Research report

Brainstem mediates diazepam enhancement of palatability and feeding: microinjections into fourth ventricle versus lateral ventricle

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Abstract

The hypothesis that benzodiazepine-induced hyperphagia is due to a specific enhancement of the palatability of foods has been supported by previous 'taste reactivity' studies of affective (hedonic and aversive) reactions to taste palatability. Diazepam and chlordiazepoxide enhance hedonic reactions of rats (rhythmic tongue protrusions, etc.) to sweet tastes in a receptor-specific fashion. A role for brainstem circuits has been indicated by a previous demonstration of the persistence of the taste reactivity enhancement by diazepam after midbrain decerebration. The present study examined whether benzodiazepine brainstem receptors are the chief substrates for palatability enhancement even in intact brains. We compared the effectiveness of benzodiazepine microinjections to elicit feeding and enhance hedonic reactions when delivered into either the lateral ventricle (forebrain) or the fourth ventricle (brainstem) of rats. The results show diazepam is reliably more effective at eliciting feeding and enhancing positive hedonic reactions to oral sucrose when microinjections are made in the fourth ventricle than in the lateral ventricle. We conclude that brainstem neural systems containing benzodiazepine-GABA receptors are likely to be the chief substrates for benzodiazepine-induced palatability enhancement.

Keywords: Brainstem; Taste; Appetite; Palatability; Food intake; Feeding; Benzodiazepine; Hyperphagia; Diazepam; Cerebral ventricle; Hindbrain; Anxiolytic

1. Introduction

The facilitating effects of benzodiazepine agonists (which promote the opening of Cl^- ion channels in response to $GABA_A$ receptor activation [32]) on food and water intake were originally proposed to be secondary consequences of their anxiolytic and sedative effects [28,31,39,52]. However, anxiolytic/sedation interpretations fail to explain important features of benzodiazepine-induced hyperphagia that indicate a facilitation specific to appetite [14,19,55]. For example, benzodiazepine agonists stimulate food intake under non-stressful conditions of the home cage, and even when the drug is delivered mixed with the animal's food: that is, under conditions of little or no stress [2,11,22,33,38]. Benzodiazepines enhance feeding to a similar extent in animals exposed to stress and in control animals, contrary to what would be expected if benzodiazepine-induced feeding depended on a reduction of anxiety [19,46,55]. Equally important has been the discovery of a class of benzodiazepine partial agonists, which produce only some of the effects of full agonists like diazepam or chlordiazepoxide. For example, the pyrazoloquinolines (CGS 9895, CGS 9896), which bind with high affinity to benzodiazepine receptors selectively [3–5], decrease behavioral measures of arousal and anxiety without increasing food intake [18,20]. Other evidence for a dissociation between the sedative and the hyperphagic effects of benzodiazepines has come from the study of the effects of a different group of benzodiazepine partial agonists (Ro 16-6028, Ro 17-1812, Ro 23-0364), which selectively increase food intake but which do not produce a strong sedative effect [15,27,44,56,57].

The hypothesis that benzodiazepines increase feeding by activating a feeding related mechanism was first proposed by Wise in the early 1970s [55]. During the late 1970s and early 1980s, Cooper and colleagues elaborated this proposal into the hypothesis that benzodiazepine agonists promote feeding by specifically *enhancing the hedonic palatability* of food [12,19], based on observations that

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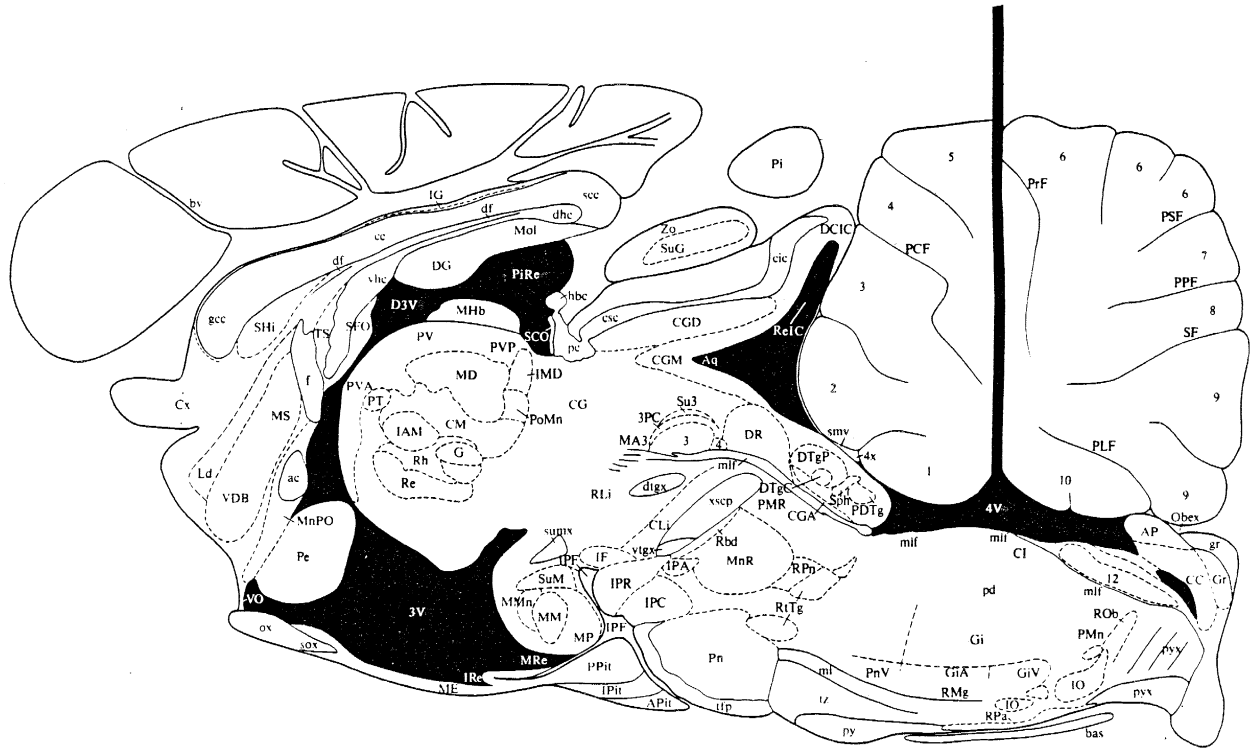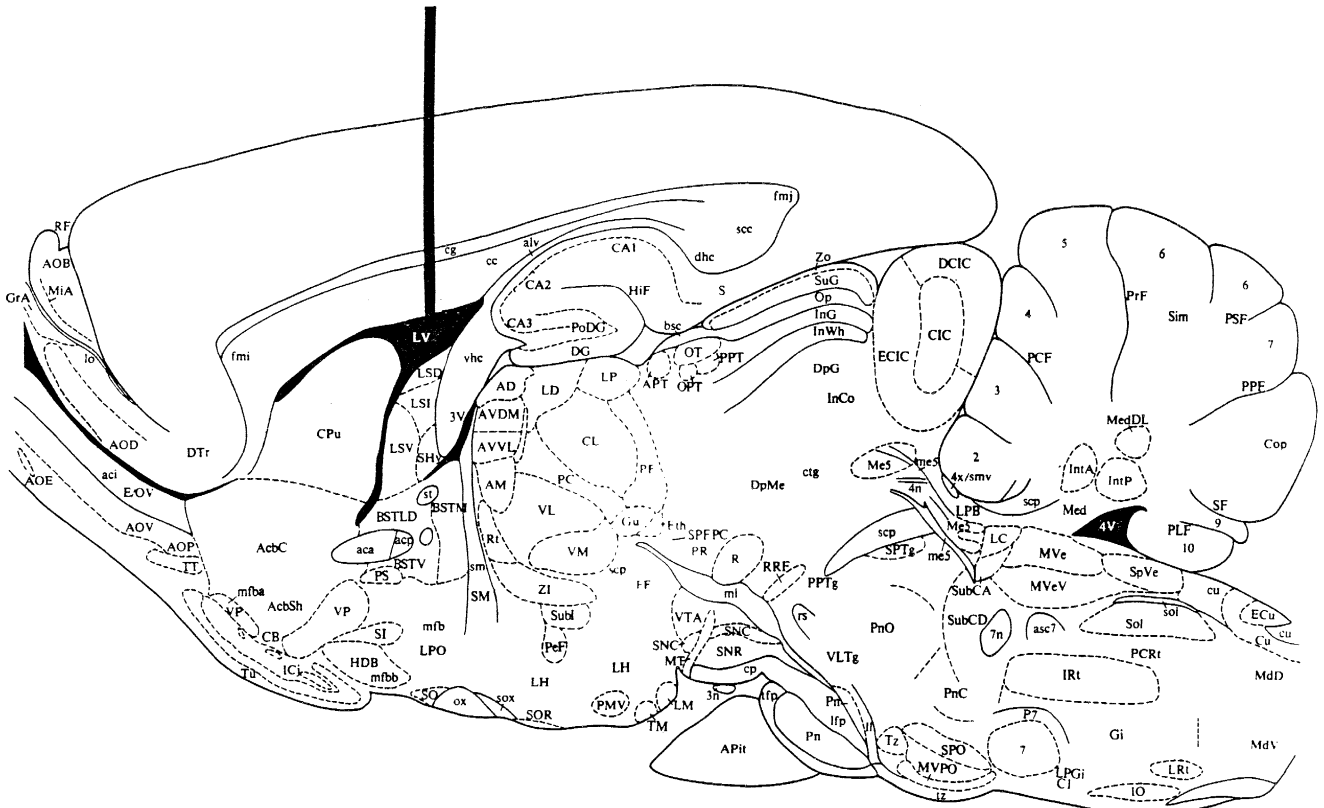
Fig. 1. Lateral and fourth ventricle targets. Placement of lateral ventricle cannulae, aimed at the forebrain, and of fourth ventricle cannulae, aimed at the hindbrain (ventricles shown in black). Adapted from the atlas of Paxinos and Watson [37].

benzodiazepines selectively increase the intake of *preferred* foods (e.g. cookies, sweetened foods or saccharin water) much more than the intake of regular chow [12,13,21]. Direct support for the 'benzodiazepine palatability hypothesis' was provided by Berridge and Treit [9] using the taste-reactivity test of hedonic reactions developed by Grill and Norgren [26]. This test measures species-typical affective reactions (such as hedonic tongue protrusions or aversive gapes), elicited by taste stimuli directly infused into the rat's mouth [26]. Systemic administration of the benzodiazepine agonist, chlordiazepoxide (10 mg/kg), selectively enhanced positive hedonic responses to sucrose infusions into the mouth. Aversive reactions elicited in response to bitter quinine tastes remained unchanged. In a later study, the selective enhancement of hedonic reactivity patterns by benzodiazepine agonists was replicated, and blocked by the administration of either the benzodiazepine antagonist Ro 15-1788 or the 'inverse agonist' CGS 8216 (which acts as an antagonist in the presence of a benzodiazepine agonist) [51]. These results were interpreted to indicate that the effects of benzodiazepines on taste palatability are benzodiazepine-receptor specific. Subsequent studies have continued to replicate benzodiazepine-induced enhancement of hedonic taste reactivity patterns [36,50]. Although the relative distribution of benzodiazepine receptors in the brain is highest in forebrain structures [41,42,54], there is evidence to suggest that the brainstem contains the particular benzodiazepine receptors and neural circuits that modulate taste palatability. Berridge [6] demonstrated that the isolated brainstem of chronic mesencephalic decerebrate rats, in which supracollicular transection isolated the forebrain from subdiencephalic structures [26], retained the ability to enhance positive taste reactions in response to systemic chlordiazepoxide administration. Thus, the midbrain and/or hindbrain appear to contain the minimum benzodiazepine receptors and neural circuitry required to enhance positive reactions to taste.

Decerebrate competence shows that the forebrain is not required for taste modulation by benzodiazepine agonists, but it does not rule out the possibility that forebrain benzodiazepine receptors are still the chief mediators of palatability enhancement *in intact brains*. Alternatively, the brainstem may contain the primary circuits for benzodiazepine-mediated palatability in both decerebrate and intact brains. The present experiment was designed to choose between these two hypotheses. We examined the relative contributions of brainstem benzodiazepine receptors to palatability enhancement and feeding in intact brains. This was done by comparing the effectiveness of microinjections of the benzodiazepine agonist, diazepam, at eliciting feeding and enhancing hedonic reactions to oral sucrose infusions, when delivered directly either into the lateral ventricle (forebrain) or the fourth ventricle (brainstem) (Fig. 1).

2. Experiment 1

2.1. Materials and methods

2.1.1. Subjects

The subjects were 21 Sprague-Dawley female rats weighing 250–300 g at the beginning of the experiment, housed in pairs on a 14:10 h light/dark cycle. Rats had free access to food and water. All testing was performed during the light phase of the cycle, between 09.00 and 13.00 h.

2.1.2. Cannula implantation

Animals were pretreated with atropine (1 mg/kg) and anesthetized with ketamine HCl (100 mg/kg) and Rompun (5 mg/kg). Each animal was surgically implanted with two intracranial guide cannulae (22 gauge), one aimed at either the right or the left lateral ventricle and the other aimed at the fourth ventricle (Fig. 1). With bregma and lambda in the same horizontal plane, skull holes were drilled and the dura was opened. A 22-gauge stainless steel guide cannula was placed using the stereotaxic coordinates from the atlas of Paxinos and Watson [37]. Coordinates were A-P = -0.8 from bregma, L = ±1.5, and V = 3.5 from skull surface for the lateral ventricle, and, A-P = -11.5, L = 0, and V = 6.5 for the fourth ventricle (the ventral coordinates were 1.0 mm dorsal to the injection site). Following surgery, dummy cannulae were placed in the guide cannulae to prevent occlusion. Animals were allowed to recover for at least 5 days before testing.

Each animal was also implanted with two chronic oral cannulae. Oral cannula (heat-flared PE 100 tubing) entered the mouth just lateral to the first maxillary molar, ascended lateral to the skull, and exited the head at the dorsal part of the skull, where they were attached to a 19-gauge steel tubing. These cannulae do not interfere with the normal eating behavior of the animal and allow the direct infusion of solutions into the mouth. All cannulae were anchored with skull screws and acrylic cement.

2.1.3. Diazepam doses and taste stimuli

The taste stimulus used for oral infusions was a 0.2 M sucrose solution. Diazepam was dissolved in a 10 μl volume of a mixture of 40% propylene glycol, 10% ethyl alcohol and 50% water. The order of vehicle and drug dose (0, 15, 25, 40, 50 and 75 μg) was randomized across animals except for the first and the last injections which were always vehicle.

2.1.4. Microinjections

Following a 3–4 day recovery period, rats were brought into the testing room in their home cages. Animals were hand held during the microinjection procedure. Dummy cannulae were removed and injector tips (28 gauge) were lowered through the guide cannulae to the site of injection

(the injector tip extended 1.0 mm below the tip of the guide cannulae). Diazepam or vehicle (10 μl) was delivered over 5 min by a microsyringe pump connected via a 20 polyethylene (PE) tubing to the 28-gauge injector. Injectors were removed after a 1 min diffusion period and dummy cannulae were replaced.

2.1.5. Taste reactivity test

2.1.5.1. Procedure. Previous to the testing period, rats underwent a 3-day habituation period during which they were exposed to the taste reactivity chamber for 10 min followed by 1 ml infusion of water. Testing began 5 days after surgery, and continued once daily for 16 days. On each test day, each rat received a single injection of either diazepam or vehicle into the fourth ventricle or the lateral ventricle in a counterbalanced order. Once chosen, each diazepam dose was administered on two consecutive days: once into the lateral ventricle and once into the fourth ventricle in counterbalanced order. Immediately after the microinjection, one of the rat's oral cannulae was connected to a stimulus delivery tube, consisting of PE 50 tubing attached to PE 10 fine tubing that can be fitted inside the cannulae. The rat was then placed in a cylindrical test chamber. After a 10 min habituation period, 1 ml of the 0.2 M sucrose solution was infused into the mouth of the animal at a constant rate (1 ml/min) during 1 min. The behavior of the rat was videotaped during testing via a mirror mounted beneath the transparent floor, which allowed the camera to zoom up so that the face and the mouth of the rat filled the entire screen.

2.1.5.2. Video analysis of taste reactivity data. The behavior of each rat was scored for the occurrence of hedonic, aversive, and 'neutral' taste reactivity components (see [7,8,25] for a discussion of taste reactivity analysis components and classification). Hedonic actions were *paw licking*; *lateral tongue protrusions*, non-rhythmic protrusions past the lip followed by forward extension, lasting about 160 ms; and *tongue protrusions*, rhythmic tongue protrusions along the midline, with a cycle length of roughly 160 ms. Neutral components were rhythmic *mouth movements* at the same or lower frequency as rhythmic tongue protrusions; and *passive dripping*, the passive leaking of fluid from the mouth. Aversive actions were *gapes*, large openings of the mandible and retraction of the lower lips lasting about 125 ms; *chin rubbing*, bringing the mouth in direct contact with the floor and projecting the body forward; *face washing*, either a single wipe over the face with the paws or a bout of several wipes; *forelimb flails*, shaking of the forelimb with a frequency of greater than 60 Hz; head shaking, at greater than 60 HZ; *paw treading*, planting of the limbs on the floor and alternating forceful strikes forward and back; and rapid *locomotion* around the chamber.

Videotapes were scored in a slow motion analysis at 1/30 to 1/10 normal speed. For the purpose of quantifying the number of responses emitted, discrete actions such as lateral tongue protrusions, gapes, chin rubs, forelimb flails and head shaking, and bouts of face washing, paw treading, and locomotion were counted each time they occurred. Continuous actions that typically persist for relatively long periods were counted as follows: paw licks, mouth movements, passive dripping, face washing and locomotion were counted in 5-s bins (any occurrence of these behaviors up to 5-s in duration was counted as a single occurrence). Rhythmic tongue protrusions were scored in the same way in 2-s bins.

2.1.6. Feeding test

2.1.6.1. Procedure. The feeding test was conducted 2 days after the completion of the taste reactivity experiment. In order to confirm that feeding was elicited by intracranial microinjections of diazepam at these doses, six non-deprived rats were selected at random from the original group used in the taste reactivity experiment. Chow pellets were removed from the animal cages and rats were microinjected with either diazepam or vehicle into the lateral or the fourth ventricle as described above (0, 15, 25, 40, 50, and 75 μg). They were immediately returned to the home cage and allowed to ingest chocolate cookies for 30 min. Amount eaten was determined by re-weighing the cookies remaining and subtracting the difference from the original weight. The order of drug administration was random except for the first and the last days which were always vehicle.

2.1.7. Verification of the cannulae placement

At the completion of the experiment, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg). Following the same microinjection procedure described for drug microinjections, ink was microinjected into the lateral and the fourth ventricles and perfused 10 min later. The brains were removed and cut midsagittally. The presence of ink in the fourth and lateral ventricles verified the correct location of the ventricular cannulae.

2.1.8. Statistical analysis

All data were analyzed using a two-factor ANOVA for repeated measures, with dose and ventricle site as the main factors. The post-hoc comparison of means used was the Newman-Keuls test.

2.2. Results

2.2.1. Feeding

Intracranial microinjections of diazepam significantly enhanced feeding. A two-way ANOVA for repeated measures (dose × ventricle) revealed that diazepam microinjections enhanced feeding, $F(5,25) = 7.97$, $P < 0.001$. A Newman-Keuls comparison revealed that feeding was en-

hanced significantly above baseline only by administration of the two highest doses (50 and 75 μg; $P < 0.05$). The site of injection was not a significant factor in determining intake, although the fourth ventricle appeared marginally more effective at these highest doses (Fig. 2).

2.2.2. Taste reactivity

Microinjections of diazepam into either the lateral or the fourth ventricles significantly enhanced the number of hedonic reactions (tongue protrusions, lateral tongue protrusions and paw licking) to an oral infusion of 0.2 M sucrose solution as revealed by a within subject, two-way ANOVA for repeated measures (ventricle × dose, $F(2,40) = 12.54$; $P < 0.0001$). Low diazepam doses (15 and 25 μg) failed to increase the number of hedonic reactions significantly over vehicle levels when administered in either ventricle (Fig. 3). Post-hoc tests showed that hedonic reactions were enhanced only after the highest doses of diazepam (50 and 75 μg). Microinjection site was also a significant factor for hedonic enhancement for doses of diazepam that were near or above the threshold for hedonic enhancement (40, 50, and 75 μg). Diazepam was significantly more effective at enhancing hedonic reactions when injected into the fourth ventricle than when injected into the lateral ventricle for these doses ($F(1,20) = 4.61$, $P < 0.05$; Fig. 3). Post-hoc paired comparisons showed that the two ventricles differed significantly for the 50 μg dose ($P < 0.05$). In other words, the threshold for hedonic enhancement appeared to be between 40 and 50 μg for the fourth ventricle, but to be between 50 and 75 μg for the lateral ventricles. Once these thresholds were exceeded, the two ventricles no longer differed in terms of hedonic enhancement induced by diazepam.

Aversive and neutral reactions did not increase or change after diazepam administration at any dose.

Fig. 3. Hedonic enhancement by intraventricular microinjections of diazepam (Expt. 1). Hedonic reactions of rats (rhythmic and lateral tongue protrusions, and paw licks) emitted to oral sucrose infusions after microinjections of diazepam or vehicle into the fourth ventricle or the lateral ventricle. Microinjections of the highest doses of diazepam (40, 50, 75 μg) into the fourth ventricle were reliably more effective at enhancing hedonic reactions (tongue protrusions, lateral tongue protrusions and paw licks) than microinjections of the same dose into the lateral ventricle ($P < 0.05$).

Histological analysis confirmed that the microinjection cannulae were placed in the correct ventricle locations for every rat.

2.3. Discussion

The results from Expt. 1 appeared to show a difference in threshold for hedonic enhancement between the fourth and lateral ventricles. The fourth ventricle appeared to be more responsive: it had a lower threshold. Microinjections of a threshold dose of diazepam were more effective here than when administered to the lateral ventricle.

However, the primacy of the fourth ventricle exceeded significance only for a single threshold dose: 50 μg. Doses much lower than this (15, 25, 40) failed to produce an hedonic effect in either ventricle. The much higher dose produced robust and equivalent hedonic enhancement in both ventricles. Possibly the two sites were equivalent at the high dose because of diffusion of the higher concentration from the lateral ventricle to a distant caudal site, or because of recruitment of secondary (less reactive) benzodiazepine/GABA forebrain circuits around the lateral or third ventricle.

In any case, the primacy of the fourth ventricle for benzodiazepine hedonic enhancement rests on its superiority at the threshold dose of 50 μg. In order to be sure that this superiority was reliable, and not a spurious effect at a single data point, a second experiment was conducted to compare the ability of diazepam doses near threshold (45, 50, and 55 μg) to enhance hedonic reactions and elicit feeding when administered to either the fourth ventricle or the lateral ventricle.
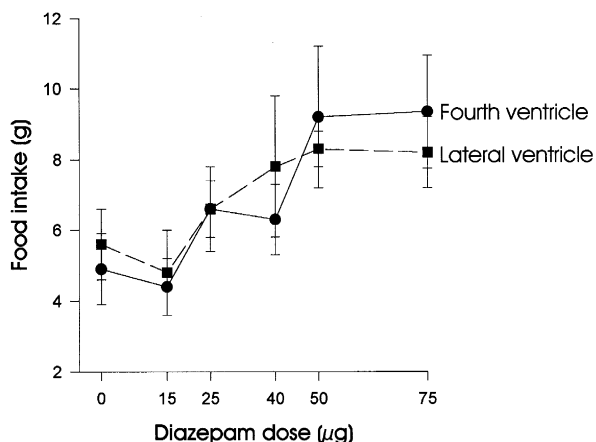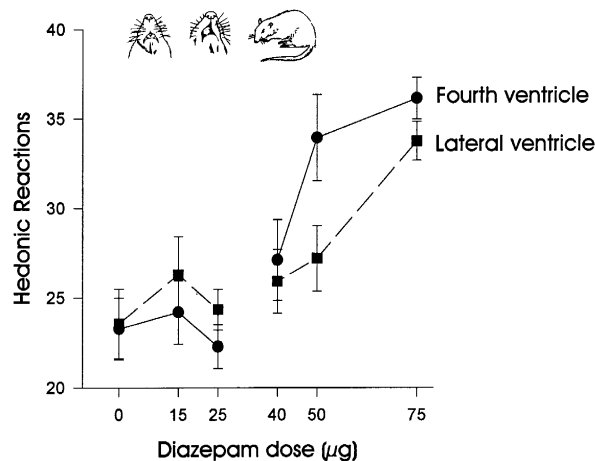

Fig. 2. Feeding elicited by intraventricular microinjections of diazepam (Expt. 1). Amount of chocolate cookies consumed after microinjections of diazepam doses or of vehicle into the lateral ventricle or the fourth ventricle. The highest diazepam doses (50 and 75 mg) significantly stimulated feeding when injected into either ventricle, $P < 0.001$.

3. Experiment 2

3.1. Materials and methods

3.1.1. Subjects and surgery

Six Sprague-Dawley female rats (weighing 250–300 g), were housed in pairs on a 14:10 h light/dark cycle throughout the experiment. Rats had free access to food and water. All testing was performed during the light phase of the cycle, between 09.00 and 13.00 h. Each rat was implanted with oral cannulae for taste reactivity, and with microinjection cannulae into the fourth ventricle and a lateral ventricle, as in Expt. 1.

3.1.2. Procedure

Three doses of diazepam or the vehicle alone (0, 45, 50 and 55 μg) were tested in each ventricle microinjection site. The order of doses was counterbalanced, except that the first and last day of testing was always vehicle alone, and that once a dose of diazepam was chosen it was administered on two consecutive days: once into the lateral and once into the fourth ventricle in counterbalanced order.

3.1.2.1. Taste reactivity test of hedonic reactions. 10 min after the microinjection, each rat received an oral infusion of 0.2 M sucrose (1 ml over 1 min) via oral cannula as in Expt. 1. Affective reactions were videotaped for later slow-motion scoring.

3.1.2.2. Feeding test. Immediately after the taste reactivity test on each day, rats were returned to their cages and presented with a premeasured amount of a familiar commercial baby cereal (one part of cereal and three parts of water). Rats were left undisturbed and allowed to ingest

Fig. 5. Hedonic enhancement by threshold doses of intraventricular diazepam (Expt. 2). Hedonic reactions of rats emitted to oral sucrose after microinjections of vehicle or of 45, 50, or 55 μg diazepam into the fourth ventricle or the lateral ventricle. Only fourth ventricle microinjections enhanced hedonic taste reactivity components at these doses ($P < 0.05$).

the palatable food. One hour later the weight of the food was measured and recorded.

3.2. Results

3.2.1. Feeding

Intraventricular microinjections of diazepam into the fourth ventricle significantly promoted feeding at all three near-threshold doses (45, 50, 55 μg; ANOVA $F(3,15) = 3.54$; $P < 0.05$; Fig. 4). By contrast, lateral ventricle administration failed to promote feeding at these doses. There was a significant main-effect difference between ventricle sites in the ability of these doses to elicit feeding ($F(1,15) = 9.21$, $P < 0.05$).

3.2.2. Taste reactivity

Fourth ventricle microinjections of diazepam were significantly more effective than lateral ventricle microinjections at enhancing hedonic reactions for all three doses ($F(1,15) = 8.31$, $P < 0.05$; Fig. 5). For every near-threshold dose, only fourth ventricle administration appeared capable of enhancing the palatability of sucrose.

3.3. Discussion

The superiority of the brainstem (fourth ventricle), compared to the forebrain (lateral ventricle), as a site for diazepam microinjections to enhance the palatability of a sweet taste was confirmed in the second experiment. For doses near the 50 μg threshold, fourth ventricle microinjections enhance hedonic reactions to sucrose but lateral ventricle microinjections do not. In addition, the primacy of the brainstem was seen to extend also to feeding elicited
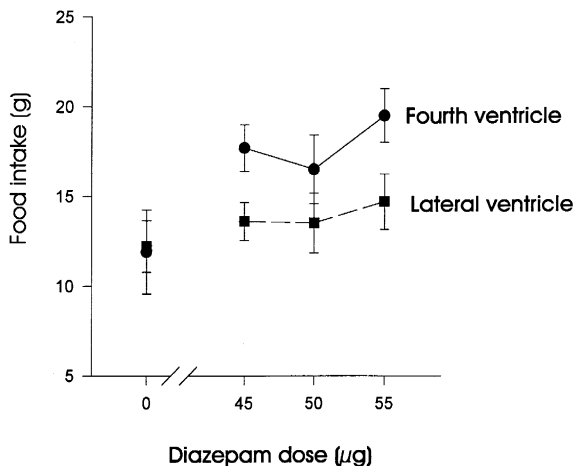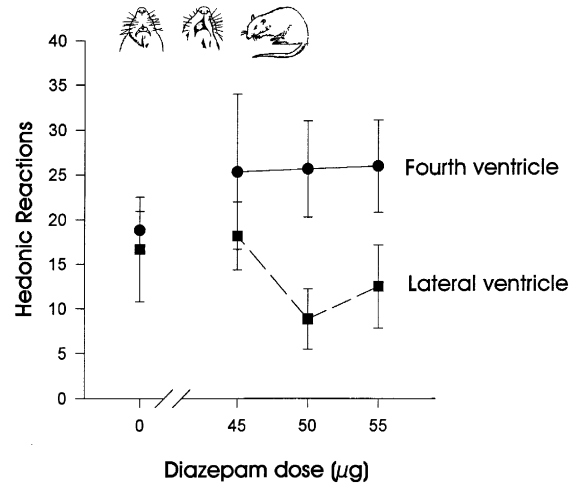

Fig. 4. Feeding elicited by threshold doses of intraventricular diazepam (Expt. 2). Amount of cereal mash consumed after microinjections of vehicle or of 45, 50, or 55 μg diazepam into the fourth ventricle or the lateral ventricle. Only fourth ventricle microinjections facilitated food intake at these doses ($P < 0.05$).
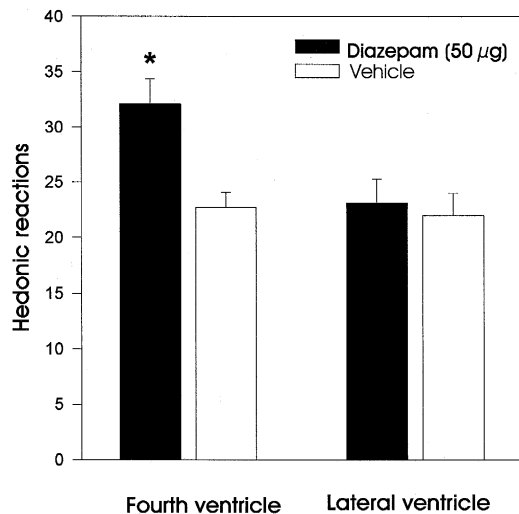
Fig. 6. Comparison of combined hedonic enhancement by 50 μg diazepam in the fourth ventricle versus the lateral ventricle. Combined data from Expt. 1 and Expt. 2 ($n = 27$ rats). Hedonic taste reactivity components elicited by sucrose after vehicle or 50 μg diazepam delivered to either the fourth ventricle or the lateral ventricle. Only fourth ventricle diazepam enhanced hedonic reactions at this dose ($P < 0.05$).

by diazepam (this difference had been only marginal in the first experiment). All three near-threshold doses elicited feeding when delivered to the fourth ventricle but not when delivered to the lateral ventricle.

It is possible to combine the results from Expt. 2 with those from Expt. 1, at least for the 50 μg threshold dose of diazepam that was used in both experiments. When the data for fourth ventricle versus lateral ventricle microinjections were compared across experiments for the 50 μg and vehicle doses, the superior effectiveness of fourth ventricle administration emerged as a clear overall effect ($F(1,26) = 5.92$, $P < 0.05$; Fig. 6).

4. General discussion

These two experiments show that microinjections of diazepam into either the lateral ventricle or fourth ventricle can elicit feeding and can enhance hedonic reactions to a sweet taste. They also show that microinjections of diazepam are consistently more effective in the fourth ventricle than in the lateral ventricle for both hedonic enhancement and feeding, when doses are near the behavioral threshold (50 μg).

Our results are consistent with reports by Cooper and colleagues that intracranial benzodiazepine administration in the parabrachial nucleus (PBN) elicit feeding [16,17,29]. Parabrachial nucleus microinjections probably enhance feeding via a brainstem substrate rather than by diffusing to act on a forebrain site. These results also constitute the first direct evidence for central mediation of benzodiazepine-induced enhancement of palatability (hedonic reactions). Finally, they indicate that palatability enhance-

ment induced by benzodiazepines, as well as feeding, may be mediated primarily by brainstem substrates even in an intact brain that has forebrain receptors available.

This does not rule out the possibility that forebrain receptors may also play a secondary role in benzodiazepine-induced hedonic enhancement. But our results indicate that forebrain benzodiazepine-systems activated by intraventricular administration of diazepam are less effective at modulating palatability than are brainstem systems.

We recognize that microinjections into a ventricle undoubtedly also stimulated receptors adjacent to other ventricles. Ink injected into either the lateral or the fourth ventricle could be detected in the other ventricle usually within 10 min. However, the drug concentration gradient between ventricles allows at least a rough comparison of the relative contribution of brainstem versus forebrain substrates, especially for doses near the behavioral threshold. Since the drug is likely to be most concentrated in the ventricle in which it was injected, receptors nearest that site would have greatest exposure. Under these conditions, receptors most accessible to the fourth ventricle clearly had the advantage.

In order to identify the actual neural sites where benzodiazepines act to enhance palatability, future studies will need to compare the effectiveness of intracranial microinjections into multiple brain structures. The ability of microinjections in the parabrachial nucleus to elicit feeding certainly highlights the possibility that it may be a chief substrate [16,17,29], but there are several sites that deserve consideration for feeding-related benzodiazepine effects. The potential candidates can be grouped in two major categories: (1) the parabrachial nucleus and the nucleus of the solitary tract, both of which belong to the primary taste pathway; and (2) other brainstem structures related to reward or feeding.

4.1. Primary taste pathway

The parabrachial nucleus of the pons contains the second gustatory relay nucleus for the rat [49]. A column of taste-responsive neurons extends from the bottom to the top of the midlateral brachium conjunctivum and above it into the dorsal parabrachial region. At least moderate densities of benzodiazepine receptor binding have been reported within the pons near the parabrachial nucleus [49]. Lesions of the parabrachial nucleus disrupt palatability-related aspects of both feeding and hedonic/aversive reactions to taste palatability [23,24,47], and, as mentioned above, microinjections of midazolam in the PBN elicit feeding. This evidence makes the parabrachial nucleus perhaps the strongest candidate to date for mediation of benzodiazepine effects on palatability and appetite.

The rostral nucleus of the solitary tract provides an even earlier opportunity for benzodiazepines to modulate taste signals within the ascending gustatory stream [34,35]. Lesions here disrupt several aspects of taste reactivity [23,24].

Moderate amounts of benzodiazepine binding sites occur within the dorsal medulla oblongata, in the vicinity of the nucleus of the solitary tract [23,24,34,35,41,42,54]. Recent evidence demonstrates the presence of both $GABA_A$ and $GABA_B$ receptors within the rostral part of the nucleus of the solitary tract [53]. Further, $GABA_A$ agonists alter the responsiveness of gustatory neurons [45,48], consistent with the possibility that benzodiazepines might modulate $GABA_A$ receptors here to influence taste processing.

Alternatively, benzodiazepine agonists might act on reward-related brainstem structures outside of the gustatory pathway to alter affective rather than sensory properties of taste. For example, the ventral tegmental area (VTA) of the midbrain is known to play a role in reward and feeding behavior [10]. In addition, GABA microinjections into this area modulate feeding behavior [1]. Although autoradiographic studies have not assigned benzodiazepine receptors specifically to the ventral tegmental area, large densities of benzodiazepine receptors have been reported in the general vicinity, and the possibility remains open [43].

The substantia nigra, the superior colliculus, and the cerebellum also have been linked to feeding behavior at least under some conditions. For example, injections of muscimol into the substantia nigra promote feeding [40]. This effect is prevented by lesions of the superior colliculus but not by administration of dopamine antagonists [40]. The superior colliculus is thought to be involved in the initiation of feeding by sensory stimulus [40] and GABA manipulations have been shown to alter perioral stimulation [40]. The cerebellum has also been implicated in aspects of feeding behavior [30]. All three structures contain moderate or large densities of benzodiazepine receptors [43].

4.2. Conclusion

Although the precise site of the brainstem circuitry responsible for the effect we have reported here still remains to be determined, we can conclude from our present results that brainstem benzodiazepine receptors and circuits are likely to be the primary substrate mediating enhancement of palatability and appetite by agonist drugs. Our results support the hypothesis that a 'brainstem benzodiazepine-GABA' neural system constitutes an important early component of the neural hierarchy that processes taste palatability, and highlight the importance of understanding hindbrain and midbrain contributions to the generation of food reward [7,25].

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