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Research report

Manipulation of GABA in the ventral pallidum, but not the nucleus accumbens, induces intense, preferential, fat consumption in rats

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HIGHLIGHTS

- Blocking GABA receptors in the VPm greatly increases preference for fat.
- The treatment yields a 4300% increase in caloric intake with 97% derived from fat.
- This macronutrient selection profile differs from that seen after food deprivation.
- Intra-AcbSh muscimol or baclofen increases intake of carbohydrate and fat equally.
- GABA receptors in the VPm, but not AcbSh, may specifically control fat intake.

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ABSTRACT

Injections of the $GABA_A$ antagonist bicuculline into the medial ventral pallidum (VPm) induce marked increases in food intake, but nothing is known about the way in which these injections alter the distribution of intake in a macronutrient selection situation. We investigated this topic by adapting rats to a diet containing independent sources of protein, carbohydrate and fat, and then examining the effects of intra-VPm bicuculline on diet selection. Under these conditions, bicuculline produced a massive, preferential increase in fat intake with subjects consuming a mean of 97% of their calories from fat. Furthermore, all treated subjects ate fat before any other macronutrient, suggesting that the animals' behavior was directed selectively toward this dietary component even before consumption had begun. Similar effects were not observed following food deprivation, which exerted its largest effect on carbohydrate intake. To compare the intra-VPm bicuculline response to that seen after activation of GABA receptors in the nucleus accumbens shell (AcbSh), a major source of projections to the VPm, we conducted similar experiments with intra-AcbSh injections of muscimol and baclofen. These injections also enhanced food intake, but did not reproduce the selective preference for fat seen after intra-VPm bicuculline. These experiments provide the first demonstration of preferential enhancement of fat intake following manipulations of a nonpeptide neurotransmitter. Since mean intakes of fat under baseline conditions and after deprivation tended to be lower than those of carbohydrates, it seems unlikely that the effects of intra-VPm bicuculline are related to the intrinsic "rewarding" properties of fat, but might rather reflect the induction of a state of "fat craving."

1. Introduction

When a variety of foods are available, animals must decide not only how much to eat, but also how to distribute their intake across the accessible items. One could imagine that the available foods could simply be ranked in an order of preference determined in part by innate factors and in part by past experiences, and that animals would always tend to consume the largest quantities of the most preferred items. Evidence against this type of "fixed hierarchy

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model" is, however, provided by observations that the relative preferences of subjects for different foods may vary depending on the internal state of the animal. For example, sodium depletion is associated with increased intake of salty foods [1], and extreme food deprivation may lead to increased intake of foods rich in fat [2].

Although much less effort has been devoted to food selection than to the control of total intake, substantial evidence suggests that a number of different brain systems may participate in mediating the relative preferences expressed by animals for different foods. For example, in animals given a choice between foods rich in fat and those rich in carbohydrates, systemic injections of opiates tend to preferentially increase the intake of fatty foods, whereas intraventricular injections of NPY tend to preferentially increase the intake of carbohydrates [2]. Some of the most convincing evidence in this regard has been obtained following manipulations of the paraventricular nucleus of the hypothalamus (PVN), a brain region from which local injections of the peptides enkephalin and galanin induce a marked preference for fat [3–5], whereas activation of NPY or norepinephrine receptors preferentially increases carbohydrate intake [6,7]. Injections of serotonin at this site also specifically decrease carbohydrate intake [8]. Such results indicate that ingestive behavior must involve more than simply gating ingestive systems and that brain mechanisms are able to influence not only how much, but also what types of food will be eaten.

Brain mechanisms controlling feeding are not restricted to the hypothalamus, and in recent years it has been shown that the nucleus accumbens (Acb), a structure located in the basal telencephalon, also exerts a powerful influence on ingestive behavior. Pronounced increases in food intake can be induced by injections of a variety of drugs into the Acb, especially its shell region (AcbSh), and these injections alter activity at a number of sites in the hypothalamus, including the PVN [9,10]. In the current context, it is important that some of these injections alter relative food preferences. For example, in animals given a choice of high-fat and high-carbohydrate diets, injections of the μ-opioid agonist D-Ala^2,N,Me-Phe^4,Gly-ol^5-enkephalin (DAMGO) into the core of the Acb induce a strong preference for fat, irrespective of the baseline preferences of the animals [11]. In contrast, rats made hyperphagic by intra-AcbSh injections of the $GABA_A$ agonist muscimol into AcbSh show similar increases in both fat and carbohydrate intake [12].

Although the circuit through which the Acb may mediate macronutrient intake has yet to be identified, it is known that projections from GABAergic medium spiny neurons in the Acb synapse on cells in the medial ventral pallidum (VPm) [13–16], and that this structure in turn can influence the lateral hypothalamus (LH) and PVN [17,18]. While the LH is well known as a brain region involved in the control of ingestion [19], the medial ventral pallidum also has a demonstrated role in the control of food intake. Injections of either $GABA_A$ antagonists, glutamate agonists, or μ-opioid agonists into the VPm induce intense feeding in satiated rats [18,20–22]. Furthermore, VPm lesions reduce the feeding induced by muscimol injections in the AcbSh through specific disruption of the AcbSh-VPm circuit [23]. It has been proposed that the Acb, VPm, and LH work together to form a functional circuit controlling some aspects of feeding behavior [10,23,24]. While the ability of chemical stimulation of the VPm to induce intense hyperphagia is well documented, nothing is known about whether these procedures also alter the relative preference of rats for different dietary components in a fashion similar to that seen after intra-Acb injections of DAMGO. In the current set of studies, we therefore investigated the effects of intra-VPm injections of the $GABA_A$ antagonist bicuculline on food selection in *ad libitum* fed rats consuming a diet containing independent sources of fat, carbohydrate and protein. We also examined deprivation-induced feeding in these same subjects in order to determine whether this manipulation produced a pattern of ingestion similar to that seen after bicuculline injections. Since certain aspects of our procedure differed from those employed by earlier investigators, we additionally examined macronutrient selection in subjects with injections of several orexigenic compounds into the AcbSh.

2. Materials and methods

2.1. Subjects

The subjects were 15 male Sprague-Dawley rats (Charles River), weighing between 290 and 350 g at the time of surgery. The rats were housed individually in plastic cages (L 43 × W 22 × H 21 cm) with wire floors on a 12-h light:12-h dark cycle at a constant room temperature (∼21 °C). Water and food were available *ad libitum*, except as noted below. For the period before surgery and for the first week after surgery, food was provided in the form of standard lab chow (Harlan Teklad), after which time animals were switched for the remainder of the experiments to a nutritionally-complete diet consisting of three separate macronutrients components, described below. Throughout the experiments, rats were handled and weighed on a daily basis. All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UIC Institutional Animal Care and Use Committee.

2.2. Surgery

Surgery was performed using standard, aseptic, flat-skull stereotaxic techniques under sodium pentobarbital (60 mg/kg, ip) anesthesia. In 8 experimental subjects, bilateral 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA), aimed so as to terminate 2.0 mm dorsal to the VPm, were implanted at coordinates of AP: −0.2, LM: ±1.8, DV: −6.7. In the remaining subjects, bilateral 22-gauge cannulae, aimed so as to terminate 2.0 mm dorsal to the AcbSh, were implanted at coordinates of AP: 1.6, LM: ±0.8, DV: −6.1. The guide cannulae were held in place using stainless steel screws and denture lining material and a stainless steel obturator was inserted into the lumen of each cannula to help maintain patency. After surgery, the rats received an injection of the analgesic carprofen (5 mg/kg, sc) and the antibiotic cefazolin (60 mg/kg, sc). Each rat was allowed to recover for at least 7 days before the start of behavioral testing.

2.3. Macronutrient diet components and adaptation

The rats were allowed to select their food from three separate sources of pure macronutrients (protein, carbohydrate, and fat) presented simultaneously in glass jars with dimensions of approximately 62 × 60 mm (diameter × height) which were placed in the corners of the animals' home cages. The detailed composition of each of the dietary components is presented in Table 1; all the ingredients were obtained from Harlan Laboratories (Indianapolis, IN) except for sucrose (Domino Foods, Chicago, IL), lard (Armour, Peoria, IL) and dibasic calcium phosphate (Sigma-Aldrich, St. Louis, MO). The dietary components were similar to those used in previous studies by other authors [2,6] except that we used custom modified versions of the AIN-93 vitamin and mineral mixes in which cellulose was substituted for sucrose. Thus our protein and fat components contained no sugar, whereas the sucrose concentrations in the mixtures used by earlier workers have been as high as 2%. Placement of the food jars containing various components was changed daily to prevent the development of position preferences. Subjects were given two weeks to adapt to the multicomponent diet before the start of drug injections. On the last three days of