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Medial Hypothalamic Nuclei Mediate Serotonin's Inhibitory Effect on Feeding Behavior

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LEIBOWITZ, S. F., G. F. WEISS AND J. S. SUH. *Medial hypothalamic nuclei mediate serotonin's inhibitory effect on feeding behavior*. PHARMACOL BIOCHEM BEHAV **37**(4) 735–742, 1990.—Previous studies have demonstrated that injection of serotonin (5-HT) into the paraventricular nucleus (PVN), specifically at the onset of the active feeding cycle, causes a strong and selective suppression of carbohydrate intake, while producing no change in fat intake and, in some cases, enhancing protein consumption. The purpose of the present investigation was to determine whether this selective inhibitory effect of 5-HT on macronutrient ingestion is localized to a specific brain region, perhaps the PVN, or whether it can also occur in other sites throughout the hypothalamus or in regions outside this structure. A total of 7 hypothalamic and 5 extrahypothalamic areas were examined in brain-cannulated, freely feeding rats maintained on pure macronutrient diets of protein, carbohydrate and fat. The effect of 5-HT, a selective suppression (−55%) of carbohydrate feeding, was replicated in the PVN with a relatively low dose of 2.5 nmoles. Tests in 11 other brain sites demonstrated that this action of 5-HT is not unique to the PVN but is anatomically localized to the medial nuclei of the hypothalamus. Sites outside the hypothalamus, namely, the amygdala, nucleus accumbens, septum, diagonal band of Broca and nucleus reuniens dorsal to the PVN, failed to exhibit any response to 5-HT injection. Within the hypothalamus, the ventromedial (VMN) and suprachiasmatic (SCN) nuclei each responded to 5-HT in a manner similar to the PVN, producing a suppression of carbohydrate intake (−50% to −70%) with little or no change in either protein, fat or total kcal intake. The dorsomedial nucleus showed a somewhat smaller response relative to these other medial hypothalamic areas. However, other hypothalamic sites, located caudal (posterior hypothalamus), rostral (medial preoptic area), and lateral (perifornical hypothalamus at different anterior-posterior levels) to these medial nuclei, were unresponsive. Based on these results and other published findings, it is proposed that the serotonergic system for inhibiting feeding exists within the medial portion of the hypothalamus, in contrast to lateral hypothalamic or extrahypothalamic regions, and these medial hypothalamic nuclei interact closely in coordinating temporal patterns of macronutrient ingestion, possibly with the PVN and VMN serving to couple the endogenous circadian generator of the SCN to the effector feeding systems.

Serotonin Medial hypothalamus Paraventricular nucleus Suprachiasmatic nucleus Feeding behavior Macronutrients Carbohydrate Protein

STUDIES of brain monoaminergic neurotransmitters have revealed the importance of these neurochemicals in the control of food ingestion, in animals and possibly in humans [e.g., (1, 8, 12, 16, 20, 46, 47)]. The anatomical focus of the studies in animals has been the hypothalamus, a structure believed to play a critical role in feeding behavior. This structure is known to receive and integrate input from a myriad of factors reflecting the animal's nutritional status. It then acts to translate this information into signals that induce appropriate adjustments in nutrient consumption and ultimately energy balance.

It is now recognized that brain serotonin (5-HT) has an inhibitory effect on food intake and that its specific function may be to control eating patterns and intake of specific macronutrients. Meal pattern analyses in rats demonstrate that hypothalamic injections of 5-HT (37), like peripheral injection of serotonergic agonists (1), reduce food intake by decreasing meal size and eating rate. Moreover, hypothalamic 5-HT is found to modulate satiety for a particular macronutrient, namely, carbohydrate, while having little effect on, or possibly increasing, preference for protein (17, 20, 37, 48).

A recent report demonstrates that this effect of hypothalamic 5-HT is linked to the light/dark cycle, apparent specifically at the onset of the active feeding period, i.e., the nocturnal cycle for the rat (18). Serotonin injection into freely feeding rats on pure macronutrient diets suppresses intake of carbohydrate only during the initial period of the dark cycle; injections in the middle and late phases of the feeding cycle, in contrast, have no impact on total food intake or on macronutrient selection. This finding has led to the proposal that hypothalamic 5-HT acts in a phasic, circadian-related manner, and its physiological function is to inhibit carbohydrate-rich meals that occur naturally at the beginning of the dark period (16).

These central injection studies with 5-HT have focused on the hypothalamic paraventricular nucleus (PVN), an area believed to have an important role in the control of satiety for carbohydrate (12, 31, 35, 44). These investigations have shown that electrolytic lesions of this nucleus enhance carbohydrate intake, while suppressing ingestion of protein. These lesion results, and the

above findings obtained with PVN 5-HT injection, suggest that 5-HT may act within the PVN, first, to stimulate the firing of the satiety-producing neurons within this nucleus and, then, to inhibit carbohydrate consumption while promoting intake of protein (16, 17, 36, 37).

What remains to be established, however, is whether the PVN is in fact a primary site in the mediation of 5-HT's action. Since other brain areas have yet to be tested with direct injections of 5-HT, it is possible that 5-HT has multiple sites of action in controlling feeding, not only within but possibly outside the hypothalamus. Thus, in order to test systematically the brain site of action for 5-HT, we conducted a mapping study to examine the relative sensitivity of a variety of hypothalamic and extrahypothalamic sites to serotonergic stimulation.

In this mapping study, 5-HT was examined in freely feeding rats maintained on pure macronutrient diets. It was centrally injected at the onset of the active (nocturnal) period, when a burst of feeding naturally occurs and when 5-HT in the PVN has been found to be most effective and selective in suppressing carbohydrate intake (18). The results of this study, reported in preliminary form (40,42), support the conclusion that specific nuclei within the medial portion of the hypothalamus have a primary role in the mediation of 5-HT's inhibitory effect on nutrient intake at the onset of the active period.

METHOD

Animals

In this experiment, 80 male albino Sprague-Dawley rats (Charles River, MA), weighing 350–400 g at the start of the experiment, were used. They were individually housed in double-sized, standard hanging wire mesh cages, in a temperature-controlled (22°C) room and under a 12:12-h light-dark cycle with lights on at 10:00 a.m. They had ad lib access to tap water and three separate sources of pure macronutrients (see below).

Surgery

Each animal was stereotaxically implanted, under methoxyflurane anesthesia, with a chronic, unilateral (23-gauge) cannula aimed at one of 12 brain areas. The stainless steel guide, which had a brass screw-on top, was fixed on top of the skull with acrylic cement and six stainless steel machine screws that penetrated the bone. The cannula tip was aimed 1.0 mm dorsal to each site, to allow the 33-gauge injection needle to penetrate this distance beyond the cannula and into the brain tissue.

With the incisor bar placed at −2.5 mm relative to the earbars, the 7 hypothalamic sites and their respective coordinates (in mm anterior to interaural line/mm lateral to midsagittal sinus/mm ventral to skull surface) were: paraventricular nucleus of the hypothalamus (PVN), +6.8/0.4/−7.4; ventromedial nucleus (VMN), +5.9/0.5/−8.4; suprachiasmatic nucleus (SCN), +8.0/0.2/−8.9; dorsomedial nucleus (DMN), +5.5/0.4/−7.9; medial preoptic area (POM), +8.4/0.4/−7.9; posterior hypothalamus (PH), +4.4/0.4/−8.0; and lateral perifornical hypothalamus (PFH), +6.0 to +7.2/1.2/−7.9. The 5 extrahypothalamic sites were: nucleus reuniens (NR), +6.8/0.4/−5.0; amygdala (AMYG), +6.6/3.9/−8.2; nucleus accumbens (NA), +10.6/1.4/−6.0; septum (SEPT), +8.0/0.7/−4.2; and diagonal band of broca (DB), +9.4/0.4/−7.9.

All rats were given approximately 10 days of postoperative convalescence before the beginning of the experiment. During this time, the animals were handled and mock-injected with the vehicle in order to habituate them to the test procedures.

Diets

Animals were maintained on three pure macronutrient diets, protein, carbohydrate and fat, which previous work (37,41) has shown to permit normal body weight gain, to yield consistent day-to-day intake scores, and to be generally palatable such that each diet accounts for at least 20% (and no greater than 50%) of the rats' total daily intake. The protein diet (3.7 kcal/g) was composed of 93% casein (granulated, enzymatic casein from the National Casein Co.), mixed with 4% minerals (USP XIV Salt Mixture Briggs, ICN Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals) and 0.03% cysteine (L-cysteine hydrochloride, ICN Pharmaceuticals). The carbohydrate diet (3.7 kcal/g) consisted of 37% sucrose (Domino sugar), 28% dextrin and 28% cornstarch (ICN Pharmaceuticals), fortified with 4% minerals and 3% vitamins. The fat diet (7.7 kcal/g) consisted of 86% lard (Armour), combined with 8% minerals and 6% vitamins.

The three diets were provided simultaneously in separate glass jars that were braced to the front of the cage with a stainless steel wire spring to minimize spillage. Water was available ad lib through an automatic water dispenser at the back of each cage. Fresh diet was provided daily, shortly after the testing was completed, and 24-h intake was measured twice weekly to verify that the average daily kcal intake of each diet accounted for at least 20% of the rats' total daily intake. Although spillage was minimal, when it did occur, the food lost under the cage was collected and added to the unconsumed total. Placement of food jars within the cage was shifted daily to prevent the formation of a position preference.

Test Procedures

The effects of 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma), on natural patterns of macronutrient intake, were investigated. The drug was dissolved in bacteriostatic saline (0.9%) immediately before the start of the test. Thirty minutes before lights-out, food jars were removed and weighed. Ten min prior to the onset of the dark period, the subjects were then centrally injected with either 5-HT (2.5 nmoles in 0.5 μl) or saline (vehicle) in counterbalanced order. Immediately following injections, the food jars were returned, and intake was measured 1 h later.

The 2.5-nmole dose of 5-HT was chosen for three primary reasons. First, dose-response studies with 5-HT injection have shown the PVN to be responsive to doses as low as 0.6 nmoles, to exhibit a maximal response at 10 nmoles, and to have nonspecific, sedative effects at doses higher than 25 nmoles (11, 18, 44). Second, in the higher range of effective doses (20–40 nmoles), the serotonergic agonists, including d-norfenfluramine and fluoxetine as well as 5-HT, are found to be less selective in their effect on macronutrient selection (11,26). Third, pilot studies with 5-HT, using the same procedures as those employed here, have shown the 2.5-nmole dose of 5-HT in the PVN to be most consistent in producing a robust as well as selective behavioral response.

The rats were injected 3–4 times/week and tested over a total period of approximately 6 weeks. The first 1–2 weeks of this period frequently yielded unstable baseline feeding scores, and thus these data were eliminated from the data analysis. The subsequent 4 weeks of testing yielded relatively stable data, approximately 6 vehicle and 6 drug scores, which were then averaged to represent the rat's final test scores. Animals (23% of original group) were eliminated from the experiment because of body weight loss or sickness (n = 5), extreme patterns of 24-h nutrient intake (n = 2), variable saline baseline scores (n = 8) and off-target cannula placements (n = 3). With the elimination of these animals, the variability (standard error of the mean) of the vehicle baseline scores, as well as the drug scores, for the different groups generally ranged from 8% to 15% of the mean.

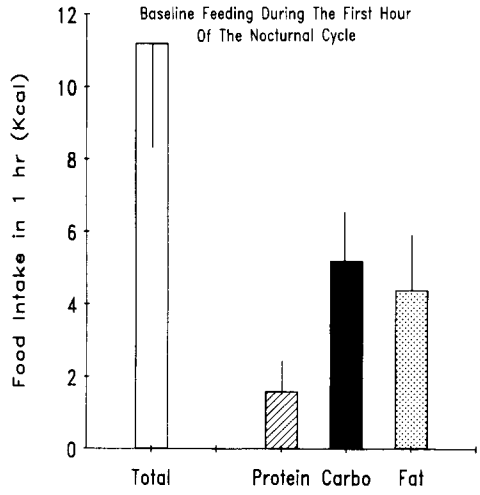

FIG. 1. Baseline pattern of nutrient intake (kcal) in brain-cannulated, freely feeding rats receiving hypothalamic injection of saline vehicle. Measurements of protein, carbohydrate and fat were taken 1 h after saline administration, which was given just prior to the onset of dark. During this 1-h test, rats exhibited a significant preference for carbohydrate and fat.

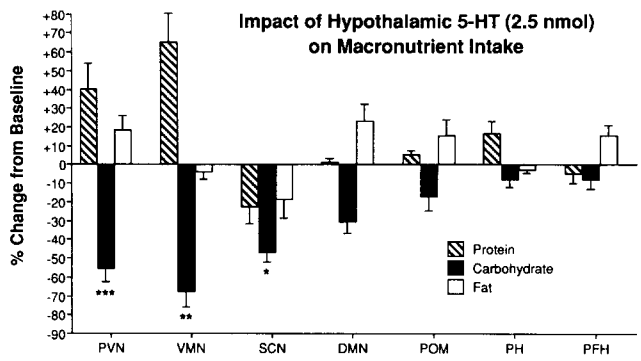

FIG. 2. Responsiveness of 7 hypothalamic sites to 5-HT injection, indicated by a percent change in feeding scores relative to vehicle control baseline. Measurements of protein, carbohydrate and fat were taken 1 h after 5-HT (2.5 nmoles) injection into the paraventricular nucleus (PVN), ventromedial nucleus (VMN), suprachiasmatic nucleus (SCN), dorsomedial nucleus (DMN), medial preoptic area (POM), posterior hypothalamus (PH) and the lateral perifornical hypothalamus (PFH), just prior to the beginning of the nocturnal cycle. The three medial hypothalamic nuclei, PVN, VMN and SCN, showed a significant response to 5-HT injection, namely, a strong suppression of carbohydrate ingestion, a tendency towards enhancement of protein intake and no change in fat consumption. The DMN exhibited a smaller response, whereas no effect was observed in the POM, PH and PFH. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ by Newman-Keuls test.

Histological Analysis

At the completion of the behavioral testing, each rat was sacrificed with an overdose of Nembutal and was intracardially perfused with isotonic saline followed by 10% buffered formalin. Their brains were removed from the skull and placed in a 30% sucrose-buffered formalin solution for at least 48 h. Frozen coronal sections of 75 μm were then cut and stained with cresyl violet. The location of the cannula tips was determined according to the atlas of Paxinos and Watson (24).

Statistical Analysis

Statistical evaluations were based on one- and two-way analyses of variance (ANOVA) for repeated measures, followed by individual mean comparisons using the Newman-Keuls procedure. "Percent change" scores were calculated by subtracting the drug score from the saline score and dividing this difference by the saline score. Correlational analyses were conducted using a Pearson Product Moment Correlation Coefficient. A two-tailed Student's *t*-test for dependent scores was used to determine diet preference and to compare percent concentration scores (percent of each diet eaten relative to total intake).

RESULTS

Saline Baseline

As shown in Fig. 1, baseline measurements under the saline conditions, averaged for all subjects independent of cannula placement, revealed in these freely feeding rats a distinct pattern of macronutrient selection during the first h of the dark cycle, $F(2,93)=31.36$, $p<0.001$. The total hourly intake score averaged 11.3 kcal, ranging from 10.5 to 13.2 kcal for the different groups tested. The nutrient selection pattern, consistently exhibited by 80–90% of the rats in each group, was characterized by a significantly greater amount ($p<0.001$) of carbohydrate and fat intake relative to protein consumption. Calculation of the percent concentration scores, which represent the percent of each diet eaten relative to total intake, revealed a significant preference ($p<0.001$) for the carbohydrate (47%) and fat (38%) diets as compared to the protein diet (15%).

Medial Hypothalamic Sites

The behavioral results, obtained with 5-HT (2.5 nmoles) injection into the 7 hypothalamic sites, are summarized in Fig. 2. This figure presents the percent change scores for each injection site, calculated by relating each group's kcal intake scores after 5-HT injection to its own saline baseline measurements. A two-way analysis of variance (drug × diet), performed on the kcal intake scores for each injection site, distinguished three medial hypothalamic sites, the PVN, VMN and SCN, as exhibiting the strongest and most selective response to local 5-HT administration. These nuclei contrasted with the DMN, which yielded a variable effect, and the POM, PH and PFH, which failed to show any behavioral change from baseline after 5-HT injection.

As described previously (18), an analysis of the saline and drug kcal intake scores for the PVN-cannulated animals ($n=6$) revealed little change in total food intake after 5-HT administration but a strong suppression in the ingestion of a specific macronutrient (Fig. 2). While the main effect for drug was statistically insignificant, $F(1,30)=0.36$, $p>0.10$, a significant drug × diet interaction, $F(2,30)=3.46$, $p<0.04$, reflected 5-HT's differential effect depending upon diet. Examination of the kcal intake scores for the different diets showed carbohydrate ingestion to be significantly suppressed [−56%, $F(1,10)=26.89$, $p<0.001$], in contrast to protein (+40%, $p>0.10$) and fat (+18%, $p>0.10$) intake which tended to be enhanced. This macronutrient selective effect of PVN 5-HT caused the rats' relative preference for carbohydrate (44% of total) after saline injection to be significantly reduced to 23% after 5-HT injection ($p<0.04$). A small increase in appetite for protein (from 11% to 19% of total, $p<0.10$) and fat

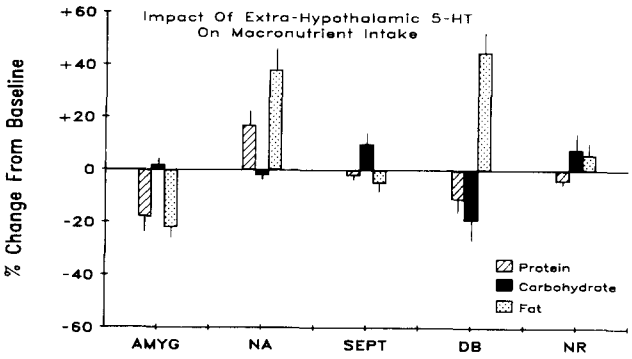

FIG. 3. Effect of 5-HT (2.5 nmoles) on 1-h macronutrient intake, in freely feeding rats, after administration into 5 extrahypothalamic sites: the amygdala (AMYG), nucleus accumbens (NA), septum (SEPT), diagonal band of Broca (DB) and nucleus reuniens immediately dorsal to the PVN (NR). No reliable change in carbohydrate, protein or fat consumption was observed in any of these extrahypothalamic sites.

(45% to 58% of total) was observed.

This effect of 5-HT in the PVN, a selective suppression of carbohydrate consumption at dark onset, was similarly detected after injection into the VMN (n=4). With total kcal intake once again unaffected, the significant drug × diet interaction, $F(2,18)=6.94$, $p<0.006$, reflected a selective suppression (−68%, $p<0.02$) of carbohydrate intake in association with some enhancement of protein ingestion (+65% $p<0.07$) and no change in fat intake. Thus, 5-HT injection into the VMN was similar to 5-HT in the PVN, in terms of both the selectivity and magnitude of its effect on carbohydrate consumption. Percent concentration scores showed VMN 5-HT to reliably reduce carbohydrate preference from 39% to 16% ($p<0.01$), while significantly enhancing preference for protein from 14% to 27% ($p<0.03$).

The SCN, which lies approximately 0.5 mm rostral to the PVN and 1.5 mm rostral to the VMN, was also responsive to 5-HT injection, although the pattern of change was somewhat different. At this site (n=5), carbohydrate ingestion was reliably suppressed (−47%, $p<0.05$), to a slightly smaller extent than that observed in the PVN and VMN. However, total kcal intake was also reduced in these animals (−32%, $p<0.05$), and the drug × diet interaction was statistically insignificant, due to the small suppression observed in both protein (−23%) and fat (−19%) intake. This lack of clear selectivity was confirmed by analyses of the percent concentration scores, which failed to reveal any significant 5-HT-induced shift in the rats' relative preference for the different macronutrients.

Statistical comparison of these data for the 7 hypothalamic sites confirmed that this inhibitory effect of 5-HT on carbohydrate consumption was site specific, $F(6,78)=2.30$, $p<0.04$. In contrast to the robust effect observed in the three medial hypothalamic nuclei, PVN, VMN and SCN, no response to 5-HT was detected in two other medial regions, the POM (n=6) which lies just rostral to the PVN and dorsal to the SCN, or the PH (n=5) situated just caudal to the VMN. The DMN (n=5), however, responded to 5-HT with a variable decrease (−31%) in carbohydrate consumption (Fig. 2). While this effect failed to reach statistical significance, 4 of the 5 rats with DMN cannulas exhibited a selective suppression of carbohydrate intake (−16%, −26%, −51% and −71%), suggesting that, in a larger set of animals, the DMN may be found to respond reliably to 5-HT.

Lateral Hypothalamic Sites

Within the lateral hypothalamus (n=10), several sites in the

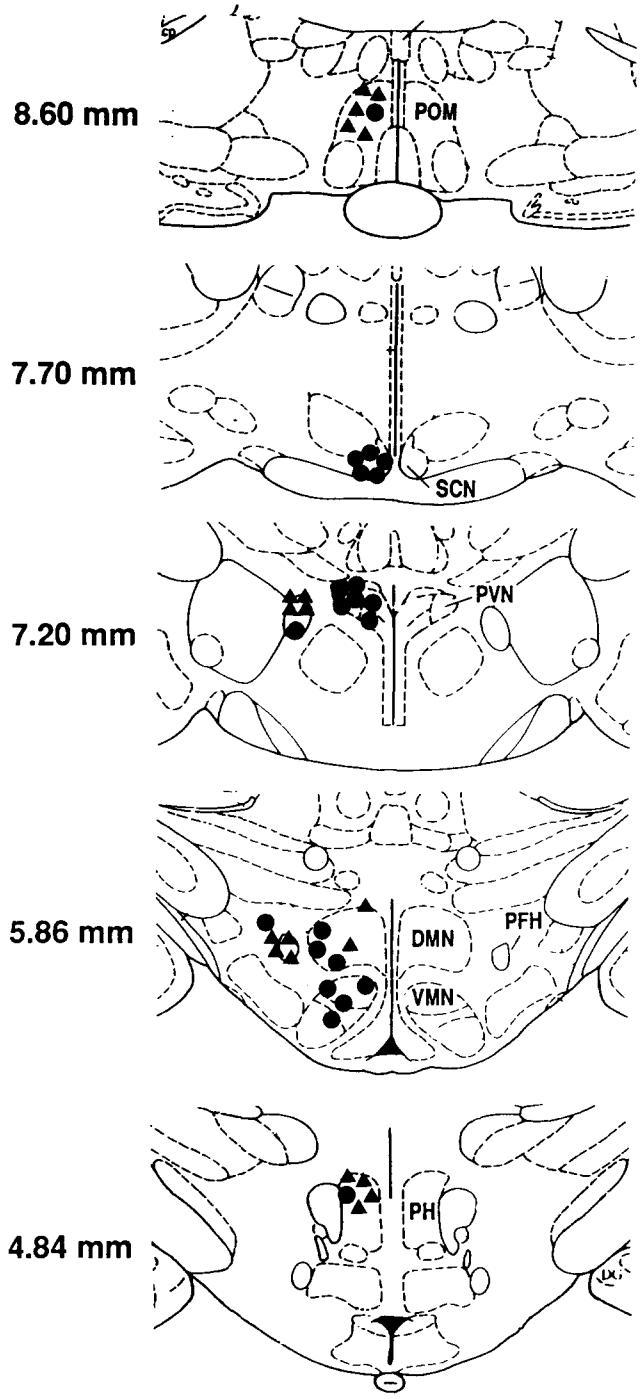

FIG. 4. Schematic diagrams of coronal sections of the rat brain (24) showing location of sites in individual subjects that exhibited a significant (circle: ≥ −25% suppression of carbohydrate intake) or insignificant (triangle: < −25% suppression) response to 5-HT (2.5 nmoles) injection. Predominantly responsive sites were located in the paraventricular nucleus (PVN), ventromedial nucleus (VMN) and suprachiasmatic nucleus (SCN); both responsive and unresponsive sites were seen in the dorsomedial nucleus (DMN); and a predominance of unresponsive sites were found in the medial preoptic area (POM), posterior hypothalamus (PH) and lateral perifornical hypothalamus (PFH).

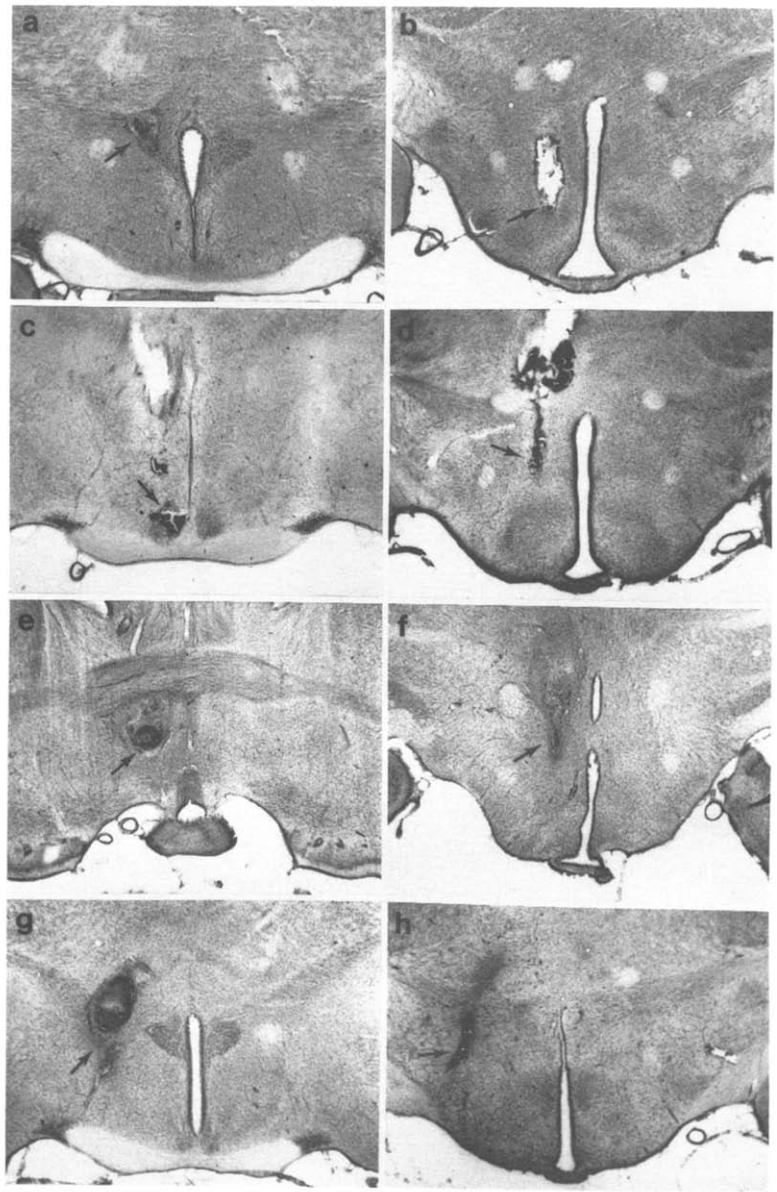

FIG. 5. Photomicrographs of coronal, cresyl violet-stained sections of the rat brain showing 8 representative injection sites as indicated by arrows. (a) PVN, paraventricular nucleus; (b) VMN, ventromedial nucleus; (c) SCN, suprachiasmatic nucleus; (d) DMN, dorsomedial nucleus; (e) POM, medial preoptic area; (f) PH, posterior hypothalamus; (g) PFH, lateral perifornical hypothalamus at the level of the PVN; and (h) PFH at the level of the VMN.

area of the fornix (PFH) were tested and consistently failed to exhibit any sensitivity to 5-HT (Fig. 2). These sites were positioned along the lateral, dorsolateral or ventrolateral edge of the fornix and were located at a variety of anterior-posterior levels, ranging from the level of the PVN to the caudal aspect of the VMN. At a dose of 2.5 nmoles, 5-HT in this lateral area failed to alter total kcal intake and, as shown in Fig. 2, had no effect on intake of the separate macronutrients.

Extrahypothalamic Sites

Five sites outside the hypothalamus (Fig. 3) were also consistently found to be unresponsive to 5-HT. These sites were the nucleus reuniens (NR, $n=6$), which lies immediately dorsal to the PVN; medial nucleus of the amygdala (AMYG, $n=3$); nucleus accumbens (NA, $n=4$); lateral septum (SEPT, $n=5$); and diagonal band of broca (DB, $n=3$). Statistical analyses of the kcal intake scores obtained with 5-HT injection into these sites showed no change in total intake or nutrient selection relative to saline baseline. The maximum reduction of carbohydrate intake detected was -18% ($p>0.10$) in the DB, and the ingestion of fat tended to be enhanced after 5-HT administration in the DB ($+55\%$, $p>0.10$) and NA ($+41\%$, $p>0.10$).

Histological Analyses

The results of the histological analyses appear in Fig. 4, which diagrammatically illustrates the hypothalamic injection sites for all animals tested, and in Fig. 5, which presents cresyl violet-stained photomicrographs of representative injection sites. The amount of damage produced at the injection site was minimized by having the injection needle (33 gauge) extend 1.0 mm beyond the tip of the cannula implant.

The stained sections for the medial hypothalamic sites that responded to 5-HT injection (PVN, VMN, SCN, DMN) were examined in detail to determine their precise location in relation to each other. The PVN injection sites (Figs. 4 and 5a) were all confined to the area of the PVN, located within or along the dorsal surface of the nucleus. In these PVN animals, the injection needle caused no apparent damage to the VMN and DMN, generally located at least 0.5 mm anterior to the rostral border of these two nuclei. Similarly, the VMN (Figs. 4 and 5b) and DMN (Figs. 4 and 5d) injection needles were located within the boundaries of these respective nuclei at the same anterior-posterior level. In the dorsal-ventral plane, these two sites were clearly distinct, separated by approximately 0.5 mm, and the injection needles produced no damage to the rostrally-located PVN. The SCN injection site (Figs. 4 and 5c) was located at least 1.0 mm directly rostral to the VMN and approximately 0.5 mm rostral and 1.0 mm ventral to the PVN. While the SCN cannula descended relatively close to the PVN, it rarely passed through or damaged this structure as it traversed ventrally. The POM injection needle (Figs. 4 and 5e) descended approximately 1.4 mm in front of the PVN, and the PH needle (Figs. 4 and 5f) was situated approximately 1.0 mm caudal to the VMN. In the PFH site (Figs. 4 and 5g,h), the targeted injection sites were distributed across a wide area along the anterior-posterior plane just lateral to the fornix. They were as far anterior as the rostral level of the PVN and as far posterior as the caudal aspect of the VMN. No significant difference in responsiveness to 5-HT was detected across these different levels within the lateral hypothalamus.

DISCUSSION

These results replicate the finding that hypothalamic injection of 5-HT modulates macronutrient intake in freely feeding rats. As previously reported (18), PVN injection of 5-HT, specifically at the onset of the active feeding cycle, causes a significant suppression of carbohydrate intake, while producing no change or an enhancement of protein or fat intake, as well as little change in total intake. This effect, previously described for doses of 5–20 nmoles in the PVN (18), is shown here to be robust at a lower, 2.5-nmole dose of 5-HT and may even be expected to occur at doses as low as 0.2 nmoles (16,44). The effectiveness of these relatively low doses is attributed, in part, to the use of freely feeding rats, which are found to be considerably more responsive to 5-HT than food-deprived animals (44).

Medial Hypothalamic Nuclei

The results of the present study demonstrate that this feeding-suppressive effect of 5-HT stimulation is anatomically localized, although not unique to the PVN. Sites outside the hypothalamus are so far found to be unresponsive to 5-HT, suggesting that this phenomenon is primarily linked to the hypothalamus, a structure long known to play an important role in the control of feeding behavior. Within the hypothalamus, the evidence indicates that 5-HT-sensitive neurons are localized specifically to the medial portion, in contrast to the lateral region. Moreover, within the medial hypothalamus, there appear to exist at least 3 and possibly 4 nuclei, the VMN, SCN and DMN in addition to the PVN, which are distinguished as being highly responsive to 5-HT stimulation.

In view of the close proximity of these 4 hypothalamic nuclei, it is possible that their responsiveness may simply reflect the spread of 5-HT from one medial hypothalamic injection site to another nearby site. There is a good deal of evidence that argues against this possibility and suggests that each of the medial hypothalamic nuclei are independently sensitive to 5-HT. Previous studies [see (10)] have suggested that, with a 1.0-μl injection volume, the radius of drug spread around the injection site is approximately 1.0 mm and probably less with a smaller volume of 0.5 μl. Despite this spread, earlier mapping studies with the monoamines have been successful in revealing differential responsiveness of hypothalamic nuclei lying within distances of less than 1.0 mm (10,15). In one study, for example, the PVN was found to be considerably more responsive than either the VMN, DMN or other sites immediately rostral, caudal or lateral to the PVN (10). Another report has distinguished the lateral perifornical hypothalamus, as opposed to other nearby hypothalamic sites, as being maximally responsive to monoaminergic injections (15). In the present study, the four responsive medial hypothalamic nuclei, the PVN, VMN, DMN and SCN, are surrounded rostrally and caudally by the POM and PH, which are totally unresponsive to 5-HT. Moreover, the lateral perifornical hypothalamus, including sites located only 0.5 mm lateral to the PVN, VMN or DMN, is also insensitive to 5-HT. This evidence, therefore, indicates that the satiety-inducing effect of 5-HT is an anatomically localized phenomenon. Any spread of 5-HT that occurs in the area of the injection site does not appear to be significant enough to preclude the demonstration of this site specificity, nor does it provide a sufficient basis for explaining the responsiveness of several nearby nuclei within the medial hypothalamus.

An additional mode of drug spread, the efflux of 5-HT dorsally along the outside of the cannula tract, also needs to be considered as a possible explanation for the responsiveness of structures lying immediately ventral to another 5-HT sensitive site. For example, the responsiveness of the SCN may be attributed to the efflux of 5-HT up along the cannula tract and into the dorsally-lying PVN. A careful analysis of the brain tissue of SCN-cannulated rats, however, fails to reveal any relationship between the magnitude of the response exhibited after SCN injection and the position of the cannula tract relative to the PVN. That is, rats with cannula implants that coursed rostrally to the PVN are equally responsive to rats with cannula implants that penetrate the PVN. With regard to the VMN and DMN, the dorsal efflux of 5-HT also appears to have had little impact, as evidenced by the finding that the ventral structure, the VMN, is considerably more responsive to 5-HT than the more dorsal DMN.

However, while the DMN-cannulated rats appear less responsive and fail to exhibit a significant group effect after 5-HT, the response observed in a majority of the DMN animals, in addition to the results of other mapping studies with serotonergic agonists (26,45), suggest that the DMN may be an additional medial hypothalamic site involved in serotonergic control of nutrient selection. The DMN-cannulated rats in the present study exhibit, as a group, a 31% suppression of carbohydrate intake. This effect reflects the responsiveness of 4 (67%) of the 6 rats in this group, two with a small to moderate response (-16% and -26%) and two others with a strong response of -51% and -71%. This suggestion of an effect in the DMN needs to be considered seriously in light of other central injection studies (16, 26, 37, 42, 45) revealing an even stronger and more consistent suppression of carbohydrate feeding (-60% to -70%) with injection of the serotonergic agonists, d-norfenfluramine (DNF) and fluoxetine (FLU), into DMN sites as much as 1.0 mm distant from the VMN or PVN. In general, the mapping studies conducted with these drugs,

which are believed to act by releasing or blocking presynaptic uptake of endogenous 5-HT (4,22), yielded very similar results to those obtained here with exogenous 5-HT, arguing strongly for anatomical specificity in the physiological action of endogenous 5-HT in the control of carbohydrate consumption.

Consistent with this evidence, revealing the importance of medial hypothalamic nuclei in serotonergic feeding mechanisms, is the finding that each of the nuclei in this area receives a moderate to dense innervation from serotonergic projections of the midbrain raphe nuclei (28) and has a relatively dense concentration of 5-HT_{1B} receptor sites (13,25) which mediate the satiety-inducing effect of serotonergic stimulation (3). Moreover, electrolytic lesions in this region, in contrast to the lateral hypothalamus, are found to attenuate or abolish the feeding suppressive effect of peripherally injected d-fenfluramine (DF) (2, 19, 43), and to produce changes in appetite for carbohydrate and also in the light/dark periodicity of feeding behavior or food-anticipatory behaviors (16, 17, 23, 31, 35, 39). This evidence, in conjunction with the mapping results obtained here with 5-HT and in other studies with DNF or FLU (16, 26, 45), suggests that these medial hypothalamic nuclei, which are anatomically and functionally linked (27, 38, 39), interact closely in coordinating temporal patterns of macronutrient choice across the circadian cycle, possibly with the PVN, VMN and DMN serving to couple the endogenous circadian generator of the SCN to the effector feeding systems. Based on the finding that serotonergic stimulation is most effective in suppressing carbohydrate feeding at the onset of the active feeding period (18), it is proposed that medial hypothalamic 5-HT acts in a phasic, circadian-related manner to inhibit carbohydrate-rich meals that occur naturally at the beginning of the dark period and then possibly to switch the animal's preference towards protein (16,18).

Lateral Hypothalamus

The lateral hypothalamus, in contrast to the medial hypothalamus, appears to be unresponsive to 5-HT injection, at least in the mid-lateral region of the area of the fornix. Animals with lateral PFH cannulas fail to respond to the 2.5-nmole dose tested here at dark onset. They are also found to be unresponsive to 2- to 10-fold higher doses tested in the light period, although considerably higher doses (25–100 nmoles) of 5-HT may suppress feeding through a non-specific sedative action [see (11)]. In the present study, several anterior-posterior levels of the lateral hypothalamus are represented, and in essentially all animals, no change in total food intake or macronutrient selection is detected after 5-HT injection. As illustrated in Fig. 4, these lateral injection sites fall within 1.0 mm of the highly responsive medial hypothalamic nuclei, the PVN and VMN, just as the unresponsive POM and PH fall as little as 0.5 mm anterior or posterior to these nuclei. Together, this evidence illustrates the effectiveness of the chronic brain-cannula technique in distinguishing the responsiveness of sites less than 1.0 mm apart (14).

This lack of response in the lateral hypothalamus agrees with other evidence revealing no effect in this region after injection of the agonists, DNF and FLU (16, 26, 45), and also no effect of lateral hypothalamic lesions on the feeding-suppressive effect of peripheral DF (2, 19, 43). While these results indicate that 5-HT in the lateral hypothalamus may have little primary effect in modulating nutrient ingestion, other evidence argues for a role in this monoamine in the lateral area in controlling other aspects of the feeding process. It has been demonstrated that both the medial and lateral regions of the hypothalamus exhibit an increase in extracellular 5-HT after peripheral injection of DF (5, 29, 32) and after refeeding in deprived rats (5,30); an increase in 5-HT metabolism in association with higher serum insulin levels (6); and a significant change in neural firing in response to 5-HT administration (7,9). Possible functions served by lateral hypothalamic 5-HT may be indicated by the findings that: 1) serotonergic stimulation, via peripheral DF injection, reduces the rewarding value of PFH self-stimulation electrodes which induce feeding; 2) hypothalamic 5-HT is increased in response to the sensory qualities (sight and smell), as well as the ingestion, of food; 3) endogenous 5-HT release in the brain appears to be necessary for the behavioral expression of a conditioned taste aversion; and 4) 5-HT metabolism and neural activity in the lateral hypothalamus is altered by stress that induces anorexia (5, 21, 33, 34). Thus, while 5-HT or 5-HT-releasing compounds within the lateral hypothalamus fail to inhibit nutrient and specifically carbohydrate consumption, this monoamine in the lateral area may control food intake, and perhaps meal duration, by causing a reduction in the general rewarding consequences of food and the feeding process. These potentially different functions of 5-HT in the medial and lateral hypothalamus may be related to their differential electrophysiological responses to 5-HT, which exerts predominantly excitatory effects in the medial region and predominantly inhibitory effects in the lateral region (7,9).

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