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Medial Hypothalamic Serotonin: Effects on Deprivation and Norepinephrine-Induced Eating

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WEISS, G. F., P. PAPADAKOS, K. KNUDSON AND S. F. LEIBOWITZ. *Medial hypothalamic serotonin: Effects on deprivation and norepinephrine-induced eating*. PHARMACOL BIOCHEM BEHAV **25**(6) 1223–1230, 1986.—Evidence to date suggests an inhibitory role for serotonin (5-HT) in the regulation of feeding behavior. In the present study, hypothalamic 5-HT was investigated for its anorexic potency under different feeding conditions. In fasted rats, 5-HT (1.1–4.4 μg) injected into the hypothalamic paraventricular nucleus (PVN), produced a reliable dose-dependent reduction in food consumption. Under satiated conditions, this inhibitory effect was significantly larger and apparent at lower doses (down to 0.1 μg), in animals induced to eat by PVN injection of norepinephrine (NE). Tests with receptor antagonists, injected into the PVN immediately prior to 5-HT, revealed a dose-dependent blockade of 5-HT's action by the serotonergic blockers, metergoline, methysergide and cinanserin. While the effect of 5-HT was somewhat attenuated by administration of certain β-adrenergic and phenothiazine-type dopamine receptor antagonists, 5-HT was totally resistant to the actions of more selective dopamine blockers and of the cholinergic and histaminergic antagonists, atropine and dexbrompheniramine. PVN injection of various serotonergic compounds, known to inhibit feeding when peripherally administered, also suppressed NE-induced feeding in a dose-related manner (fluoxetine = *dl*-norfenfluramine > quipazine > chlorimipramine > *dl*-fenfluramine). Further tests with PVN administration of the dextro isomer and metabolite of fenfluramine showed a considerably stronger inhibitory effect with *d*-norfenfluramine as compared to dexfenfluramine, and a particular effectiveness of peripherally injected dexfenfluramine in NE-injected rats, at doses at least 10-fold higher than centrally effective doses. Taken together, these and related findings converge to support the hypothesis that the PVN, and perhaps the local α_2-noradrenergic receptor system, is a possible site and mechanism involved in the mediation of serotonergic inhibition of eating.

Paraventricular nucleus Feeding Norepinephrine Serotonin agonist Serotonin antagonist

A variety of evidence suggests that serotonergic systems, possibly in the brain, may exert an inhibitory influence on the expression of feeding behavior [3, 33, 50]. Peripheral injections of serotonergic receptor agonists are found to suppress food intake, while serotonergic antagonists block this effect and may themselves potentiate feeding [2,3]. Drugs which release serotonin (5-HT) from presynaptic terminals also reduce food intake [3], suggesting a role for endogenous 5-HT in feeding behavior. While these effects have been observed in a variety of species, the central site(s) and mechanism of 5-HT's action have yet to be elucidated.

Very few studies have been conducted to investigate the feeding changes induced by centrally injected 5-HT. Ventricular administration of 5-HT is known to be effective in suppressing food consumption, albeit at quite high doses of 30–100 μg [17,27]. While hypothalamic injection of 5-HT (18–20 μg) has also been found to produce a significant reduction in food intake [29], other investigators have reported lower doses of hypothalamic 5-HT (approximately 5 μg) to be generally ineffective in suppressing feeding [57,58]. Furthermore, higher doses, above 10 μg, may be suspected of producing anorexia as a consequence of 5-HT's general suppressing effect on behavior (see below).

The present study examined the impact of medial hypothalamic injection of 5-HT on the ingestive behavior of rats under food-deprived, as well as satiated, conditions. The site of injection was the medial paraventricular nucleus (PVN), which is known to have an important function in the control of eating behavior [32,33]. In particular, this nucleus is most sensitive to noradrenergic stimulation, which is known to elicit a robust feeding response [30]. This response, elicited by norepinephrine (NE) and epinephrine at near physiological doses [31], is mediated by α_2-noradrenergic receptors [22] and relies on the integrity of the PVN for its expression [34,39]. This information, and additional biochemical results indicating an antagonistic interaction between hypothalamic 5-HT and α_2-noradrenergic receptor systems [19], have led us to examine the direct impact of PVN-injected 5-HT upon the α_2-noradrenergic feeding response. If endogenous 5-HT does indeed act within the medial hypothalamus to affect appetite, one possible mode of action may be via its inhibitory interaction with endogenous noradrenergic function in this brain area.

The results of the present study, obtained through tests with various centrally injected 5-HT agonists and antagonists [35], support the conclusion that 5-HT acts in part via satiety

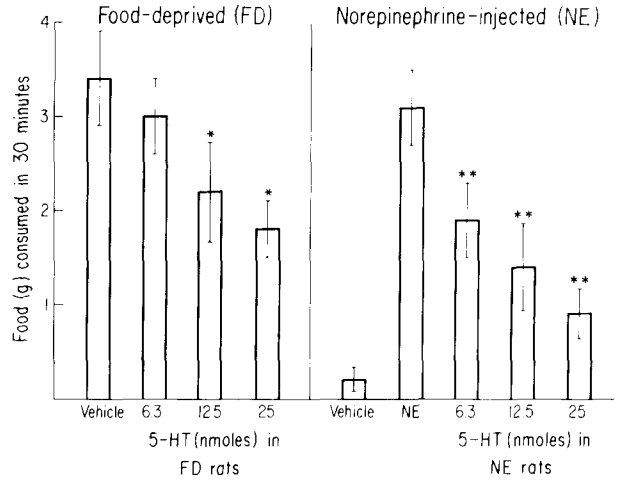

FIG. 1. Dose response analysis of feeding suppression elicited by serotonin (5-HT) administration into the paraventricular nucleus of food-deprived and norepinephrine-injected rats. A reliable inhibition with 5-HT, compared to vehicle baseline, was obtained at all doses (*$p<0.05$; **$p<0.01$), except for 6.3 nmoles in the food-deprived group.

TABLE 1

SUPPRESSIVE EFFECT OF 5-HT ON FOOD INTAKE IN NE-INJECTED RATS

5-HT (nmoles)	5-HT (μg free base)	Food Intake	% Suppression*
0.0	0.0	3.8 ± 0.3	—
20.0	3.5	0.9 ± 0.4	−76
10.0	1.8	0.8 ± 0.4	−78
5.0	0.9	1.3 ± 0.5	−65
2.5	0.4	1.6 ± 0.6	−58
1.3	0.2	2.7 ± 0.5	−30
0.6	0.1	2.9 ± 0.3	−23
0.3	0.05	3.7 ± 0.4	− 3

*Percent suppressions were statistically significant ($p<0.05$) at all doses, except for 0.3 nmoles.

mechanisms of the medial hypothalamus to suppress deprivation- and NE-induced feeding. Furthermore, this study demonstrates a particularly strong antagonism between 5-HT and NE specifically within the PVN, thus suggesting a potential site of action and mechanism through which 5-HT and drugs which release 5-HT, may act to control satiety.

METHOD

Male, albino Sprague-Dawley rats (Charles River, MA) weighing 350–400 g at the start of the experiment were used. The animals were individually housed in wire mesh cages and maintained in a temperature-controlled environment (22°C), with a 12 hr light/12 hr dark cycle and lights on at 7:00 a.m. Subjects (N=8–12/group) were maintained and tested on either Purina lab chow pellets (Experiments 1–4) or on a sweetened milk-mash diet (Experiment 5) consisting of 46% Purina lab chow powder, 37% sucrose, and 17% Carnation evaporated milk. Water was provided ad lib. The tests were generally conducted in the afternoon, between 1:00 and 4:00 p.m.

Surgery

Rats requiring central drug injections were stereotaxically implanted, under pentobarbital anesthesia (Nembutal, 40 mg/kg, Abbott Laboratories), with a chronic, unilateral 26-gauge stainless steel guide cannula aimed at the PVN. With the incisor bar placed 3.1 mm above the interaural line, the following coordinates were employed: 0.4 mm caudal to bregma, 0.3 mm lateral to midline, and 7.2 mm beneath the skull surface. The injection needle (33-gauge) penetrated the brain 1.0 mm beyond the tip of the cannula to reach within 0.3 mm of the PVN. Each implant was secured with acrylic cement which was held in place by stainless steel screws drilled into the skull. A 3–4 day recovery period was permitted, during which time the animals were frequently handled and mock injected.

Test Procedure

In Experiment 1, two groups of rats were used to examine the feeding-suppressive effect of PVN injection of serotonin creatinine sulfate (5-HT, Sigma). One group was tested under food-deprived conditions, whereas the other was induced to eat with PVN injection of l-norepinephrine-d-bitartrate (NE, Sigma). In both groups, 5-HT was administered directly into the PVN at 3 dose levels, 6.3, 12.5 and 25 nmoles. For the specific procedures, the first group of subjects was deprived of food for 18 hr and then tested with vehicle or 5-HT. The drug was dissolved in 0.05% ascorbic acid and injected directly into the PVN in a volume of 0.5 μl. Immediately following the injection, preweighed food pellets were placed into the rat's cage, and food intake measurements were taken 60 min post-injection. These tests were conducted every 2–3 days, with the vehicle (5–6 tests) and drug doses (3 tests/dose) administered according to a Latin square design.

The second group of rats, as well as all other rats in this study (Experiments 2–5), were tested under food-satiated conditions after PVN administration of NE. In each of these experiments, food intake measurements were taken 60 min post-drug injection. For these experiments, subjects were first pre-screened for their responsiveness to PVN NE injection and then tested for the impact of PVN 5-HT on noradrenergically-stimulated eating. Norepinephrine (40 nmoles) was dissolved in 0.9% bacteriostatic saline and injected in a volume of 0.5 μl. Those animals (approximately 70–80%) which consumed greater than 1.5 g after NE, relative to 0.4–0.6 g after saline vehicle, were used in the study. The precise procedures included a one-hour satiation period with fresh diet just prior to the test, to ensure that the animals were indeed satiated. Subsequent to this pretreatment period, the rats were injected with 5-HT directly into the PVN at the same doses as the food-deprived group. This was immediately followed by NE administration into the PVN and then a food intake measurement 60 min later.

In Experiments 2–5, rats induced to eat with NE were

subjected to various additional pharmacological manipulations. As an extension of the first study, the subjects in Experiment 2 were tested in a more complete dose-response analysis, with 5-HT doses ranging from 0.3–20.0 nmoles (0.5–3.5 μg free base). In the third experiment, the impact of receptor-blocking agents on 5-HT's action in the PVN was examined. For these tests, the receptor blockers or their respective vehicles were injected directly into the PVN, 10 min before administration of 5-HT (13 nmoles) followed by NE.

The following groups of antagonists were used and tested at doses which are known to be centrally effective and to leave intact the NE-induced feeding response [32]: (1) the 5-HT blockers, methysergide maleate (Sandoz, 3.7–60 nmoles); cinanserin hydrochloride (Squibb, 3.7–60 nmoles); and metergoline (Farmitalia, 7.5–60 nmoles); (2) the β-adrenergic antagonists, *dl*-alprenolol hydrochloride (Hassle, 50–400 nmoles) and *dl*-propranolol hydrochloride (Ayerst, 50–400 nmoles); (3) the dopaminergic antagonists of the phenothiazine-type, fluphenazine hydrochloride (Squibb, 1.3–10 nmoles) and chlorpromazine hydrochloride (SK&F, 10–60 nmoles), and the more specific dopamine antagonists, haloperidol (McNeil, 5–20 nmoles) and pimozide (Janssen, 10–40 nmoles); (4) the cholinergic-muscarinic blocker, atropine methylnitrate (Sigma, 2.5–20 nmoles); and (5) the histaminergic antagonists, dexbrompheniramine maleate (Schering, 5–20 nmoles). To increase their solubility, haloperidol, pimozide, methysergide and metergoline were dissolved in 0.1–10% tartaric acid and injected in 1–2 μl. The remainder of these blockers were dissolved in 0.9% bacteriostatic saline or sterile water and injected in a routine volume of 0.5 μl. In addition to their being tested in combination with 5-HT, these receptor blockers in separate tests were injected prior to ascorbic acid vehicle, to examine their impact on baseline feeding. At the doses tested in this study, none of these blockers when injected alone into the PVN produced any change in the animals' baseline feeding scores.

In Experiment 4, the impact of several PVN-injected serotonergic agonists was investigated in noradrenergically-stimulated rats. All agents were administered directly into the PVN immediately prior to NE administration. They included: (1) the 5-HT-releasing drugs, *dl*-norfenfluramine hydrochloride (NORFEN, A. H. Robins, 3.2–200 nmoles) and *dl*-fenfluramine hydrochloride (FEN, A. H. Robins, 50–200 nmoles); (2) the receptor agonist, quipazine hydrochloride (Miles, 6.3–200 nmoles); and (3) the 5-HT uptake inhibitors, fluoxetine hydrochloride (Lilly, 1.6–100 nmoles); and chlorimipramine hydrochloride (CIBA-GEIGY, 25–100 nmoles). These agonists were eached dissolved in bacteriostatic saline and injected in a volume of 0.5 μl.

In Experiment 5, the dextro isomers of the 5-HT-releasing compounds, *d*-norfenfluramine (*d*-NORFEN, Servier) and dexfenfluramine chlorohydrate (DEXFEN, Servier), were tested in combination with NE. In two sets of animals, these drugs were administered directly into the PVN, in doses of 12.5–200 nmoles (3.2 to 54 μg/rat) just prior to NE. In a third group of rats, DEXFEN was tested after intraperitoneal (IP) administration, at doses of 0.06–2.0 mg/kg (approximately 31–1000 μg/rat). This experiment was conducted to determine the effectiveness of the dextro isomers of FEN and NORFEN, which have been found to be more behaviorally potent than the *dl*-isomer, to be more selective for 5-HT, and to avoid the anti-dopaminergic ("neuropeptide-like") effect of *l*-fenfluramine [15, 20, 21, 25, 26]. This experiment was also designed to compare the relative effectiveness of peripheral versus central injection of this 5-HT stimulant.

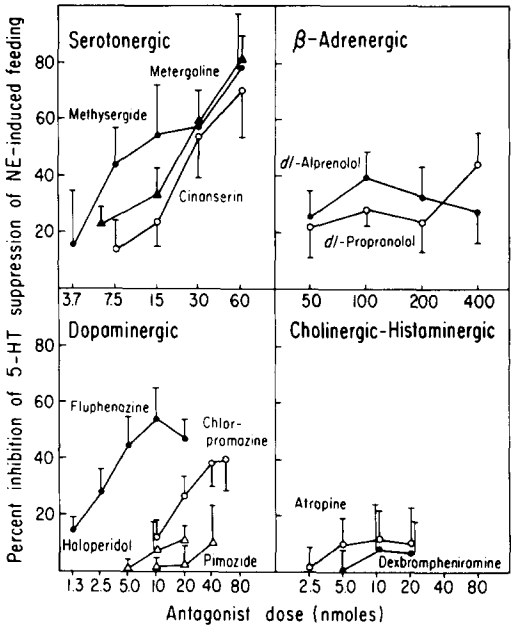

FIG. 2. A summary of the effects of various receptor blockers on the inhibitory effect of serotonin (5-HT) on the feeding elicited by norepinephrine (NE) injection into the paraventricular nucleus (PVN). Percent inhibition was calculated by taking the difference between the blocker + 5-HT response and the saline + 5HT response and dividing the result by the saline + 5-HT response × 100. As shown, the serotonergic antagonists, when injected into the PVN, produced a near total block of 5-HT inhibition of NE-elicited feeding in a dose-dependent manner ($p<0.001$).

Histological and Statistical Analysis

Following the completion of these experiments, histological verification of cannula placement was performed. The rats, anesthetized with a 60 mg/kg dose of Nembutal, were transcardially perfused with saline followed by a 10% buffered formalin solution. The brains were then removed and placed in a 30% sucrose-buffered formalin solution for at least 2 days. Frozen sections (50 μm), taken in the coronal plane, were mounted and stained with cresyl violet. Sections were viewed relative to the stereotaxic atlas of Pellegrino *et al.* [46]. The injection needle was generally denoted by discrete tissue damage either within the PVN or along its dorsolateral border. Animals (less than 5%) with cannulas more than 0.3 mm from the borders of the PVN were eliminated from the study.

The dose-response results for each group were evaluated by a one-way analysis of variance (ANOVA). A significant effect revealed by an ANOVA was followed by a planned post-hoc comparison using the Dunnett's test to evaluate differences between specific vehicle and drug scores at each drug dose.

RESULTS

Experiment 1

The results of this experiment showed that in food-

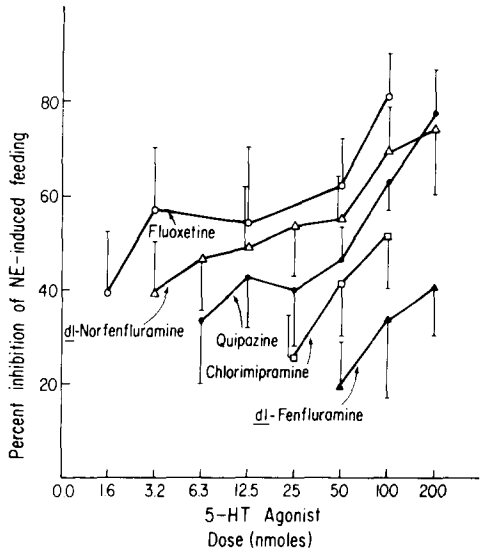

FIG. 3. A dose-response study of the effects of various serotonergic agonists on feeding elicited by paraventricular nucleus (PVN) administration of norepinephrine. All agonists, when injected directly into the PVN, demonstrated a statistically reliable dose-related suppression of feeding, with the order of potency of fluoxetine = *dl*-norfenfluramine > quipazine > chlorimipramine > *dl*-fenfluramine.

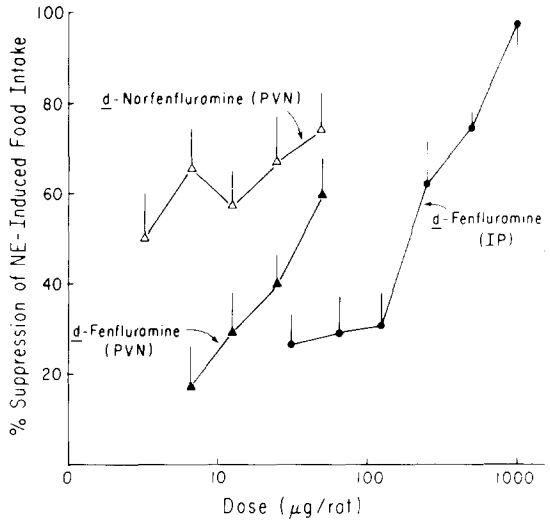

FIG. 4. Dose-response analysis of the feeding suppression caused by the dextro isomers of fenfluramine (DEXFEN) and its metabolite, norfenfluramine (*d*-NORFEN). Paraventricular (PVN) nucleus administration of the compounds, with *d*-NORFEN more potent than DEXFEN, showed a statistically reliable suppression of norepinephrine-induced feeding at all doses. Peripheral injection of DEXFEN required doses approximately 10-fold higher, to produce effects of comparable magnitude to those caused by PVN DEXFEN injection.

deprived (n=9), as well as in satiated NE-treated (n=10) rats, PVN injection of 5-HT, in a dose-dependent manner, was effective in suppressing food consumption. As illustrated in Fig. 1, rats deprived of food for 18 hr consumed an average of 3.3 g after vehicle injection. Following 5-HT administration, however, a reliable inhibition of food intake was observed ($p<0.05$), with the higher doses of 12.5 and 25 nmoles showing the strongest effect. Satiated rats induced to eat by PVN NE injections exhibited a similar response (Fig. 1), except that they were more strongly affected by 5-HT than the food-deprived rats. In these NE-injected animals, each of the three doses (6.3, 12.5 and 25 nmoles) caused a statistically reliable suppression of food intake ($p<0.01$). This occurred in the absence of any sedative or behaviorally depressive effects, which actually started to become apparent at doses of 5-HT above 25 nmoles. Furthermore, the magnitude of this suppression across doses in NE rats (40, 50 and 74%) was significantly greater ($p<0.01$) than that observed in food-deprived animals (9, 33 and 47%).

Experiment 2

The results of a more extensive dose-response analysis, of 5-HT's effectiveness as a feeding suppressant after PVN administration, can be seen in Table 1. This table summarizes the mean food intake scores of an additional group of satiated animals (n=12) induced to eat by NE. These animals were first selected for their responsiveness to a relatively high dose of 5-HT (20 nmoles) injected into the PVN. Those rats (n=8) that exhibited at least a 50% suppression of NE-elicited feeding at this dose were given further tests with lower doses of 5-HT. As shown in Table 1, these animals exhibited a significant and dose-related suppression of NE-elicited feeding ($p<0.001$). The threshold dose for this effect was 0.6 nmoles or 0.1 μg ($p<0.05$), with a 20-fold higher dose (10 nmoles or 1.8 μg) yielding a suppressive effect as high as −78% ($p<0.001$).

Experiment 3

This experiment, in which tests were conducted in 6 groups of rats (n=8/group) with the various receptor-blocking agents (Fig. 2), showed that the 5-HT-induced suppression of NE-elicited feeding was sharply diminished by prior PVN injection of the 5-HT receptor antagonists, metergoline, methysergide and cinanserin. In each case, this effect was dose-dependent ($p<0.001$), achieving at the highest dose a near total blockade of 5-HT-induced anorexia. A different pattern of effects was observed with the β-adrenergic antagonists. At the relatively high doses tested, these blockers caused a small attenuation (20–40%, $p<0.05$) of 5-HT's feeding suppressive action. This attenuation occurred independent of dose, thus possibly reflecting a nonspecific antagonism. The selective dopaminergic receptor blockers, haloperidol and pimozide, had no apparent impact on 5-HT's action. However, the less selective phenothiazine-type antagonists (fluphenazine and chlorpromazine) caused some attenuation of the feeding-suppressive response, ranging from 10–15% ($p<0.02$) depending upon dose. The cholinergic antagonist, atropine, and the histaminergic antagonist dexbrompheniramine, tested at centrally effective doses were without effect on the 5-HT response.

Experiment 4

As revealed in Fig. 3, 5 groups of rats (n=8–12/group) were tested with one of six serotonergic stimulants, at doses ranging from 1.6–200 nmoles. These agonists, when adminis-

tered into the PVN immediately prior to NE, produced a reliable and dose-dependent ($p<0.01$) inhibition of NE-elicited eating. The agents of highest potency were fluoxetine and *dl*-NORFEN, which are known to block the uptake or enhance the release of 5-HT. These compounds caused a 40% suppression ($p<0.001$) of NE eating at a dose as low as 1.6 nmoles (0.5 μg) and 3.2 nmoles (0.8 μg), respectively, and a maximum suppression of 70–80% ($p<0.001$) at the highest dose of 100 nmoles. Quipazine, a direct 5-HT receptor agonist, also dose-dependently suppressed NE-induced feeding ($p<0.001$) at doses above 6.3 nmoles, while chlorimipramine and *dl*-FEN were effective only at considerably higher doses, ranging from 50–200 nmoles ($p<0.05$).

Experiment 5

In tests similar to those of the previous experiment, 3 separate groups of rats (n=8–12/group) received either PVN injections of DEXFEN (25–200 nmoles), or of its active metabolite *d*-NORFEN (12.5–200 nmoles), or systemic injections of DEXFEN (.06–2.0 mg/kg), just prior to PVN NE administration. As indicated in Fig. 4 on a semi-log graph, a reliable, dose-dependent ($p<0.01$) decrease in noradrenergically-stimulated feeding was observed with each of these agents, revealing a similar but somewhat stronger or consistent suppression than that obtained with the racemic compounds, *dl*-FEN and *dl*-NORFEN (Fig. 3). Consistent with Experiment 4, PVN injection of *d*-NORFEN was found here to be considerably more potent than PVN DEXFEN injection ($p<0.001$, Fig. 4); *d*-NORFEN yielded a reliable reduction (−51%, $p<0.05$) in NE feeding at a dose of 12.5 nmoles (3.1 μg/rat), in contrast to a significantly smaller suppression of −28% ($p<0.05$) obtained with DEXFEN at a 4-fold higher dose (50 nmoles or 12.5 μg/rat). To acheive a 50% suppression, DEXFEN required a dose between 100 and 200 nmoles as compared to the ED50 dose of 12.5 nmoles for *d*-NORFEN.

When systemically administered, DEXFEN produced a potent and dose-related ($p<0.01$) inhibition of feeding, which ranged from −28% ($p<0.05$) at a dose of 0.13 mg/kg (approximately 60 μg/rat) to −98% ($p<0.001$) at a higher dose of 2 mg/kg (1000 μg/rat). As shown in Fig. 4, these effective doses with systemically administered DEXFEN were at least 10-fold higher than the doses of PVN-injected DEXFEN that had equal potency in antagonizing the NE-elicited feeding response.

DISCUSSION

The present series of experiments utilized the PVN noradrenergic feeding response to examine the influence of central 5-HT in the control of food intake. Whereas α_2-noradrenergic stimulation of the PVN has been shown to potentiate feeding [22], the present investigation demonstrates that 5-HT administration into this nucleus produces the opposite response, namely, a strong and dose-dependent suppression of feeding. This suggests a potential brain site of action for 5-HT in the induction of anorexia and further supports a role for this neurotransmitter in the inhibitory control of feeding behavior [3, 33, 50].

Previous studies have explored the impact of peripherally administered serotonergic agonists on food intake [2, 3, 21, 50]. This includes work with 5-HT itself and with the racemic and dextro isomers of FEN, which are believed to act indirectly via endogenous 5-HT. Evidence for the involvement of 5-HT in the anorexic action of FEN is provided by studies which show that suppression of food intake by peripheral injection of this compound is attenuated by 5-HT receptor antagonists and by drugs or central lesions which interfere with 5-HT synthesis or synaptic transmission [2,3]. The 5-HT precursor 5-hydroxytryptophan is also effective in inhibiting feeding [4,8], further supporting a physiological role for endogenous 5-HT in control of food intake. These studies with systemic injections, however, have unfortunately left unanswered the question of whether 5-HT and its receptors exist peripherally or centrally to control appetite for food and, if centrally, where in the brain they may be acting.

While there is some evidence to suggest a peripheral site of action [16], other evidence argues for the existence of central 5-HT receptors that control feeding behavior. For example, systemic injection studies have demonstrated that the 5-HT antagonist xylamidine, which acts predominantly on peripheral 5-HT receptors, is ineffective in blocking FEN anorexia [11]. Intraventricular injection studies have confirmed the effectiveness of central 5-HT, at doses of 30–100 μg, in reducing food intake in different species [17, 18, 27]. While these doses are high for central injection studies and may, in part, be acting in a behaviorally nonspecific manner, centrally injected 5-HT is found to have a dramatic effect at doses at least 10-fold lower than peripherally effective doses [47]. Unfortunately, there are only a few studies which have administered 5-HT directly into brain tissues, and those which exist have observed either little change in feeding [57,58] or a small to moderate feeding suppression with a high dose of 5-HT (18–20 μg) which might be expected to produce a nonspecific behavioral depression [7, 29].

In light of this paucity of information and the lack of essential dose-response studies, the present investigation appears to provide the first clear evidence for a central effect of 5-HT on feeding. In the first experiment, 5-HT doses of 12.5 and 25 nmoles (approximately 2.5 and 5.0 μg free base) injected into the medial hypothalamus were effective in reducing food intake in food-deprived rats, by up to 47%. While doses of 10 μg or higher caused an even stronger anorexia, these doses were also associated with sedative effects, leaving in question the significance of the feeding suppression. This narrow range of effective and behaviorally specific doses in food-deprived rats is indicative of the ongoing problems investigators have had to address in their research with 5-HT.

In contrast to the studies in food-deprived animals, we found PVN-injected 5-HT to be especially effective in reducing food intake induced by local NE injection. The greater effectiveness of 5-HT in this particular paradigm can be clearly seen in the dose-response curve, where 5-HT at a dose as low as 0.1 μg produced a reliable (23%) suppression of NE-elicited eating, comparable in magnitude to the suppressive response observed with 5 μg of 5-HT in food-deprived rats. This differential sensitivity may be attributed to the fact that deprivation-induced eating has multiple and diverse neural substrates and thus is more difficult to suppress; this is in contrast to the NE-elicited response, which has a clearly defined neural substrate [30, 34, 39, 60] that may be directly influenced by serotonergic terminals [32,33]. The medial hypothalamus is known to be densely innervated by 5-HT-containing neurons [54], which may interact closely, in an inhibitory fashion, with local noradrenergic innervation [53]. Although it is possible that 5-HT and NE may be exerting thier opposing effects through quite separate or independently functioning systems, we propose that they

are interacting more directly through closely related systems in the PVN. In support of this idea are the results of studies showing that PVN lesions attenuate or abolish the feeding effects of NE and peripheral injections of the α-agonist clonidine and the 5-HT agonist fenfluramine.

Autoradiographic studies have demonstrated the existence of serotonergic receptors in the medial hypothalamus [43,45], which may provide the neural substrates for 5-HT's actions on feeding. The findings of the present study, that 5-HT receptor antagonists effectively and dose-dependently block the inhibitory feeding effect of PVN 5-HT, supports this suggestion. Certain other antagonists, of dopaminergic, cholinergic and histaminergic receptors, were ineffective, thus arguing for a specific role of serotonergic receptors in this system. The results indicating some attenuation of 5-HT's action, with high doses of β-adrenergic blockers and with less selective dopaminergic antagonists, presumably reflect the ability of these antagonists to bind to 5-HT receptors [14, 23, 40, 41, 42, 44] and thus block the behavioral effects of this indoleamine [13,59].

Further support for the role of endogenous hypothalamic 5-HT in control of feeding is obtained from the results of other experiments, showing an inhibitory effect of PVN 5-hydroxytryptophan on feeding [35], and of the present study, showing a reliable and dose-dependent effect of PVN injection of a variety of direct- and indirect-acting 5-HT agonists on food intake. Of the compounds tested in Experiment 3, fluoxetine and *dl*-NORFEN were found to be the most potent in reducing food intake. When administered into the PVN, doses at least as low as 1.6 nmoles (0.4 μg) and 3.2 nmoles (0.8 μg), respectively, were effective in suppressing NE-elicited eating by 40%, indicating that even lower doses of these compounds might also produce an inhibitory response. Quipazine was somewhat less effective, requiring 12.5 nmoles to induce a comparable response, and chlorimipramine and *dl*-FEN produced the weakest response, at doses of 25–200 nmoles. The effective doses of each of these presynaptically acting compounds fall between 50 to 500-fold lower than peripherally effective doses, [49, 51, 52]. This argues strongly for a central site of action for these compounds, as well as the involvement of presynaptic 5-HT in their behavioral response.

Our findings that PVN injection of *dl*-NORFEN is considerally more potent than *dl*-FEN is consistent with the results of peripheral injection studies showing the former to be an active metabolite of FEN [20,21]. These studies have demonstrated a stronger anorexic effect with NORFEN than its parent compound, as well as a greater effect of this metabolite on the release of presynaptic 5-HT. Numerous investigations have now established that, in their effects on endogenous 5-HT, DEXFEN is more potent than the levo isomer, which has additional effects on dopamine as well as other neurotransmitter systems [20, 21, 25]. This led us to examine in Experiment 5, both DEXFEN and *d*-NORFEN in our present test paradigm. In this dose-response study, we established that these compounds were effective in reducing food intake after injection into the PVN, and, once again, that by this central route of administration the metabolite was considerably more potent than its parent compound. We further demonstrated that DEXFEN, when systemically administered, was remarkably potent in causing a suppression of PVN NE-induced feeding, requiring approximately 10-times higher doses than PVN-injected DEXFEN. Thus, once again, we reveal a close and specific interaction between 5-HT and NE in the PVN. It is interesting to note that this testing paradigm with PVN-injected NE enabled us to reveal a feeding suppressive effect with DEXFEN at considerably lower doses than are required in food-deprived rats [25].

Since we have yet to conduct a systematic search for the specific hypothalamic site(s) involved in 5-HT's action, we can only tentatively conclude that the medial hypothalamus, and specifically the PVN, is one of the sites sensitive to serotonergic receptor stimulation. Other evidence supporting a medial hypothalamic site of action for 5-HT is indicated by meal pattern analyses, conducted with central administration of 5-HT, as well as of the 5-HT releasing drug *dl*-NORFEN. These compounds have been shown to suppress food intake through a decrease in meal size and feeding rate, without affecting the latency to meal initiation or producing a change in meal frequency [56]. These findings are consistent with studies demonstrating a similar pattern of meal-taking in animals receiving *systemic* injection of drugs presumed to facilitate 5-HT synaptic activity [5, 6, 12, 16, 24, 28], and the opposite pattern after systemic administration of the 5-HT antagonist, cyproheptadine [1]. Moreover, with PVN injection of 5-HT or *dl*-NORFEN, a selective inhibition of carbohydrate occurs in conjunction with a sparing or even enhanced consumption of protein intake. A similar pattern of diet selection is apparent after peripheral administration of FEN [10, 38, 63, 64]. These diet selection and meal-taking patterns observed with both PVN and peripheral serotonergic agonists appear to be in direct opposition to the effects of PVN α_2-noradrenergic stimulation. Specifically, NE has been shown to enhance feeding, particularly of carbohydrate, primarily through an increase in meal size and duration, and a simultaneous suppression of protein intake [36, 37, 48, 55]. These contrasting effects of the two aminergic systems strengthen the suggestion that 5-HT and NE in the medial hypothalamus may interact in a close and antagonistic manner to control eating.

The similarities in diet preference and meal patterns observed in animals after centrally and peripherally administered 5-HT stimulants suggest that these compounds may share to some extent a common site of action. In support of this proposal is a preliminary finding that electrolytic lesions in the PVN significantly attenuate, by 30–40%, the effectiveness of peripheral DEXFEN-induced anorexia [61]. In contrast to the impact of this lesion, damage to the lateral, anterior, dorsomedial and ventromedial hypothalamic areas have been found to leave intact the behavioral effect of this anorectic compound [3, 7, 9, 62]. While the partial attenuation of this response suggests the involvement of other brain areas in addition to the PVN, all evidence taken together indicates that this nucleus may have a specific function in mediating the influence of serotonergic agents on appetite for specific foods.

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