Behavioural Brain Research 216 (2011) 514–518

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Opposite effects on the ingestion of ethanol and sucrose solutions after injections of muscimol into the nucleus accumbens shell

Thomas R. Stratford*, David Wirtshafter

Laboratory of Integrative Neuroscience, Department of Psychology (m/c 285), University of Illinois at Chicago, 1007 West Harrison Street, Chicago, IL 60607-7137, USA

ARTICLE INFO

Article history:
Received 26 June 2010
Received in revised form 13 August 2010
Accepted 20 August 2010
Available online 8 September 2010

Keywords:
Alcohol
GABA
Muscimol
Food intake
Taste
Calories
Reward

ABSTRACT

Injection of the $GABA_A$ receptor agonist muscimol into the nucleus accumbens shell (AcbSh) elicits robust feeding in satiated rats, but has no effect on water intake. The current study was designed to examine whether intra-AcbSh muscimol injections influence the intake of ethanol solutions in rats trained to drink using a limited access paradigm. We confirmed that bilateral injections of muscimol (100 ng) into the AcbSh produce large increases in the intake of sucrose solutions and of the chow maintenance diet but found in two independent experiments that these injections potently reduce the intake of a 10% ethanol solution. Furthermore, intra-AcbSh muscimol significantly increased intake of an ethanol–sucrose mixture. These results demonstrate that activating $GABA_A$ receptors in the vicinity of the AcbSh can have opposite effects on the intake of different caloric substances and are consistent with the possibility that GABAergic circuits in the AcbSh may play a role in mediating voluntary ethanol intake.

1. Introduction

The nucleus accumbens shell (AcbSh) has been implicated as a key component of a neural system involved in the mediation of feeding behavior [1]. Inhibition of neurons in the AcbSh with GABA agonists or glutamate antagonists elicits intense feeding in satiated rats, with short-term intakes that are among the highest reported in the literature [2–4]. Importantly, inhibition of the AcbSh appears to affect feeding behavior specifically, as such treatments do not increase water intake, non-ingestive gnawing, or locomotor activity when food is present [5,6]. Furthermore, although inhibiting neurons in the AcbSh does not alter water intake even in water-deprived rats that are primed to drink [3,5], the treatment does increase intake of certain concentrations of sucrose solutions [5,7], demonstrating that AcbSh-mediated hyperphagia is not limited to ingestion of solid food or maintenance diets. The AcbSh is believed to play an important role in reward or reinforcement mechanisms [8,9], however, the behavioral specificity of the feeding response suggests that a general alteration in reward processing does not underlie the effect. Rather, amino acid-coded circuits in the AcbSh appear to be preferentially involved in the control of food intake.

Several lines of evidence suggest that the AcbSh may also be involved in the control of ethanol intake. For instance, intracerebroventricular or intraperitoneal administration of ethanol increases Fos expression in the AcbSh [10–12], and voluntary ingestion of ethanol elicits a number of physiological changes in the AcbSh, including increases in dopamine [13] and opioid levels [14–16], expression of CaM IV kinase and CREB phosphorylation [17], and changes in neuronal firing rates [18] and glucose utilization [19]. Additionally, alcohol-preferring rats have higher densities of GABA terminals in the Acb than rats bred for low alcohol consumption [20,21].

In the present study, we investigated whether local injections of the $GABA_A$ receptor agonist muscimol into the AcbSh, a treatment that potently stimulates food intake, would alter intake of a 10% ethanol solution or a 10% ethanol solution sweetened with 10% sucrose. The effects of the treatment on the intake of lab chow and a 10% sucrose solution were also examined in the same rats as positive controls.

2. Methods

2.1. Subjects

Nineteen male Sprague–Dawley rats (Harlan, Madison, WI) weighing between 305 and 385 g at the time of surgery served as subjects. The rats were housed individually in wire-mesh 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.

* Corresponding author. Tel.: +1 312 355 0848; fax: +1 312 413 4122.
E-mail address: ratdoc@uic.edu (T.R. Stratford).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.08.032

2.2. Test solutions and acquisition of ethanol drinking

The ethanol solution was prepared by diluting 95% ethanol with tap water to yield a 10% ethanol (w/v) solution, the sucrose solution by dissolving sucrose to a concentration of 10% (w/v) in tap water, and the sweetened ethanol solution by dissolving sucrose to a concentration of 10% (w/v) in the 10% ethanol solution. Rats were trained to drink ethanol over a four-week period using a limited-access protocol. During the ethanol drinking acquisition phase, rats were placed in test cages for 1 h each day, beginning 4 h after the start of the light period, where they were given access to an ethanol solution. The rats were water-deprived for 20 h before the first exposure to encourage sampling of the ethanol solution and neither food nor water was available during the ethanol presentation. The concentration of the ethanol solution was increased from 3% during the first week of acquisition to 6% during the second week and to 10% during the third and fourth weeks. At the end of four weeks, baseline intakes of 10% ethanol had stabilized. The ten rats in Experiment 1 received several exposures to the sucrose solution or lab chow in the test cages interspersed with the ethanol trials, while the nine rats in Experiment 2 received several interspersed exposures to the sucrose or the sweetened ethanol solution. Two rats consistently failed to drink any unsweetened ethanol during the presentations and were excluded from the remainder of the studies.

2.3. Surgery

At the end of the four-week ethanol acquisition phase, the rats were anesthetized with sodium pentobarbital (60 mg/kg) and bilateral 26-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted using standard, flat-skull stereotaxic techniques. The guide cannulae were aimed so as to terminate 2.0 mm dorsal to the AcbSh using the following coordinates: anteroposterior: 1.5, mediolateral: ±0.8, and dorsoventral: −6.1 (mm from bregma). 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. Each rat was allowed to recover for at least seven days during which time the daily presentations of ethanol continued.

2.4. Intracerebral injections

In order to acclimate the rats to the test procedure, they were restrained gently, the obturators removed, and a 32-gauge injection cannula, extending 2.0 mm beyond the ventral tip of the guide, was inserted into each guide cannula on three consecutive days. On each day, the obturators were replaced and the rats were placed in the test cages for 60 min with ethanol present. On the final acclimation day, each rat received bilateral 0.25 μl intracerebral injections of sterile 0.15 M saline. On test days, each rat received simultaneous bilateral 0.25 μl injections of 100 ng muscimol or the sterile saline vehicle into the ventromedial AcbSh at a rate of 0.25 μl/min (the dose of muscimol we have found to elicit maximal food intake when administered into the AcbSh [3] and the use of which allows us to maximize comparability with our previous results). After the infusion, the injection cannulae were left in place for an additional 60 s in order to minimize leakage up the cannula track. In Experiment 1, the rats then were placed in test cages for 1 h with either 10% sucrose, 10% ethanol, or chow available. In Experiment 2, the rats were placed in test cages with either 10% sucrose, 10% ethanol, or a sweetened 10% ethanol solution containing 10% sucrose. In both experiments, the ethanol and sucrose solutions were presented in a counterbalanced order in burettes calibrated to 0.1 ml. Intake of chow was evaluated in Experiment 1 and intake of the sweetened ethanol solution in Experiment 2 after the other tests had been completed. At least 72 h were allowed between injections.

2.5. Histology

After the completion of behavioral testing, each of the rats was deeply anesthetized using sodium pentobarbital and perfused transcardially with 50 ml of a 0.15 M saline solution followed immediately by 500 ml of a 10% buffered formalin solution. The brains were removed and stored in fixative for at least one week, after which they were frozen and 60 μm-thick coronal sections were taken throughout the extent of the AcbSh. The sections were stained with cresyl-violet and the injection sites were examined for placement accuracy and evidence of excessive damage.

3. Results

Histological examination showed that all microinjector cannulae terminated in the medial AcbSh at placements similar to those used in our previous studies (e.g., [3,22,23]). Fig. 1 is a schematic illustration of the 17 bilateral AcbSh injection sites.

Mean intakes in Experiment 1 can be seen in Fig. 2 which shows that injections of muscimol into the AcbSh produced a significant *decrease* in ethanol intake as compared to saline injections ($F_{(1,7)} = 45.6$; $p < .001$). In contrast, muscimol injections in these same animals produced significant *increases*, relative to saline injections, in the intakes of both a 10% sucrose solution ($F_{(1,7)} = 35.8$; $p < .001$) and of their regular chow maintenance diet ($F_{(1,7)} = 41.9$; $p < .001$).

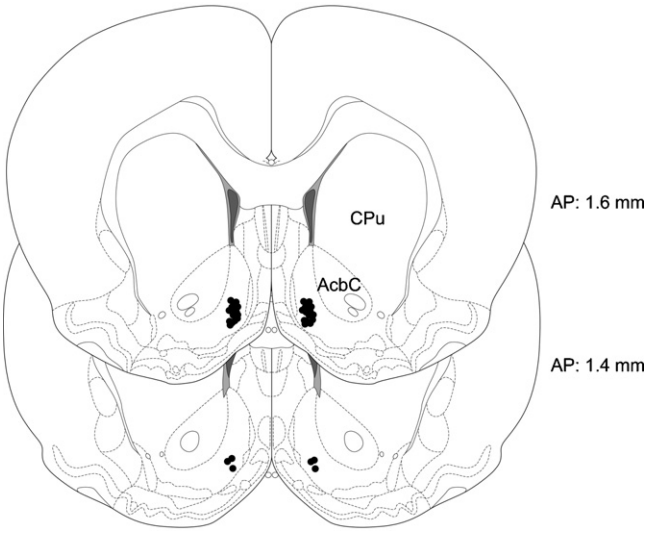

Fig. 1. Schematic representation of the bilateral AcbSh injection sites (AcbC: nucleus accumbens core; CPu: caudate-putamen).

Mean total intakes in Experiment 2 are shown in Fig. 3a (and, for convenience, are expressed as dose of ethanol received in Fig. 3b). Again, intra-AcbSh muscimol resulted in a marked suppression of ethanol intake ($F_{(1,8)} = 24.6$; $p < .001$), while producing increases of similar magnitude in the intakes of both the sucrose solution and the sucrose + ethanol mixture. The data for the two sucrose containing solutions were analyzed by means of a 2 × 2 (ethanol × muscimol) repeated measures ANOVA which indicated a significant effect of muscimol ($F_{(1,8)} = 26.1$, $p < .001$). The effect of ethanol was also significant ($F_{(1,8)} = 7.4$; $p < .03$), reflecting the fact that intakes of the mixture were slightly lower than intakes of the sucrose solution following injections of either saline or muscimol. The ethanol × muscimol interaction ($F < 1$) was not significant, however, indicating that muscimol produced statistically indistinguishable increases in the intakes of the two solutions. This result is striking because the sweetened ethanol solution was much more calorically dense than the sucrose solution alone. Mean calories consumed are shown in Fig. 3c, where it can be seen that muscimol actually tended to produce a larger increase in caloric

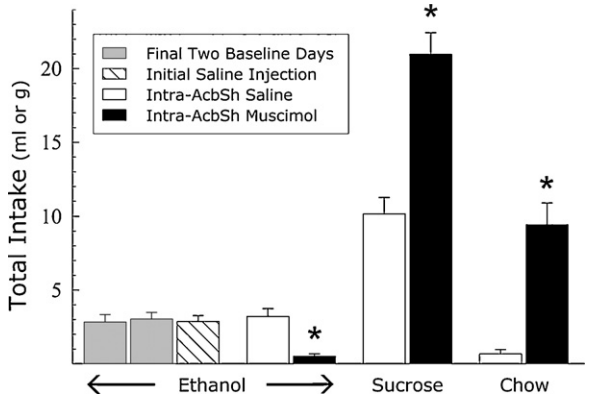

Fig. 2. Experiment 1: Effects of intra-AcbSh injections of saline or muscimol (100 ng/side) on the mean 60 min intakes of a 10% ethanol solution, a 10% sucrose solution, or lab chow. *$p < .001$ vs. saline injections.