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Hyperphagia induced by direct administration of midazolam into the parabrachial nucleus of the rat

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Abstract

Benzodiazepine receptor agonists increase food intake in many different species, yet there has been little investigation of the central site of actions of these drugs on ingestive behaviour. In the present experiments, direct administration of the benzodiazepine receptor agonist midazolam (3–30 μg/μl) into the parabrachial nucleus of the pons significantly increased the consumption of a wet mash diet and a 3% sucrose solution in adult non-deprived rats. The hyperphagic response was blocked by pre-treatment with the selective benzodiazepine receptor antagonist flumazenil. Injection of midazolam into the parabrachial nucleus had no effect on locomotor activity, despite the fact that in the same animals an increase in mash intake was observed following intra-parabrachial midazolam. These data suggest that benzodiazepine receptors located in the parabrachial nucleus may be an important site of action for the effects of benzodiazepines specifically on ingestive behaviour.

Keywords: Benzodiazepine; Hyperphagia; Brain stem; Parabrachial nucleus; Palatability; (Rat)

1. Introduction

The potent stimulation of appetite by benzodiazepine receptor agonists is a well-understood phenomenon, which has been comprehensively documented in terms of pharmacological and behavioural characteristics (Cooper, 1980, Cooper, 1989). The hyperphagic effect exhibits stereospecificity (Cooper and Yerbury, 1988), is produced by a wide range of both full and partial benzodiazepine receptor agonists, and is blocked by the selective benzodiazepine receptor antagonist flumazenil (Cooper et al., 1985). The increase in food intake caused by these drugs stems from a selective enhancement of taste-related palatability (Berridge and Pecina, 1995; Cooper and Higgs, 1994). For example, benzodiazepine receptor agonists have a selective effect in taste preference tests, increasing the consumption of a preferred saccharin solution without causing a concomitant increase in water intake (Cooper and Green, 1993; Cooper and Yerbury, 1988; Roache and Zabik, 1986). In addition, using the taste reactivity paradigm, Berridge and Treit (1986) have shown that chlordiazepoxide increases hedonic responding to taste stimuli without affecting aversive or neutral reactions.

The identification of the central site(s) of action for benzodiazepine-induced hyperphagia is now of primary importance. Berridge (1988) has shown that chlordiazepoxide retains its ability to enhance positive taste reactions in the chronic decerebrate rat preparation. This suggests that caudal brainstem site(s) may be important for effects of chlordiazepoxide in the taste reactivity paradigm. In support of this, Berridge and Pecina (1995) have shown that direct administration of the benzodiazepine receptor agonist diazepam into the IVth ventricle in rats enhances the positive hedonic reactions elicited by a 7% sucrose solution. Further support for brainstem mediation of benzodiazepine effects on ingestive responses derives from recent work showing that direct administration of benzodiazepine receptor agonist midazolam into the IVth ventricle significantly increased the consumption of a palatable wet mash in non-deprived rats (Higgs and Cooper, 1996). This hyperphagic response was blocked by pretreatment with flumazenil, indicating that the effect was mediated by specific benzodiazepine receptors. Therefore, benzodiazepines appear to act in, or near, the IVth ventricle both

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to enhance taste palatability and increase food consumption.

The aim of the present experiments was to identify more precisely the brainstem structures involved in benzodiazepine-induced increases in food intake. The target for this work was the parabrachial nucleus of the pons which contains the second relay for the taste projection system (Norgren and Leonard, 1973; Norgren, 1978). Taste is an important determinant of food intake (Pfaffmann, 1982), and so the parabrachial nucleus is well placed to influence food ingestion. Recently, we have found evidence of a population of benzodiazepine receptors in the region of the parabrachial nucleus, in contrast to the general sparsity of these receptors in the lower brainstem (Higgs et al., 1993). It is also well established that the parabrachial nucleus makes extensive connections to many other areas of the brain involved in the control of ingestive behaviour, such as the hypothalamus (Krukoff et al., 1993; Norgren, 1976; Saper and Loewy, 1980) and amygdala (Norgren, 1976).

The aim of the first experiment was to establish whether an injection of midazolam into the parabrachial nucleus is capable of eliciting a hyperphagic response. Experiment 2 was designed to check if the response to midazolam is mediated by specific benzodiazepine receptors.

The parabrachial nucleus can be subdivided up into a number of distinct regions (Krukoff et al., 1993; Saper and Loewy, 1980). Guide cannulae were targeted either to medial or lateral areas of the parabrachial nucleus to examine whether a functional dissociation between these two areas could be demonstrated.

Systemic administration of benzodiazepine receptor agonists can enhance the consumption of liquid foods such as sucrose solutions and solid foods or wet mash (Cooper and Greenwood, 1992; Cooper et al., 1987). The aim of experiment 3 was to determine whether the effect of midazolam injected directly into the parabrachial nucleus mimics the effects of systemically administered benzodiazepine receptor agonists. Non-deprived rats were trained to consume a 3% sucrose solution and the potential hyperphagic effect of intra-parabrachial midazolam was investigated.

Benzodiazepine receptor agonists affect locomotor activity and have sedative effects at large doses (File, 1981). In a fourth experiment, an activity monitor was used to measure locomotion following administration of intra-parabrachial midazolam. As a control experiment, the intake of a sweetened wet mash was also measured on a separate occasion in the same animals to ensure that any result obtained was not a false negative due to failure of the drug to reach the receptors.

Virtually all previous work on the parabrachial nucleus and ingestive responses has involved lesion techniques which have obvious drawbacks and limitations (Spector, 1995). The aim of these studies was to employ a pharmacological approach with the specific purpose of determining if the parabrachial nucleus is a principle site of action for benzodiazepine-induced hyperphagia.

2. Materials and methods

2.1. Animals

Adult male hooded rats (General strain bred in the School of Psychology, University of Birmingham) weighing 300–400 g at the beginning of experimentation were used. Rats were housed individually in plastic cages in a room with a constant temperature of 22 ± 2°C, and were maintained on a 12 h light:dark cycle (lights on at 8:00). Standard laboratory food pellets (Pilsbury 41B, Heygate and Sons, UK) and water were available at all times. Behavioural testing was conducted in the light phase.

2.2. Drugs

The water soluble benzodiazepine receptor agonist midazolam maleate (Roche, Basel, Switzerland) was prepared for injection by dissolving in isotonic saline. The range of doses used in these experiments was 3, 10 and 30 μg/μl of midazolam. The choice of doses was based on previous studies. A dose range of 3–30 μg/μl has been shown to increase intake of mash following injection into the IVth ventricle of rats (Higgs and Cooper, 1996). The vehicle used in control injections was isotonic saline. The selective benzodiazepine receptor antagonist flumazenil (Roche, Basel, Switzerland) was prepared for injection by ultrasonic dispersion in distilled water to which Tween 80 (BDH Chemicals, Poole, UK) had been added. The dose used was 20 mg/kg which has been shown to block the hyperphagic effect of benzodiazepine receptor agonists (Cooper and Moores, 1985; Cooper et al., 1985). A repeated-measures design was used and 24 h elapsed between successive injections.

2.3. Surgery

For implantation of stainless steel guide cannulae, rats were anesthetized with medetomidine, 250 μg/kg (Domitor) and ketamine, 60 mg/kg (Vetelar) combination anesthetic. Effects of the medetomidine were reversed using atipmazole (Antisedan) at a dose of 1 mg/kg. The analgesic buprenorphine (Temgesic) was also administered prior to recovery at a dose of 0.03 mg/kg. Guide cannulae were targeted bilaterally to either the medial or lateral parabrachial nucleus (coordinates for medial parabrachial nucleus: L +1.4 A-P −9.4 V −5.8, coordinates for lateral parabrachial nucleus: L +2.2 A-P −9.4 V −6.2). The cannulae tips were implanted 2 mm dorsal to the intended injection site. Bregma was used as a reference point and the coordinates were taken from the atlas of Paxinos and Watson (1982). Dental acrylic and three screws were used to fix the cannulae to the skull. Stylets were placed in the guide cannulae to prevent occlusion, and the animals were allowed seven days to recover before behavioural testing occurred. Postoperative care involved

weighing the animals daily and applying an antibiotic wound powder to the headmount if necessary.

2.4. Injection procedure

Central microinjection of drugs was performed using an injection cannula connected by a polyethylene tube to a micrometer-driven 10 μl Hamilton syringe. The injection needles protruded 2 mm beyond the tip of the guide cannula and accuracy of injections was ensured by observing the progress of an air bubble in the tubing. The volume infused was 0.5 μl, each side, injected over a period of 30 s. Each animal was then placed immediately in the test cage and food intake over 30 min was measured. For pretreatment with flumazenil, the antagonist was administered via the intraperitoneal route 15 min prior to central injection of midazolam. The volume injected was 1 ml/kg. Two days prior to testing each animal received a sham injection of isotonic saline to familiarize it with the microinjection procedure.

2.5. Food intake measurement

2.5.1. Mash intake

Rats were first adapted to eating a sweetened wet mash for a period of 10 days. The meal was made up daily according to the following formula: 100 ml sweetened condensed milk, 400 ml ground maintenance diet (Special Diet Services, Essex, UK) and 200 ml distilled water. It has previously been shown that this mash is readily consumed by non-deprived rats (Cooper et al., 1985). Each rat was given 30 min access to 50 g portions of the diet placed in a clear plastic dish inside an individual stainless steel test cage. The consumption of the sweetened mash was measured every 10 min to the nearest 0.1 g with corrections made for any spillage.

2.5.2. Sucrose intake

Familiarisation with a 3% sucrose solution occurred over a period of seven days. Rats were transferred from their home cages to testing cages in which they had access to a single drinking spout attached to a 50 ml graduated cylinder. Sucrose intake was recorded volumetrically to the nearest ml after the 20 min test period.

2.6. Locomotor activity

A photo-cell activity monitor was used to measure locomotor activity. This consisted of a black plastic cylindrical container 30 cm in height. An outer circle of diameter 42 cm enclosed an inner circle of diameter 18 cm. The arrangement created a 12 cm wide corridor which allowed the animals to move in a circular path. Three photobeams, positioned near to the floor, monitored the activity of the animal. When a beam was interrupted this registered as one count. Each rat was familiarised with the test procedure over a period of five days. Individual rats were exposed to a 20 min session in the photocell activity monitor.

2.7. Histology

At the end of each experiment rats were deeply anaesthetized with pentobarbital and a small quantity of methylene blue dye was injected through the cannula. Each animal was then perfused transcardially with isotonic saline followed by a 10% formalin solution. After decapitation, the excised brains were fixed in a 10% formalin solution for one week. The fixed brains were then frozen and sectioned sagitally on a freezing microtome and the correct placement of the cannulae was verified histologically. The histological work was conducted blind with respect to the behavioural results. Rats with cannulae placements further than 1 mm from the intended injection site were judged to be non-parabrachial placements and so were analyzed separately from parabrachial placements. This criterion was chosen based on previous studies showing that an injection volume of 0.5 μl diffuses to spread over a 1 mm area (Myers, 1966).

2.8. Statistical analysis

The data were analyzed using a one-way analysis of variance (ANOVA) for repeated-measures. Comparisons between means were carried out using Dunnett's *t*-test or a Newman-Keuls multiple comparisons test. Statistical tests were performed using Statview SE + graphics (Abacus Concepts, Berkeley, CA, USA, 1987) and a result was considered statistically significant if $P < 0.05$.

3. Results

3.1. Experiment 1: the effect of direct injection of midazolam into the parabrachial nucleus on intake of a palatable mash

Histological analysis showed that not all the placements were targeted at the parabrachial nucleus and so the data for animals where the cannulae were located more than 1 mm outside the parabrachial nucleus ($n = 5$) were analyzed separately from the parabrachial placements ($n = 6$) (Fig. 1). No difference was observed between the responses of animals receiving lateral as opposed to medial injections of midazolam into the parabrachial nucleus, and so their data were pooled.

3.1.1. Parabrachial placements

A one-factor repeated-measures ANOVA revealed a significant effect of the drug after 30 min ($F(3,15) = 9.78$, $P < 0.001$). Midazolam (3–30 μg/μl in 0.5 μl) significantly increased consumption of the mash relative to the

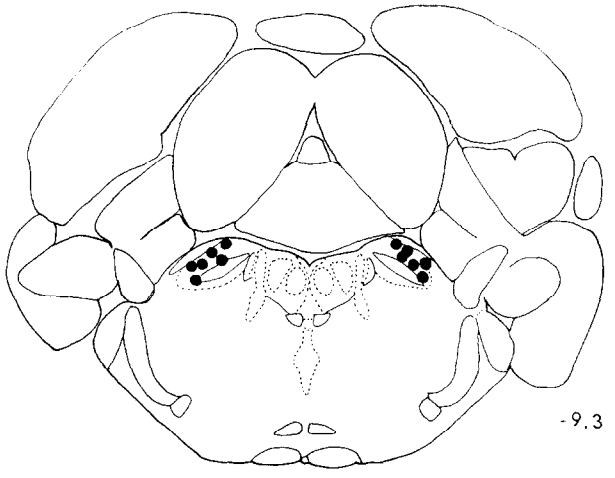

Fig. 1. The distribution of injection sites in the parabrachial nucleus for rats used in Experiment 1. Sites are shown bilaterally. Sections are redrawn from Paxinos and Watson (1982). Section numbers refer to mm from bregma.

control condition. The baseline intake of 8.6 g was almost doubled to 15.5 g (Fig. 2a). Individual comparisons with a Dunnett's *t*-test indicated that the 10 μg/μl and 30 μg/μl conditions differed significantly from the vehicle condition ($P < 0.01$).

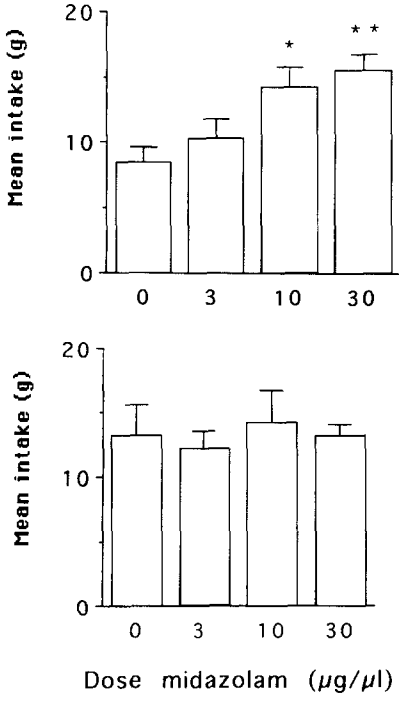

Fig. 2. (a) The effect of direct injection of midazolam into the parabrachial nucleus on ingestion of a sweet mash in non-deprived rats. $n = 6$. Levels of significance for individual dose comparison against the vehicle control * $P < 0.05$, ** $P < 0.01$ (Dunnett's *t*-test). (b) The effect of direct injection of midazolam into areas around the parabrachial nucleus on ingestion of a sweet wet mash in non-deprived rats. $n = 5$. Results are shown as mean intake (g) after 30 min.

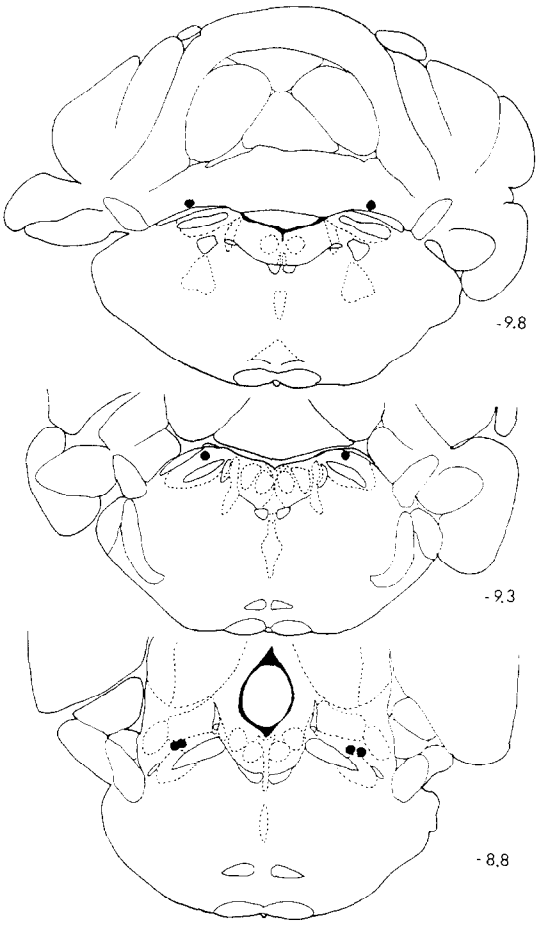

Fig. 3. The distribution of injection sites in the parabrachial nucleus for rats used in Experiment 2. Sites are shown bilaterally. Sections are redrawn from Paxinos and Watson (1982). Section numbers refer to mm from bregma.

3.1.2. Non-parabrachial placements

A one-factor repeated-measures ANOVA revealed that injections which were not within 1 mm of the parabrachial nucleus had no significant effects on intake ($F(3,12) = 0.17$, $P = 0.9$). The baseline intake of 13.3 g was not affected by administration of midazolam into areas around the parabrachial nucleus (Fig. 2b).

3.2. Experiment 2: the effect of pretreatment with flumazenil on hyperphagia induced by injection of midazolam into the parabrachial nucleus

Following histological examination it was found that in four cases the guide cannulae were located further than 1 mm away from the intended area and so the data for these animals were analyzed separately from the parabrachial nucleus placements ($n = 4$) (Fig. 3). The histology for two animals showed extensive damage such that it was impossible to determine the placement site, and so the data for these animals were discarded. No difference was observed in the behaviour of animals receiving lateral as opposed to medial injections of midazolam into the parabrachial nucleus, and so the data were pooled for analysis.

3.2.1. Parabrachial placements

A one-factor repeated-measures ANOVA revealed a significant effect of treatment after 10 min ($F(3,9) = 8.16$, $P < 0.01$). Intra-parabrachial injection of midazolam dose dependently enhanced food consumption relative to saline injection. Pairwise comparisons with a Newman-Keuls multiple comparison test revealed that the 30 μg/μl dose significantly increased ingestion of sweet wet mash after 10 min ($P < 0.01$) (Fig. 4a). Flumazenil (20 mg/kg) administered alone did not significantly effect ingestion of sweet wet mash after 10 min. However, flumazenil did significantly attenuate the increase in ingestion caused by midazolam as indicated by a significant pretreatment × drug interaction ($F(1,6) = 10.96$, $P < 0.05$). By 30 min, no significant effect of drug treatment was observed in the parabrachial placements. This was despite the fact that the baseline intake of 12.8 was nearly doubled following administration of midazolam alone to 21.8.

3.2.2. Non-parabrachial placements

Analysis of the data from animals with guide cannulae more than 1 mm away from the parabrachial nucleus did not reveal a significant effect of drug treatment after 10 min ($F(3,9) = 0.57$, $P = 0.6$). The baseline intake of 9 g was not significantly affected (Fig. 4b). There was still no drug effect evident after 30 min ($F(3,9) = 1.1$, $P = 0.36$).

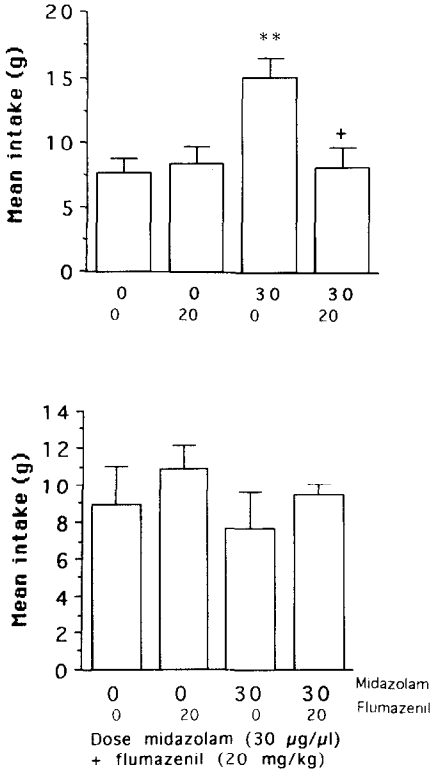

Fig. 4. (a) The effect of pretreatment with flumazenil on the hyperphagia induced by injection of midazolam into the parabrachial nucleus. $n = 4$. Levels of significance for pairwise comparisons ** $P < 001$ significantly different from control condition. $^{+}$ $P < 0.01$ significantly different from vehicle/midazolam condition. (b) The effect of direct injection of midazolam into areas around the parabrachial nucleus on ingestion of a sweet wet mash in non-deprived rats. $n = 4$. Results are shown as mean intake (g) after 10 min.

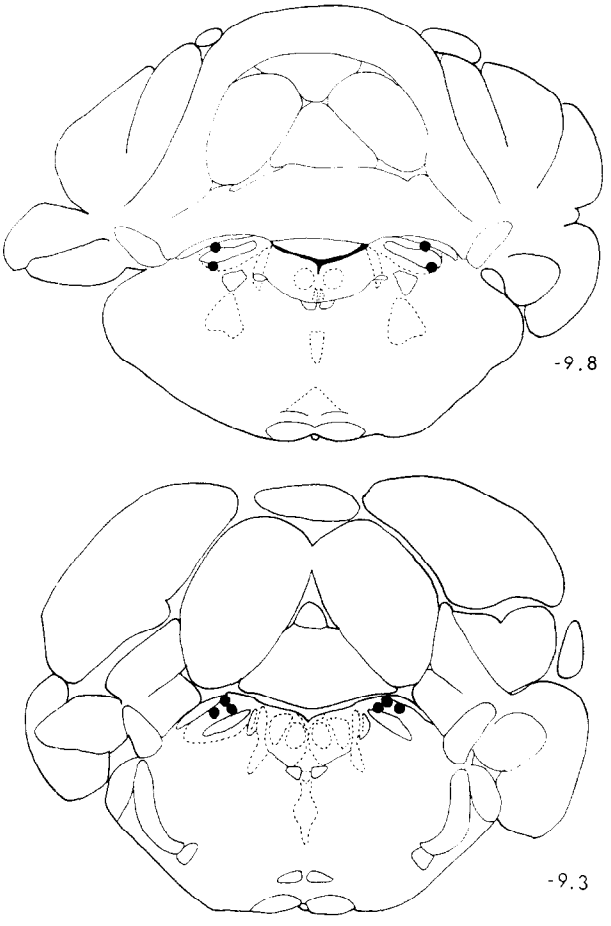

Fig. 5. The distribution of injection sites in the parabrachial nucleus for rats used in Esperiment 3. Sites are shown bilaterally. Sections are redrawn from Paxinos and Watson (1982). Section numbers refer to mm from bregma.

3.3. Experiment 3: the effect of direct injection of midazolam into the parabrachial nucleus on intake of a 3% sucrose solution

Histological analysis showed that not all the placements were correctly targeted. Five animals had cannulae which were correctly targeted (Fig. 5). In one animal the cannulae missed the target area by more than 1 mm and so the data from this animal was discarded. Although cannulae were targeted at both the lateral and medial parabrachial nucleus, no difference was observed between the results for these groups and so the data were pooled.

3.3.1. Parabrachial placements

A one-factor repeated-measures ANOVA revealed a significant effect of the drug in the parabrachial nucleus group ($F(3,12) = 12.81$, $P < 0.001$). Midazolam (3–30 μg/μl in 0.5 μl) significantly increased consumption of

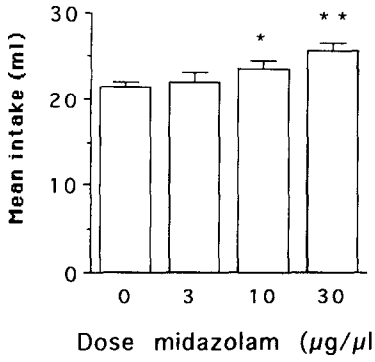

Fig. 6. The effect of direct injection of midazolam into the parabrachial nucleus on ingestion of a 3% sucrose solution in non-deprived rats. $n = 5$. Levels of significance for individual dose comparison against the vehicle control * $P < 0.05$, ** $P < 0.01$ (Dunnett's t-test). Results are shown as mean intake (g) after 20 min.

the 3% sucrose solution relative to the control condition. The high baseline intake of 21.4 ml was increased to 25.6 ml after 20 min (Fig. 6). Individual comparisons with a Dunnett's t-test indicated that the 10 μg/μl condition differed significantly from the vehicle condition ($P < 0.05$) and the 30 μg/μl was also significantly different ($P < 0.01$).

3.4. Experiment 4: the effect of direct injection of midazolam into the parabrachial nucleus on locomotor activity

3.4.1. Locomotor activity

The data from one animal had to be discarded from the analysis due to a fault in the equipment. In six cases the cannulae were correctly targeted (Fig. 7). There was one case where the guide cannula missed the intended target by more than 1 mm and so this animal was not included in the statistical analysis.

3.4.1.1. Parabrachial placements. Results are shown in Fig. 8a. A one-factor repeated-measures ANOVA revealed no significant effect of treatment after 20 min ($F(3,15) = 2.19$, $P = 0.14$). The mean count for the vehicle-treated group was 224.6 in 20 min and this was not significantly affected by drug administration.

3.4.2. Mash intake

A paired t-test revealed that there was a significant effect of the drug ($P < 0.05$). Midazolam (30 μg/μl in 0.5 μl) significantly increased consumption of the mash

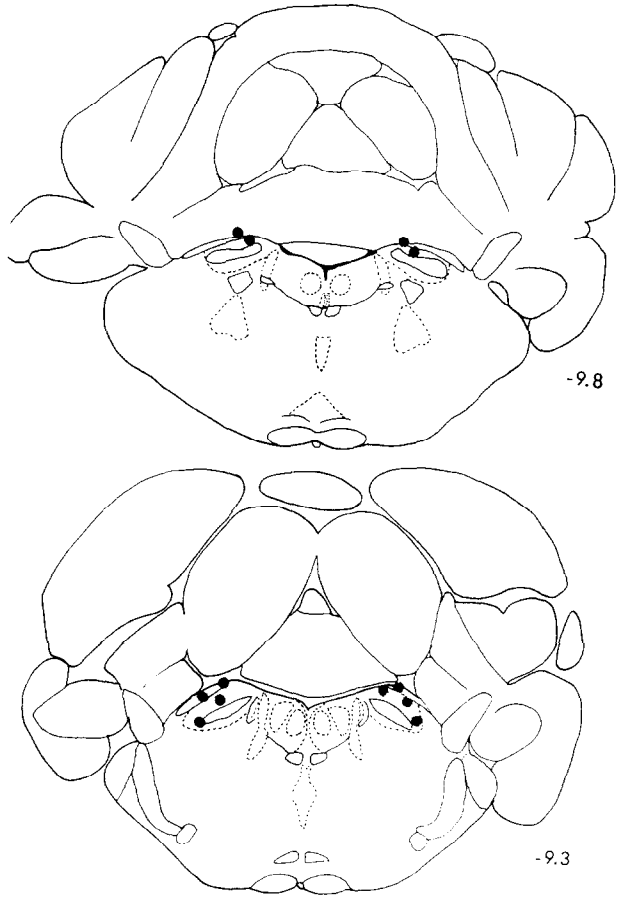

Fig. 7. The distribution of injection sites in the parabrachial nucleus for rats used in Experiment 4. Sites are shown bilaterally. Sections are redrawn from Paxinos and Watson (1982). Section numbers refer to mm from bregma.

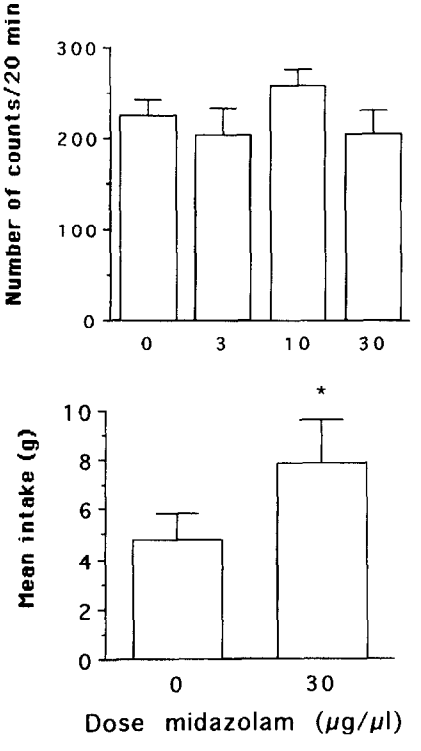

Fig. 8. (a) The effect of direct injection of midazolam into the parabrachial nucleus on locomotor activity. $n = 6$. Results are shown as mean counts in 20 min. (b) The effect of direct injection of midazolam into the parabrachial nucleus on ingestion of a sweet wet mash in non-deprived rats. $n = 6$. Levels of significance for individual dose comparison against the vehicle control * $P < 0.05$, ** $P < 0.01$ (paired t-test). Results are shown as mean intake (g) after 30 min.

after 30 min. The baseline intake of 7.6 g was substantially increased to 12.1 g (Fig. 8b).

4. Discussion

The experiments showed that direct administration of midazolam (10 and 30 μg/μl) into the parabrachial nucleus significantly increased the consumption of a palatable diet in non-deprived rats. The finding that the benzodiazepine receptor antagonist flumazenil attenuated this hyperphagia suggests that the effect was mediated by specific benzodiazepine receptors located in the parabrachial nucleus, and argues against the effects of midazolam being due to any artifact of pH, osmolarity or hydraulic pressure. Injection of midazolam into the parabrachial nucleus (3–30 μg/μl) also significantly increased the consumption of a 3% sucrose solution, despite the fact that the animals were consuming high baseline levels. These data are in close agreement with the effects of peripherally administered benzodiazepines on ingestive behaviour (Cooper et al., 1985).

Injection of midazolam (3–30 μg/μl) into the parabrachial nucleus did not affect locomotor activity, although in the same animals, the highest dose of 30 μg/μl significantly increased the consumption of a palatable mash. In small systemic doses benzodiazepine receptor agonists cause hyperlocomotion and in large doses produce sedation (File, 1981). The hyperlocomotion or sedative effects of midazolam observed following systemic administration were completely absent in the present experiments. The absence of any effect on locomotion cannot be accounted for by a general lack of drug effect since in the same animals, a significant increase in mash intake was observed. These data suggest that benzodiazepine receptors in the parabrachial nucleus may be specific for ingestional behaviour.

Benzodiazepine receptors are associated with the $GABA_A$ receptor complex. This is supported by evidence that these receptors co-immunoprecipitate (Schoch et al., 1985). The $GABA_A$ receptor complex comprises subunits which have multiple isoforms, and varying the combination of $GABA_A$ receptor subunits has been shown to determine the resultant pharmacology of benzodiazepine receptor ligands (for review see Luddens et al., 1995). Different populations of $GABA_A$ receptors are likely to exist in vivo, and stimulation of a particular population could result in selective behavioural effects. The benzodiazepine receptor population identified in the parabrachial nucleus may constitute a subpopulation specific for the effects of these compounds on ingestive behaviour. The development of subtype specific ligands would help to resolve this issue.

We have shown that injection of midazolam into the IVth ventricle significantly increases food consumption (Higgs and Cooper, 1996). The data from the present experiments suggest that this effect may have been due to rapid diffusion of the drug from the IVth ventricle to receptors in the parabrachial nucleus. This provides the most likely explanation of the data for several reasons. First, no increase in intake was observed in animals where the guide cannulae were more than 1 mm away from the parabrachial nucleus. This is wholly consistent with our finding that benzodiazepine receptors are present in the parabrachial nucleus but not in adjacent areas (Higgs et al., 1993). Second, the injection volume used, was 0.5 μl which is reported to spread 1 mm from the injection site (Myers, 1966). This means that diffusion from the parabrachial nucleus injection site to the IVth ventricle is unlikely to be able to account for the results. Nevertheless, it might be predicted that the behaviourally effective dose for parabrachial nucleus injection compared with IVth ventricle injection should be lower if receptors in the parabrachial nucleus were responsible for the IVth ventricle effect. We found no difference in the doses which increased food intake for both the previously reported IVth ventricle experiments and the current experiments. However, the volumes injected in both cases were different. The volume injected into the IVth ventricle was 3 μl, compared with 0.5 μl into the parabrachial nucleus. If the results obtained when injecting into the parabrachial nucleus were due to a ventricular site of action, it might be expected that when injecting a much larger volume into the IVth ventricle (3 μl), a lower dose would be effective. Since this was not so, the IVth ventricle results might be better explained by diffusion from the ventricle to receptors in the parabrachial nucleus. Taken together, this evidence indicates that the hyperphagic effect depends upon benzodiazepine receptors located in the parabrachial nucleus.

No difference was found between injections into the medial versus the lateral regions of the parabrachial nucleus and so the data from both sets of animals were combined. The reason for our failure to distinguish between medial compared with lateral injection sites, despite the differential connections of these two areas, may have been due to spread of the injection volume through the entire parabrachial nucleus. Future experiments investigating the contribution of sub-regions within the parabrachial nucleus would involve injecting smaller volumes of drug to allow more accurate distinctions between the functioning of different regions to be made.

The effect of lesioning the parabrachial nucleus on the behavioural response to taste stimuli has been studied by several investigators. The results of these studies have shown that an intact parabrachial nucleus is important for the execution of a variety of taste-guided behaviours. For example, parabrachial nucleus-lesioned animals are unable to form conditioned taste aversions, have blunted re-

sponses to taste stimuli, and do not reliably express a depletion-induced sodium appetite (for review see Spector, 1995). However, parabrachial nucleus lesions do not simply render animals ageusic or unable to detect taste stimuli. Despite blunted responses to unconditioned tastants, parabrachial lesioned animals can discriminate between taste stimuli and show concentration-dependent responses (Flynn et al., 1991a,b; Spector et al., 1993). It is unlikely that the decreased responsiveness to taste stimuli in parabrachial lesioned animals results from a reduction in the perceived intensity of the stimulus. Spector and colleagues have shown that parabrachial lesioned rats which fail to learn a conditioned taste aversion can nevertheless use taste stimuli as signals for other reinforcing events. For example, such animals perform competently in a taste-signalled shock avoidance task (Spector et al., 1995). This suggests that parabrachial nucleus lesions may result in an impairment of the affective response to taste stimuli rather than a purely sensory deficit. Therefore, the parabrachial nucleus may be involved in mediating the affective response to taste stimuli. Integrating the data gathered from lesion studies with the present results suggests that the increase in food intake caused by midazolam injected into the parabrachial nucleus results from an increase in hedonic responding mediated by receptors in this area. This conclusion is consistent with evidence from systemic injections of benzodiazepine receptor agonists which suggests that these drugs increase ingestive behaviour by increasing perceived palatability (Berridge and Pecina, 1995; Cooper and Higgs, 1994). Further experiments examining the effect of intra-parabrachial midazolam in sham feeding and taste preference tests are required to examine this hypothesis.

The parabrachial nucleus may not be the only important structure for the effects of benzodiazpines on ingestive behaviour. Investigation of the effects of lesions of the parabrachial nucleus on benzodiazepine-induced hyperphagia could help to resolve this issue. If an intact parabrachial nucleus is necessary for the effects of benzodiazepines on ingestive behaviour then lesioning the parabrachial nucleus would be predicted to abolish the hyperphagic effect of systemically administered benzodiazepine receptor agonists. However, if the parabrachial nucleus is sufficient for benzodiazepine effects on ingestion, then lesioning the parabrachial nucleus would not be expected to block all forms of benzodiazepine-induced enhancement of ingestive behaviour. Other brain sites involved in controlling feeding behaviour such as the nucleus of the solitary tract, hypothalamus and amygdala are possible candidates for mediation.

In conclusion, the present studies suggest that the neural substrate for benzodiazepine-induced hyperphagia may be located in the brainstem, specifically in the parabrachial nucleus. Stimulation of receptors located in the parabrachial nucleus may affect ingestive behaviour without affecting locomotor behaviour.

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