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

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DiPatrizio NV, Simansky KJ. Inhibiting parabrachial fatty acid amide hydrolase activity selectively increases the intake of palatable food via cannabinoid CB1 receptors. Am J Physiol Regul Integr Comp Physiol 295: R1409–R1414, 2008. First published September 3, 2008; doi:10.1152/ajpregu.90484.2008.—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 4 h during the light period, with compensatory decreases in the consumption of standard chow during the ensuing 20 h. These diet-selective changes were repeated on the next day, indicating a shift in feeding toward the more palatable diet that lasted for 48 h 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 4-h access period, but substantial increases in the intake of high-fat/sucrose during the following 20-h interval. When presented with only high-fat/sucrose diet for 24 h, AA5HT increased 24-h food intake. In contrast, when given 24-h 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.

parabrachial nucleus; endocannabinoids; feeding; reward

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 brain stem where agonists act. Further, no studies have directly compared the influence of the 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-arachidonoylglycerol were anatomically specific, as off-target anatomical controls with infusions ~500 μm 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 parabrachial nucleus has a role, though not exclusive, 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

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AA5HT into this brain stem 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 375 g at time of surgery were used for these experiments. Animals were housed individually in plastic hanging cages with wire-mesh floors (43 × 22 × 18 cm) and maintained on a standard 12:12-h light-dark cycle (lights on at 0700) at 23 ± 2°C. 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 sodium (35 mg/kg) and chloral hydrate (160 mg/kg) anesthesia (Equithesin), with bilateral 26-gauge stainless-steel guide cannulas (3.8-mm center-to-center; Plastics One, Roanoke, VA) aimed centrally within the lateral parabrachial nucleus (PBN). Guide cannulas 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 cannula immediately following surgery to prevent occlusion. Stereotaxic coordinates for cannula placement were determined from Paxinos and Watson’s rat brain atlas (24a) using standard flat skull technique (from bregma to lambda: 9.5–9.8 mm caudal to bregma, 1.9 mm lateral to the midline suture, and 4.8 mm ventral. For pain management, animals were administered ketoprofen [1 mg/kg at 2 mg/ml USP grade (Sigma Aldrich, St. Louis, MO)] just prior to and 24 h 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-isodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; mol. wt. = 555], from Tocris Cookson (Ellisville, MO). Because of the highly lipophilic nature of the compounds, AA5HT was first solubilized in DMSO (Sigma Aldrich), then 0.9% (wt/vol) 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-gauge injector with polyethylene (PE)-20 tubing (Becton Dickinson, Sparks, MD). Injector tips extended 2.5 mm past the tips of the guide cannulas. Bilateral infusions were made over 90 s, beginning between 0900 and 1000, with the injector left in place for 30 s following infusion of drug or vehicle to minimize backflow of liquid.

Experimental procedure. Experiments began following 1 wk 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 3 days of this habituation. Following infusion of vehicle or test compound (1000), standard pelleted rodent chow (3.34 kcal/g (12% kcal from fat, 28% protein, 60% carbohydrate); Purina 5001, St. Louis, MO) was removed from the home cages and replaced with preweighed quantities (30 g) of high-fat/sucrose pellets [5.56 kcal/g (58% kcal from fat, 16% protein, 26% carbohydrate); Research Diets #D12331; New Brunswick, NJ]. Animals were given access to the test diet for 4 h; then upon completion of the 4-h test, remaining food was removed and replaced with free access to 60 g of standard chow for the subsequent 20 h 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-h access to standard chow, then high-fat/sucrose pellets for the ensuing 20 h. 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 4 h and 24 h following infusion of the test compound.

AA5HT, at a dose of ~2 nmol/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 (2 nmol) for testing the behavioral responses to inactivating parabrachial FAAH on 4-h intakes of high-fat/sucrose pellets. The 2-nmol dose failed to alter intake of the test diet compared with baselines obtained after vehicle infusion 24 h earlier (n = 4; means ± SE; from 58.7 ± 10.5 to 57.5 ± 6.0 kcal). Therefore, we increased the dose of AA5HT infused to 4 nmol and monitored the animals’ subsequent 4-h high-fat/sucrose intakes. This test was conducted 3 days following that with the lower dose. We found a robust increase in the consumption of the test diet during the 4-h test compared with vehicle treatment 24 h earlier (means ± SE; 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-Palmer Instrument, Vernon Hills, IL). Brains were removed, immersed in phosphate-buffered formalin for 1 h, then transferred to 0.1 M PBS containing 30% (wt/vol) sucrose for 48 h. Brain stems were blocked and frozen at -16°C, 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 10 min each, we incubated sections in PBS containing 10% normal donkey serum (Vector Laboratories, Burlingame, CA) and 0.3% Triton X-100 for 30 min at room temperature to reduce background staining. Sections were incubated with the FAAH primary antibody for 24 h at room temperature. The rabbit anti-human FAAH polyclonal antibody was directed against a 17-amino acid sequence toward the N-terminus of the receptor (1:50 dilution in 4% normal donkey serum/PBS; Alpha Diagnostics International, San Antonio, TX). After 30-min washes with PBS, sections were incubated with a secondary antibody for 2 h 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). Fluorescently labeled sections were visualized with a fluorescent microscope (Leitz Asiptoplan), 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 killed by guillotine, and brains were removed and blocked to isolate the hindbrain, then immediately frozen to -18°C. Thirty-micrometer
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) for anatomical verification. The white outline in Fig. 1 indicates the boundaries in which the injector tips were located for infusion.

Statistical analysis. For experiments in which animals were given 4-h access to either high-fat/sucrose pellets or standard chow (then reversed for the subsequent 20 h), statistical comparison of conditions (vehicle and AA5HT) at days following infusion (0, 1, 2, and 3) were analyzed by ANOVA separately for 4-h intakes and for subsequent 20-h period on the day of infusion (Fig. 2, middle); means ± SE; from 43.2 ± 6.4 to 18.1 ± 4.1 kcal; P < 0.01) and again, 24 h following infusion (Fig. 2, middle); means ± SE; from 45.8 ± 3.8 to 21.3 ± 6.0 kcal; P < 0.01). Thus, total 24-h intakes in kcal (4-h high-fat/sucrose + 20-h standard chow) were not affected by AA5HT, indicating that the animals regulated their daily energy consumption (Fig. 2, right; means ± SE; from 91.4 ± 4.0 to 86.8 ± 6.0 kcal).

Next, we reversed the feeding schedule, giving animals access to standard chow for 4 h and high-fat/sucrose pellets for 20 h. Parabrachial infusions of AA5HT only nominally stimulated their feeding of standard chow (Fig. 3, left; n = 7/treatment group; means ± SE; 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 20 h (Fig. 3, middle); means ± SE; from 94.5 ± 12.5 to 136.6 ± 10.3 kcal; P < 0.01) and cumulatively at 24 h on the day of infusion (Fig. 3, right; means ± SE; 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 4-h test period (Fig. 4, left; n = 7/treatment group; values are means ± SE; from 8.3 ± 1.8 to 19.0 ± 4.6 kcal; P < 0.05) and cumulatively at 24 h on the day of testing (Fig. 4, right; values

RESULTS

Immunofluorescence identifies the presence of FAAH throughout the PBN. FAAH-like immunoreactivity can be seen throughout the PBN. Immunoreactivity is absent in the tissue sections incubated without the primary antibody (NO1, inset). Infusion sites for all test compounds were located within the white oval. BrC, brachium conjunctivum; LPBN, lateral parabrachial nucleus; MPBN, medial parabrachial nucleus.

Fig. 1. Immunofluorescence identifies the presence of fatty acid amide hydrolase (FAAH) throughout the parabrachial nucleus (PBN). Red FAAH-like immunoreactivity (Tetramethyl Rhodamine Iso-Thiocyanate conjugate) can be seen throughout the PBN. Immunoreactivity is absent in the tissue sections incubated without the primary antibody (NO1, inset). Infusion sites for all test compounds were located within the white oval. BrC, brachium conjunctivum; LPBN, lateral parabrachial nucleus; MPBN, medial parabrachial nucleus.

Fig. 2. Arachidonoyl serotonin (AA5HT) persistently stimulates feeding of high-fat/sucrose (HFS) pellets by 4 h after infusion into the PBN, with compensatory decreases in the 20-h intake of standard chow (SC). Parabrachial infusions of AA5HT (4 nmol/side) stimulated the intake of high-fat/sucrose pellets within 4 h, an effect persisting 24 h later when the test diet was presented again (left; n = 7/treatment group). Middle: compensatory decreases in 20-h standard chow intake on the day of AA5HT infusions and 1 day later. Total 24-h caloric intakes were unaffected on all test days (right). *P < 0.05 and **P < 0.01 indicate significant differences between value for AA5HT vs. vehicle; means ± SE; ANOVA followed by Student-Newman-Keuls test.
are means ± SE; from 110.1 ± 9.6 to 139.9 ± 13.3 kcal; *P < 0.01. In contrast, AA5HT failed to alter standard chow intake at 4 h (Fig. 4, right; *n = 5/treatment group; values are means ± SE; 1.9 ± 1.0 to 1.6 ± 1.0 kcal) and cumulatively at 24 h (Fig. 4, right; values are means ± SE; 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 Fig. 2, AA5HT stimulated feeding of the high-fat/sucrose pellets during the 4-h test period following infusion compared with vehicle-treated animals (Fig. 5, left: *n = 8/treatment group; values are means ± SE; from 47.4 ± 4.7 to 71.5 ± 7.8 kcal; *P < 0.01). Importantly, coadministration of the CB1R-selective antagonist, AM251, completely blocked the orexigenic responses to AA5HT, implicating CB1Rs in the actions of the FAAH inhibitor (Fig. 5, left: values are means ± SE; from 71.5 ± 7.8 to 47.5 ± 5.9 kcal; *P < 0.01). AM251 did not alter 4-h intake from vehicle treatment (Fig. 5, left: values are means ± SE; from 47.4 ± 4.7 to 43.5 ± 4.1 kcal). There were compensatory decreases, though nonsignificant, in 20-h standard chow intake following AA5HT treatment (Fig. 5, middle: values are means ± SE; from 39.5 ± 6.7 to 26.9 ± 5.2 kcal). This decrease was blocked by coadministration of AM251 (Fig. 5, middle: values are means ± SE; from 26.9 ± 5.2 to 40.9 ± 4.0 kcal). Total 24-h intakes were not significantly affected by any compound (Fig. 5, right: values are means ± SE; 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 because of the highly lipophilic nature of AM251 and underscores the technical difficulties when infusing these and other cannabinoid compounds into the brain. Compared with their 20-h standard chow baseline intakes, vehicle treatments reduced intake (means ± SE; 54.4 ± 2.4 to 39.5 ± 6.7 kcal), thereby suggesting that, although 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 ± SE; from 54.7 ± 5.0 to 41.4 ± 5.0 kcal) and for the AA5HT + AM251 treatment group (means ± SE; 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 PBN 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 24-h 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 20 h. 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 20 h or more during testing. Interestingly, under these conditions, animals overconsumed their total 24-h caloric intake, although 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 reestablished.

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, Δ⁹-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 PBN 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 coadministration 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 (i.e., 20 h or more), animals were unable to regulate their total energy intake as they did when given access to the palatable diet for only 4 h. Thus, 24-h 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 reestablished 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 4 h daily, as the rats compensated calorically by decreasing their subsequent 20-h 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, noncovalent 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 0.5 μl into the central lateral region (Fig. 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 that implicates cannabinoids in stimulating food intake in human and nonhuman animals. Furthermore, anecdotal and increasingly empirical evidence has established a role for these compounds in enhancing consumption of 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 brain stem in food selection, we show that parabrachial endocannabinoid receptors may vary in their oral stimulus properties and post-ingestive consequences.

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