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Injectons of muscimol into the paraventricular thalamic nucleus, but not mediodorsal thalamic nuclei, induce feeding in rats

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Abstract

The paraventricular thalamic nucleus (PVT) is a component of the midline thalamic group that is interconnected with several brain regions known to play important roles in the control of food intake, including the lateral hypothalamus and nucleus accumbens shell, suggesting that the PVT itself may be involved in mediating feeding behavior. In the current study, we examined whether inhibition of cells in the PVT with the GABA_A agonist muscimol could alter food intake in non-deprived rats. To control for possible spread of the drug, we also observed food intake after injections of muscimol into the overlying ventricle or laterally adjacent mediodorsal thalamic nuclei (MD). We found that muscimol injections into the central PVT dose-dependently increased food intake. In contrast, intra-MD injections of muscimol resulted in a potent dose-dependent suppression of food intake, while those into the overlying ventricle had no effect. These results support the proposal that the PVT is a component of the neural circuitry controlling feeding behavior.

Keywords

Feeding behavior; Thalamus; GABA; Paraventricular thalamic nucleus; Mediodorsal thalamic nuclei; Food intake

1. Introduction

The investigation of diencephalic control of food intake has largely centered on the hypothalamus, but evidence is available which suggests that the paraventricular nucleus of the thalamus (PVT) may also play a role. This nucleus projects heavily to the shell region of nucleus accumbens, a structure which is able to exert a powerful influence on feeding behavior (Stratford, 2007), as well as to a number of feeding related sites in the medial hypothalamus (Kiss et al., 2011; Moga et al., 1995). Conversely, the PVT receives inputs from a number of sites known to be involved in the control of food intake including the nucleus of the solitary tract, the parabrachial nucleus, the lateral hypothalamus and several medial hypothalamic nuclei (Brog et al., 1993; Chen and Su, 1990; Christie et al., 1987; Cornwall and Phillipson, 1988; Groenewegen et al., 1982; Ruggiero et al., 1998). The possibility that these connections may be involved in ingestive behavior is reinforced by findings that many of them contain substances which have been shown to influence food intake; for example, projections to the PVT from the nucleus of the solitary tract contain glucagon-like peptide 1 (GLP-1) (Llewellyn-Smith et al., 2011), those from the arcuate and

dorsomedial hypothalamic nuclei contain cocaine and amphetamine regulated transcript peptide (CART) and cholecystokinin (CCK), respectively (Kirouac et al., 2006; Otake, 2005), and those from the lateral hypothalamic region contain orexin (Kirouac et al., 2005). Although the transmitters involved have not been identified, it has also been shown that dorsomedial hypothalamic projections to the PVT originate from cells expressing receptors for the adipocyte hormone leptin (Gautron et al., 2010). Additionally, the PVT contains high levels of neuropeptide Y (NPY) and its receptors as well as the MC-4 melanocortin receptor (Freedman and Cassell, 1994; Gelez et al., 2010; Nichol et al., 1999).

Although these neuroanatomical and neurochemical data certainly suggest a role for the PVT in controlling food intake, functional data are far more limited, but still suggestive. Fos expression within the PVT has been shown to be altered both by ingestive behavior and by the administration of glucoprivic agents (Horn and Friedman, 1998; Nakahara et al., 2004; Warne et al., 2007). Additionally, lesions of the PVT have been reported to increase food intake and weight gain in rats (Bhatnagar and Dallman, 1999), although a similar effect was not obtained in hamsters (Purvis and Duncan, 1997).

Given the fragmentary nature of the experimental evidence linking the PVT to food intake, we, in the current study, investigated the effects of acute inhibition of PVT neurons with the GABA_A receptor agonist muscimol on short-term feeding behavior in satiated rats. As controls for spread of the drug, we also evaluated the effects of muscimol injections aimed at the laterally adjacent mediodorsal thalamic nucleus (MD) and the overlying ventricle. The results of these studies support the view that the PVT is able to influence ingestive behavior.

2. Results

2.1. Experiment 1: Response to muscimol injections into the PVT and third ventricle

Three animals were excluded for inappropriate placements, and, in the remaining 13 subjects histological analysis revealed that the ventral tip of each cannula track terminated within the boundaries of the PVT (Fig. 1, round symbols; Fig. 2a). In the three animals in which Fluoro-Gold injections were made dorsal to the PVT, diffusion of the dye into the third ventricle and cerebral aqueduct could be observed through a fluorescence microscope.

As can be seen in Fig. 3, injections of muscimol into the PVT induced a pronounced increase in food intake ($F_{(2,12)}=21.5$; $p<0.001$). Post hoc comparisons showed that food intake was increased by both the 50 ng and 100 ng doses of muscimol ($p<0.001$ for each) as compared to saline injections. In contrast, food intake was not altered by injection of 100 ng muscimol immediately dorsal to the PVT ($p>0.6$).

2.2. Experiment 2: Response to muscimol injections into the MD

Cannulae were aimed at the MD in six animals and all cannula tips terminated within the boundaries of that structure (Fig. 1, square symbols; Fig. 2b). Fig. 4 illustrates that injections of muscimol into the MD strongly suppressed the total number of pellets consumed during the test ($F_{(2,5)}=7.3$; $p<0.011$). *Post hoc* comparisons indicated that intake was suppressed significantly by both the 50 ng and 100 ng doses of muscimol ($p<0.013$ and $p<0.005$, respectively) as compared to saline injections.

3. Discussion

The current results demonstrate that injections of the GABA_A receptor agonist muscimol into the paraventricular thalamic nucleus (PVT) significantly increase food intake in non-deprived rats. These findings are consistent with the anatomical and gene expression data reviewed in the introduction and provide strong support for previous speculation that this

structure may play a role in the control of feeding (Kelley et al., 2005; Kirouac et al., 2006; Parsons et al., 2006; Stratford, 2007). In contrast, similar hyperphagia was not seen following injections placed dorsal to the PVT or lateral to the PVT in the mediodorsal nucleus (MD), suggesting that the effect did not result from diffusion of the drug to adjacent thalamic sites or up along the cannula tract into the third ventricle. The PVT injections in the current study were aimed at the central/posterior portion of the nucleus: since different levels of the PVT may differ in their connectivity, further work will be required to determine if effects similar to those observed here can be obtained with injections into the rostral or caudal poles of the nucleus.

The neural circuitry through which the PVT effects changes in food intake is currently unknown. It is possible that the effect could be mediated through projections to the arcuate, ventromedial and dorsomedial hypothalamic nuclei (Vertes and Hoover, 2008), as these structures are well known to play a role in the control of ingestion. Very little direct evidence is available as to the functional role of these projections, but it is possible that they exert a mixture of excitatory and inhibitory effects (Alamilla and Aguilar-Roblero, 2010). Another plausible mediator of the influence of the PVT on feeding is the medial shell of the nucleus accumbens (AcbSh) (Stratford, 2007). In non-deprived rats, injections of glutamate antagonists and agonists into the AcbSh induce pronounced increases and decreases, respectively, in feeding (Maldonado-Irizarry et al., 1995; Stratford et al., 1998), suggesting that glutamate at this site plays a major role in the control of ingestion. The PVT sends a large glutamatergic projection to the AcbSh (Berendse and Groenewegen, 1990; Bubser and Deutch, 1998; Hartig et al., 2003; Parsons et al., 2006; Van der Werf et al., 2002; Wright and Groenewegen, 1995) which arises from cells innervated by orexin and CART containing afferents (Parsons et al., 2006). It has been suggested that the glutamatergic projections to the AcbSh which influence feeding are likely to arise in telencephalic limbic structures (Faure et al., 2011), but the current findings suggest that glutamatergic afferents from the PVT are also likely to play an important role. The PVT may also be able to affect AcbSh glutamate release by indirect routes, since it projects directly to the infralimbic cortex, ventral subiculum, and basolateral amygdala (Moga et al., 1995; Vertes and Hoover, 2008), which, in turn, are the major telencephalic sources of glutamate input to the AcbSh. It is striking that feeding behavior has been reported to be accompanied both by a reduction in the activity of PVT cells (Nakahara et al., 2004) and by decreases in accumbens glutamate release (Rada et al., 1997).

We examined the effects of muscimol injections into the MD to control for the possibility that the increased feeding obtained from the PVT might be mediated by diffusion to nearby structures and were surprised to observe a significant, dose-dependent suppression of food intake after injections at this site. (Indeed, it is possible that diffusion of the drug to the MD actually acts to limit the magnitude of PVT hyperphagia). Future work will be required to determine the conditions under which this effect occurs and whether it is specific to feeding behavior, or reflects a more general alteration in motivational or motor systems. Injections of muscimol into the MD have been reported to produce mild increases in locomotion (Churchill et al., 1996), so it is unlikely that the observed effects were due to sedation. Interestingly, the MD receives a large projection from the ventral pallidum (Haber et al., 1985; O'Donnell et al., 1997; Zahm et al., 1996), a brain region known to be involved in the control of food intake that has been demonstrated to be a component of the AcbSh feeding circuit (Stratford et al., 1999; Stratford and Wirtshafter, 2012). It should be noted that experimental lesions of the MD do not appear to reduce food intake (Ferreira et al., 1987; Luyten et al., 2009), although some clinical studies have suggested that abnormalities in this region may play a role in eating disorders [e.g., (Conrad et al., 2008)]. The prefrontal cortex, which is the major projection target of the MD, appears to also exert an influence on feeding (Kolb and Nonneman, 1975, Richard and Berridge, in press), so it is possible that the effects

of muscimol injections in the MD might be mediated through this cortical region. Further studies are needed to investigate this possibility.

In summary, the current results strongly support the possibility that the PVT may be a component of the neural circuitry controlling food intake. A similar conclusion has recently been reached by Choi et al. (2012). Since the PVT receives inputs from feeding related structures in the brainstem and hypothalamus and projects to the AcbSh, this structure may play a major role in providing ingestion-related information to the AcbSh. Since cells in the PVT are strongly affected by administration of neuroleptic drugs (Deutch et al., 1995), this structure may be involved in the weight gain that is sometimes clinically associated with the use of these compounds. Cells in the PVT are also very sensitive to a variety of stressors, and one could speculate that this structure might play a role in mediating stress effects on ingestive behavior.

4. Experimental procedures

4.1. Subjects

Male Sprague-Dawley rats (Charles Rivers) weighing between 320 and 406 g at the time of surgery served as subjects. The rats were housed individually in plastic cages on a 12 h light: 12 h dark cycle at a constant room temperature (~21 °C) with food (Harlan Teklad) and tap water available *ad libitum*, except as noted below. All experiments conformed to the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Chicago.

4.2. Surgery

Surgery was performed using standard, aseptic, flat-skull stereotaxic techniques. In Experiment 1, the rats were anesthetized with sodium pentobarbital (60 mg/kg) and a 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA), aimed so as to terminate 2.0 mm dorsal to the PVT, was implanted at coordinates: AP: -3.3, LM: 0.0, DV: -4.1 (mm from bregma). In Experiment 2, bilateral 22-gauge cannulae, aimed so as to terminate 2.0 mm dorsal to the MD, were implanted bilaterally at coordinates AP: -3.3, LM: $\pm$ 0.9, DV: -4.5. The guide cannulae were held in place using stainless steel screws and denture lining material and stainless steel obturators were inserted into the lumen of each cannula to help maintain patency. After surgery, the rats received an injection of carprofen (5 mg/kg, sc) to help alleviate postoperative pain. Each rat was allowed to recover for at least seven days before the start of behavioral testing.

4.3. Feeding measurements

Test chambers (pelletometers) consisted of plastic “shoebox” cages (L 43 X W 22 X H 21 cm) equipped with metal troughs into which individual 45 mg grain based pellets similar in macronutrient composition to the maintenance diet (Precision Dustless pellets, Bio-Serve, Frenchtown, NJ) could be deposited by automated dispensers (Med-Associates, St. Albans, VT) under computer control. Removal of the pellet unblocked an infrared photobeam; the time of these events was recorded to the nearest 0.1 s, after which another pellet was delivered. Examination of the test apparatus revealed that all of the pellets were consumed during the test period. Test sessions lasted for 60 min after introduction of the animal to the pelletometer. In order to acclimate the rats to the apparatus and procedure, they were placed in the pelletometers and allowed to eat for 60 min on at least five days before the start of testing.

4.4. Intracerebral injections

During the intracerebral injections, the rats were restrained gently, the obturators removed, and 28-gauge injection cannulae, extending either 1.5 mm or 2.0 mm beyond the ventral tip of the guide, were inserted into each guide cannula. All injections were made in a volume of 0.5 μ l at a rate of 0.33 μ l/min. The injectors were connected to microsyringes (Hamilton Co., Reno NV) through a length of fluid filled polyethylene tubing attached to a model PHD Ultra infusion pump (Harvard Apparatus, Holliston, MA). After the infusions, the injection cannulae were left in place for an additional 30 s in order to minimize leakage up the cannula track. The obturators then were replaced and the rats were placed in the test cages for 60 min.

4.5. Procedure

Sixteen rats were used in Experiment 1; each animal received 0.5 μ l injections of either sterile saline or of 50 ng or 100 ng muscimol through 2 mm injectors aimed at the PVT or of 100 ng muscimol through 1.5 mm long injectors aimed to terminate immediately dorsal to the PVT. Drug dose and injection site were counterbalanced and at least 48 h were allowed between injections. Six rats were used in Experiment 2, and each subject received simultaneous bilateral 0.5 μ l injections of either sterile saline or 50 or 100 ng muscimol aimed at the MD.

4.6. Histology

When behavioral testing was completed, each of the rats was deeply anesthetized with sodium pentobarbital (100 mg/kg) and perfused transcardially with a 0.15 M saline solution followed by 10% buffered formalin. 50 μ m-thick coronal sections were then taken through the injection sites, mounted on chrome-alum coated slides, air-dried, and stained with cresyl violet. The injection sites were mapped and examined for placement accuracy and evidence of excessive damage.

4.7. Statistical analysis

Data from intrathalamic injections in Experiments 1 and 2 were analyzed by means of one-way repeated measures analyses of variance (ANOVAs). Data were log transformed prior to analysis in order to more closely approximate homogeneity of variance. Where appropriate, single degree of freedom *post hoc* contrasts were conducted using the Bonferroni technique to adjust significance levels for multiple tests. Feeding data from the ICV injection targets in Experiment 1 were analyzed separately using a repeated-measures Student's t-test. All alpha values were two-tailed.

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Fig. 1. Schematic illustration of the location of paraventricular thalamic (circles) and mediodorsal thalamic (squares) injection sites in the current study. Section modified from (Paxinos and Watson, 2007), Plate 60.

Fig. 2.
Photomicrographs of coronal sections of the rat brain showing infusion cannula tracks terminating in the (a) PVT and (b) MD.

Fig. 3. Mean ($\pm$ SEM) food intake after muscimol injections into the PVT or the overlying ventricle. Intra-PVT muscimol dose-dependently increased food intake while intraventricular injections had no effect. * $p < 0.001$ vs. saline injection.

Fig. 4. Mean ($\pm$ SEM) food intake after muscimol injections into the MD. Intra-MD muscimol significantly suppressed spontaneous food intake. * $=p<0.02$ vs. saline injections.