in the PVN, which occur in response to two drugs, idazoxan (IDA) and fluoxetine (FLU). Pharmacologically, IDA is a potent α_2-adrenergic blocker that antagonizes noradrenergic transmission, both at the presynaptic or postsynaptic level (7,8,42). Fluoxetine, in contrast, is a 5-HT reuptake blocker (11,51) that enhances serotonergic transmission by increasing synaptic concentration of endogenous 5-HT (22,39,50). With regard to the behavioral actions of these drugs, FLU has been shown to suppress carbohydrate intake (32,49,52), consistent with the action of 5-HT itself (26). While the feeding effects of IDA have received little attention, there is one study that indicates that this antagonism may suppress food intake (9). Based on the evidence that NE potentiates carbohydrate intake (29), one would expect this suppressive effect of IDA to be specific to carbohydrate, a prediction to be tested in the present study in animals fed pure macronutrients diets.

The question to be examined further is whether these behavioral effects of the two drugs after peripheral administration can be linked to specific neurochemical changes in the PVN. Based on their known pharmacological action, FLU

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would be predicted to stimulate serotonergic activity within the PVN, while IDA might activate PVN noradrenergic activity via its impact on presynaptic α_2-noradrenergic receptors. To examine these possibilities, we used the microdialysis technique, in combination with high performance liquid chromatography with electrochemical detection (EC-HPLC), which allows one to monitor extracellular levels of monoamines and metabolites in the brains of awake and freely moving rats (19,35,47).

METHOD

Animals

Male albino Sprague-Dawley rats were singly housed in a reverse 12 D : 12 L cycle. These animals included 17 rats (310–380 g) for the microdialysis experiments and 16 rats (250–300 g) for the behavioral experiments.

Diets

The animals in the microdialysis experiments were given Purina rat chow pellets. Those used for the behavioral experiments were maintained on a self-selection feeding paradigm with separate sources of protein, carbohydrate, and fat freely available (49). The protein consisted of 93% casein (National Casein Co.) and 0.03% cysteine (L-cysteine hydrochloride, I.C.N. Pharmaceuticals). The carbohydrate was composed of 28% dextrin, 28% corn starch (I.C.N. Pharmaceuticals), 37% sucrose (Domino). Both diets (3.7 kcal/g) were mixed with 4% minerals (U.S.P. XIV Salt Mixture Briggs, I.C.N. Pharmaceuticals) and 3% vitamins (Vitamin Diet Fortification Mixture, I.C.N. Pharmaceuticals). The fat diet (7.7 kcal/g) consisted of 86% lard (Armour) mixed with 8% minerals and 6% vitamins. These diets were placed in separate glass jars on scales situated next to openings in front of the cage. Presentation of fresh diets, as well as jar rotation to prevent position preferences, was done daily 5 h before dark onset. Water was available ad lib for all the animals.

Behavioral Test Procedures

The animals maintained on the three macronutrient diets received two to three tests each with intraperitoneal (IP) injections of vehicle, IDA (1 mg/kg), or FLU (10 mg/kg) in counterbalanced order, immediately before the onset of the dark cycle. Food was removed and weighed before injection and then returned immediately after drug or vehicle injection. Food intake was measured 120 min after dark onset. Data are expressed and analyzed in terms of kcal intake and also percent of total diet (kcal intake for a specific nutrient/total kcal intake).

Surgery

The rats (n = 17) were anesthetized with pentobarbital (40–60 mg/kg, IP) and placed in a Kopf stereotaxic apparatus. A 12-mm, 21-gauge guide tube was implanted 1.5 mm above the dorsal border of the PVN, according to the atlas of Paxinos and Watson (38) (AP: 6.9 mm; L: 0.4 mm; V: 6.9 mm from flat skull surface). The guide cannula was plugged with a stylet, and the rats were returned to their cages for a minimum recovery period of 6 days. The animals were handled daily to allow adaptation to the microdialysis procedure.

Microdialysis Probe

The concentric removable dialysis probe (18) had a working surface of 2-mm long regenerated cellulose membrane (Spectrum) 6000 Da cut off, o.d.: 170 μm, i.d.: 150 μm. The middle of the functional part was targeted in the PVN (at 8.4 mm from skull). The probes were kept wet in the refrigerator between perfusions.

Microdialysis Test Procedures

The test sessions were carried out between 0630 h (1 h before dark onset) and 1300 h. The dialysis probe was inserted through the guide tube in the awake rat. The animal was placed into a Plexiglas cage (11 × 11 × 26″), with food or water absent during the experiment. The dialysis solution (NaCl 142 mM, KCl 2.4 mM, and $CaCl_2 \cdot 2\ H_2O$ 1.2 mM) was infused at 1 μl/min by Razel syringe pump attached to a fluid swivel system. The dialysate obtained during the first 60 min before dark onset was discarded. Successive 20-min samples were then taken over the next 4 h: three samples before and seven samples after IP injection of IDA (5–20 mg/kg), FLU (10 mg/kg), or vehicle (water) in a counterbalanced manner. Each animal had two to three test sessions with either drug or vehicle, each separated by an interval of 4–5 days.

Analytical Procedure

The mobile phase composition was 75 mM $NaH_2PO_4 \cdot H_2O$ (Sigma); 1.4 mM 1-octanesulfonic acid sodium salt (OSA) HPLC grade (Kodak) (or 1.8 mM OSA in IDA 5 mg/kg experiment to improve separation of the amines); 9.5% acetonitrile HPLC grade (Fisher); 0.01–0.05 mM EDTA (Kodak), pH 3.1 adjusted with O-Phosphoric acid 85% HPLC grade (Fisher). The mobile phase, degassed constantly with Helium, was delivered by PM-48 BAS pump at a flow rate of 1 ml/min onto a Hypersil ODS 3 μm, 15 cm L × 4.6 mm i.d. column (Alltech).

Samples were manually injected (model 7125 Rheodyne injector) immediately after collection and analyzed using a electrochemical detector (COULOCHEM II, 5011 analytical cell, ESA). The system was set with a guard cell (+400 mV of applied potential). The detection was carried out with an analytical cell with two working electrodes, an applied potential o.d. −40 mV in the first one and +320 mV in the second one. The sensitivity of the detector was 10 nA in all the experiments.

Identification (by retention times) and quantitation of the compounds (by peak size) in the samples was achieved by comparison to standard solutions containing 20 pg/20 μl of NE, 5-HT, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5HIAA), 4-hydroxy-3-methoxy-phenylacetic acid (HVA). All chemicals were analytical grade or HPLC grade when possible. They were dissolved in deionized purified 18.2 MΩ water (Alfa Q Millipore Corp.) The monoamines and metabolites were obtained from Sigma and prepared as 1 mg/ml stock solution in 0.1 M HCl with 100 μM EDTA.

Drugs

Idazoxan hydrochloride (RX 781094) was purchased from Research Biochemical Incorporated. Fluoxetine hydrochloride (Ly 110140) was generously supplied by Eli Lilly & Co.

Statistical Analyses

For the dialysis experiments, the values are expressed in pg/20 μl sample (Figs. 1 and 2). In Table 1, the results for the 5 mg/kg dose of IDA are expressed as percent change from

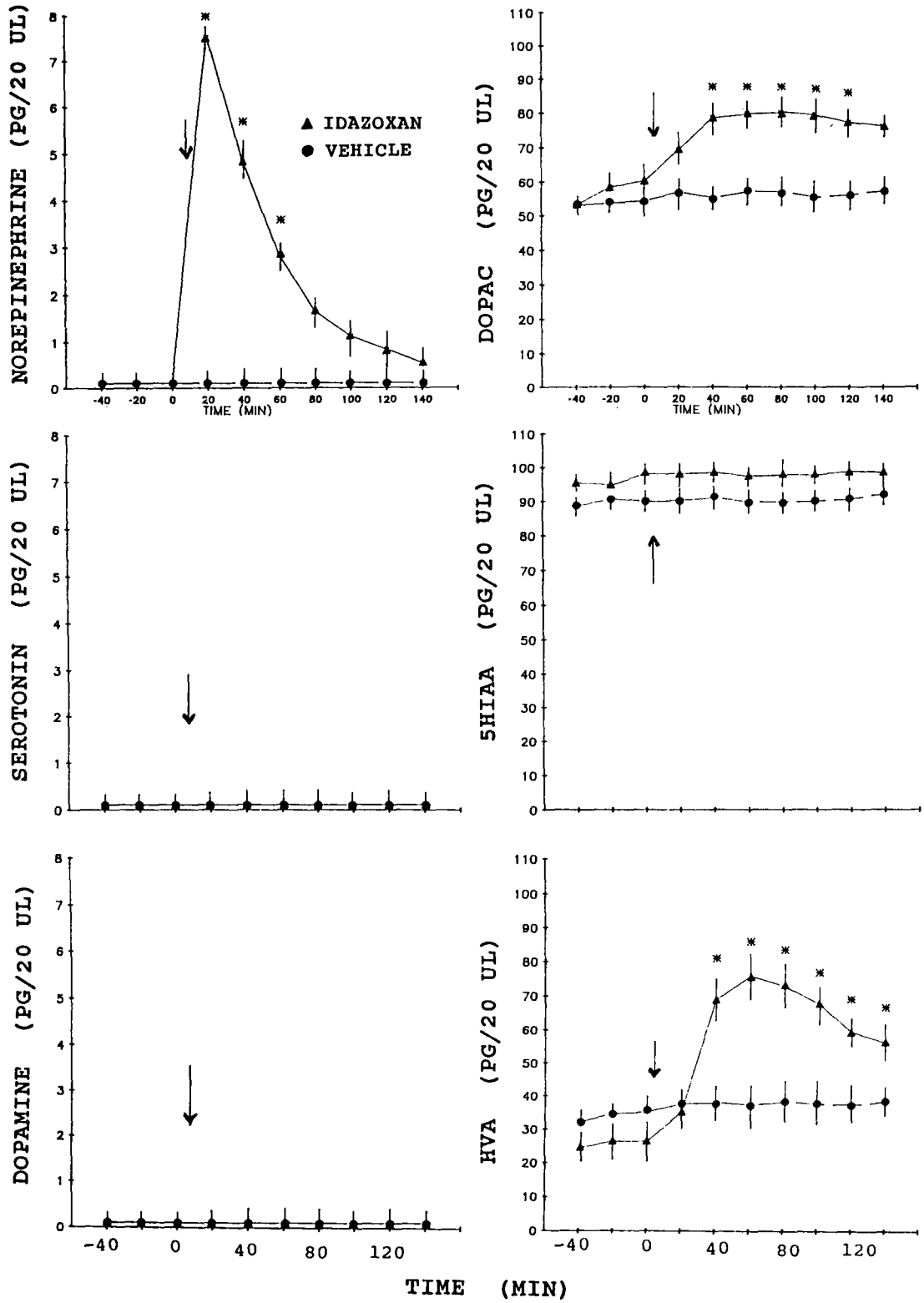

FIG. 1. Mean ± SEM (pg/20 μl) extracellular content of the monoamines and metabolites in dialysates from PVN, before (−40 to 0 min) and after (20 to 140 min) 20 mg/kg IP idazoxan. Arrow indicates the time of injection; *$p < 0.01$ for drug vs. vehicle.

each animal's baseline pretreatment score (i.e., mean of the three preinjection samples). The data were analyzed by analysis of variance with a repeated measurement design. Simultaneous multiple comparisons were based on the least significant difference test. The null hypothesis was rejected at the 0.05 level.

Histology

Animals were perfused intracardially with 0.9% NaCl first and then with 10% buffered formalin. The brains were removed and sliced with a freezing microtome and then stained with cresyl violet to verify the track of the guide and probes. All rats were found to have their probes either within the PVN or along its borders.

RESULTS

Idazoxan Microdialysis Experiment

The α_2-receptor blocker IDA, at doses of 5 and 20 mg/kg, produced a large and relatively selective increment in extracellular NE levels in the PVN. These results are presented in Fig. 1 for the 20 mg/kg dose, in terms of absolute amounts (pg) of NE/ 20-min sample, and in Table 1 for the 5 mg/kg dose, expressed as percent change relative to preinjection baseline. For both doses, the stimulatory effect of IDA on NE was detected in the first 20-min postinjection sample. It was maximum during this and the subsequent 20-min period [20 mg/kg, $F(1, 80) = 109.2$, $p < 0.01$; 5 mg/kg, $F(1, 24) = 22.75$, $p < 0.01$] and was still apparent in the third 20-min sample but not in the subsequent periods. With regard to DA, IDA at 20 mg/kg had no effect on this amine (Fig. 1), while 5 mg/kg IDA evoked a small, transient increment, $F(1, 20) = 13.63$, $p < 0.01$, lasting only 20 min (Table 1). No significant change in 5-HT was seen with either dose of IDA.

With respect to the metabolites, IDA at 20 mg/kg evoked a significant elevation of DOPAC, $F(1, 79) = 38.1$, $p < 0.01$, and HVA, $F(1, 79) = 18.8$, $p < 0.01$, from 40 min after injection to the end of the experiment (Fig. 1). Similar changes in DOPAC, $F(1, 20) = 15.4$, $p < 0.01$, and HVA, $F(1, 23) = 34.5$, $p < 0.01$, were seen with 5 mg/kg of IDA (Table 1), although they appeared to have a shorter duration at this lower dose. No change in 5-HIAA was seen with either dose.

Fluoxetine Microdialysis Experiment

Peripheral administration of the 5-HT uptake blocker FLU (10 mg/kg), compared to its vehicle, induced a significant increment in PVN levels of the three monoamines, although its effect on 5-HT appeared strongest (Fig. 2). The stimulatory effect of FLU on 5-HT, $F(1, 49) = 30.5$, $p < 0.01$, was apparent during the first 20 min after injection and reached a peak at 60 min. Somewhat smaller stimulatory effects were also seen in the case of NE, $F(1, 70) = 46.6$, $p < 0.01$, and DA, $F(1, 59) = 35.8$, $p < 0.01$. While HVA (Fig. 2) increased significantly, $F(1, 60) = 12.9$, $p < 0.01$, no changes in 5-HIAA and DOPAC levels were detected after FLU injection.

TABLE 1

MEAN ± SE PERCENT CHANGES FROM PREINJECTION BASELINE IN MONOAMINES AND METABOLITES IN THE PVN AFTER IP INJECTION OF IDAZOXAN (5 mg/kg) OR VEHICLE

	Time			
Compound/Treatment	20 Min	40 Min	60 Min	120 Min
NE				
Vehicle	5.6 ± 4.8	7.9 ± 7.1	7.9 ± 7.1	5.6 ± 4.8
IDA	143.7 ± 33.5*	128.0 ± 13.5*	74.1 ± 8.9†	24.0 ± 13.0
DOPAC				
Vehicle	4.1 ± 4.5	7.3 ± 5.8	9.1 ± 8.4	8.1 ± 8.8
IDA	48.8 ± 14.9	109.6 ± 35.7†	106.6 ± 35.7†	105.3 ± 47.8
DA				
Vehicle	−2.1 ± 1.4	−28.3 ± 11.6	−33.3 ± 16.6	−13.3 ± 13.3
IDA	75.8 ± 31.0†	137.7 ± 66.6	108.0 ± 53.8	33.4 ± 34.2
HVA				
Vehicle	8.2 ± 4.3	13.4 ± 4.2	12.3 ± 6.8	19.1 ± 7.5
IDA	34.9 ± 5.9	140.5 ± 33.8†	178.4 ± 41.5†	124.5 ± 42.4
5HT				
Vehicle	−10.1 ± 13.2	−23.6 ± 7.8	−27.2 ± 11.4	−26.6 ± 26.1
IDA	−29.0 ± 23.3	−33.1 ± 8.9	−26.4 ± 12.2	−16.0 ± 11.7
5HIAA				
Vehicle	0.3 ± 2.1	−2.5 ± 1.5	1.4 ± 1.8	2.4 ± 1.6
IDA	−2.5 ± 1.3	1.4 ± 0.8	0.3 ± 1.1	1.5 ± 1.9

Baseline value is the mean of three pretreatment samples for each rat. These values (pg/20 μl sample) ranged as follows: for norepinephrine (NE), 2.4–3.5; dopamine (DA), 0.5–0.6; homovanillic acid (HVA), 37–49; 3,4 dihydroxyphenylacetic acid (DOPAC), 35–60; serotonin (5HT), 1–2; 5-hydroxyindoleacetic acid (5HIAA), 79–91.

* < 0.01, † $p < 0.05$ drug vs. vehicle.

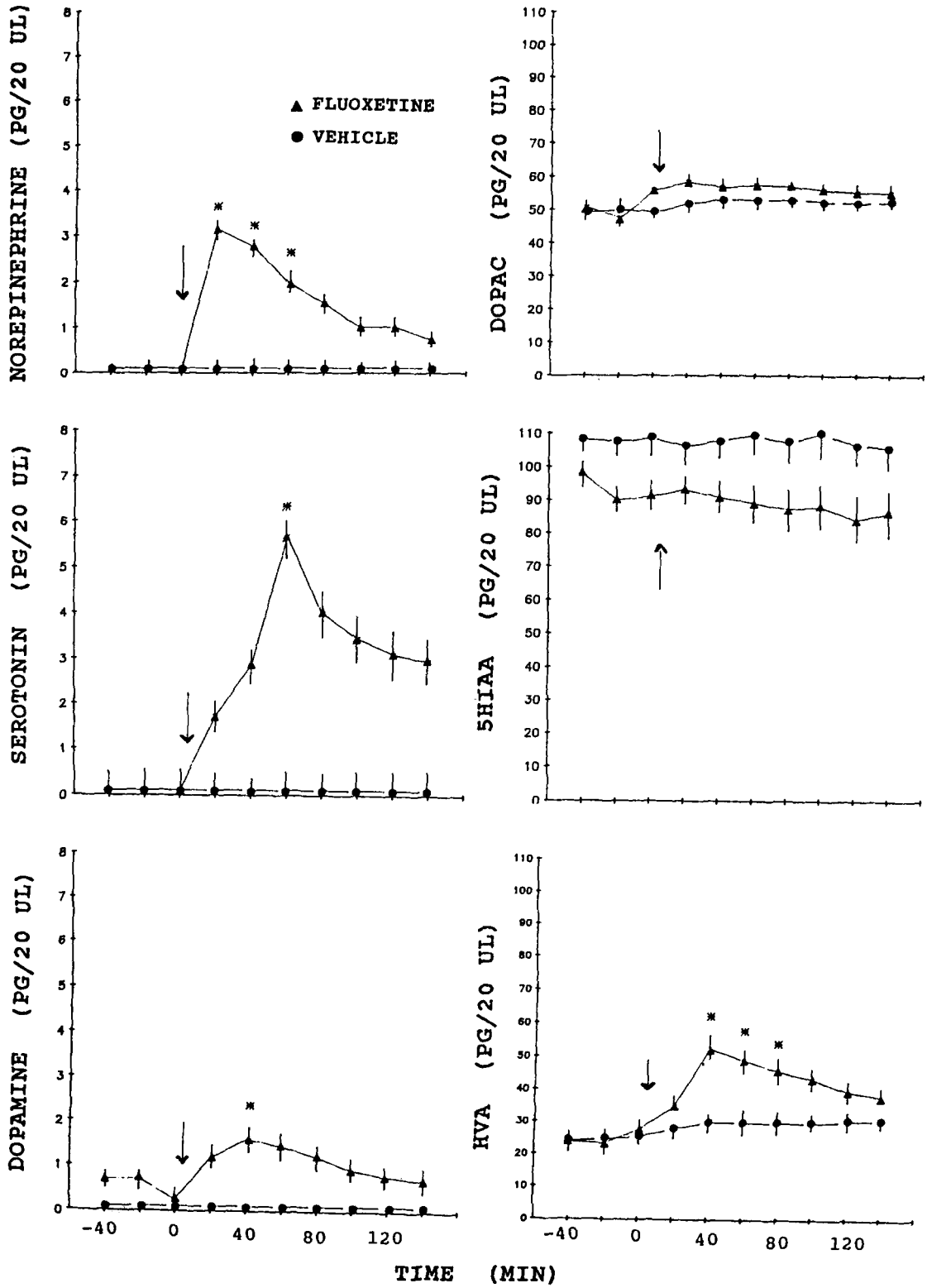

FIG. 2. Mean ± SEM (pg/20 μl) extracellular content of monoamines and metabolites in dialysates from PVN, before (−40 to 0 min) and after (20 to 140 min) 10 mg/kg IP fluoxetine. Arrow indicates the time of injection; *$p < 0.01$ drug vs. vehicle.

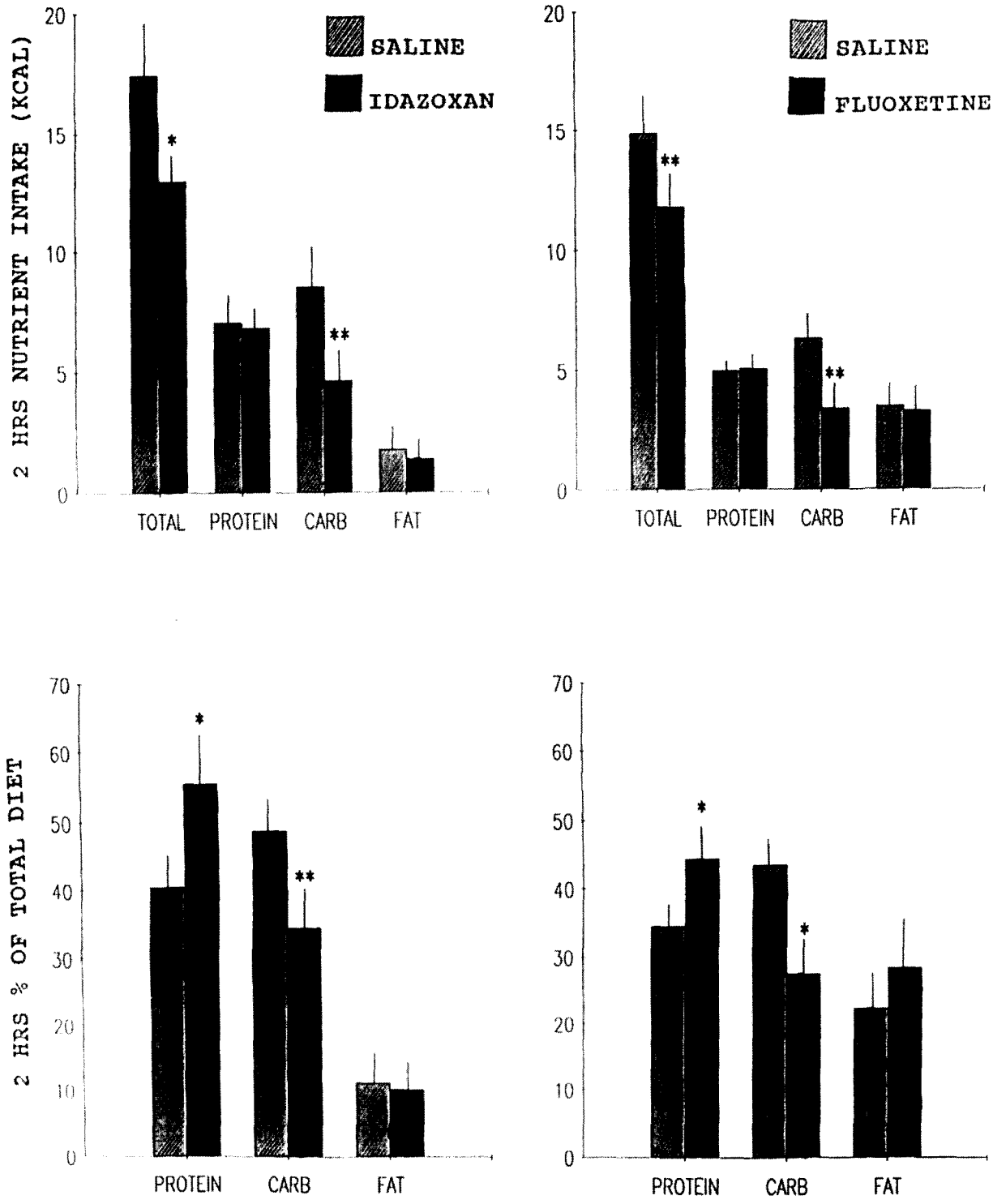

FIG. 3. Top: mean ± SEM (kcal) of food intake after idazoxan (1 mg/kg IP) (n = 7) and FLU (10 mg/kg IP) (n = 9). Bottom: mean ± SEM of percent concentration of one nutrient relative to total food intake. *$p < 0.01$, **$p < 0.001$ for drug vs. vehicle.

Feeding Experiment

The results in Fig. 3 show that IDA (1 mg/kg) and FLU (10 mg/kg) had similar effects on nutrient intake. Both drugs caused a significant reduction (20–30%) in total food intake ($p < 0.01$) in the 120-min period after injection. In both cases, there was a reliable and selective decrease (30–40%) in carbohydrate intake ($p < 0.01$), which significantly reduced

the concentration of carbohydrate in the total diet from approximately 45% after vehicle to 30% after the drugs ($p < 0.01$). While no change in kcal intake of protein was seen with either drug, the selective reduction in carbohydrate intake and in percent of carbohydrate in the diet resulted in a significant rise in the protein concentration of the rats' diet, from 37% to 50% ($p < 0.01$). No change in fat intake or preference for this diet was seen after IDA or FLU.

DISCUSSION

Peripheral administration of IDA and FLU induced significant changes in extracellular levels of monoamines in the PVN. Idazoxan, an α_2 adrenoreceptor blocker (7,8), evoked a dose-dependent rise in NE. Previous studies, using microdialysis in the cerebral cortex (6), posterior hypothalamus (42), and hippocampus (45), have shown a similar increase in extracellular NE, suggesting a blockade of presynaptic α_2 receptors that are inhibitory towards NE turnover (6,37). In the present study, IDA also induced a significant rise in DOPAC. In NE-rich areas, such as the PVN, this increase in DOPAC may possibly reflect an increase in turnover of NE (3,46).

Fluoxetine, a 5-HT reuptake blocker (11,51), caused an elevation of extracellular 5-HT in the PVN. The enhanced release of 5-HT, after acute peripheral and central administration of FLU, has been seen previously in other brain areas, namely, the hippocampus (22,50), nucleus accumbens (17), posterior hypothalamus (1), striatum (39). In some of these studies, as in the present report, no changes in 5-HIAA were observed. It has been suggested that 5-HIAA may reflect primarily intraneural metabolism and, thus, may not be a good indicator of synaptic activity (1,21).

The present results showed an additional effect of IDA and FLU on dopaminergic activity, with both compounds increasing DA and HVA. Recently, a rise of DA and HVA during dialysis has been reported after injection of yohimbine, another α_2 adrenoreceptor blocker (4). These results may reflect an indirect effect, through the impact of these compounds on noradrenergic and serotonergic systems, which, in turn, modulate DA. A possible direct effect of these drugs on dopaminergic systems, however, is suggested in an electrophysiological study that showed IDA to modulate DA-containing neurons in the ventral tegmental area (16). The rise in NE levels, in addition to 5-HT, after injection of FLU is also difficult to interpret as either a direct or indirect effect on noradrenergic transmission. These results simply reaffirm numerous observations indicating that drugs, while preferentially affecting one neurochemical system, are also likely to influence other systems as well.

Idazoxan and FLU produced similar changes in feeding, namely, a reduction of total caloric intake, associated with a decrease specifically in carbohydrate intake and an increment in preference for protein. This effect with IDA is consistent with other evidence showing α_2 antagonists to reduce food intake (5). Moreover, it is predicted on the basis of the evidence that NE in the PVN increases total caloric intake and carbohydrate intake, an effect mediated via postsynaptic α_2 receptors (15,28). Together, this evidence supports a possible physiological role for these receptors in natural carbohydrate feeding. The additional finding, that IDA through blockade of presynaptic α_2 receptors strongly and relatively selectively enhances NE levels in the PVN, is at least consistent with this proposed role for endogenous NE and its α_2 receptors in feeding behavior. However, this interpretation of the data is clearly complicated by the fact that the doses of IDA used in the biochemical study (5–10 mg/kg) were higher than that tested in the behavioral experiment (1 mg/kg) and also by the recent evidence that IDA, at doses of 3 and 10 mg/kg, may, in fact, stimulate feeding through its actions on nonadrenergic sites (20).

Fluoxetine, by blocking 5-HT reuptake, increases the extracellular levels of 5-HT and the availability of 5-HT for acting on postsynaptic 5-HT receptors in the PVN. Serotonin is believed to be involved in mechanisms of satiety within the medial hypothalamus and specifically in the selective reduction of carbohydrate intake (2,23,26,31,32,34,47,48,52). Moreover, recent studies have shown that inhibition of the serotonergic system, with postsynaptic receptor antagonists (12) or presynaptic agonists (10,33), produces the opposite effect, namely, a stimulation of food and carbohydrate intake. Thus, the anorectic effect of FLU on carbohydrate intake in the present study, along with the stimulatory effect of FLU on 5-HT in the PVN, support a role for endogenous 5-HT in controlling natural feeding of carbohydrate.

It has been proposed that NE and 5-HT in the PVN have an antagonist function in modulating carbohydrate ingestion (30). The present behavioral results support this suggestion, showing that an antagonist of the α_2 receptors mediating NE's actions modulates nutrient intake in a manner identical to that seen with a 5-HT agonist. This common behavioral effect agrees with the main biochemical changes detected in the PVN after drug administration. Moreover, this proposed interaction between NE and 5-HT in a specific hypothalamic area is consistent with evidence for an interaction between these systems in other brain areas (13,14,40,41). The behavioral and biochemical evidence together support the proposal that the drugs, FLU and IDA, may be producing their effects on macronutrient consumption, in part, through their action on monoaminergic systems in the PVN.

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