Metabolic and Hormonal Responses to Hypothalamic Administration of Norfenfluramine in Rats

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SCHEURINK, A. J. W., H. LEUVENINK AND A. B. STEFFENS. Metabolic and hormonal responses to hypothalamic administration of norfenfluramine in rats. PHYSIOL BEHAV 53(5) 889-898, 1993.—The effects of intrahypothalamic administration of norfenfluramine (NFFL), an anorectic agent that increases serotonergic transmission, on plasma concentrations of glucose, free fatty acids (FFA), and their regulating hormones were investigated in resting and exercising rats. Infusion of 5 μg NFFL in 0.125 μl aCSF/min into the nucleus paraventricularis of the hypothalamus (PVN) caused a significant increase of blood glucose, plasma epinephrine (E), and corticosterone concentrations. Plasma levels of FFA, insulin, or norepinephrine (NE) remained unchanged. Lower doses of NFFL (0.5 and 0.05 μg/min) did not affect peripheral metabolism. The effects of NFFL in the PVN were completely prevented by prior administration of a 5-HT₁ antagonist, (S)-(-)propranolol. The exercise-induced increase of plasma NE was reduced after prior administration of 5 μg NFFL/min into the PVN. Plasma E responses tended to be increased. The exercise-induced alterations in glucose, FFA, corticosterone, and insulin were not affected by NFFL infusion into the PVN. The data suggest that activation of serotonergic mechanisms in the PVN might change the neurohormonal response to a stressor favouring the release of adrenal hormones above activation of the neuronal branch of the sympathetic nervous system.

FENFLURAMINE (NFFL) is an anorectic agent that enhances serotonergic transmission. It increases the release and it inhibits the reuptake of serotonin (5-HT) in serotonergic nerve terminals (11,13,27). It also directly activates postsynaptic serotonergic receptors when administered in high concentrations (27). The anorectic effects of NFFL, i.e., inhibition of total food intake and changes in food preference, are mediated by 5-HT receptors in the brain, in particular postsynaptic 5-HT₁ receptors in the paraventricular nucleus of the hypothalamus (PVN) (21–23,42). Furthermore, effects of NFFL on peripheral energy metabolism also contribute to the anorectic action of NFFL (10,29,30).

The effects of 5-HT and other serotonergic agents on peripheral energy metabolism are well documented. In general, 5-HT influences peripheral metabolism mainly by parallel activation of the sympathoadrenal system and the pituitary–adrenocortical axis. For example, intravenous administration of the 5-HT₁₄ agonists, buspirone and ipsapirone, caused a marked increase of blood glucose and concomitant increases of plasma epinephrine (E), norepinephrine (NE), and corticosterone concentrations (6,18). Also, IV injection of the 5-HT₁₄ agonist, 8-hydroxy-(di-n-propylamino)tetralin (8-OH-DPAT), caused a marked increase in baseline blood glucose, plasma E, and corticosterone concentrations (2,7,8).

Recent experiments by Korte et al. (17) suggest that the effects of peripherally administered serotonergic agents might be mediated by centrally located mechanisms. In these experiments, local infusion of minute amounts of 8-OH-DPAT into the PVN resulted in consistent increases of both plasma E and corticosterone concentrations. An increase in blood glucose levels occurred with some delay. Also, experiments in which depletion of 5-HT in the hypothalamus completely blocked the adrenocortical responses following afferent neural stimuli (12) suggest that the hypothalamus might play an important role in the effects of 5-HT on peripheral metabolism.

To summarize, substantial evidence indicates that the effects of NFFL on food intake behavior and nutrient preference are mediated via serotonergic mechanisms in the hypothalamus (PVN). The effects of serotonergic agonists on peripheral metabolism seem also to be mediated via hypothalamic 5-HT receptors, most likely located in the PVN (12,17). Therefore, it is conceivable that hypothalamic pathways might be involved in the supposed effects of NFFL on peripheral energy metabolism.
In the present study, we investigated this by monitoring the effects of hypothalamic administration of NFFL on the peripheral concentrations of the main energy substrates glucose and free fatty acids, and their regulating hormones such as insulin, catecholamines, and corticosterone in resting and exercising rats. The results reveal that central administration of NFFL markedly affects peripheral energy metabolism, probably by activation of 5-HT \textsubscript{1} receptors in the PVN.

**METHOD**

**Animals and Housing**

Male Wistar rats, weighing 300-330 g at the beginning of the present experiments, were used. The animals were individually housed in Plexiglas cages (25 × 25 × 30 cm) at room temperature (20 ± 2°C), and had continuous access to food (Hope Farm chow) and water unless otherwise stated. The rats were maintained on a 12:12 h light:dark regime (0700-1900 h light), and housed in Plexiglas cages (25 X 25 X 30 cm) at room temperature (20 ± 2°C), and had continuous access to food (Hope Farm chow) and water unless otherwise stated. The rats were maintained on a 12:12 h light:dark regime (0700-1900 h light), and they were handled and weighed every day at 0900 h.

**PVN and Heart Cannulation**

Surgery was performed under ether anesthesia. All animals were provided with permanent cannulas in the heart and in the hypothalamus. Bilateral permanent stainless steel cannulas (in mm: 21.0 length, 0.3 o.d., 0.1 i.d.) for drug infusion were stereotactically implanted into the PVN [in mm: anterioposterior (AP) 7.2, ventral (V) 2.3, lateral (L) ±0.5] according to the coordinates of Paxinos and Watson (31). A sterile stainless steel obturator, flush with the tip of the cannula, was inserted into each cannula between experiments to ensure that the cannula remained patent and pyrogen free. The cannula end protruding from the skull was protected with a 2 IG protective sleeve. A polyethylene cap was placed at the protruding end of the protective sleeve to cover the cannula. The PVN infusion procedure was similar to techniques described earlier (39). After termination of the experiments, brain cannula placement was histologically verified in 40-μm brain slices under a light microscope. Only animals with correct cannula placement were included in the experiments.

All animals were also provided with a silicon heart catheter (0.95 mm o.d., 0.50 mm i.d.), inserted into the heart through the right jugular vein and externalized on the top of the skull according to techniques described earlier (45). This method allows frequent repeated blood sampling in unanesthetized, undisturbed, freely moving animals (44). The rats were accustomed to the brain infusion and blood-sampling procedures three to five times before the start of the experiments. The experiments started 1 week after insertion of the heart catheter when the rats were above their preoperative weights.

**Infusion Solutions**

In all experiments, norfenfluramine (NFFL, Servier, France) was infused into the PVN. The drug was dissolved in sterile artificial cerebrospinal fluid (aCSF) containing (in mM) 127.64 NaCl, 2.55 KCl, 1.26 CaCl\textsubscript{2}, and 0.93 MgCl\textsubscript{2}·6H\textsubscript{2}O. Fenfluramine was bilaterally infused into the PVN for 17 min at a rate of 0.05, 0.5, or 5 μg in 0.125 μl aCSF per minute. The two lower doses mimicked concentrations of NFFL that may reach the brain after oral administration of doses of NFFL that influence food intake and peripheral metabolism. The highest (5 μg) dose was comparable to the doses used in previous studies in which feeding behavior was studied (21,22,42). In Experiment 2, 0.3 mg/kg of the 5-HT \textsubscript{1} antagonist, (S)-(−)-propranolol (Sigma, St. Louis, MO) (13,16), dissolved in 0.5 ml saline, was intravenously injected prior to and during intrahypothalamic infusion of the highest dose of NFFL.

**Exercise**

Exercise was performed in a pool made of stainless steel (length 3.00 m, width 0.40 m, and depth 0.90 m) filled 70% with water at 33 ± 2°C. The pool was equipped with a starting platform (33 × 37 cm) placed 2 cm above the water level. This starting platform could be lowered into the water down to the bottom of the swimming pool. A water pump (Loewe Silenta, Germany) provided a counter current of 0.22 m/s that forced the animal to swim continuously. At the end of the exercise period, a removable resting platform (20 × 37 cm) at the upstream side of the swimming pool was offered to the swimming rat. The rats readily learned to climb on this lighted and warmed platform within 2 min after presentation. Rats were accustomed to the exercise conditions before the onset of the actual experiments.

**Experimental Procedure**

All experiments were performed in the light period between 0900 and 1200 h. On the experimental day, food was removed from the home cages 1.5 h before the start of the experiment. Then, 40 min before the first blood sample was taken, the animals were connected to two polyethylene PVN infusion tubings (in mm: 400 length, 0.61 o.d., and 0.20 i.d.) and one blood-sampling tubing (in mm: 300 length, 1.25 o.d. and 0.75 i.d.). Throughout the experiment, 10 blood samples (0.6 ml each) were taken for determination of the glucose, insulin, catecholamine, and corticosterone levels in blood. After each sample a transfusion of 0.6 ml of heparinized donor blood was given to avoid diminution of the blood volume with related changes in hemodynamics. Donor blood was obtained from undisturbed rats with permanent heart catheters.

In all experiments, two blood samples in a 10-min interval were taken in the home cage of the rat to measure baseline levels of the blood components. Then, at \( t = 0 \) min, different doses of norfenfluramine or control solution (aCSF) were infused into the PVN. The infusion was discontinued at \( t = 17 \) min. In Experiments 1 and 2, blood samples were taken before, during, and after hypothalamic infusion of NFFL at the time points \( t = -11, -1, 5, 10, 15, 21, 25, 35, 45, \) and 55 min relative to the start of the infusion.

In Experiment 3, the animal had to perform strenuous exercise immediately after the infusion period. For this, the rat was transferred from the home cage to the starting platform of the swimming pool after termination of the infusion at \( t = 17 \) min. At \( t = 20 \) min relative to the start of the infusion, the starting platform was lowered down to the bottom of the swimming pool, and the rat had to swim vigorously against the counter current for 15 min. At the end of the exercise period (at time point \( t = 35 \) min) the resting platform was offered, and the animal was transferred back into his homecage. In this experiment, blood samples were taken at the time points \( t = -11 \) and \(-1 \) (baseline), 5 and 15 (during infusion), 21, 25, 30, and 35 (during exercise), 45 and 55 min (postinfusion and postexercise).

**Chemical Determinations**

Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.1% EDTA as antioxidant and 10 μl heparin solution (500 U/ml) as anticoagulant. Blood glucose was measured by the ferricyanide method of Hoffman (Tech-
nicon Auto Analyzer TMII) with 0.05 ml blood taken from the sample. The remaining part was centrifuged for 15 min at 5000 rpm at 4°C. The supernatant was divided into three parts: 100 μl was immediately stored at -80°C for catecholamine measurements, 100 μl was used for the FFA assay, and the remaining plasma was stored at -30°C for the corticosterone and insulin assays.

Determination of plasma catecholamine concentrations was performed by high pressure liquid chromatography (HPLC) in combination with electrochemical detection (ECD) as previously described (32) with some minor modifications. The HPLC-ECD system included an LKB 2150 pump (LKB Instruments, Bromma, Sweden), a Rheodyne injection valve with a 100 μl loop, a reversed-phase nucleosil C18 column (length 25 cm, i.d. 4 mm) (Gimex, The Netherlands) held at 30°C by a column stove (LKB), an ESA 5100 A electrochemical detector with a 5011 high-sensitive analytical cell and a 5020 guard cell (ESA), and a BD 41 two-channel flat recorder (Kipp). Guard cell potential in front of the injection valve was +450 mV; the potentials of the working electrodes were -50 mV and +350 mV, respectively. The mobile phase contained 0.05 M Na-acetate, 0.08% heptane sulfonic acid, 0.01% EDTA, 0.01% NaCl, and 5% methanol 95% H2O (pH 4.75). The limit of the detection level for epinephrine was 0.010 ng/ml and 0.005 ng/ml for norepinephrine. Intra- and interassay coefficients for the catecholamine measurements were 2.25% and 5.36%, respectively.

Plasma FFA was determined according to the method of Antonis (1) by adapting it for small volumes. Plasma was immediately extracted and the evaporated extracts were stored at -30°C until determination. Rat-specific plasma immunoreactive insulin was determined by means of a radioimmunoassay kit (NOVO, Denmark). Guinea pig serum MR8309 served as antisem. Duplicate assays were performed on 25 μl samples. The bound and free 125I-labeled insulin was separated by means of a polyethylene glycol solution (23.75% w/w) as described by Henquin et al. (14).

Measurement of plasma corticosterone concentrations was performed by HPLC in combination with UV detection. Corticosterone was extracted from 30 μl plasma according to the techniques used by Shimuzu et al (41) with minor modifications and adaptations for small volumes.

**Experiments 1 and 2: Infusion of Norfenfluramine Into the PVN**

The aim of Experiments 1 and 2 was to investigate the effect of hypothalamic administration of different doses of norfenfluramine (NFFL) on peripheral concentrations of the energy substrates glucose and free fatty acids (FFA), and their regulating hormones insulin and catecholamines, in freely moving, undisturbed rats. Therefore, in Experiment 1, three doses of NFFL (0.05, 0.5, and 5 μg/min) were bilaterally infused into the PVN at a rate of 0.125 μl/min/cannula for 17 min. In the control experiment aCSF was infused into the PVN. Blood was sampled as described. The concentrations of blood glucose and plasma FFA were determined in all blood samples in all experiments. Plasma catecholamine concentrations were determined in the blood samples taken at the time points t = -11, -1, 5, 15, 25, and 45 min. Likewise, plasma corticosterone concentrations were measured at t = -1, 10, 21, and 55 min, and plasma insulin concentrations at the time points t = -11, 5, 15, 25, and 45 min.

In Experiment 2, the 5-HT1 antagonist, (S)-(-)propranolol (27,31), was intravenously injected immediately after the blood samples at the time points t = -11, -1, 5, and 10 min to characterize the serotonergic receptors involved in the effects of intrahypothalamic infusion of the highest dose of NFFL on peripheral energy substrate metabolism. The experimental design was similar to that in Experiment 1, except for the IV administration of the 5-HT1 antagonist. Blood samples were taken for determination of blood glucose, plasma catecholamines, insulin, and corticosterone at the same time points as in Experiment 1. Three procedures served as controls: the experiment in which the high dose of NFFL was infused into the PVN without administration of the 5-HT1 antagonist, the control experiment in which aCSF was infused into the PVN, and a control experiment in which aCSF was infused into the PVN together with intravenous administration of 0.3 mg/kg of the 5-HT1 antagonist, (S)-(−) propranolol.

**Experiment 3: Exercise and NFFL Infusion Into the PVN**

In Experiment 3, the effect of hypothalamic administration of the high dose of norfenfluramine (NFFL) on the exercise-induced changes in peripheral concentrations of the energy substrates glucose and free fatty acids (FFA), and their regulating hormones insulin and catecholamines, was investigated. Norfenfluramine (5 μg/min) was bilaterally infused into the PVN at a rate of 0.125 μl/min/cannula for 17 min. Thereafter, the animals were subjected to strenuous exercise in the swimming pool. In the control experiment aCSF was infused into the PVN. Blood glucose and plasma FFA concentrations were determined in all blood samples taken. Plasma catecholamines were determined in blood samples taken at the time points t = -11, -1, 15, 25, 35, and 45 min. Plasma insulin concentrations were measured at the time points t = -11, 15, 25, and 45 min.

**Data Analysis and Statistics**

Data were expressed as average changes ± SE from baseline values at t = -1 min before infusion. Within each experiment, Wilcoxon matched-pairs signed rank test was used to compare the levels of the blood components at each time point relative to the baseline level at t = -1 min. Differences between NFFL-infused rats and the control group were determined by applying a Mann–Whitney U-test for the changes recorded at each sample point. Significance was set at p < 0.05.

**RESULTS**

**Experiment 1**

Baseline concentrations of all the blood components are presented in Table 1. No differences between the NFFL-treated animals and the control group were found for any of the baseline measurements. The changes in blood glucose and FFA concentration as response to intrahypothalamic infusion of various doses of NFFL are depicted in the Figs. 1 and 2. Infusion of two low doses of NFFL into the PVN failed to influence blood glucose concentrations. Infusion of the high dose (5 μg/kg/min) of NFFL caused an immediate increase in blood glucose from a baseline level of 109.3 ± 3.0 mg/dl at t = -1 min up to a maximum of 142.5 ± 6.1 mg/dl at t = 10 min relative to the start of the infusion. After termination of the infusion, blood glucose concentrations slowly declined and reached control values at t = 55 min. The increase above control values was significant at the time points t = 5, 10, 15, 21, 25, 35, and 45 min. Plasma levels of FFA did not change during or after infusion of NFFL or aCSF into the PVN.

The changes in plasma epinephrine (E) and norepinephrine (NE) levels during infusion of different doses of NFFL and aCSF are presented in the Figs. 3 and 4. Intrahypothalamic infusion...
TABLE 1
BASELINE CONCENTRATIONS OF BLOOD GLUCOSE, PLASMA FFA, CATECHOLAMINES,
AND CORTICOSTERONE AT TIME POINT \( t = -1 \text{ min} \) IN THE HOME CAGE

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Blood Glucose (mg/dl)</th>
<th>Plasma FFA (( \mu \text{Eq/ml} ))</th>
<th>Plasma E (ng/ml)</th>
<th>Plasma NE (ng/ml)</th>
<th>Plasma Corticosterone (( \mu \text{g/ml} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCSF [6]</td>
<td>114 ± 2</td>
<td>0.18 ± 0.04</td>
<td>0.06 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>NFFL1 [8]</td>
<td>116 ± 4</td>
<td>0.12 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.44 ± 0.14</td>
<td>--</td>
</tr>
<tr>
<td>NFFL2 [6]</td>
<td>107 ± 2</td>
<td>0.19 ± 0.05</td>
<td>0.07 ± 0.02</td>
<td>0.30 ± 0.07</td>
<td>--</td>
</tr>
<tr>
<td>NFFL3 [8]</td>
<td>109 ± 3</td>
<td>0.13 ± 0.02</td>
<td>0.03 ± 0.01</td>
<td>0.23 ± 0.03</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Prop [5]</td>
<td>109 ± 3</td>
<td>0.17 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>0.27 ± 0.05</td>
<td>28 ± 6</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCSF [6]</td>
<td>106 ± 3</td>
<td>0.17 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.35 ± 0.04</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>NFFL3 [8]</td>
<td>110 ± 2</td>
<td>0.20 ± 0.04</td>
<td>0.02 ± 0.01</td>
<td>0.31 ± 0.04</td>
<td>35 ± 4</td>
</tr>
</tbody>
</table>

Number of rats in brackets.

of the high dose of NFFL altered the concentration of E in plasma. The other doses and the control solution failed to have any effect. In the experiment in which the high dose of NFFL was infused, plasma E concentrations increased from baseline values of 34 ± 8 pg/ml up to a maximum of 395 ± 93 pg/ml at \( t = 5 \text{ min} \). Thereafter, E levels remained moderately increased at all time points during and after the infusion of 5 \( \mu \text{g/min} \) NFFL. Plasma levels of E were significantly increased above the values in the control experiment at the time points \( t = 5, 15, 25, \) and 45 min. Plasma levels of NE did not change during or after infusion of NFFL or aCSF into the PVN.

The changes in plasma insulin and corticosterone levels were only determined in the experiments in which the high dose of NFFL and aCSF were infused into the PVN. The results are presented in Table 2 and Fig. 5. Plasma levels of insulin did not change during or after infusion of NFFL or aCSF into the PVN. Plasma corticosterone concentrations increased during infusion of NFFL from baseline concentrations of 30.3 ± 2 ng/ml to a maximum of 49.6 ± 5 ng/ml at \( t = 55 \text{ min} \). The differences with the control experiment were significant at the time points \( t = 10, 21, \) and 55 min.

Experiment 2

Baseline concentrations of the blood components measured in Experiment 2 are also presented in Table 1. The changes from baseline levels are depicted in Figs. 6–10 and Table 2. Injection of the 5-HT\(_1\) antagonist, \((S)-(-)\)propranolol, had no effect on baseline concentrations of the blood components measured. Furthermore, no changes in any of the blood components measured were observed after infusion of either aCSF, or the combination of \((S)-(-)\)propranolol and intrahypothalamic aCSF. Administration of \((S)-(-)\)propranolol prior to a high dose of intrahypothalamic NFFL infusion completely prevented the increase in E as seen in Experiment 1 during and after intrahypothalamic infusion of NFFL. Blood glucose levels increased only moderately during NFFL infusion after prior administration of \((S)-(-)\)propranolol. Finally, prior administration of \((S)-(-)\)propranolol could not prevent the NFFL-induced increase in plasma corticosterone levels. The differences between the ex-
FIG. 3. Plasma epinephrine (E) concentrations in ng/ml before, during, and after infusion of different doses of norfenfluramine (NFFL) or aCSF into the PVN. Data are expressed as in Fig. 1.

Experiments in which NFFL was infused with and without the serotonergic antagonist were significant at the time points $t = 5$ and 10 min for glucose, and at $t = 5$ and 45 min for E.

Experiment 3

In Experiment 3, the effect of hypothalamic administration of the high dose of norfenfluramine (NFFL) or aCSF on the exercise-induced changes in peripheral concentrations of glucose, FFA, insulin, and catecholamines was investigated. Baseline measurements are depicted in Table 1; the changes from baseline are presented in Figs. 11-14 and Table 2. Plasma concentrations of any of the blood components measured did not change during infusion of aCSF into the PVN. Exercise induced an increase in blood glucose, plasma FFA, NE, and E concentrations. Plasma insulin levels were decreased during swimming. The exercise-induced changes in the blood components were very similar to the changes described for the control animals in previous studies from our laboratory (36,37). Intrahypothalamic infusion of NFFL before exercise increased blood glucose and plasma E concentrations, and failed to influence plasma concentrations of FFA, NE, and insulin. These results were equal to the data obtained in Experiment 1. Exercise following NFFL infusion further enhanced blood glucose and plasma E levels. Plasma FFA and NE levels were also increased during exercise after NFFL infusion. To compare the exercise-induced changes in the aCSF- and NFFL-treated groups, the effects of swimming on glucose, E, and corticosterone concentrations were calculated as changes from the data at corresponding time points in the nonexercising animals in Experiment 1. It means that, for each time point, the value in the nonexercising animals receiving aCSF or NFFL was subtracted from the value at the corresponding time point in the exercising animals receiving aCSF or NFFL. The results of the calculations for glucose and E are presented in Figs. 15 and 16. No differences between the NFFL- and the aCSF-treated animals with regard to the exercise-induced changes in glucose, FFA, E, or insulin were found. In contrast, the exercise-induced increase in plasma NE levels was significantly lower in the NFFL-treated animals when compared with the aCSF-infused control rats. In the latter group, plasma NE levels increased up to $2.7 \pm 0.3$ ng/ml at $t = 45$ min, which is similar to control values obtained in previous studies in our lab (36). On the other hand, the exercise-induced increase in plasma NE remained relatively low in the NFFL-treated animals; the maximum of $2.0 \pm 0.3$ ng/ml after 15 min of swimming was markedly lower than in the control group.

TABLE 2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-11</th>
<th>5</th>
<th>15</th>
<th>25</th>
<th>45</th>
</tr>
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<tr>
<td>Experiments 1 and 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCSF [5]</td>
<td>30 ± 6</td>
<td>34 ± 5</td>
<td>42 ± 9</td>
<td>40 ± 7</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>NFFL3 [5]</td>
<td>38 ± 5</td>
<td>42 ± 5</td>
<td>44 ± 6</td>
<td>46 ± 6</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>Prop [5]</td>
<td>37 ± 7</td>
<td>38 ± 9</td>
<td>39 ± 8</td>
<td>42 ± 8</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aCSF [5]</td>
<td>37 ± 5</td>
<td>- -</td>
<td>39 ± 6</td>
<td>22 ± 5</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>NFFL3 [5]</td>
<td>36 ± 7</td>
<td>- -</td>
<td>32 ± 2</td>
<td>26 ± 11</td>
<td>19 ± 4</td>
</tr>
</tbody>
</table>

Number of rats in brackets.
FIG. 6. Blood glucose concentrations in mg/dl before, during, and after intrahypothalamic infusion of 5 μg of norfenfluramine (NFFL) with or without previous intravenous administration of the 5-HT₁ antagonist, (S)-(−)-propranolol. Data are expressed as average changes ± SE from baseline values at the time point t = −1 min in the home cage. * Denotes a significant change from the values in the animals in which the 5-HT₁ antagonist was infused. Infusion time is indicated by horizontal bar. The absolute control experiment in which aCSF was infused is also depicted in this graph.

FIG. 7. Plasma free fatty acid (FFA) concentrations in μEq/ml before, during, and after intrahypothalamic infusion of 5 μg of norfenfluramine (NFFL) with or without previous intravenous administration of the 5-HT₁ antagonist, (S)-(−)-propranolol. Data are expressed as in Fig. 6.

FIG. 8. Plasma epinephrine (E) concentrations in ng/ml before, during, and after intrahypothalamic infusion of 5 μg of norfenfluramine (NFFL) with or without previous intravenous administration of the 5-HT₁ antagonist, (S)-(−)-propranolol. Data are expressed as in Fig. 6.

FIG. 9. Plasma norepinephrine (NE) concentrations in ng/ml before, during, and after intrahypothalamic infusion of 5 μg of norfenfluramine (NFFL) with or without previous intravenous administration of the 5-HT₁ antagonist, (S)-(−)-propranolol. Data are expressed as in Fig. 6.

DISCUSSION

The anorectic drug, norfenfluramine (NFFL), acts via enhancement of serotonergic transmission. It increases the release and inhibits the reuptake of serotonin (5-HT) in serotonergic nerve terminals (11,13,30). In the present experiments, intrahypothalamic infusion of a relatively high dose of NFFL led to a marked increase of blood glucose and plasma concentrations of the adrenal hormones epinephrine (E) and corticosterone. Administration of the 5-HT₁ antagonist, (S)-(−)-propranolol, prior to intrahypothalamic infusion of NFFL completely prevented the NFFL-induced increases of E and, partly, glucose. Prior administration of (S)-(−)-propranolol did not prevent the NFFL-induced increase of plasma corticosterone concentrations. Plasma norepinephrine (NE) and free fatty acid (FFA) levels remained at baseline values during and after infusion of NFFL into the PVN. The results are in agreement with the effects observed by Korte et al. (17), who found that intrahypothalamic administration of a 5-HT₆ selective agonist, 8-OH-DPAT, resulted in immediate increases of both plasma E and corticosterone concentrations. They also observed an increase in blood glucose levels, no changes in plasma NE concentrations, whereas plasma FFA and insulin concentrations were not measured in...
that study (17). The present data on the effects of relatively low doses of centrally administered 8-OH-DPAT and NFFL on peripheral energy substrate metabolism were remarkably similar to the effects observed by others after peripheral administration of much higher doses of 5-HT and other serotonergic agents (2,6-8,18), suggesting that in particular central serotonergic mechanisms are involved (23).

Based on these data, the following underlying mechanism for the NFFL-induced increases of plasma E and glucose might be hypothesized. First, hypothalamic administration of NFFL will lead to increased turnover of serotonin in the PVN, leading to stimulation of, particularly, postsynaptic 5-HT₁ receptors. Activation of these PVN 5-HT₁ receptors causes the activation of a hypothalamic–adrenal-medullary pathway, leading to an increased release of the adrenal hormone epinephrine. As a consequence, blood glucose levels increased, caused by the stimulatory effect of increased E levels on hepatic glucose production (37,40,43).

Hypothalamic administration of NFFL increased plasma corticosterone concentrations in the present study. This confirms earlier findings (12,17) that serotonergic mechanisms in the PVN may play a role in the activation of the hypothalamic–pituitary–adrenal (HPA) axis. The increase of corticosterone outflow is presumably mediated by 5-HT₁ receptors in the PVN, because Korte et al. (17) showed that intrahypothalamic infusion of the selective agonist, 8-OH-DPAT, leads to increased levels of corticosterone in plasma. However, prior administration of the 5-HT₁ antagonist, (S)-(-)propranolol, could not prevent the NFFL-induced increase of plasma corticosterone levels in the present study. This suggests that besides 5-HT₁ receptors, also other subtypes of hypothalamic serotonergic receptors might play

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**FIG. 10.** Plasma corticosterone concentrations in µg/dl before, during, and after intrahypothalamic infusion of 5 µg of norfenfluramine (NFFL) with or without previous intravenous administration of the 5-HT₁ antagonist, (S)-(-)propranolol. Data are expressed as in Fig. 6.

**FIG. 11.** Blood glucose concentrations in mg/dl before, during, and after infusion of 5 µg of norfenfluramine (NFFL) or aCSF into the PVN and exercise. Data are expressed as average changes ± SE from baseline values at the time point t = -1 min in the home cage. * Denotes a significant change from the values in the control animals in which aCSF was infused. Infusion time is indicated by horizontal bar. Exercise period is indicated by dotted area.

**FIG. 12.** Plasma free fatty acid (FFA) concentrations in µEq/ml before, during, and after infusion of 5 µg of norfenfluramine (NFFL) or aCSF into the PVN and exercise. Data are expressed as in Fig. 11.

**FIG. 13.** Plasma epinephrine (E) concentrations in ng/ml before, during, and after infusion of 5 µg of norfenfluramine (NFFL) or aCSF into the PVN and exercise. Data are expressed as in Fig. 11.
a role in the NFFL-induced increase in plasma corticosterone concentrations. Data in the literature suggest that 5-HT2 receptors might be involved (47).

Now the question arises as to which neuronal or hormonal connections, arising from the 5-HT2 receptors in the PVN and activating both the sympathetic nervous system and the pituitary–adrenocortical axis, might be involved. The pathways containing corticotropin releasing hormone (CRH) as the main neurotransmitter might be the major candidate for this function for several reasons. First, neuroanatomical studies revealed that CRH-containing neurons with cell bodies in the PVN synaptically interact with serotonergic axons (24). The CRH-containing neurons in the PVN directly project to areas in the brain such as the central amygdala, the autonomic areas in the brain stem, as well as the locus coeruleus (35,46). These areas are directly connected with the motor neurons of the sympathetic nervous system in the intermediolateral column in the spinal cord and might play an important role in the regulation sympathoadrenal outflow. In addition, the CRH-containing neurons also project to the median eminence, the main (neuro)hormonal pathway between the hypothalamus and the pituitary (28,33,34). Second, physiological evidence for a role of CRH-containing neurons in the PVN in the control of E and corticosterone studies has emerged from a number of studies in which stimulation or lesioning of the PVN have led to alterations in the outflow of CRH and ACTH (5,25,26), and from studies in which intracerebral and/or peripheral administration of CRH specifically changed plasma levels of E and corticosterone (3,4). Finally, recent experiments by Le Feuvre et al. (20) revealed that pretreatment with CRH antibodies could prevent the thermogenic and anorectic effects of centrally administered dexfenfluramine.

Hypothalamic infusion of NFFL increased plasma E levels without any increase of plasma NE concentrations. This means that activation of serotonergic mechanisms in the PVN selectively activates the adrenal medullary branch of the sympathoadrenal system. It might be hypothesized that hypothalamic serotonergic mechanisms particularly play a role in the central nervous response to emotional stress. Direct evidence for this emerged from a study in which lesioning of the PVN reduced the normal stress-induced increase of plasma E but not NE (19). Indirect evidence is provided by studies in which administration of CRH mediated the E response to several stressful stimuli (4), and studies that revealed that emotional stress causes a selective activation of the adrenal medulla without any increase of the outflow of NE from the sympathetic nerve endings (9,38). Further evidence for a role of serotonergic mechanisms in the PVN in the sympathoadrenal response to stress derives from Experiment 3, in which infusion of NFFL into the PVN markedly reduced the exercise-induced increase of plasma NE levels. These results are in concert with our recent findings that emotional stress leads to a reduction in the outflow of neuronal NE during exercise (38).

In Experiment 3, intrahypothalamic infusion of NFFL resulted in increases of blood glucose and plasma E concentrations that were similar to the changes observed in Experiment 1. Exercise further enhanced the levels of these blood components. However, when calculated as changes from nonexercising controls in Experiment 1, the actual exercise-induced changes in glucose and E levels were not different between the NFFL- and the aCSF-treated
animals. This suggest that serotonergic mechanisms in the PVN are not directly involved in the normal exercise-induced changes in the aforementioned blood components.

In summary, the results of the present study reveal that infusion of the anorectic agent, norfenfluramine (NFFL), into the nucleus paraventricularis of the hypothalamus (PVN) markedly increases plasma E, corticosterone, and blood glucose concentrations in unanesthetized, freely moving, and undisturbed rats. In addition, intrahypothalamic administration of NFFL reduces the normal exercise-induced increase in plasma norepinephrine (NE) concentrations. It is concluded that activation of 5-HT receptors in the PVN leads to a parallel activation of the sympathetic-adrenocortical system and the pituitary-adrenocortical axis. Corticotropin releasing hormone (CRH)-containing pathways arising from the PVN and projecting to the pituitary and the sympathetic motor nuclei in the brain stem might serve as an intermediate factor. A dissociation occurs within the sympathoadrenal response to hypothalamic NFFL infusion, leading to selective activation of the adrenal medullary branch and concomitant reduction of neuronal outflow of NE. It is hypothesized that serotonergic mechanisms in the PVN might be involved in the central nervous response to emotional stress.

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