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Pharmacology, Biochemistry and Behavior 82 (2005) 379 – 387
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Nasal administration of the calcium channel blocker diltiazem decreases food intake and attenuates weight gain in rats

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Received 8 March 2005; received in revised form 5 August 2005; accepted 15 September 2005
Available online 7 October 2005

Abstract

Food intake is normally influenced by a multitude of complex endogenous neurochemical systems, in addition to numerous external environmental stimuli, including olfaction. Since most olfactory neurons process odorant exposures through Ca^{2+} -mediated mechanisms via Ca^{2+} channels, a novel approach at influencing the ingestive behaviors of animals might therefore involve altering olfactory acuity via Ca^{2+} channel blockade. We tested the ability of a Ca^{2+} channel blocker, diltiazem, to alter food intake in hyperphagic rats when administered using the intranasal (i.n.), intraperitoneal (i.p.), oral (p.o.) or intracerebroventricular (i.c.v.) routes of administration. Male Sprague Dawley rats, which had been food-deprived for 4 h at the beginning of the dark cycle, were administered different doses of diltiazem (0 – 8 mg/ animal or 0–40 mg/kg) and the amounts of food consumed were measured. While food intake at 1, 2 and 4 h post drug administration was significantly decreased in a dose-dependent manner after i.n. administration, the i.p., p.o., and i.c.v. routes did not affect food intake. In another experiment, rats trained to eat their daily meal during the first 4 h at the onset of the dark cycle and treated daily with i.n. diltiazem (0 – 8 mg/ animal) prior to food introduction exhibited a significantly decreased rate of weight gain in a dose-dependent manner over a 14-day period. Both i.n. and i.p. diltiazem significantly increased the plasma drug concentration at 1 h, however there was no significant difference between these routes of administration. Additional studies failed to demonstrate any detrimental effects of i.n. diltiazem (0– 8 mg/ animal) on conditioned taste aversion, locomotion or gross neurological/behavioral competence using the rota-rod test. While a local action on the nasal odorant receptors is most likely the site of diltiazem's action, further studies are needed to determine the exact mechanism of action of i.n. diltiazem.

Keywords: Diltiazem; Food intake; Nasal administration; Weight gain

1. Introduction

Olfaction is believed to play a role in food intake regulation. Olfactory dysfunction is associated with decreased enjoyment of food, and adding odors to food can increase intake in some individuals. Changes in palatability or sensory quality of food, a main determinant of food consumption, can be ascribed to declining perception of odors (Griep et al., 1996). It is also believed that human chemosensory receptors of taste and smell have similarities in molecular detection, transduction-related processes, organizational complexities, and regulatory roles related to food intake (Poothullil, 1995). It has been reported that olfactory neurons respond to certain odorants with alterations in cyclic adenosine mono phosphate (cAMP), which elicits opening of cation channels directly gated by cAMP; cyclic nucleotide-gated (CNG) channels (Nakamura and Gold, 1987), and this creates an inward transduction current carried by Ca^{2+} and sometimes Na^{+} (Restrepo et al., 1990; Zufall et al., 1997). This odorant-induced calcium increase controls both excitation and adaptation of the olfactory neurons (Schild and Restrepo, 1998; Menini, 1999).

Overweight and obesity have increased dramatically in the past ten years in the United States, and have reached epidemic proportions worldwide. An etiologic classification suggests four causes of obesity: genetic transmission, hypothalamic injury, endocrine disorders, and simply energy intake in excess of energy output. Taken as a group, the obese have increased morbidity and mortality rates when compared to normal-weight individuals. Some consequences of obesity include increased degenerative joint disease, cardio-pulmonary dysfunction, diabetes and other endocrine diseases, in addition to adverse

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doi:10.1016/j.pbb.2005.09.008

social/psychological consequences (Mokdad et al., 2000; Aronne, 2001).

The overwhelming cause of obesity in the majority of people is most likely energy intake in excess of energy output, and a number of contributing factors are associated with this imbalance. Several clinical and epidemiological studies have documented that obese individuals frequently underestimate their caloric intake, and carefully conducted laboratory studies have documented greater caloric intake among obese and overweight individuals (Flier, 2001).

Diltiazem, a benzothiazepine derivative, is a calcium channel blocker that has been used clinically as an anti-hypertensive, anti-anginal, and anti-arrhythmic agent (Abernethy and Schwartz, 1999). Many epidemiological studies have demonstrated associations between elevated body mass index and elevated blood pressure, and there is evidence to suggest that obesity is a causal factor in the development of hypertension in obese individuals. The risk for hypertension in obese individuals has been reported to be 50% to 300% higher than for normal weight individuals (Mikhail et al., 1999).

In addition to its anti-hypertensive effect, diltiazem has been reported to block the CNG channels of rods and cones and olfactory sensory neurons in a voltage-dependent fashion (Stern et al., 1986; Quandt et al., 1991; Kaupp and Seifert, 2002). Since the long-term outcomes of weight management programs for obesity are generally poor, and many hypertensive patients will require anti-hypertensive medications, a novel approach at influencing ingestive behaviors might therefore involve altering olfactory acuity via Ca^{2+} channel blockade using the antihypertensive calcium antagonist, diltiazem. The primary goal of this study was to determine the ability of intranasally applied diltiazem to influence food intake in rats.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the MCPHS Institutional Animal Care and Use Committee (IACUC) prior to commencement of the studies. To determine the effects of diltiazem on food intake in food-deprived rats, male Sprague–Dawley rats, 175–225 g (Charles River Laboratories, Wilmington, MA) were housed individually in suspended wire mesh bottom cages. For the acute food-deprived and long-term weight gain experiments, animals were maintained on a reversed-lighting schedule (lights-on at 7 pm, lights-off at 7am); and acclimated to our climate controlled animal facility and to the test diets for 5 days prior to experimentation. As these experiments were performed during the dark cycle, when rats normally eat, a dim red light (25 watt) was used during the dark cycle to help the investigators perform the experimental procedures (Hull and Maher, 1990). Since the time of daily lighting exposure was reversed (for the convenience of the investigators), an initial re-entrainment period of 5 days was included.

For experimental studies on the effects of diltiazem on conditioned taste aversion (CTA), rota-rod, and diltiazem concentrations in plasma, rats were housed in polycarbonate cages with stainless steel lids and maintained on a regular light/dark cycle (lights-on at 7 am, lights-off at 7 pm).

2.2. Diet and treatment

A “mash” diet used on test days for food-intake experiments was composed of equal parts by weight of ground rodent chow (Rodent Laboratory Chow No. 5001-meal form, Purina, Richmond, IN) and of a 4% nutrient agar solution (Harlan-Teklad Diets, Madison, WI) and was presented to the animals in pre-weighed glass jars. The use of this agar-based mash diet minimizes spillage and allows for a more accurate determination of food intake. Additionally, it has been shown previously in our laboratory that this mash diet is sufficient for maintaining normal rat growth (Hull and Maher, 1990). For the CTA experiment, the rats were water deprived 24 h before the first session and fluid access was from then on restricted to daily experimental sessions of 30 min. Food was freely available throughout the procedure, but was not available during the experimental sessions. A 0.1% W/V saccharin solution was used for the CTA (as described below). Rats had ad libitum access to tap water and food (Rodent Laboratory Chow No. 5001-pellet form, Purina, Richmond, IN) on non-testing days, and for all other experiments.

Diltiazem ((+)-cis-diltiazem hydrochloride, Sigma, St Louis, MO) was dissolved in normal saline solution and the pH was adjusted to 5.5 with 0.5 N HCl. Many nasal spray solutions that are currently marketed for human use are often formulated in this pH range, and even at lower pH values. The drug solution was prepared on the day of the experiment and was administered i.n. in a volume of 20 μL/ nare, and i.p., or p.o. in a volume of 0.5 or 1 mL/ animal, respectively. Because the major focus of the study involved nasal administration, a topical application, the volume of the application was kept constant so as to minimize any influences due to differences in the residency time of the agent in the nasal cavity. In order to make the most appropriate comparisons, i.p. and p.o. dosing were based on per animal doses in a similar fashion to that used for i.n. dosing. Intracerebroventricular dosing is frequently administered as an absolute amount per animal, and not based on body weight. Thus, for the i.c.v. route of administration the drug in a volume of 2 μL of the vehicle (artificial cerebrospinal fluid; CSF) was administered slowly over a 2 min period into the left lateral cerebral ventricle through a pre-placed guide cannula (as described below).

2.3. Experimental procedures

2.3.1. Effect of diltiazem administration on food intake in food-deprived rats

On the day of an experiment, food was removed at 7 am just prior to lights-off and 4 h later (11 am) the drug was administered using the i.n., i.p., p.o. or i.c.v. routes of administration. One hour later (12 noon) pre-weighed food jars were introduced into

the cages and a sheet of clean white paper was placed under each suspended wire mesh bottom cage to catch any spillage. The amount of food consumed after 1, 2 and 4 h was determined to the nearest 0.01 g and expressed per 100 g of body weight. Water was available at all times.

Nasal administration involved direct application of the drug solution (0%, 0.2%, 2% or 20%) in a volume of 20 μL/ nare to four groups of rats, $n=16–26$ for the drug-treated groups and 32 for the vehicle-treated group (0, 0.08, 0.8, or 8 mg/animal). This is equivalent to an average dose of 0, 0.4, 4, or 40 mg/kg. To instill the treatments nasally, the rat was gently restrained in one hand and turned over to expose the underside of the nasal cavities. The solutions were then administered in one naris with an eppendorf pipette in the other hand and the animal allowed to remain in this position for 20 s before repositioning for the administration of drug to the other naris. This procedure allowed the solution administered to remain in contact with the nasal mucosa for at least 20 s. For i.p. administration, diltiazem was administered to three groups of rats, $n=12$ (0, 0.8, or 8 mg/animal), which is equivalent to a dose of 0, 4, or 40 mg/kg. Diltiazem (0 or 8 mg/animal, equivalent to a dose of 0 or 40 mg/kg) was administered p.o. to two groups of rats, $n=10$.

For the i.c.v. administration of diltiazem, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Sigma, MO, USA), and were placed in a stereotaxic frame (Stoelting, IL, USA) to fix a guide cannula (CMA/12, CMA Microdialysis, MA, USA) in the left lateral cerebral ventricle at the following stereotaxic coordinates: AP −0.8 mm (posterior to bregma), LR+1.5 mm (lateral to the midline), and DV −3.6 mm (below the dura mater) (Paxinos and Watson, 1982). The guide cannula was fixed to the skull with bone screws and dental cement. Eye drops were used to prevent corneal dryness during the surgical procedure and rats were allowed to recover for 5 days before experimentation. On the day of the experiment, diltiazem (0 or 100 μg/animal) was administered i.c.v. using a Hamilton micro-syringe to two groups of rats ($n=5$). The artificial CSF was prepared by mixing 1 mL of the following stock solutions: 1450 mM NaCl, 27 mM KCl, 10 mM $MgCl_2.6H_2O$, 12 mM $CaCl_2.2H_2O$ (Sigma, MO, USA), the mixture was diluted to 10 mL using electrochemical grade water and was filtered through a 0.22 μm pore size nylon filter (Varian, CA, USA).

2.3.2. Effect of long term i.n. administration of diltiazem on weight-gain

Three groups of rats, $n=6$, were trained for seven days to eat their daily meal during the first 4 h at the onset of the dark cycle. Rodent chow pellets were introduced daily at the beginning of the dark cycle (7 am) and were removed four hours later (11 am), while water was available at all times. Laboratory rats generally show very stable diurnal patterns of intake in which both food and fluid intake are at a maximum at the beginning one-third of the dark phase and at a minimum level during the light phase (Clifton, 2000). Additionally, rats can be easily trained to consume their normal food intake at the beginning of the dark cycle (Smith, 2000).

On testing days, a volume of 20 μL/ nare of diltiazem (0%, 2%, or 20% solution, equivalent to 0, 0.8, or 8 mg/animal) was administered daily before introducing the food for 14 days. Rats were weighed daily, and changes in weight were expressed as the percentage of the individual baseline (rat weight on the first day of administering the drug).

2.3.3. Effect of acute i.n. administration of diltiazem on motor coordination

Since the rota-rod test is a very useful technique that has been employed previously to determine the effects of drugs on the central nervous system that control gross motor coordination and balance, we used this test to determine if diltiazem adversely affected motor function thereby possibly affecting ingestive behaviors (Ingram et al., 1994; Quang et al., 2002). Two groups of male Sprague–Dawley rats, $n=12$, were administered diltiazem solution (0 or 20%) i.n. in a volume of 20 μL/ nare (0 or 8 mg/animal). Each rat was exposed to 4 sessions on the rota-rod at a constant speed of 10 rpm: 1 session before drug administration (time 0), and 3 sessions after drug administration (time 1, 2, and 4 h). For each trial, the rat was lifted by its tail and placed onto the rotating rod (Rota-Rod/RS, Letica Scientific Instruments, Spain) with its body placed horizontally on the cylinder. The rat was then released and a digital timer was activated. The timer was automatically deactivated when the rat stopped log-rolling and fell off onto a soft pad or passed the test. Rats were considered to have passed the test when they remained on the rotating rod for at least ten consecutive seconds at a given test period. The apparatus was cleaned with soap and water after each trial.

2.4. Conditioned taste aversion (CTA) experiment

A modification of the method described by De Beun et al. (1996) was followed. Animals designated to the same experimental group ($n=8$ per group) were run in parallel and were water deprived 24 h before the first session and fluid access was from then on restricted to daily experimental sessions of 30 min. During the first four sessions, both bottles contained plain tap water. This phase of the procedure gave the animals the opportunity to learn to drink a reasonable amount of water in a short period of time and to establish a relatively stable baseline value of water intake. For the fifth session (conditioning session), both bottles were filled with a novel fluid (i.e., a 0.1% W/V saccharin solution) and immediately after completion of this session intranasal diltiazem (0, 2, or 20%, 20 μL/ nare) was administered. Between the conditioning session and the final test session for CTA, the animals were left undisturbed for 72 h (wash-out period) and during the first 48 h of this period they had free access to tap water in their cages after which time they were again deprived of water. During this last session, one bottle contained the saccharin solution used for conditioning and the other bottle was filled with tap water. To control for location bias, the saccharin solution was presented in the left bottle for half of the animals in each group and in the right bottle for the other half. By measuring

the amount of fluid consumed from both bottles separately, drug-induced CTA could be determined by comparison of the relative saccharin intake in the drug-treated groups and their vehicle-treated controls.

2.5. Diltiazem concentrations in plasma

Blood samples were collected from tail veins immediately before treatment and at 60 min after drug administration to two groups of rats ($n=7–8$) that received diltiazem, 8 mg/ animal, i.n. or i.p. Blood was collected in 13 × 75 mm blood collection tubes containing sodium heparin (Becton Dickinson Vacutainer System, Rutherfurd, NJ, USA), and immediately centrifuged for 5 min. The supernatant fluid was removed to microcentrifuge tubes using a pipette and the tubes were frozen at −80 °C.

Plasma samples were analyzed for diltiazem using a high performance liquid chromatography (HPLC) method that was tested in our laboratory for sensitivity, specificity, accuracy, precision and linearity. The HPLC system consisted of an autosampler; ESA model 540, an ESA 580 pump (ESA, MA, USA), and a Spectra-Physics SP8940 UV detector (Thermo-Finnigan, CA, USA) set at 237 nm. The data was acquired with PC/Chrom+software (H and A Scientific, NC, USA). The column used was a Zorbax Eclipse XDB-C18 (250 × 4.6 mm, 5 μm particle size, 80 Å pore size, MAC-MOD Analytical, PA, USA). The mobile phase consisted of acetonitrile/ methanol/ 0.025 M KH_2PO_4 (300 : 180 : 550; Sigma, MO, USA). The buffer was prepared using electrochemical grade water, and the mixture pH was adjusted to 3.0 with H_3PO_4, filtered and degassed before being pumped at a flow rate of 1 mL/min in an isocratic mode at 25 °C. Plasma samples were prepared by adding 60 μL of mobile phase and 2 μL of 70% perchloric acid to 40 μL of plasma to precipitate proteins. The mixture was vortexed and frozen at −80 °C. After thawing, the mixture was again vortexed and centrifuged at 14,000 rpm for 5 min. Twenty μL of the supernatant were injected for analysis. Standards were prepared as above by adding 60 μL of mobile phase and 2 μL of 70% perchloric acid to 40 μL of different concentrations of diltiazem. The mixture was vortexed and frozen at −80 °C, thawed, centrifuged, and 20 μL injected for analysis to develop the calibration curve.

2.6. Statistical analysis of data

In the food deprivation experiment, cumulative food intake (g of food/100 g of rat body weight) is expressed as group means±S.E.M. Differences between groups were determined using independent one-way ANOVA and post hoc Student–Newman–Keuls testing (minimum significance level was set at $p<0.05$), with separate analyses performed at each measurement time point. For the weight gain experiment, comparisons were made between the percentages of the rat weights on each day to its starting baseline value (the rat weight on the first day of treatment). The percentage of starting body weight is expressed as group means±S.E.M. Differences between groups were determined using independent one-way ANOVA or ANOVA on Ranks when the normality test failed, and post hoc Student–Newman–Keuls testing (minimum significance level was set at $p<0.05$), with separate analyses performed for each day. For the CTA experiment, saccharin preference was expressed as the amount of 0.1% saccharin consumed relative to the total fluid intake during the test session. Differences between groups were determined using one-way ANOVA and post hoc Student–Newman–Keuls testing (minimum significance level was set at $p<0.05$). Comparisons between plasma levels of the drug at 1h when the drug was administered using the two routes was determined using the *t*-test (minimum significance level was set at $p<0.05$). The rota-rod test results are expressed as the percentages of rats passing the test within each group in each trial and the data are expressed as group means±S.E.M. Differences between the groups in each trial were determined using the non-parametric Mann–Whitney Rank Sum Test (minimum significance level was set at $p<0.05$).

3. Results

Intranasal diltiazem significantly and dose-dependently decreased food intake in this food-deprived, hyperphagic rat model at 1, 2 and 4 h (Fig. 1); ANOVA- F (3,86)=8.046, 6.146, and 9.121 (all $p<0.001$) at 1, 2, and 4 h, respectively. When compared to the vehicle control group, both 2% and 20%, (0.8 and 8 mg/animal) diltiazem significantly reduced food intake at all time points measured ($p<0.05$ or 0.01) as determined by Student–Newman–Keuls test. However, the lowest diltiazem dose tested, 0.08 mg/animal, failed to reach statistical significance ($p>0.05$). On the other hand, similar doses of diltiazem failed to produce a similar anorectic effect when administered i.p. (Fig. 2); ANOVA- F (2,33)=1.419, 1.693, and 1.528 ($p=0.256$, 0.199, and 0.232) at 1, 2, and 4 h, respectively. Diltiazem also failed to affect food intake when it was administered p.o. (Fig. 3; the *t*-test yielded $p=0.39$, 0.078, and 0.638 at 1, 2, and 4 h, respectively), or i.c.v. (Fig. 4; the *t*-test yielded $p=0.47$, 0.20, and 0.17 at 1, 2, and 4 h, respectively). No observable adverse effects were noted in any of the animals at any dose tested.

Rats treated daily with i.n. diltiazem prior to food introduction exhibited a significant attenuation of weight gain in a dose-dependent manner over the duration of the experiment (14 days) (Fig. 5). When compared to the vehicle control group, 20% diltiazem (8 mg/animal) significantly attenuated the rate of weight gain compared to vehicle controls from day 2 to day 14 ($p<0.05$ or <0.001) as determined by Student–Newman–Keuls test. The weight gain rate attenuation effect of 2% diltiazem (0.8 mg/animal) reached statistical significance only at day 14 ($p<0.05$).

In the CTA experiment, both the treatment and vehicle-treated groups demonstrated 50–60% saccharin preference with no statistically significant difference among the groups as determined by ANOVA- F (2,23)=0.364 ($p=0.699$) (Fig. 6).

Diltiazem attained a plasma level of 619±87 and 418±71 ng/mL at 60 min post i.n. or i.p. administration of the drug (8

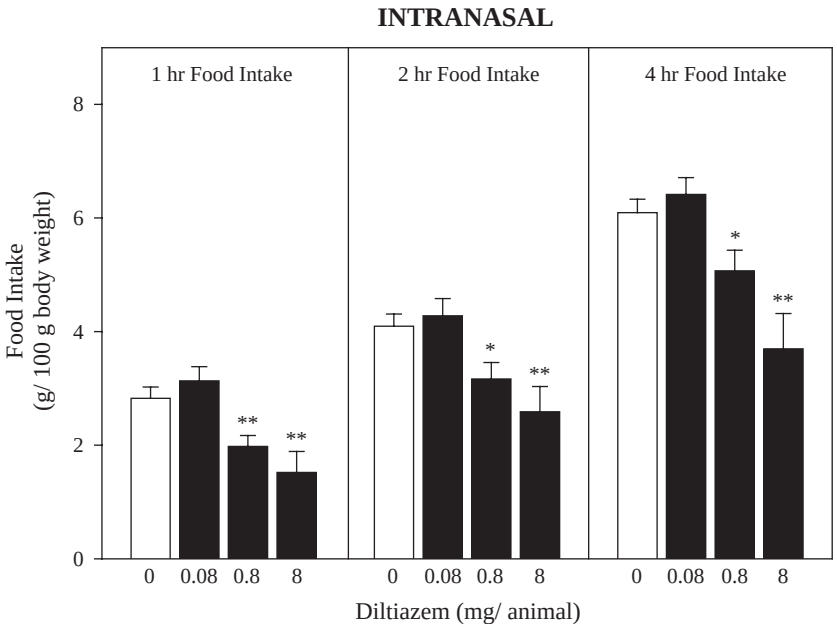

Fig. 1. *Effect of intranasal (i.n.) administration of diltiazem on food intake in food-deprived rats*: Mean ± S.E.M. cumulative food intake at 1, 2 and 4 h in 4 h fasted rats administered various doses of diltiazem (i.n.). *$p<0.05$, **$p<0.01$ when compared to the vehicle treated control group as determined by ANOVA and post hoc Student–Newman–Keuls test.

mg/animal), respectively (Fig. 7). *T*-test analysis failed to reveal any significant difference ($p=0.104$) between the two routes of administration.

Finally, the highest dose of diltiazem used in the previous experiments (8 mg/animal) failed to alter the rats' performance on the rota-rod test at any of the three post administration sessions when the drug was administered i.n. and compared to the vehicle treated control group (data not shown; Mann–Whitney Rank Sum Test no statistically significant difference, $p=0.943$).

4. Discussion

These data demonstrate that intranasal administration of diltiazem, a calcium channel blocker that has been used clinically as an anti-hypertensive agent, significantly and dose-dependently decreased food intake in our food-deprived, hyperphagic rat model, and attenuated body weight gain when administered daily before food introduction to rats trained to consume their daily food allotment within the first four hours of the dark cycle. To our knowledge, such an effect of diltiazem

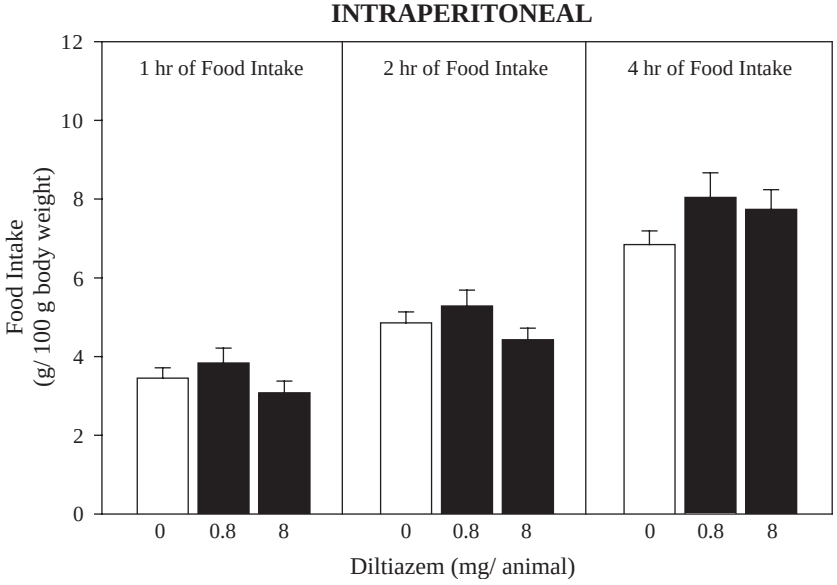

Fig. 2. *Effect of intraperitoneal (i.p.) administration of diltiazem on food intake in food-deprived rats*: Mean ± S.E.M. cumulative food intake at 1, 2 and 4 h in 4 h fasted rats administered various doses of diltiazem (i.p.). No statistically significant differences were observed ($p>0.05$) when the diltiazem treated groups were compared to the vehicle treated control group as determined by ANOVA.

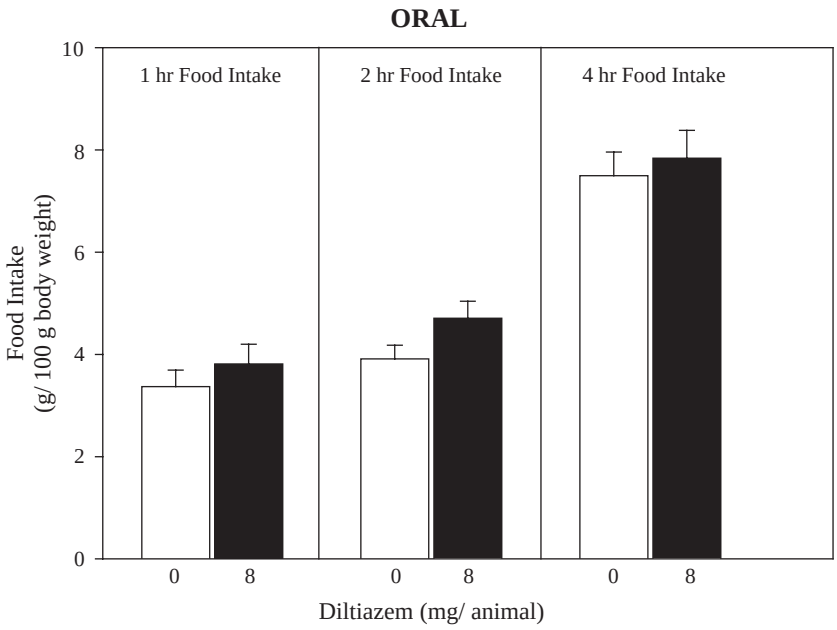

Fig. 3. *Effect of oral (p.o.) administration of diltiazem on food intake in food-deprived rats*: Mean ± S.E.M. cumulative food intake at 1, 2 and 4 h in 4 h fasted rats administered the vehicle or diltiazem (p.o.). No statistically significant differences were observed ($p > 0.05$) when the diltiazem treated group was compared to the vehicle treated control group as determined by *t*-test.

administered nasally on animals' ingestive behavior has not been reported previously.

We also started to examine possible mechanisms of this anorectic effect of diltiazem. We found that similar or higher doses of diltiazem that had an anorectic effect when administered intranasally, failed to show such a similar effect when administered orally or intraperitoneally. Diltiazem also failed to show any anorectic effect after intracerebroventricular administration using a dose that was previously reported to lower blood pressure and decrease heart rate in rats after similarly being administered centrally (Iyoda et al., 1985; Rabkin, 1991; Roychowdhary et al., 1991). While the above study by Roychowdhary et al. utilized the intracisternal administration of diltiazem, there is a paucity of literature on i.c.v. diltiazem dosing in rats. Thus the doses we chose were based on those studies where diltiazem was administered centrally. Additional

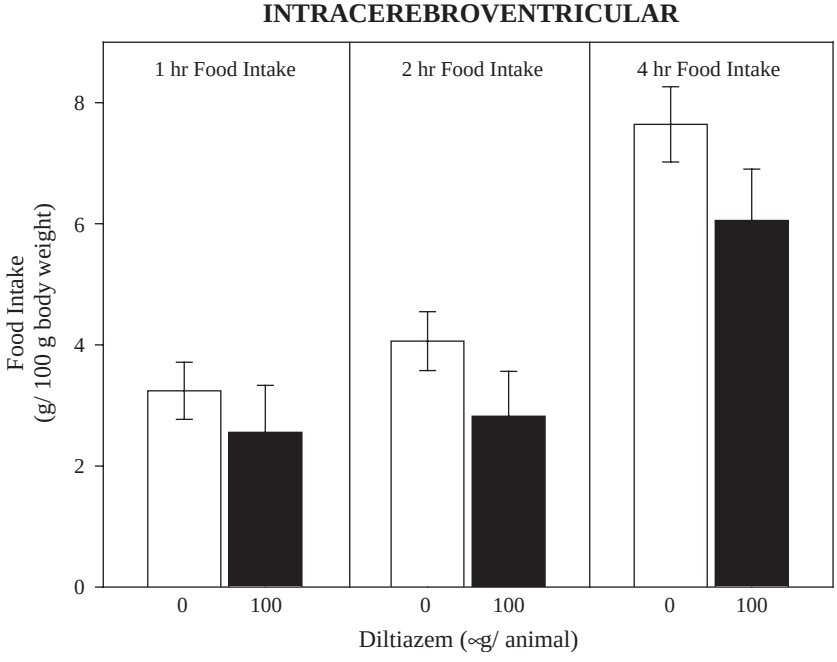

Fig. 4. *Effect of intracerebroventricular (i.c.v.) administration of diltiazem on food intake in food-deprived rats*: Mean ± S.E.M. cumulative food intake at 1, 2 and 4 h in 4 h fasted rats administered the vehicle (artificial cerebrospinal fluid) or diltiazem (i.c.v.) directly into the lateral ventricle (in a volume of 2 uL). No statistically significant differences were observed ($p > 0.05$) when the diltiazem treated group was compared to the vehicle treated control group as determined by *t*-test.

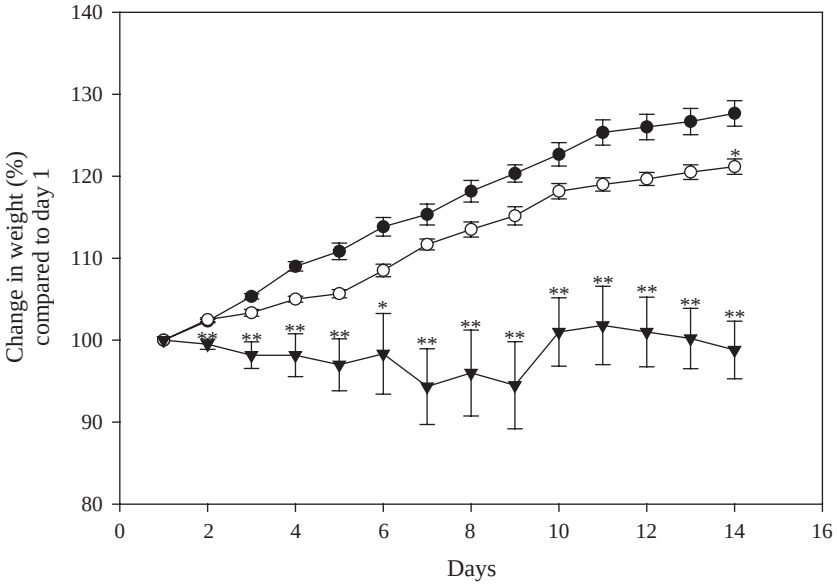

Fig. 5. *Change in body weight of rats treated daily with intranasal (i.n.) diltiazem over 14 days*: Mean ± S.E.M. of body weights (percent of starting baseline) on each day. Three groups of rats were trained to eat their daily meals in 4 h and were administered various doses of diltiazem (i.n.) daily before introducing food: 0 mg/ animal (the vehicle; closed circles), 0.8 mg/ animal; open circles, 8 mg/ animal; closed triangles. $*p<0.05$, $**p<0.001$ when compared to the vehicle treated group as determined by ANOVA and post hoc Student–Newman–Keuls test.

studies are needed utilizing various doses and various central routes of administration to definitively determine the role of diltiazem present in the CNS on food intake behaviors. The findings in the i.c.v. dosing study suggests that the decrease in food intake observed when diltiazem was administered via the i.n. route was most likely not due to a central effect as the result of any of the drug that would have entered the CNS directly via the olfactory nerve.

Another experiment tested the possibility that the anorectic effect was a consequence of drug-induced aversive effects, such as malaise. We found that nasal diltiazem did not attenuate saccharin-preference in a standard CTA paradigm. Thus, the reduction in food intake seen after i.n. diltiazem was most likely not the result of a malaise produced by the drug treatment. However, additional studies with other doses of diltiazem in other CTA paradigms are needed to further address this possibility.

A statistically similar plasma concentration at 1h after i.n. versus i.p. administration of diltiazem indicates that the drug had been readily delivered to the systemic circulation after nasal administration. However, at the time when food intake was initially measured (1 h), there was no significant difference between the plasma levels, suggesting that the lack of an anorectic effect following i.p. administration compared to the i.n. route was most likely not due to differences in plasma concentrations of the drug. Future studies should be designed

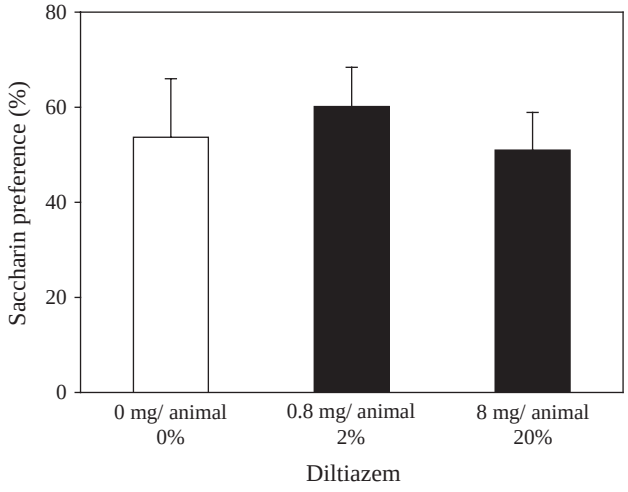

Fig. 6. *Effect of nasal diltiazem in a conditioned taste aversion (CTA) paradigm*: Saccharin preference was expressed as the ratio of saccharin intake divided by total fluid intake, multiplied by 100, during the test session for CTA. Diltiazem was administered i.n., 72 h before the 30-min test. $n=8$ per dose. Data presented as group mean ± S.E.M.

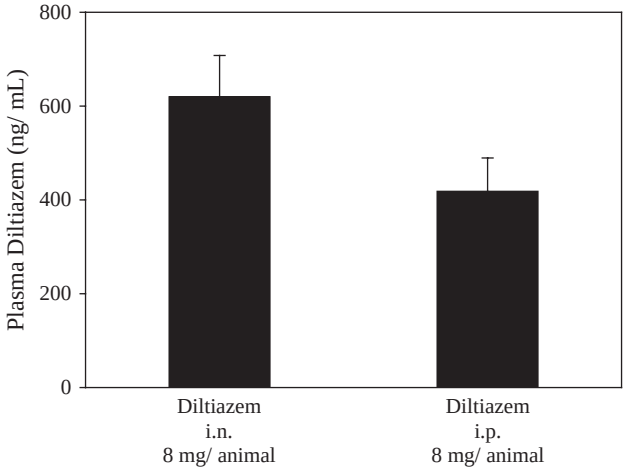

Fig. 7. *Diltiazem concentrations in plasma 60 min after intranasal (i.n.) or intraperitoneal (i.p.) administration*: Mean ± S.E.M. diltiazem concentrations in plasma 60 min after two groups of rats received the drug (8 mg/animal) i.n. or i.p. There was no significant difference between the two groups as determined by the *t*-test ($p>0.05$). Groups' $n=7–8$.

to measure diltiazem concentrations in a pharmacokinetically elegant fashion not only in plasma, but also in brain tissue (e.g., using cerebral microdialysis) at various time points to better understand the pharmacokinetics of diltiazem administered intranasally.

Further, these results suggest that after i.n. administration of diltiazem, the observed anorectic effect is likely not mainly attributed to a systemic effect. This is also supported by the lack of anorectic behavior after p.o. administration of similar doses of diltiazem. Again, this behavior was most likely not due to a gastrointestinal effect after the nasally administered diltiazem was ingested due to mucociliary clearance to the nasopharynx; otherwise, p.o. diltiazem would have also been expected to have had a similar anorectic behavior in this model. While diltiazem has a relatively poor oral bioavailability, the lack of an anorectic effect following i.p. diltiazem, but not i.n. diltiazem, argues against a lack of systemic absorption as the explanation for the differences between the efficacy of the two routes of administration. However, this study does not rule out any potential role that the "first-pass" effect might have played following p.o. and i.p. administration. Further investigations are required to more fully explore these many possibilities.

Additional studies failed to demonstrate any detrimental effects of i.n. diltiazem on locomotion or gross neurological/behavioral competence using the rota-rod test. While this test does not detect subtle alterations in motor behaviors following drug treatments, it is still a useful screening test for overt motor effects of drugs. Additional studies are needed to determine if fine motor coordination or performance on more involved tasks are altered by i.n. diltiazem.

These findings are consistent with a direct action of diltiazem on the calcium current mediated by the CNG cation channels in the olfactory sensory neurons. Evidence has previously been provided that oro-sensory stimuli, in particular the odor of a food, are involved in the metabolic control of meal size (Le Magnen, 1999) and in food preference by rats (Ramirez, 1993). Some have also suggested that human chemosensory receptors of taste and smell have similar regulatory roles with regard to food intake (Poothullil, 1995).

Although the pore of the CNG channels resembles the voltage-gated K^+ channels most closely in terms of its amino acid sequence (Heginbotham et al., 1992), the CNG channel permeation properties are more like those of the voltage-gated L-type Ca^{2+} channels. In both CNG channels and voltage-gated L-type Ca^{2+} channels, a set of glutamate residues represents the cation-binding site and resides in the conduction pathway of the channels (Yang et al., 1993; Root and MacKinnon, 1994; Zagotta and Siegelbaum, 1996; Dzeja et al., 1999). When these glutamate residues were neutralized or mutated into other amino acid residues, properties of the expressed channels were drastically affected (Eismann et al., 1994; Gavazzo et al., 2000). Additionally, the list of CNG channel blockers includes some Ca^{2+} channel antagonists such as diltiazem and pimozide (Stern et al., 1986; Quandt et al., 1991; Nicol, 1993; Kaupp and Seifert, 2002). These observations suggest that the CNG channels may share some of the pharmacological properties with voltage-gated Ca^{2+} channels.

The use of diltiazem via the intranasal route of administration in obese patients may offer certain therapeutic advantages, including ease of administration. Also, it may be possible to use this route of administration for the dual purposes as an antihypertensive medication to not only lower blood pressure, but also as an anorectic to suppress excessive food consumption in obese hypertensive individuals. Many more carefully designed studies are needed to investigate this possibility.

Because these studies were designed to determine the effects of locally administered diltiazem on food intake, we kept the volume of the nasal instillation constant. This approach has the advantage of trying to keep constant the amount of drug in contact with the nasal mucosa as the residency time in the nose would be significantly influenced by the volume (e.g., a large volume might pass through the nasal cavity into the esophagus very quickly). However, the disadvantage of this approach involves the slight differences in the mg/kg doses animals received. On the other hand, the animals used were of similar weights and thus an average mg/kg dose (0–40 mg/kg) could be calculated.

As nasally administered drugs can act locally on the nasal mucosa, peripherally after systemic absorption, and/or centrally via a preferred entry into the brain along the olfactory nerve, one or more of these mechanisms may have accounted for the observed anorectic activity of diltiazem. However, the lack of anorectic activity of i.p., p.o., and i.c.v. diltiazem in these experiments strongly suggests that the anorectic activity observed following i.n. administration most likely results from a local effect on the nasal mucosa where the chemosensory receptors are located. Furthermore, the ability of i.n. diltiazem to suppress ingestive behaviors is likely not the result of altered blood pressure or locomotor effects since the i.p., p.o. and i.c.v. route which might be expected to more potently alter the cardiovascular and centrally controlled motor functions, failed to exhibit any anorectic activity. Further studies are needed to determine the exact mechanism and site of action of nasally administered diltiazem. Additional studies should determine the effects of other L-type Ca^{2+} channel antagonists, as well as graded doses of diltiazem administered nasally on behaviors that could alter food intake via non-olfactory nerve mediated mechanisms.

Acknowledgements

The authors wish to thank Dr. Mark Bholke for his analytical expertise in analyzing the diltiazem plasma samples, Mr. Naser Mahmoud for his surgical expertise in the intracerebroventricular studies and Chris Adams and Karl Weinrich for their valuable input in the design of these experiments.

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