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Intra-accumbens baclofen, but not muscimol, mimics the effects of food withdrawal on feeding behaviour

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ABSTRACT

Intra-accumbens stimulation of GABA receptors results in a robust increase in food intake. However the differential consequences of stimulating $GABA_A$ and $GABA_B$ receptors in the nucleus accumbens have not been extensively explored with respect to feeding behaviour. Here we compare the effects of the $GABA_B$ receptor agonist baclofen and $GABA_A$ receptor agonist muscimol, infused into the nucleus accumbens shell, on food intake and related behavior patterns. Baclofen (110–440 ρmol) dose dependently enhanced intake and delayed the onset of satiety within the test period as did the effects of 4–8 h food withdrawal. Muscimol (220–660 ρmol) enhanced intake but also disrupted the sequence of associated behaviours at every dose tested. We conclude that $GABA_B$ receptors in the nucleus accumbens shell may play a role in relation to feeding motivation whereas $GABA_A$ receptors may, as previously suggested, have a more restricted role in relation to the motor components of approach to food and ingestion.

1. Introduction

Stratford and Kelley (1997) reported that a robust feeding response is induced by intra-accumbens shell administration of the $GABA_A$ receptor agonist muscimol or of the $GABA_B$ receptor agonist baclofen. The GABA transaminase inhibitor γ-vinyl–GABA, which reduces the metabolism of GABA, also increases food intake, which would be consistent with a role for endogenous GABA in the modulation of feeding (Stratford and Kelley, 1997). These manipulations are thought to locally inhibit the activity of medium spiny neurons within the accumbens and thus to release downstream centres, particularly in the lateral hypothalamus, which could drive consummatory components of feeding behaviour without necessarily increasing motivation for food (Kelley et al., 2005). This hypothesis is consistent with the observation that the onset of consummatory responses to food is characterized by inhibition of a population of neurons within the nucleus accumbens (Taha and Fields, 2005) which could permissively gate goal directed sequences of behaviour (Taha and Fields, 2006).

Behavioural support for this interpretation comes from the observation that intra-accumbens administration of baclofen selectively increased intake of solid chow, but not generalized gnawing behaviour or drinking (Ward et al., 2000). In addition intra-accumbens shell administration of muscimol increased intake of caloric diets regardless of macronutrient content but did not increase intake of palatable non-caloric solutions (Basso and Kelley, 1999). Further evidence for this view, in the case of muscimol, is provided by studies of instrumental responding for food. Thus, muscimol failed to increase lever pressing for sucrose pellets in rats trained on a progressive ratio schedule (Zhang et al., 2003). In addition intra-accumbens muscimol failed to enhance the initial acquisition of lever pressing for sucrose pellets whereas food withdrawal was effective in this regard (Hanlon et al., 2004).

The effects of intra-accumbens infusions of muscimol and baclofen on *ad libitum* consumption of food have only previously been measured in terms of total intake. Standard paradigms used to explore drug effects on appetite and satiety have not been employed. One candidate paradigm is the Behavioural Satiety Sequence (BSS) which tracks the transition from feeding to post-prandial behaviours. Richter (1922) first described the predictable pattern of activity that follows feeding in rats. He observed a distinct temporal profile of behaviours following access to food characterized by an initial period of feeding followed by exploration, a period of grooming and, eventually, 'rest' or sleep. The BSS has been used to characterise the effects of a wide variety of drug and other manipulations (Halford et al., 1998). Here we use the method to characterise the early stages of feeding behaviour and its progression towards inactivity.

The data from experiments 1 and 2 characterise the BSS elicited by intra-accumbens administration of baclofen or muscimol in pre-fed animals. The hypothesis suggested by Stratford and Kelley (1997)

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predicts that an increase in motor behaviours specifically directed at food consumption would limit and disrupt the expression of the rest of BSS behavioural repertoire. This is supported by their observation that temporary inactivation of excitatory inputs to the accumbens also reduces "exploratory-like motor responses" (Kelley et al., 2005). The third experiment provides a reference profile for a 'natural' manipulation of the BSS under the same conditions as the previous experiments. Animals were systematically exposed to short but varied periods of food withdrawal prior to a free-feeding test period. The effects of hunger and presatiation have been demonstrated using a mash meal (Ishii et al., 2003a) and it was anticipated that the same general effects would be observed here although the timings of the transition from feeding to inactive behaviour might be different because of the use of solid chow. This food type was chosen to be in line with previous studies investigating the effects of baclofen and muscimol on feeding (Stratford and Kelley, 1997; Ward et al., 2000).

2. Methods

2.1. Animals

Male Lister hooded rats (Harlan, UK), weighing 250–275 g at the start of each experiment were initially housed in groups of 3. Separate groups of animals were used in each experiment. In Experiments 1 and 2, at least 7 days prior to surgery, and, in Experiment 3, 7 days prior to testing, they were habituated to single housing. All animals were maintained on an *ad libitum* diet of chow (SDS expanded diet) and water. The animals were held in rooms with controlled temperature (20–22°) and humidity (40–60%RH) on a 12:12 h light cycle (lights on: 07.00 h). In Experiment 2 the animals were trained on an instrumental schedule prior to surgery and were then tested on this schedule (data not reported here) prior to the BSS test. These animals were habituated to the BSS test procedure prior to and post instrumental testing and no difference in intake was observed. In Experiments 1 and 2, using centrally administered drugs, test sessions ran between 10.00–16.00 h and in Experiment 3 at 15.30 h for fasted animals.

All experimental protocols were in accordance with the Home Office Animals (Scientific Procedures) Act 1986, and were also approved by the University of Sussex Local Ethical Review Committee.

2.2. Surgery

Anaesthesia was induced with 4% isoflurane in 0.5 L/min N_2O and 0.5 L/min O_2, and then maintained by adjusting the isoflurane concentration to 1.5–2.5%. Thin wall 26ga, 16 mm stainless steel cannulae (Coopers Needleworks, UK) were implanted bilaterally aimed 2.2 mm dorsal to the target site in the accumbens shell using the coordinates anteroposterior (AP), +1.2 mm, mediolateral (ML), ±1.5 mm relative to bregma (β) and dorsoventral (DV),−5.8 mm relative to the flat skull surface (Paxinos and Watson, 1998). The cannulae were secured with three small screws, Geristore dental resin and finished with a cap of Simplex dental acrylic. Cannula patency was maintained by 33 g wire obdurators. The incision was treated with Cicatrin (GlaxoSmithKline) and the animals were administered an antibiotic (oxytetracycline 10 mg/kg) and a non-steroidal analgesic (meloxicam 2 mg/kg) immediately, and then at 24 and 48 h after surgery. These drugs were mixed into a small pot of palatable wet mash, to which the animals had been accustomed prior to surgery.

2.3. Histological verification

Brains were sectioned coronally at 60 μm on a freezing microtome. Relevant sections were mounted on gelatinized slides and, following alcohol dehydration, run through a standard Nissl staining procedure using Thionin. The location of infusion sites was determined from Paxinos and Watson (1998).

2.4. Drugs and drug administration

Baclofen (Sigma, UK) was dissolved in 0.9% sterile saline and the pH of the solution adjusted to around 7.5 using 1 M sodium hydroxide and administered in doses of 110, 220, and 440 ρmol per μl. This dose range was chosen to avoid motor side effects that were observed in pilot experiments at higher doses, including 880 ρmoles per side, the most effective dose, in terms of intake, used by Stratford and Kelley (1997). Muscimol (Sigma, UK) was dissolved in 0.9% sterile saline, which gave a solution with a pH of 7.5 without further adjustment and administered at doses of 220, 440 and 660 ρmol per μl. Baclofen increases intake across a lower dose range than muscimol and a total infusion of 176 ρmol muscimol has been shown to have no effect on total intake of chow during a 2 h test session (Stratford and Kelley, 1997) so a dose of 110 ρmol muscimol was not used.

On test days bilateral infusions of drug or vehicle were made simultaneously into the accumbens shell at a rate of 0.5 μl per side over 30 s (1 μl of drug solution infused in total). Injectors were left in for a further minute to allow diffusion of drug away from the tip. The infusions were given using 31 g stainless steel infusors which extended 2.2 mm beyond the tip of the guide cannulae to reach the target structure. These injectors were connected via number 10 PPE tubing to 10 μl Hamilton syringes. A microinfusion syringe pump model 802 (Univentor, Malta) which held two syringes allowed bilateral infusions to be made simultaneously. Behavioural testing followed immediately after the infusions were completed.

2.5. Behavioural testing

The method was based on procedures previously described (Clifton et al., 1989; Vickers et al., 1996). 1 h prior to daily test sessions food was removed from the home cage and replaced with 5 g of fresh chow pellets to which the rats had access for 30 min. Pre-fed animals were then transferred to the test cage for a 30 min acclimation period. Finally, they were presented with a pre-weighed pot of chow for a 30 min test session. As expected, consumption was initially low and habituation continued until there was no significant difference in the mean meal size over four consecutive days. The average habituation period was 7 days. Water was available throughout.

During the subsequent test sessions in Experiments 1 and 2 infusions were made following the 30 min acclimation period in the test cage and in all 3 experiments chow was then presented and the BSS was recorded using the method described by Vickers et al. (1996). The behavioural categories were: *Ingest*: retrieval of food with mouth or paws, holding chewing and ingesting food; *Active:* moving around cage, rearing, sniffing, standing alert and any other behaviour not already defined; *Groom:* grooming, biting or licking of head, body or tail using mouth or limbs; *Inactive:* absence of movement in a resting posture (head and / or body lowered) with, or without, eye closure. Test sessions were separated by at least 48 h. In Experiments 1 and 2 animals were tested in pairs (5 s inter-observation interval) following infusions. In Experiment 3 animals were tested as a single cohort of 12 (30 s inter-observation interval) following i.p. injections of saline to provide a degree of handling stress equivalent to infusing. A 30 min test session for Experiments 1 and 2 was chosen to minimise time of day effects.

2.6. Statistics

Food intake was expressed as mean (±SEM) intake of chow (g) and analysed using repeated measures ANOVA with dose or food withdrawal as the repeated measure factor. Each of the four mutually