Inhibiting parabrachial fatty acid amide hydrolase activity selectively increases the intake of palatable food via cannabinoid CB1 receptors

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Running head: Parabrachial FAAH inhibition and hedonic eating

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ABSTRACT

These studies investigated feeding responses to indirect activation of parabrachial cannabinoid CB1 receptors. Arachidonoyl serotonin (AA5HT), an inhibitor of the endocannabinoid degradative enzyme, fatty acid amide hydrolase (FAAH), was infused into the parabrachial nucleus of Male Sprague-Dawley rats and intakes of high-fat/sucrose pellets and standard rodent chow were subsequently evaluated under various feeding schedules. FAAH blockade stimulated the intake of high-fat/sucrose pellets that were presented daily for 4hr during the light period, with compensatory decreases in the consumption of standard chow during the ensuing 20hr. These diet-selective changes repeated on the next day, indicating a shift in feeding towards the more palatable diet that lasted for 48hrs after a single infusion. The cannabinoid CB1 receptor antagonist, AM251, blocked the orexigenic actions of AA5HT, implicating CB1 receptors in mediating the feeding responses to FAAH inactivation. When the feeding schedule was reversed, AA5HT produced nominal increases in the consumption of standard chow for the 4hr access period, but substantial increases in the intake of high-fat/sucrose during the following 20hr interval. When presented with only high-fat/sucrose for 24hr, AA5HT increased 24hr food intake. In contrast, when given 24hr access only to standard chow, AA5HT failed to affect intake. Therefore, indirectly activating parabrachial CB1 receptors by blocking the degradation of native ligands selectively stimulates the intake of palatable food, with differential actions on total energy intake depending upon the feeding schedule. Our results support a role for parabrachial cannabinoid mechanisms in providing physiological regulation to neural substrates modulating feeding, energy balance and behavioral responses for natural reward.

Keywords: parabrachial nucleus, endocannabinoids, feeding, reward
INTRODUCTION

Cannabinoid mechanisms have been widely reported to be involved in the regulation of feeding and energy balance (17). The endocannabinoid system, which includes native ligands (endocannabinoids) (5, 18, 27), their receptors (4, 20) and proteins modulating synthesis and degradation (25), has been found throughout the central nervous system and in the periphery. Evidence suggests that central endocannabinoids play an important role in modulating food intake (6, 14, 26). Nonetheless, specific brain sites and mechanisms for their central actions contributing to the physiological control of feeding and energy balance are only beginning to be elucidated. Infusion of cannabinoid receptor agonists into several areas of the forebrain, including the nucleus accumbens shell (14, 26) and multiple nuclei of the hypothalamus (1, 11, 28), increased consumption of standard chow. Similarly, endocannabinoids administered into the fourth ventricle of the hindbrain increased feeding when a more palatable diet of sweetened condensed milk was used (19). Ventricular administration, however, fails to reveal specific loci in the brainstem where agonists act. Further, no studies have directly compared the influence of test diet on the actions of endocannabinoids in the hindbrain on feeding.

We have reported the presence and functional capacity of CB1 receptors (CB1Rs) in the parabrachial nucleus to couple to their G-proteins, in vitro, following incubation of tissue sections with the endocannabinoid, 2-arachidonoylglycerol (7). Furthermore, we have demonstrated that parabrachial infusions of 2-arachidonoylglycerol stimulated feeding. The actions of the endocannabinoid were selective for palatable foods, including fat and/or sugar, as 2-arachidonoylglycerol failed to affect intake of standard rodent chow. In addition, the actions of 2-arachidonoyl glycerol were anatomically specific, as
off-target anatomical controls with infusions ~500\(\mu\)m caudal to those that successfully stimulated feeding, failed to affect the intake of the palatable food. These results suggested that hedonically-positive sensory properties of food enable endocannabinoids at parabrachial CB1Rs to initiate feeding.

The parabachial nucleus serves a role, though not exclusively, in gating gustatory neurotransmission and communicates this information with many brain regions controlling feeding behavior, including hypothalamic nuclei regulating energy balance and ventral striatal regions associated with reward (8, 16, 21-23). Therefore, parabrachial CB1Rs may provide an additional layer of regulation to the neural substrates modulating feeding, energy balance and behavioral responses for natural reward.

Recent identification of proteins that modulate endocannabinoid synthesis and degradation (25) has led to indirect pharmacological approaches for investigating physiological roles for endocannabinoid mechanisms in feeding. Indeed, pharmacologically inhibiting one of the endocannabinoid degradative enzymes, fatty acid amide hydrolase (FAAH), by administering the FAAH inhibitor, arachidonoyl serotonin (AA5HT), into the nucleus accumbens shell increased the intake of standard chow (26). The selective CB1R antagonist, AM251, blocked this increase, thereby implicating CB1Rs in the orexigenic responses to AA5HT.

The present series of studies characterized potential physiological roles for cannabinoid mechanisms in the parabrachial nucleus in modulating food intake. Specifically, we infused AA5HT into this brainstem site to compare the effects of CB1R activation in modulating the intakes of hedonically-positive (pellets high in fat and sucrose) or hedonically-neutral (standard rodent chow) foods. We also tested the prediction that pretreatment with AM251 would block any orexigenic action of FAAH
inhibition to indirectly stimulate these receptors in the parabrachial nucleus. We report a robust increase in the consumption of a test diet high in fat and sucrose content, but not of standard chow after parabrachial FAAH inhibition. This effect was prevented by CB1R blockade.

MATERIALS AND METHODS

Animals. Thirty-two male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing between 300 and 375g at time of surgery were used for these experiments. Animals were housed individually in plastic hanging cages with wire-mesh floors (43 x 22 x 18cm) and maintained on a standard 12h light/dark cycle (lights on at 0700 hrs) at 23±2ºC. Standard laboratory chow (see Behavioral testing) and water were provided ad libitum, unless otherwise noted. All experimental procedures complied with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research of the National Research Council (2003) and were approved by the Institutional Animal Care and Use Committee of Drexel University.

Surgical procedures. Rats were bilaterally implanted, under pentobarbital (35mg/kg) and chloryl hydrate (160mg/kg) anesthesia (Equithesin®), with bilateral 26-ga stainless steel guide cannulae (3.8mm center-to-center; Plastics One, Roanoke, VA) aimed centrally within the lateral PBN. Guide cannulae were secured to the skull using three stainless steel screws (Small Parts, Miami Lakes, FL) and orthodontic resin (Dentsply, Milford, DE). Twenty-eight gauge obturators (Plastics One, Roanoke, VA) were placed into the guide cannulae immediately following surgery to prevent occlusion. Stereotaxic coordinates for cannulae placement were determined from (Paxinos and Watson, 1998)
using standard flat skull technique (from bregma to lambda): 9.5-9.8mm caudal to bregma; 1.9mm lateral to the midline suture and 4.8mm ventral. For pain management, animals were administered ketoprofen [1mg/kg at 2mg/ml USP grade (Sigma Aldrich, St. Louis, MO)] just prior to and 24hrs after surgery. All animals were allowed 7-10 days to recover from surgery before testing commenced.

Drugs. The FAAH inhibitor, AA5HT (arachidonoyl serotonin; for. wt. = 462), was obtained from Cayman Chemical (Ann Arbor, MI) and the CB1R antagonist, AM251 [N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; mol. wt. = 555], from Tocris Cookson Inc. (Ellisville, MO). Due to the highly lipophilic nature of the compounds, AA5HT was first solubilized in dimethyl sulfoxide [DMSO (Sigma Aldrich, St. Louis, MO), then 0.9% (wt/v) NaCl was slowly added to yield a final concentration of 50% DMSO for the vehicle. AM251 was solubilized in 100% DMSO. When AM251 was used to block the effects of AA5HT, all test compounds and vehicle contained 100% DMSO. Drugs were prepared freshly at the appropriate concentration just prior to experimentation. Infusions were made in a total volume of 0.5 µl with a Harvard infusion pump (Harvard Apparatus, Cambridge, MA) using a 10-µl Hamilton microsyringe (Hamilton, Reno, NV) attached to a 33-ga injector with PE20 polyethylene tubing (Becton Dickinson, Sparks, MD). Injector tips extended 2.5mm past the tips of the guide cannulae. Bilateral infusions were made over 90sec, beginning between 0900 and 1000 hrs, with the injector left in place for 30sec following infusion of drug or vehicle to minimize backflow of liquid.
Experimental procedure. Experiments began following one week of daily habituation to the feeding schedules. At this time, baseline intakes were stable and did not vary by more than 10% during the last three days of this habituation. Following infusion of vehicle or test compound (1000 hrs), standard pelleted rodent chow [3.34kcal/g (12% kcal from fat, 28% protein, 60% carbohydrate); Purina 5001, St. Louis, MO] was removed from the home cages and replaced with pre-weighed quantities (30g) of high-fat/sucrose pellets [5.56kcal/g (58% kcal from fat, 16% protein, 26% carbohydrate); Research Diets #D12331; New Brunswick, NJ]. Animals were given access to the test diet for 4hr, then upon completion of the 4-hr test, remaining food was removed and replaced with free access to 60g of standard chow for the subsequent 20hr of the day and this schedule was repeated until the end of the study (for 3 days following infusion of test compounds). The same feeding schedule was used in a separate group of animals to test the ability for the CB1R antagonist, AM251, to block the hyperphagic responding to AA5HT. For the next group of animals, the feeding schedule was reversed and animals were given 4-hr access to standard chow, then high-fat/sucrose pellets for the ensuing 20hr. To test the diet-selective effects of AA5HT, two more groups of animals were given free access to either high-fat/sucrose pellets or standard chow for the length of the study. All test diets were simply placed on the floor in the front of the cage. Intakes, adjusted for spillage, were measured at 4hr and 24hr following infusion of the test compound.

AA5HT, at a dose of ~2nmol/side, has been reported to stimulate feeding of standard chow when infused into the nucleus accumbens of rats (26). We chose this dose of AA5HT (2nmol) for testing the behavioral responses to inactivating parabrachial FAAH on 4-hr intakes of high-fat/sucrose pellets. The 2nmol dose failed to alter intake of the test-diet when compared to baselines obtained after vehicle infusion 24hrs earlier.
Therefore, we increased the dose of AA5HT infused to 4nmol and monitored the animals’ subsequent 4-hr high-fat/sucrose intakes. This test was conducted three days following that with the lower dose. We found a robust increase in the consumption of the test diet during the 4-hr test when compared to vehicle treatment 24hrs earlier (mean ± SEM; from 54.8±5.9 to 90.8±7.8 kcal; p=0.01, Student’s t-test for repeated measures). Thus, we chose to analyze the actions of this dose in all subsequent experiments.

**Immunohistochemistry.** Three rats were deeply anesthetized and perfused transcardially with 10% phosphate-buffered formalin (pH 7.4; Fischer, King of Prussia, PA) using a peristaltic pump (Cole Parmer Instrument, Vernon Hills, IL). Brains were removed, immersed in phosphate-buffered formalin for 1hr, then transferred to 0.1 M sodium phosphate buffer (PBS) containing 30% (wt/vol) sucrose for 48hr. Brainstems were blocked and frozen at -16ºC, with 30-µm thick sections (Leica cryostat model CM3050, Deerfield, IL) containing the PBN collected in PBS. After washing sections in PBS three times for 10min each wash, sections were incubated in PBS containing 0.3% Triton X-100 for 30min at room temperature to reduce background staining. Sections were incubated with the FAAH primary antibody for 24hrs at room temperature. The rabbit anti-human FAAH polyclonal antibody was directed against a 17-amino acid sequence towards the N-terminus of the receptor (1:50 dilution in 4% normal donkey serum/PBS; Alpha Diagnostics International, San Antonio, TX). After three 10-min washes with PBS, sections were incubated with a secondary antibody for two hours at room temperature (diluted 1:200 in 4% normal donkey serum/PBS; donkey anti-rabbit IgG conjugated with
TRITC; Jackson Immunoresearch Laboratories, West Grove, PA). The secondary antibody was cross-adsorbed by the manufacturer to ensure specificity for primary antibodies raised in rabbit. After three 10-min washes in PBS, sections were mounted onto chrome-alum subbed glass slides and placed under coverslips with Vectashield® mounting medium (Vector Laboratories, Burlingame, CA). Fluorescently labeled sections were visualized with a fluorescent microscope (Leitz Asistoplan) and digital pictures were obtained using a Leica DC-200 camera linked to Leica DC Viewer software.

**Histology.** Upon completion of all experiments, animals were sacrificed by guillotine, brains removed and blocked to isolate the hindbrain, then immediately frozen to -18º C. Thirty-µm sections were obtained containing the infusion site, then mounted on slides. Slides containing the tissue sections were projected onto templates of coronal sections of the brain with a Camera Lucida (Bausch and Lomb, Rochester, NY, USA) for anatomical verification. The white outline in Figure 1 indicates the boundaries in which the injector tips were located for infusion.

**Statistical analysis.** For experiments where animals were given 4-hr access to either high-fat/sucrose pellets or standard chow (then reversed for the subsequent 20hrs), statistical comparison of conditions (vehicle and AA5HT) at days following infusion (0, 1, 2 and 3) were analyzed by ANOVA separately for 4-hr intakes and for subsequent 20-hr intakes. The same statistical analysis was made for the group receiving AM251 and AA5HT, except the conditions included: vehicle, AA5HT, AM251, AA5HT and AM251 together. For the experiments investigating diet-selective effects for AA5HT when animals had free access to either high-fat/sucrose pellets or standard chow, 4-hr and 24-hr cumulative
intakes were separately analyzed by ANOVA at days following infusion as mentioned above. Post-hoc evaluations were made for comparison among means by Student-Newman-Keuls test. An alpha level of \( p < 0.05 \) was the threshold for significance for all statistical tests.

RESULTS

Immunofluorescence identifies the presence of FAAH throughout the parabrachial nucleus

FAAH-like immunoreactivity can be seen throughout the parabrachial nucleus (Figure 1) and is absent when the primary antibody is left out of the incubation medium (Figure 1; inset).

Inactivating parabrachial FAAH with AA5HT selectively stimulates feeding of palatable foods

Parabrachial infusions of AA5HT stimulated the feeding of high-fat/sucrose pellets during the 4-hr test [Figure 2, left panel: \( n=6 \)/treatment group (all separate groups of animals for all conditions throughout all studies); means ± SEM; from 48.2±3.8 to 68.7±7.5 kcal; \( p<0.05 \)]. This increase re-occurred 24hrs following infusion, when the animals were presented again with 4-hr access to the test diet, and was absent by 48hrs following infusion (Figure 2, left panel: means ± SEM; from 49.3±4.6 to 69.9±6.1 kcal; \( p<0.05 \)). Rats compensated for the increases in high-fat/sucrose pellet intake by consuming less standard chow during the ensuing 20hr period on the day of infusion (Figure 2, middle panel; means ± SEM; from 43.2±6.4 to 18.1±4.1 kcal; \( p<0.01 \)) and again, 24hrs following infusion (Figure 2, middle panel; means ± SEM; from 45.8±3.8 to
21.3±6.0 kcal; p<0.01). Thus, total 24hr intakes in kcal (4hr high-fat/sucrose + 20hr standard chow) were not affected by AA5HT, indicating that the animals regulated their daily energy consumption (Figure 2, right panel: means±SEM; from 91.4±4.0 to 86.8±6.0 kcal).

Next, we reversed the feeding schedule, giving animals access to standard chow for 4hr and high-fat/sucrose pellets for 20hr. Parabrachial infusions of AA5HT only nominally stimulated their feeding of standard chow (Figure 3, left panel: n=7/treatment group; means ± SEM; from 1.6±0.9 to 3.8±0.8 kcal; p<0.05), while robustly stimulating the intake of high-fat/sucrose pellets for the subsequent 20hr (Figure 3, middle panel: means ± SEM; from 94.5±12.5 to 136.6±10.3 kcal; p<0.01) and cumulatively at 24hr on the day of infusion (Figure 3, right panel: means ± SEM; from 96.1±12.8 to 140.4±10.0 kcal; p<0.01).

To test potential diet-selective actions for AA5HT in the parabrachial nucleus, we gave separate groups of animals ad libitum access to either high-fat/sucrose pellets or standard chow. AA5HT stimulated high-fat/sucrose intake during the 4hr test period (Figure 4, left panel: n=7/treatment group; means ± SEM; from 8.3±1.8 to 19.0±4.6 kcal; p<0.05) and cumulatively at 24hr on the day of testing (Figure 4, left panel: means ± SEM; from 110.1±9.6 to 139.9±13.3 kcal; p<0.01). In contrast, AA5HT failed to alter standard chow intake at 4hr (Figure 4, right panel: n=5/treatment group; means ± SEM; 1.9±1.0 to 1.6±1.0 kcal) and cumulatively at 24hr (Figure 4, right panel: means ± SEM; 88.8±4.5 to 86.8±13.8 kcal).

**Orexigenic feeding responses to AA5HT are mediated by CB1Rs**
In a similar manner to the feeding responses in Figure 2, AA5HT stimulated feeding of
the high-fat/sucrose pellets during the 4-hr test period following infusion when compared
to vehicle treated animals (Figure 5, left panel: n=8/treatment group; means ± SEM; from
47.4±4.7 to 71.5±7.8 kcal; p<0.01). Importantly, co-administration of the CB1R-selective
antagonist, AM251, completely blocked the orexigenic responses to AA5HT, implicating
CB1Rs in the actions of the FAAH inhibitor (Figure 5, left panel: means ± SEM; from
71.5±7.8 to 47.5±5.9 kcal; p<0.01). AM251 did not alter 4-hr intake from vehicle
treatment (Figure 5, left panel: means ± SEM; from 47.4±4.7 to 43.5±4.1 kcal). There
were compensatory decreases, though non-significant, in 20-hr standard chow intake
following AA5HT treatment (Figure 5, middle panel: means ± SEM; from 39.5±6.7 to
26.9±5.2 kcal). This decrease was blocked by co-administration of AM251 (Figure 5,
middle panel: means ± SEM; from 26.9±5.2 to 40.9±4.0 kcal). Total 24hr intakes were
not significantly affected by any compound (Figure 5, right panel: means ± SEM;
vehicle=86.9±4.9, AA5HT=98.4±5.2, AM251=84.9±4.2, AA5HT+AM251=88.3±7.1
kcal).

It should be noted that in this group of animals, all test compounds were administered in
100% DMSO as opposed to the 50% DMSO vehicle for all other groups. This was
necessary due to the highly lipophillic nature of AM251 and underscores the technical
difficulties when infusing these and other cannabinoid compounds into the brain. When
compared to their 20-hr standard chow baseline intakes, vehicle treatments reduced
intake (means ± SEM; 54.4±2.4 to 39.5±6.7 kcal), thereby suggesting that, while the
changes did not reach significance, infusions of 100% DMSO had actions on their own.
This reduction also occurred for the AM251 treatment group (means ± SEM; from
54.7±5.0 to 41.4±5.0 kcal) and for the AA5HT+AM251 treatment group (means ± SEM;
from 53.0±4.0 to 40.9±4.0 kcal). Importantly, however, AM251 blocked the orexigenic responses to AA5HT on the day of infusion. This result identified CB1Rs as mediating the actions of AA5HT.

**DISCUSSION**

In this report, we manipulated endogenous mechanisms to evaluate potential physiological roles in feeding for the endocannabinoid system in the parabrachial nucleus of rats. Parabrachial infusion of the FAAH inhibitor, AA5HT, increased the consumption of a palatable high-fat/sucrose diet when compared with the intake of standard chow. These actions were indirectly mediated through CB1Rs as evidenced by the complete blockade of the orexigenic responses with a selective CB1R antagonist. Importantly, animals were able to maintain their total 24hr caloric intake at baseline levels by compensating for their orexigenic responses to the palatable food with decreases in the intake of standard chow when presented for the subsequent 20hrs. These feeding responses to AA5HT occurred again the next day when presented with the same feeding schedule. This is in contrast to when the palatable diet was presented for 20hrs or more during testing. Interestingly, under these conditions, animals overconsumed their total 24hr caloric intake, though only on the day of infusion. Therefore, the actions of AA5HT in the parabrachial nucleus appear to be determined by the properties of the diet. In addition, the amount of time animals have access to the diets influences total caloric consumption and the time until regulation of overall caloric intake is re-established.

Directly activating CB1Rs in discrete brain loci has been reported previously to increase feeding. Few analyses, however, have been performed regarding the influence of the test diet on this orexigenic action. Koch and Matthews (15) reported that infusions of
the natural cannabinoid receptor agonist, $\Delta^9$ tetrahydrocannabinol, into the lateral ventricles of rats increased the intake of a chocolate cake batter over standard rodent chow. This might imply that cannabinoid receptors in the forebrain selectively enhance the consumption of palatable foods. However, administration of cannabinoid agonists into discrete loci in the nucleus accumbens and hypothalamus (1, 11, 14, 26, 28) did increase intake of standard chow; palatable foods were not tested. In contrast, fourth ventricular infusion of a CB1R agonist enhanced the intake of sweetened condensed milk (19).

We observed that discretely targeting parabrachial CB1Rs by infusing 2-arachidonoyl glycerol specifically increased the intake of hedonically-positive diets (7). The present study, by using an inhibitor of the degradation of endogenous cannabinoids, strongly implicates a physiological role for these fatty acid derivatives in the parabrachial nucleus in controlling consumption of desirable foods.

The novel strategy to use enzyme inhibitors for investigating physiological roles for the endocannabinoid system in discrete brain loci in feeding has been reported only once in a study involving the nucleus accumbens in rats (26). In that study, directly activating CB1Rs with the endocannabinoid, anandamide, or indirectly, by blocking the FAAH-mediated enzymatic degradation of endocannabinoids in the nucleus accumbens of rats with AA5HT, increased the intake of standard chow on the day of infusion. The actions of the FAAH inhibitor were correlated with elevated local concentrations of endocannabinoids. Although AA5HT has no direct actions at CB1Rs (2), orexigenic responding to the inhibitor was blocked by central co-administration with AM251, thereby implicating CB1Rs in the feeding responses to FAAH inactivation. That
investigation, however, failed to analyze in detail either the temporal components of the actions of AA5HT or the effect of diet on these responses.

We report differential actions on total energy intake for inactivating parabrachial FAAH depending upon the feeding schedule. When the feeding schedule consisted of high-fat/sucrose pellets for extended periods of time (ie., 20hrs or more), animals were unable to regulate their total energy intake as they did when given access to the palatable diet for only 4hr. Thus, 24-hr caloric intakes increased. This energy imbalance occurred only on the day of AA5HT infusion. It is plausible that the hedonic value of the test diet superseded the brake of homeostatic regulatory processes under these feeding schedules. Subsequently, homeostatic mechanisms orchestrated by the hypothalamus, or other regions, overrode the hedonically-driven feeding and re-established baseline levels of intake on the following day. A regulatory imbalance did not occur when animals were presented with high-fat/sucrose pellets for only 4hr daily, as the rats compensated calorically by decreasing their subsequent 20hr intake of standard chow. In all of these studies, one possibility for the orexigenic action of the parabrachial endocannabinoids is that these fatty acid derivatives enhanced the reinforcing characteristics of the palatable food. As a consequence, responding for the desirable flavor or somatosensory properties of the diet was maintained at a higher level during the meal.

AA5HT has been reported to be a tightly binding, non-covalent inhibitor of FAAH in vitro (2). This suggests that in our study, AA5HT still inhibits FAAH the day following infusion. It is possible, though highly unlikely, that the feeding schedules themselves altered FAAH expression and therefore sensitivity to pharmacological inactivation. Instead, it is more likely that the properties of the test diet dictated whether
endocannabinoid levels, which were presumably elevated, acted to increase food consumption. These biochemical issues remain to be evaluated in future studies.

CB1Rs of the parabrachial nucleus appear to selectively modulate the intake of foods with hedonically-positive sensory properties. This role aligns with the known functions for the parabrachial nucleus which include, but are not limited to, the gating of gustatory neurotransmission derived from the tongue and mouth. This information reaches the parabrachial nucleus via Cranial Nerves X, IX and VII and is transmitted to other brain regions for processing (3, 9, 10, 23). These areas include multiple brain sites modulating feeding, including the hypothalamus, amygdala and the ventral striatum. Of particular relevance, ventral striatal regions have been proposed to assign reward value to the afferent sensory information transmitted by the parabrachial nucleus (22). Gustatory neurotransmission in the parabrachial nucleus involves the waist region across the brachium conjunctivum and columns of cells bordering this structure in both lateral and medial aspects of the nucleus (12, 13, 24). It is likely that our infusions of 0.5µl into the central lateral region (Figure 1) diffused and accessed substantial portions of the gustatory parabrachial nucleus. Therefore, our results suggest that parabrachial endocannabinoid mechanisms may assume a modulatory role in transmitting information associated with the sensory properties of foods to the mentioned regions in the forebrain.

PERSPECTIVES AND SIGNIFICANCE

Understanding the mechanisms that govern the choice of food on the basis of its oral stimulus properties and post ingestive consequences is critical if we are to develop novel, effective therapies for treating and preventing energy imbalance leading to obesity. Scientific evidence has existed for several decades implicating cannabinoids in
stimulating food intake in human and non-human animals. Furthermore, anecdotal and increasingly empirical evidence has established a role for these compounds in enhancing consumption of foods palatable foods. Often, of course, these foods are calorically dense. The present studies demonstrate a role for endocannabinoids in the parabrachial nucleus in specifically promoting the intake of such desirable foods. In the past, major attention has been given to sites within the forebrain for this function. By identifying a region of the pontine brainstem in food selection, our data add to the anatomical range of the network that influences hedonically-based eating. Moreover, we show that endocannabinoids serve this physiological function in the brainstem. This function may involve mechanisms of reward.

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**Figure 1:** Immunofluorescence identifies the presence of FAAH throughout the PBN. Red FAAH-like immunoreactivity (TRITC conjugate) can be seen throughout the PBN. Immunoreactivity is absent in the tissue sections incubated without the primary antibody (NO1º, inset). Infusion sites for all test compounds were located within the white oval. BrC, brachium conjunctivum; LPBN, lateral parabrachial nucleus; MPBN, medial parabrachial nucleus.

**Figure 2:** AA5HT persistently stimulates feeding of high fat/sucrose pellets (HFS) by 4hr after infusion into the PBN, with compensatory decreases in the 20hr intake of standard chow (SC). Parabrachial infusions of AA5HT (4nmol/side) stimulated the intake of high-fat/sucrose pellets within 4hr, an effect persisting 24hrs later when the test
diet was presented again (left panel; n=7/treatment group). The middle panel shows compensatory decreases in 20hr standard chow intake on the day of AA5HT infusions and one day later. Total 24hr caloric intakes were unaffected on all test days (right panel). *p<0.05 and **p<0.01 indicates significant differences between value for AA5HT versus vehicle; means ± SEM; ANOVA followed by Student-Newman-Keuls test.

Figure 3: AA5HT nominally stimulates feeding of standard chow (SC) by 4hr after infusion into the PBN and robustly stimulates the 20hr intake of high-fat/sucrose (HFS) pellets acutely. Parabrachial infusions of AA5HT (4nmol/side) only nominally stimulated the intake of standard chow by 4hrs (left panel; n=6/treatment group). The middle panel shows a robust stimulation of 20hr high-fat/sucrose pellet intake on the day of AA5HT infusion. Total 24hr caloric intakes were increased on the day of AA5HT infusion (right panel). *p<0.05 and **p<0.01 indicates significant differences between value for AA5HT versus vehicle; means ± SEM; ANOVA followed by Student-Newman-Keuls test.

Figure 4: AA5HT stimulates 4hr and 24hr high-fat/sucrose (HFS) pellet intake following infusion into the PBN and fails to alter the 24hr intake of standard chow (SC) at any timepoint. Parabrachial infusions of AA5HT (4nmol/side) stimulated the intake of high-fat/sucrose pellets on the day of infusion by 4hr and cumulatively at 24hr (left panel; n=6/treatment group). AA5HT did not alter the intake of standard chow at any timepoint (right panel; n=5/treatment group). **p<0.01 indicates significant differences between value for AA5HT versus vehicle; means ± SEM; ANOVA followed by Student-Newman-Keuls test.
Figure 5: CB1 receptors mediate the stimulatory actions of AA5HT on intake of high-fat/sucrose pellets (HFS).

Co-administration of the CB1R selective antagonist, AM251 (4nmol/side), completely blocked the orexigenic responding to high-fat/sucrose pellets elicited by AA5HT (4nmol/side) at 4hr (left panel; n=8/treatment group). The apparent trend towards compensatory decreases in the 20hr intake of standard chow following infusion of AA5HT and blockade of this response with AM251 was not significant. Total 24hr caloric intakes were unaffected on all test days (right panel). **p<0.01 indicates significant differences between value of AA5HT and vehicle (VEH). ##p<0.01 indicates significant differences between value for arachidonoyl serotonin and AM251 (BOTH) versus AA5HT alone. Means ± SEM; ANOVA followed by Student-Newman-Keuls test.