



Parabrachial benzodiazepine receptor antagonist effects on licking for sucrose



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INTRODUCTION

- Benzodiazepines such as midazolam induce hyperphagia by enhancing the hedonic acceptance of taste stimuli.
- Previous studies have implicated the hindbrain parabrachial nucleus (PBN) as a site for benzodiazepine stimulation of hyperphagia (Higgs & Cooper, 1996; Soderpalm & Berridge, 2000).
- Benzodiazepine infusions to the PBN reverse potent anorexia induced by ablation of AGRP/NPY/GABA neuron input to the PBN (Wu et al., 2009).
- To further clarify the role of the PBN in benzodiazepine-mediated modulations of feeding and taste evaluation, we tested the effectiveness of PBN injections of the benzodiazepine receptor antagonist, flumazenil (FLZ).

METHODS

- 48 Male Sprague-Dawley rats were fitted with guide cannulae aimed bilaterally to the PBN, or to the PBN and to the 4V.
- 4V cannula placement was confirmed with 4V 5 thio-d-glucose (5TG; 120 µg/2.0 µl; 60 µl/h) induced hyperglycemia or Angiotensin II induced hyperdipsia > 5ml (3V).
- Rats were trained to consume sucrose solutions for 90 min per day. For the drug dose experiments, drug injections were made every third test day.
- Experiment 1: On injection days, habituated rats were injected with the following concentrations of FLZ diluted in 0.4µL of dimethyl sulfoxide (DMSO) in a counterbalanced design: 3000ng, 300ng, 30 ng or 0.0nmol (DMSO) at a rate of 13.3µL/hr. Rats were placed in the licking apparatus immediately after injection, for 90 minutes with access to 0.2M sucrose.
- Experiment 2: Naive rats were injected in counterbalanced orders with a range of midazolam maleate doses into the 4V (60, 30, 15, 7.5 µg/2µl isotonic saline) using a design identical to experiment 1. To identify a hyperphagic dose of midazolam we thereafter tested midazolam HCl (60 µg/2µl) and midazolam maleate (60 µg/2µl) into the 4V offering rats a more palatable 0.5M sucrose solution under otherwise identical test conditions.
- Experiment 3: Naive rats received 4 counterbalanced injections where each rat received each of the following drug conditions: FLZ-MDZ, FLZ-Saline, DMSO-MDZ and DMSO-Saline (FLZ: 3000ng/0.4µl/PBN; MDZ: 60 µg/2µl/4V). Testing conditions were identical to the prior experiment and rats were offered 0.5M sucrose.
- Licking patterns were recorded and analyzed using established protocols (Davis & Smith, 1992). The initial rate of licking (first minute) and mean burst size were regarded as measures associated with taste evaluation. Meal duration and number of bursts in the meal are measures associated with post-ingestive feedback.

RESULTS

Experiment 1: The intra PBN benzodiazepine antagonist FLZ did not suppress licking for sucrose.

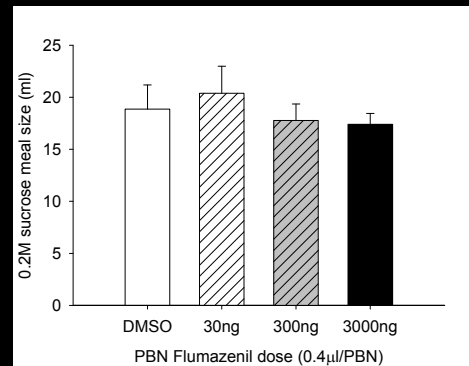


FIGURE 1: FLZ had no effect on intake or several measures of licking microstructure including first minute lick rate, mean lick-burst size, mean lick-burst duration, number of bursts in the meal, or meal duration (n=13; most F values <1). Data for confirmed bilateral PBN placements shown (n=7).

Experiment 2: 4V benzodiazepine agonist increases for 0.5M sucrose but not 0.2M sucrose intake.

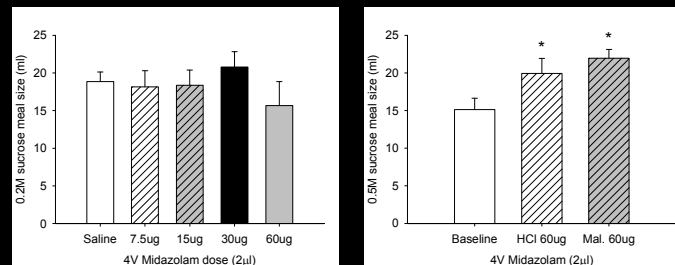


FIGURE 2: Left: the benzodiazepine agonist midazolam maleate had no significant effect on licking for 0.2M sucrose across a range of doses (F < 1; n=5). **Right:** Two different midazolam salts (MDZ maleate and MDZ HCl) both significantly increased meal size when rats were offered a sweeter 0.5M sucrose solution. *p<0.02; n=5.

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Experiment 3: Preliminary results. IntraPBN benzodiazepine antagonist may block 4V benzodiazepine agonist-induced hyperphagia

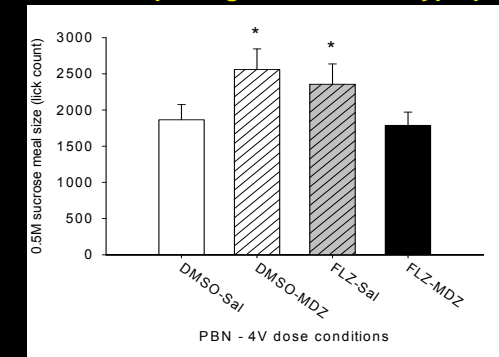


FIGURE 3: Preliminary analysis of responses to combined 4V benzodiazepine agonist injection of midazolam maleate (60µg/2µl) or vehicle (saline) after PBN preinjection of the benzodiazepine antagonist flumazenil (3000 ng/0.4µl/PBN), or vehicle (DMSO). Both MDZ and FLZ alone increased meal size, however combined application reduced intake to baseline values. *p<0.05; n=14, histological confirmations pending.

DISCUSSION

- FLZ failed to influence feeding behavior when administered into the PBN on its own, suggesting that the present doses are subthreshold with respect to an influence on feeding behavior. This result is consistent with the inability of i.c.v FLZ in influencing feeding when administered alone (Kokare et al., 2006).
- Both MDZ maleate and MDZ HCl proved to be effective hyperphagic BDZ agonists in the 4V, replicating previous findings.
- Preliminary analysis of the results of Experiment 3 indicate that FLZ significantly increased meal size when administered alone, however blocked MDZ induced hyperphagia. The unexpected hyperphagic effect of FLZ could be attributed to the intrinsic effect of the drug, which under some conditions has been shown to act as a partial benzodiazepine agonist (Kapczinski et al, 1994). Concerns of FLZ activity varying with stress levels have been raised in previous literature (Moy et al, 1997) and should not be overlooked in interpreting the results of Experiments 1 & 3.

- Overall, the results provide preliminary evidence that intraPBN flumazenil may block 4V MDZ-induced hyperphagia. However, no definite conclusions can be drawn with respect to the effectiveness of FLZ treatment at present, as the preliminary data are pending histological confirmation.

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