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BRIEF COMMUNICATION

Injecting 5-HT Into the PVN Does Not Prevent Feeding Induced by Injecting 8-OH-DPAT Into the Raphe

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FLETCHER, P. J. AND D. V. COSCINA. *Injecting 5-HT into the PVN does not prevent feeding induced by injecting 8-OH-DPAT into the raphe.* PHARMACOL BIOCHEM BEHAV 46(2) 487–491, 1993.—The selective 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) activates raphe somatodendritic autoreceptors, leading to an inhibition of 5-HT neuronal activity and reduced synthesis and release of 5-HT in forebrain terminal areas. One behavioural consequence of this is increased feeding in satiated rats. Because injections of 5-HT agonists into the medial hypothalamus suppress feeding, it has been proposed that 8-OH-DPAT-induced feeding may involve a reduction of 5-HT release within this area. This hypothesis was tested by examining the ability of 5-HT injected into the medial hypothalamus to reverse the feeding-stimulant action of 8-OH-DPAT following injection into the dorsal raphe or median raphe. Two groups of rats, maintained with free access to food at all times, were used. Each was prepared with two cannulae, one aimed at the paraventricular nucleus (PVN) of the medial hypothalamus and the other at either the dorsal raphe nucleus or median raphe nucleus. Food intake over the next hour was increased following dorsal raphe or median raphe injections of 8-OH-DPAT (1 and 0.5 μg, respectively). These effects were not blocked by injections of 7.5 or 15 μg 5-HT into the PVN. However, 15 μg 5-HT did attenuate the feeding-stimulant action of 10 μg norepinephrine injected into the PVN. These results do not support the hypothesis that a reduction in 5-HT release within the medial hypothalamus is responsible for the feeding-stimulant action of 8-OH-DPAT.

Feeding 8-OH-DPAT 5-Hydroxytryptamine Midbrain raphe nuclei Paraventricular nucleus

PHARMACOLOGICAL manipulations that alter 5-hydroxytryptamine (5-HT; serotonin) neuronal activity alter food intake and feeding behaviour of laboratory animals, with drugs that enhance 5-HT activity reducing food intake. Such drugs include *d*-fenfluramine, 5-HT reuptake inhibitors such as fluoxetine and sertraline, 5-HT precursors and 5-HT$_{1B/1C}$ agonists [for reviews, see (2,22)]. Injection of direct and indirect 5-HT receptor agonists into the medial hypothalamus, particularly the paraventricular nucleus (PVN), reduces food intake (10, 14,17,18,24,28). Thus, it has been proposed that 5-HT neuronal activity within the PVN is an important mechanism in the control of food intake (17,18,24,28).

Despite the consistency of the finding that increasing 5-HT function suppresses feeding, it has been harder to demonstrate increased feeding following acute reductions in 5-HT activity by various pharmacological manipulations. Systemic administration of 5-HT receptor antagonists such as methysergide and metergoline have been reported to enhance food intake under some experimental conditions (4,6). Agonists at the 5-HT$_{1A}$ receptor subtype, including 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT), ipsapirone, and gepirone, have been shown consistently to increase food intake in satiated rats (1,5,7–9,11,20). The effects of these drugs can be abolished by 5-HT depletion (1,9,15) and can be reproduced by microinjections into the midbrain raphe nuclei (1,7–9,15). Thus, this class of compounds appears to increase short-term feeding by stimulating somatodendritic 5-HT$_{1A}$ receptors located within the midbrain raphe nuclei. Stimulation of these autoreceptors suppresses 5-HT neuronal activity (25), leading to reduced 5-HT synthesis and release in terminal areas (13).

Both the dorsal and median raphe nuclei project to the PVN (26,27), and 5-HT fibres within the PVN have been

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shown to originate in both raphe nuclei (23). In light of these anatomic connections, and the observations that PVN injections of 5-HT agonists reduce feeding, it has been proposed that 8-OH-DPAT-induced feeding may result from a reduction in the endogenous release of 5-HT within the medial hypothalamus (17). Because direct evidence to support this hypothesis is lacking, experiments were conducted to test this possibility. The strategy used was to attempt to reverse the feeding-stimulant effect of dorsal or median raphe injections of 8-OH-DPAT by simultaneously infusing 5-HT into the medial hypothalamus at the level of the PVN.

METHOD

Subjects

Twenty-three adult, male Sprague–Dawley rats weighing 270–310 g at the time of surgery were used. They were housed singly in hanging wire mesh cages with free access to food (Purina lab chow) and water at all times. The colony room was maintained at a temperature of 22 ± 2°C and with a 12 L : 12 D cycle (light on at 0800 h).

Surgery

All rats were anaesthetized with 40 mg/kg sodium pentobarbitol (Somnotol) and placed in a stereotaxic frame. Two stainless steel guide cannulae (22 ga, 15 mm length; Plastic Products, Roanoke, VA) were implanted in each rat. One cannula was aimed at the left PVN (all rats) and the other at either the dorsal raphe (n = 12) or the median raphe (n = 11). Co-ordinates for each site were as follows: PVN, AP −1.5, L +0.3, V −4.8 (relative to bregma); dorsal raphe, AP +1.2, L −1.5, V +7.5 (relative to interaural zero); median raphe, AP +1.2, L −1.1, V +5.5 (relative to interaural zero). The cannulae for raphe placements were implanted at an angle of 20° from the vertical to avoid damage to the midsagittal sinus and, in the case of median raphe placements, avoid penetrating the dorsal raphe. All cannulae were implanted so that their tips were 4 mm dorsal to the intended target sites. Stylets (28 ga) were used to ensure that the cannulae remained patent. At least 7 days of recovery were allowed before behavioural testing began, by which time rats had fully recovered their preoperative body weights and were gaining weight normally. During this recovery period, animals were habituated on three occasions to the handling procedures associated with testing.

Drug Injection and Behavioural Testing

All testing was carried out between 1300 and 1500 h during the light phase of the light/dark cycle. One hour prior to drug infusion, fresh food pellets were placed on the floors of the home cages to ensure satiation at the time of testing. Drugs were infused into the intended sites by inserting an ultrafine glass microinjection needle, housed inside a 15-mm length of 28-ga stainless steel tubing, into the guide cannula. The needle was then extended 4 mm beyond the cannula tip to terminate in the intended site. Drugs were infused manually in a volume of 0.5 μl, over a period of 1 min, using a 5-μl Hamilton syringe (Hamilton Co., Reno, NV) and a length of plastic tubing connected to the end of the needle. Two such assemblies were used to infuse solutions simultaneously into the PVN and either of the raphe nuclei. Immediately after drug infusions, the stylets were replaced and animals returned to their home cages together with preweighed amounts of food. One hour later, food intakes were determined by reweighing the remaining food plus any spillage, collected on paper towels placed beneath each cage.

Each animal was tested with four combinations of PVN 5-HT or saline vehicle, plus raphe injection of 8-OH-DPAT or vehicle. The combination pairs, with the PVN infusion listed first, were (a) saline + saline, (b) saline + 8-OH-DPAT, (c) 7.5 μg 5-HT + 8-OH-DPAT, and (d) 15 μg 5-HT + 8-OH-DPAT. For the dorsal raphe injection, 8-OH-DPAT was given at a dose of 1 μg; for median raphe injection, the dose was 0.5 μg. These doses were chosen on the basis of previous experiments showing equivalent increases in food intake (7) from these two sites. The four tests were administered at 3- to 4-day intervals in a randomized order.

One week following these tests, rats were tested two more times in a similar fashion following infusion into the PVN of 10 μg norepinephrine (NE) and 10 μg NE + 15 μg 5-HT. These tests were included to confirm the accuracy of the placements as well as to confirm previous observations that PVN injection of 5-HT attenuates NE-induced feeding (28).

Drugs

Drugs used were 5-HT bimaleate, NE bitartrate (Sigma Chemical Co., St. Louis, MO) and 8-OH-DPAT (Research Biochemicals, Inc., Natick, MA).

Histology and Verification of Injection Sites

Following completion of the studies, approximately half the rats from each raphe group were given an overdose of Somnotol and injected as described above with 0.5 μl of fast green dye to aid in visualizing the injection sites. They were then perfused transcardially with 10% formalin and their brains removed and stored in formalin. After fixation, brains were cut at 40-μm sections that were stained with cresyl violet. Rats with injection sites located outside those intended (21) were excluded from the data analysis. Previous work using the same surgical and testing procedures as in these studies has shown that raphe injections of 8-OH-DPAT elicit feeding of at least 1 g over that seen after saline (control) injections (7,8) while PVN injections of NE elicit at least 1.5 g intake above control levels (10). Thus, for the remaining animals, these criteria were used for inclusion in statistical analyses.

RESULTS

All rats with dorsal raphe implants were found to have appropriate injection sites in both the raphe and the PVN. The remaining rats in this group met the feeding criteria mentioned above, so data from all were included in this study. However, before NE testing was completed two rats had lost their cannulae; hence, only 10 subjects could contribute data to this portion of the study. Of rats with median raphe implants, one was found to have an inaccurate placement, so its data were discarded. An additional rat lost its cannula before testing began. All remaining rats in this group met the feeding criteria; hence, their data were used.

The left panel of Fig. 1 shows the effects of combined injections of 8-OH-DPAT into the dorsal raphe and 5-HT into the PVN. A one-way analysis of variance revealed an overall significant main effect of treatment, $F(3, 33) = 8.05$, $p < 0.001$. Post hoc comparisons using Tukey's test confirmed that dorsal raphe injections of 8-OH-DPAT increased food intake regardless of whether saline or 5-HT was infused

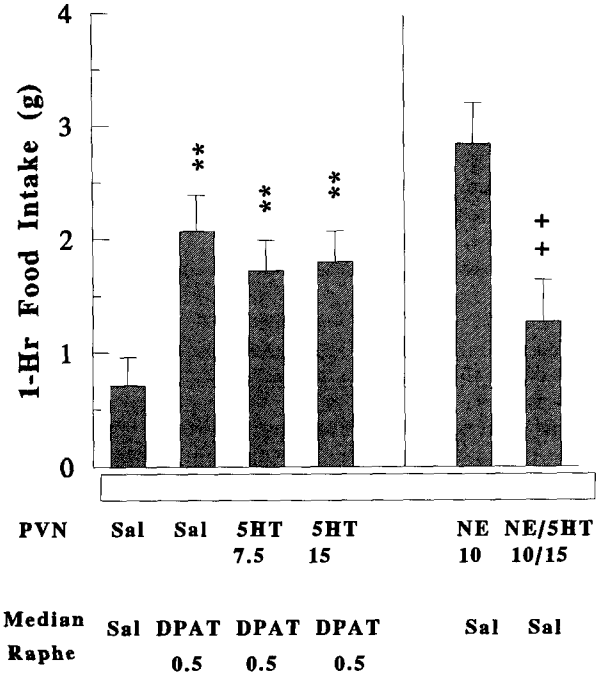

FIG. 1. Effects of paraventricular nucleus (PVN) injections of 5-hydroxytryptamine (5-HT) or saline (sal) on the feeding response elicited by 1 μg 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) injected into the dorsal raphe (DR). The right panel shows the effects of 5-HT injected into the PVN on the feeding-stimulant action of PVN injected norepinephrine (NE). Values represent mean (± SEM) food intakes over 1 h for 12 animals; only 10 animals were used for the NE conditions. $^{**}p < 0.01$ compared to sal-sal condition. $^{++}p < 0.01$ compared to NE + 5-HT condition.

into the PVN. The 5-HT treatment did not significantly affect the magnitude of the feeding response to 8-OH-DPAT. The right panel of Fig. 1 shows that PVN injection of 5-HT significantly attenuated the eating elicited by PVN injection of NE, $t(11) = 3.58, p < 0.01$.

The same pattern of results was found when 8-OH-DPAT was injected into the median raphe (see Fig. 2, left panel). The overall main effect of treatment was significant, $F(3, 24) = 12.2, p < 0.001$, showing that median raphe-injected 8-OH-DPAT stimulated feeding and this effect was not altered by PVN injection of 5-HT. In these same rats, 5-HT injected into the PVN significantly reduced feeding in response to PVN injection of NE, $t(8) = 3.51, p < 0.03$.

DISCUSSION

In line with previous results, injections of 8-OH-DPAT into the dorsal raphe or median raphe elicited feeding in satiated rats (1,7,8,15). If reduced 5-HT input to the PVN underlies the expression of this effect, then it would be predicted that 5-HT injection into the PVN should prevent this increased eating. However, simultaneous injection of 5-HT into the PVN failed to alter the magnitude of this increased food intake regardless of whether 8-OH-DPAT was injected into the dorsal or median raphe. Contrasting to this, 5-HT reduced the ability of PVN-injected NE to stimulate feeding in both groups of rats. This result is important because it provides a functional test of the accuracy of the medial hypothalamic injection sites and confirms that 5-HT was pharmacologically active at the doses used. Therefore, it is unlikely that the lack of effect of 5-HT on 8-OH-DPAT-induced feeding is related to inaccurate cannulae placements or an insufficient dose of 5-HT. Together, these results fail to support the hypothesis that the feeding-stimulant action of 8-OH-DPAT is related to reduced 5-HT activity within the PVN.

It has been reported that dorsal raphe-injected 8-OH-DPAT, at doses up to 5 μg, fail to alter hypothalamic 5-HT neuronal activity as measured by 5-HTP accumulation in rats pretreated with a decarboxylase inhibitor (16). This same study showed that injections of 8-OH-DPAT into the median raphe also failed to reduce hypothalamic 5-HT neuronal activity at doses that reliably induce feeding (7). In both cases, though, evidence for reduced 5-HT neuronal activity in other brain regions was noted. While these results suggest that raphe injections of 8-OH-DPAT do not modify hypothalamic 5-HT activity, and are consistent with the failure of 5-HT to reverse 8-OH-DPAT-induced feeding, they do not agree with other findings that dorsal raphe 8-OH-DPAT can reduce hypothalamic 5-HT turnover as measured by a reduction in the ratio of endogenous 5-hydroxyindoleacetic acid (5-HIAA)/5-HT (12). Clearly, additional work is needed to clarify the capacity of raphe-injected 8-OH-DPAT to modify the functional status of 5-HT neurotransmission.

Feeding induced by peripheral or raphe injections of 8-OH-DPAT has been shown to be reversed by dopamine receptor antagonists (7,8,20), suggesting that increased dopamine activity may be involved in the expression of this effect. Because

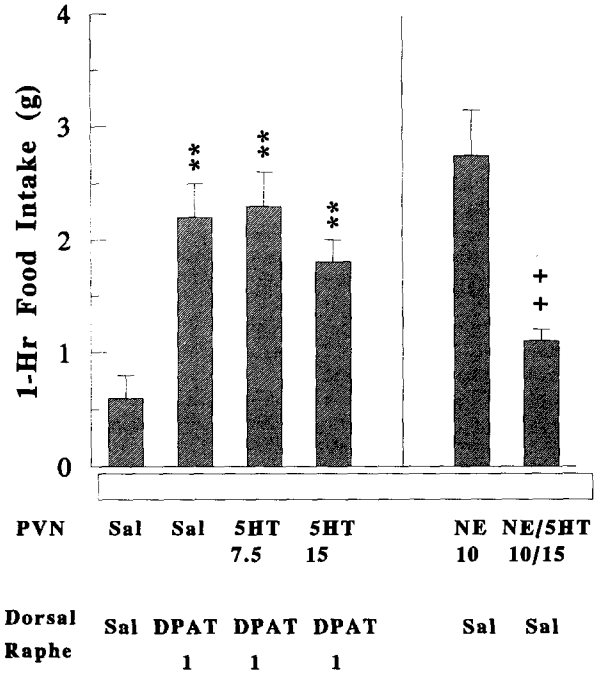

FIG. 2. Effects of paraventricular nucleus (PVN) injections of 5-hydroxytryptamine (5-HT) or saline (sal) on the feeding response elicited by 0.5 μg 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) injected into the median raphe (MR). The right panel shows the effects of 5-HT injected into the PVN on the feeding-stimulant action of PVN-injected norepinephrine (NE). Values represent mean (± SEM) food intakes over 1 h for nine animals. $^{**}p < 0.01$ compared to sal-sal condition. $^{++}p < 0.01$ compared to NE + 5-HT condition.

5-HT is thought to modulate the activity of dopaminergic neurons [see (19) for review], it has been proposed that the feeding elicited by 8-OH-DPAT results from a removal of the inhibitory influence that 5-HT neurons exert of dopamine neurons (7,8,20). Blockade of dopamine receptors within the nucleus accumbens attenuates feeding induced by dorsal raphe- or median raphe-injected 8-OH-DPAT, and this effect deriving from the dorsal raphe is also attenuated by dopamine receptor blockade within several striatal subregions (7). All of these results indicate that an interaction between 5-HT systems and both mesolimbic and nigrostriatal dopamine neurons is involved in mediating the increased feeding that follows 8-OH-DPAT injection. Thus, it would appear that reduced 5-HT input to dopamine pathways, rather than to hypothalamic areas, is likely to be the important neurochemical event underlying 8-OH-DPAT-induced feeding. It is unclear, however, whether this interaction takes place at the level of the dopamine cell bodies (ventral tegmental area and substantia nigra) or in terminal areas (nucleus accumbens and dorsal striatum). In this regard, it is interesting to note that 5-HT synthesis in both the nucleus accumbens and striatum is reduced by raphe-injected 8-OH-DPAT (16) at doses that reliably increase feeding (7).

Although reductions in food intake can be induced by increasing 5-HT activity within the PVN (10,14,18,24,28), it has not been demonstrated yet that the converse holds true, that is, that selective decrements in PVN 5-HT necessarily enhance feeding [see also (3)]. Perhaps of particular note in this regard, the nonselective 5-HT antagonist metergoline can increase food intake after peripheral (4,6), intraventricular (Coscina, Feifel, Nobrega, and Currie, submitted), or dorsal raphe (8) injection and also reverses the anorectic effect of PVN-injected 5-HT (28). However, when injected into the PVN metergoline has been reported ineffective in enhancing food intake [(28); Coscina, Feifel, Nobrega, and Currie, submitted]. This result is consistent with the present report of a lack of effect of medial hypothalamic injections of 5-HT on 8-OH-DPAT-induced feeding.

In conclusion, the present results do not support the hypothesis that reducing the release of 5-HT within the medial hypothalamus is responsible for the feeding-stimulant action of 8-OH-DPAT. Viewed in conjunction with the other research cited, these findings suggest that the release of 5-HT within the PVN may suppress feeding in instances where intake has been otherwise stimulated. However, it does not appear to be the case that suppressing 5-HT release in this site alone is a sufficient condition to stimulate feeding in its own right. As such, this implies that 5-HT neuronal elements within the PVN do not operate in a bidirectional fashion in controlling food intake.

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