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Hyperphagic effect of novel compounds with high affinity for imidazoline I_2 binding sites

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Abstract

Previous studies have suggested that imidazoline I_2 receptors play a role in feeding control in rats. The effect of subcutaneous (s.c.) injections of four novel imidazoline I_2 ligands, 2-naphthalen-2yl-4,5-dihydro-1*H*-imidazole hydrochloride (benazoline), 2-styryl-4,5-dihydro-1*H*-imidazole oxalate (tracizoline), *o*-nitro-tracizoline and *o*-methyl-tracizoline (metrazoline) on food intake during the light phase was now evaluated in freely feeding male Wistar rats. Their effect was compared to that of idazoxan, a high-affinity ligand at imidazoline I_2 binding sites, but also a potent α_2-adrenoceptor antagonist. Compared to idazoxan, metrazoline exhibits a higher pK_i for imidazoline I_2 binding sites in rat liver, while the other compounds have a slightly lower pK_i; on the other hand, the novel compounds have much lower affinity than idazoxan at α_2-adrenoceptors. Idazoxan stimulated drinking at a dose as low as 1 mg/kg, and evoked feeding at a higher dose (30 mg/kg). The selective α_2-adrenoceptor antagonist 2-methoxy-idazoxan (RX821002), with negligible affinity at imidazoline I_2 binding sites, significantly increased drinking but failed to stimulate feeding at doses of 10–50 mg/kg. Metrazoline induced hyperphagia and water drinking at doses of 50 mg/kg or higher. Its dipsogenic effect was secondary to the hyperphagic effect, since it was not observed in rats without access to food. Benazoline significantly increased feeding only in response to 30 mg/kg, but its effect was less pronounced than that of metrazoline. Tracizoline and *o*-nitro-tracizoline were inactive. Following injection into the lateral cerebroventricle at doses up to 100 μg/rat, and into the third or fourth brain ventricle at doses up to 50 μg/rat, neither idazoxan nor metrazoline induced hyperphagia. The present results support the idea that imidazoline I_2 ligands influence feeding in rats, and suggest that their site of action is not in the central nervous system. The finding that idazoxan elicits a more potent hyperphagic effect than metrazoline and benazoline, although its affinity for imidazoline I_2 binding sites is lower than that of metrazoline and similar to that of benazoline, raises the question whether its hyperphagic effect might also be due to interaction with other receptors.

Keywords: Imidazoline I_2 receptor ligand; Idazoxan; RX821002; Metrazoline; Benazoline; Tracizoline; Food intake; Water intake

1. Introduction

Several imidazoline/guanidine compounds exhibit high affinity not only for α_2-adrenoceptors, but also for non-α_2-adrenergic binding sites, referred to as imidazoline binding sites (Bousquet, 1995; Bousquet et al., 1984; Ernsberger et al., 1987; Bricca et al., 1989; Escriba' et al., 1996; Regunathan and Reis, 1996; Molderings et al., 1997). The imidazoline binding sites have been divided into two subtypes: the imidazoline I_1 binding sites, labeled with [^{3}H]clonidine and its derivatives, and the imidazoline I_2 binding sites, traditionally labeled with [^{3}H]idazoxan (Ernsberger, 1992). The subtypes exhibit different tissue and subcellular distribution, as well as different pharmacological profiles (Brown et al., 1990; Bousquet, 1995; Ernsberger et al., 1995; Reis et al., 1995; Tesson et al., 1995; Olmos et al., 1996; Parini et al., 1996; Alemany et al., 1997).

Idazoxan, which shows high affinity for both adrenergic α_2 and imidazoline I_2 binding sites (Michel et al., 1989), has been reported to increase food and water intake in freely feeding rats during the daylight phase (Sleight et al., 1988; Jackson et al., 1991; Hartley et al., 1994). Other ligands at imidazoline I_2 binding sites have also been reported to stimulate food intake (Garcia-Sevilla et al.,

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1993; Menargues et al., 1994; Nutt et al., 1995; Jackson and Nutt, 1996). On the other hand, analogues of idazoxan such as 2-ethoxy-idazoxan (RX811059) and 2-methoxy-idazoxan (RX821002), that are selective α_2-adrenoreceptor antagonists with negligible affinity for imidazoline I_2 sites, induce a dipsogenic response and have been reported either to have no effect on food intake or to produce only modest hyperphagia (Jackson et al., 1991; Miralles et al., 1993). Thus, it has been proposed that the hyperphagic effect of idazoxan may be mediated by imidazoline I_2 receptors. Interestingly, imidazoline I_2 binding sites have been found in regions of the rat brain, such as the hypothalamus and the area postrema (Mallard et al., 1992; French, 1995; MacKinnon et al., 1995), that are known to be involved in feeding control (Bernstein et al., 1985; Sugrue, 1987). However, evidence in favour of a role of imidazoline I_2 receptors in feeding control is still rather weak, since only a limited number of imidazoline I_2 ligands have been tested and nothing is known about the mechanism and the site of action of their hyperphagic effect. On the other hand, there is still controversy about the structure and localization of imidazoline I_2 binding sites (Eglen et al., 1998). The majority of I_2 sites are widely accepted as being allosteric sites on monoamine oxidase (Tesson et al., 1995; Limon-Boulez et al., 1996). However, it has also been proposed that they are molecular entities distinct from monoamine oxidase in rat liver, and human platelets (Ozaita et al., 1997; Soto et al., 1999). Moreover, imidazoline receptors have also been found in axon terminals of the rat central nervous system (Ruggiero et al., 1998). Lastly, no biological test is available to study the intrinsic activity of imidazoline I_2 ligands, so that it is unknown whether they increase food intake by an agonist or antagonist action.

Table 1
Affinity of the tested compounds at imidazoline I_2, I_1 and adrenergic α_2 binding sites

The affinity of idazoxan and of the four novel compounds for imidazoline I_2 binding sites was determined by Parini et al. (personal communication) in the rat liver, using [^{3}H]idazoxan in the presence of norepinephrine. The affinity at α_2 binding sites was taken from Carrieri et al., 1997, Pigini et al., 1997, 1998. The values concerning the affinity of RX821002 for imidazoline I_2 and α_2 binding sites and of tracizoline at imidazoline I_1 binding sites are personal communication from Bousquet et al. The affinity of benazoline, idazoxan and RX821002 at imidazoline I_1 binding sites was taken, respectively, from Ernsberger et al., 1997; Eglen et al., 1998; Bruban et al., 1999.

NA = not available.

I_2/α_2 is the antilog of the difference between pK_iI_2 and $pK_i\alpha_2$ values.

Compound	pK_iI_2	pK_iI_1	$pK_i\alpha_2$	I_2/α_2
Tracizoline	8.67	7.72	4.85	7762
Metrazoline	9.55	NA	6.04	2455
o-Nitro-tracizoline	8.53	NA	5.49	1585
Benazoline	8.82	8.70	4.80	18,621
Idazoxan	8.90	5.90	7.72	4.5
RX821002	5.87	6.40	8.74	0.0018

Several compounds recently synthesized in our laboratories are endowed with high affinity for imidazoline I_2 binding sites and high imidazoline I_2/α_2-adrenergic binding sites selectivity (Pigini et al., 1997, 1998). The present study evaluated, in comparison to that of idazoxan and RX821002, the effect of four novel imidazoline I_2 ligands (Table 1) on food intake in freely feeding rats, following subcutaneous (s.c.) or central administration.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Italia), weighing 350–400 g, were used. They were housed in individual stainless-steel cages in a room with a 12:12 h light:dark cycle (lights off at 6:00 p.m.), controlled temperature (20–21°C) and humidity (45–55%). The rats had free access to food pellets (4RF18, Mucedola, Settimo Milanese, Italy) and tap water.

All animal testing was carried out according to the European Communities Council Directive of November 24, 1986 (86/609/EEC).

2.2. Surgery

For intracranial surgery, the rats were anesthetized by intramuscular injection of 100–150 μl/100 g body weight (b.w.) of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml).

A group was stereotaxially implanted with a guide cannula for injections into the lateral cerebroventricle. The following coordinates, taken from the atlas of Paxinos and Watson (1986), were used for the guide cannula: AP = 1.0 mm behind the bregma, L = 2.0 mm from the sagittal suture, V = 2.0 mm from the surface of the skull. Injections were made by means of a stainless-steel injector 2.5 mm longer than the guide cannula.

Another group was implanted with a guide cannula for injections into the third cerebroventricle. The following coordinates were used: AP = 1 mm behind the bregma, L = 1 mm from the sagittal suture, V = 7.6 mm from the surface of the skull. The cannula was introduced at an angle of 10° from the sagittal plane, to avoid damage to the sagittal sinus. Drug injections were made by means of a stainless-steel injector 0.5 mm longer than the cannula.

A third group was implanted with a guide cannula for injections into the fourth cerebroventricle. The following coordinates were used for the guide cannula: AP = 11.5 mm behind the bregma, L = 0 mm from the sagittal suture, V = 7.1 mm from the surface of the skull. Injections were made by means of a stainless-steel injector 0.5 mm longer than the guide cannula.

2.3. Drugs

The drugs, RX821002 (Stillings et al., 1985), 2-naphthalen-2yl-4,5-dihydro-1*H*-imidazole hydrochloride (benazoline) and 2-styryl-4,5-dihydro-1*H*-imidazole oxalate (tracizoline) (Pigini et al., 1997), *o*-nitro-tracizoline and *o*-methyl-tracizoline (metrazoline) (Pigini et al., 1998) were synthetized in the Department of Chemical Sciences of the University of Camerino. Idazoxan was purchased from RBI, Natick, Ma, USA.

2.4. Experimental protocol

2.4.1. Effect of s.c. injections of idazoxan, RX821002 or other ligands at imidazoline I_2 binding sites on food and water intake in freely feeding rats

Six groups of freely feeding rats were used. A within-subject design was adopted in which each group received the different doses of a single compound, as well as vehicle (distilled water). The different treatments were given according to a Latin Square design at intervals of 7 days. The following drugs were tested: idazoxan (1, 10, 30 mg/kg b.w.), RX821002 (10, 30, 50 mg/kg), benazoline (10, 30, 50 mg/kg), tracizoline (10, 50 mg/kg), *o*-nitro tracizoline (10, 50 mg/kg), metrazoline (10, 30, 50, 70, 90 mg/kg). The different doses were given by s.c. injection in a constant volume of 6 ml/kg. Drug administration took place at 9:00 a.m. (daylight phase). Food and water intake was measured 30, 60, 120, 240 min, as well as 24 h after drug injection. Food intake was determined by weighing the food containers to the nearest 0.01 g and taking into account the spillage. Water intake was determined to the nearest 0.1 ml by means of graduated drinking tubes.

2.4.2. Effect of s.c. injections of metrazoline on water intake in rats without access to food

In the previous experiment, rats had simultaneous access to food and water. Thus, the observed increase in water intake following metrazoline might have been the expression of a primary dipsogenic effect or of food-associated drinking secondary to the orexigenic effect. The present experiment was aimed at evaluating whether metrazoline displays a primary dipsogenic effect.

One group of freely feeding rats received s.c. injections of metrazoline (50 mg/kg) or vehicle at intervals of 7 days. Food was removed just before the s.c. injection. Water intake was measured 30, 60, 120 and 240 min after metrazoline administration, which took place at 9:00 a.m.

2.4.3. Effect of intraperitoneal (i.p.) injections of idazoxan or metrazoline on food intake in freely feeding rats

Idazoxan or metrazoline, the agents that most effectively increased food intake by s.c. injection, were also tested following i.p. injection.

Two groups of freely feeding rats were used. Each group received the various doses of either idazoxan (1, 3, 10 mg/kg b.w.) or metrazoline (3, 10, 30 mg/kg b.w.), as well as vehicle (isotonic NaCl). The different treatments were given according to a Latin Square design at intervals of 7 days. The different doses were given by i.p. injection in a constant volume of 3 ml/kg. Drug administration took place at 9:00 a.m. (daylight phase). Food and water intake was measured 30, 60, 120, 240 min, as well as 24 h after drug injection.

2.4.4. Effect of central injections of idazoxan or metrazoline on food intake in freely feeding rats

To evaluate whether idazoxan or metrazoline exert their hyperphagic effect at a central site of action, they were tested following central administration.

Three groups of freely feeding rats were used: one group for injections into the lateral cerebroventricle, one for injections into the third ventricle and one for injections into the fourth ventricle. According to a within-subject design, each group received isotonic saline (vehicle), as well as the different doses of idazoxan and metrazoline; the different pharmacological treatments were given at intervals of 3 days. In the third and fourth ventricles, idazoxan and metrazoline were administered in 3 μl/rat; in the lateral ventricle, idazoxan and metrazoline were administered in 6 μl/rat. The volume injected was given at a rate of 1 μl/10 s. Food intake was measured 30, 60, 120 and 240 min after the central injection.

2.4.5. Validation of cannula placement

After completion of the experiments, 1 μl of India ink was injected into the ventricle (either lateral, third or fourth ventricle) just before killing and ink diffusion into the ventricular space was evaluated. Only data from animals with correct cannula placement (about 85% of the rats) were submitted to statistical analysis.

2.5. Statistical analysis

Data are reported as means ± S.E.M. Statistical analysis of data was performed by means of multifactorial analysis of variance (ANOVA), with repeated measurements. Pairwise comparisons were made by means of Dunnett's test. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect of s.c. injections of idazoxan, RX821002 or other ligands at imidazoline I_2 binding sites on food and water intake in freely feeding rats

Idazoxan, given by s.c. injection at doses of 1–30 mg/kg, increased feeding in the 4 h after administration. ANOVA revealed a highly significant treatment effect [$F(3,24) = 25.7$; $P < 0.001$] and time effect [$F(3,24) = 66.7$; $P < 0.001$]. A statistically significant increase in

food intake was observed in response to 30 mg/kg (Fig. 1A), but not at the doses of 1 or 10 mg/kg. In response to 30 mg/kg, food intake was significantly increased at 60, 120 and 240 min, but not in the first 30 min. The cumulative 24-h food intake of rats given 30 mg/kg (65.2 ± 3.3 g/kg) was not significantly different from that of controls (72.6 ± 5.8 g/kg).

ANOVA revealed a highly significant effect of idazoxan on water intake [$F(3,24) = 5.01$; $P < 0.01$] (Fig. 1B). Pairwise comparisons showed a statistically significant increase in water intake at the dose of 1 mg/kg during the whole period of observation. The s.c. doses of 10 and 30 mg/kg also increased water intake, but their effect was statistically significant only at the end of the observation period.

The selective α_2-adrenoceptor antagonist, RX821002, did not significantly increase feeding, at s.c. doses of 10–50 mg/kg (Fig. 2A). Indeed, food intake was decreased at doses of 30 and 50 mg/kg, which induced immobility in treated rats.

The ANOVA revealed a non significant effect of RX821002 on water intake [$F(3,21) = 0.7$; $P > 0.05$], but a significant treatment-time interaction [$F(9,63) = 4.1$; $P < 0.001$]. Pairwise comparisons showed that water drinking was significantly increased at 4 h following 10 mg/kg (Fig. 2B).

Metrazoline, given at s.c. doses of 10–90 mg/kg, evoked a pronounced hyperphagic effect in the 4 h following administration. The ANOVA revealed a highly significant treatment effect [$F(5,40) = 8.3$; $P < 0.001$] and time effect [$F(3,24) = 106.6$; $P < 0.001$]. The effect of metrazoline on food intake was statistically significant in response to 50 mg/kg or higher doses. The maximum increase in food intake was observed in response to 50 mg/kg (Fig. 3A); at this dose no effect on the rat's

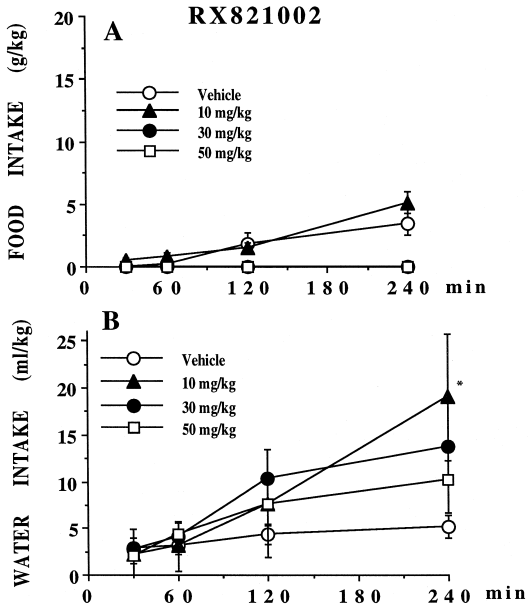

Fig. 2. Cumulative food (Panel A) and water (Panel B) intake in 4 h after s.c. injection of RX821002, 10–50 mg/kg, or vehicle in freely feeding rats. Data are means ± S.E.M. for eight rats. Difference from controls as in Fig. 1.

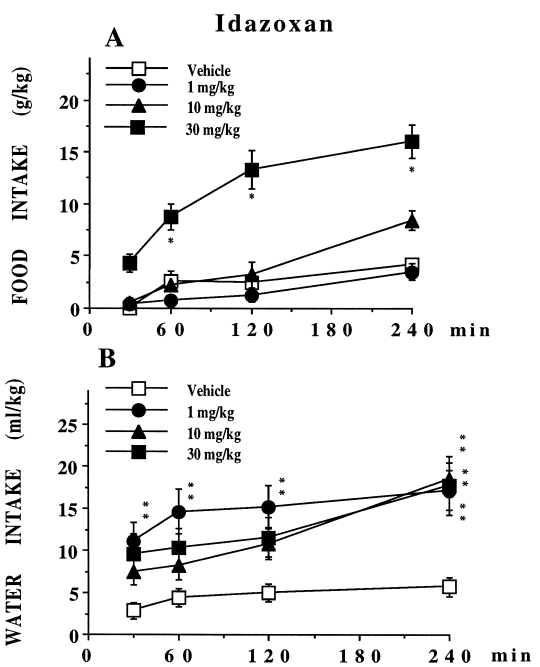

Fig. 1. Cumulative food (Panel A) and water (Panel B) intake in 4 h after s.c. injection of idazoxan, 1–30 mg/kg, or vehicle in freely feeding rats. Data are means ± S.E.M. for nine rats. Difference from controls: $^{*}P < 0.05$, $^{**}P < 0.01$, where not indicated, difference from controls was not statistically significant.

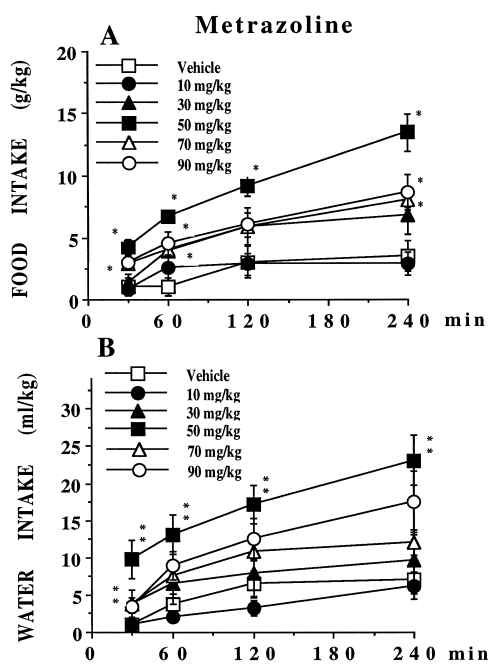

Fig. 3. Cumulative food (Panel A) and water (Panel B) intake in 4 h after s.c. injection of metrazoline, 10–90 mg/kg, or vehicle in freely feeding rats. Data are means ± S.E.M. for nine rats. Difference from controls as in Fig. 1.

behaviour was visible. Higher doses also were able to stimulate food intake, but their effect was less intense, probably owing to the induction of slight sedation in treated rats. The 24-h food intake of rats treated with 50 mg/kg (69.0 ± 3.5 g/kg) was not significantly different from that of controls (74.8 ± 4.3 g/kg).

The ANOVA revealed a highly significant effect of metrazoline on water intake [$F(5,40) = 6.3$; $P < 0.001$] (Fig. 3B). Pairwise comparisons showed a statistically significant increase in water intake at the dose of 50 mg/kg during the whole period of observation. Moreover, a statistically significant effect was also observed in response to 70 mg/kg, but only during the first 30 min following injection.

Benazoline increased feeding in the first 4 h after administration, but its effect was less pronounced than that of metrazoline. Overall ANOVA revealed a significant treatment effect [$F(3,21) = 3.6$; $P < 0.05$] and time effect [$F(3,21) = 14.3$; $P < 0.001$] (Fig. 4A). Pairwise comparisons showed a statistically significant effect at the dose of 30 mg/kg only at the end of the 4-h period of observation.

The ANOVA showed no significant effect of benazoline on water intake [$F(3,21) = 1.86$; $P > 0.05$], but a significant treatment-time interaction [$F(3,24) = 66.7$; $P < 0.01$] (Fig. 4B). Pairwise comparisons showed that drinking was significantly increased by 30 mg/kg at 4 h.

Both tracizoline and *o*-nitro-tracizoline, 10 or 50 mg/kg, failed to significantly modify food intake (Table 2).

Water intake following tracizoline or *o*-nitro-tracizoline injection was very modest; the mean values ranged from 2.3 to 4.9 ml/rat in 4 h and were not significantly different from those of the controls.

Table 2
Effect of s.c. injections of tracizoline (TZ) or *o*-nitro-tracizoline (NTZ) on food intake in freely feeding rats
Food intake is expressed as g/kg. Data are means ± S.E.M. from 6 (tracizoline) and 6 (*o*-nitro-tracizoline) rats. Difference from controls was not statistically significant.

Compound	30 min	60 min	120 min	240 min
vehicle	0.06 ± 0.04	0.24 ± 0.17	1.09 ± 1.02	1.21 ± 1.07
TZ (10 mg/kg)	0.79 ± 0.51	2.32 ± 0.98	2.57 ± 1.14	3.24 ± 1.12
TZ (50 mg/kg)	0.24 ± 0.15	0.34 ± 0.18	0.51 ± 0.21	4.75 ± 1.81
vehicle	0.40 ± 0.13	0.50 ± 0.10	0.71 ± 0.13	1.26 ± 0.35
NTZ (10 mg/kg)	0.40 ± 0.26	0.92 ± 0.25	1.23 ± 0.22	1.75 ± 0.36
NTZ (50 mg/kg)	1.03 ± 0.34	1.47 ± 0.44	1.58 ± 0.48	1.78 ± 0.64

3.2. Effect of s.c. injections of metrazoline on water intake in rats without access to food

Control rats took a small amount of water in the 4-h period of observation (1.4 ± 0.6 ml/rat). Rats treated with metrazoline, 50 mg/kg, showed a 4-h water intake of 1.8 ± 0.4 ml/rat, which was not significantly different from that of the controls.

3.3. Effect of intraperitoneal injections of idazoxan or metrazoline on food intake in freely feeding rats

As shown in Fig. 5, both idazoxan and metrazoline increased food intake following their i.p. injection. Ida-

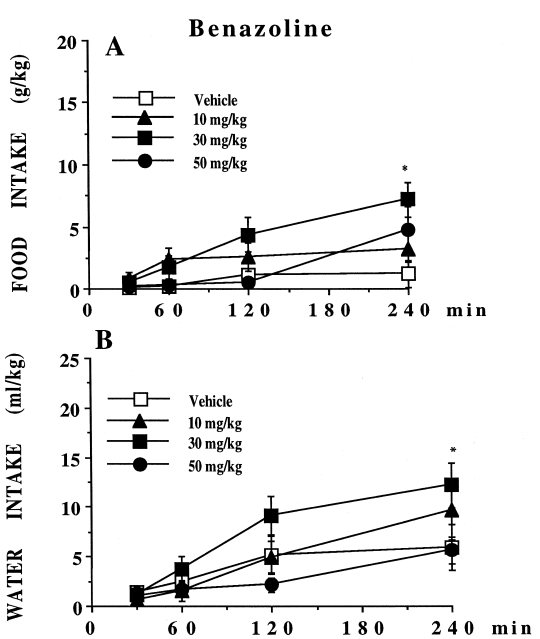

Fig. 4. Cumulative food (Panel A) and water (Panel B) intake in 4 h after s.c. injection of benazoline, 10–50 mg/kg, or vehicle in freely feeding rats. Data are means ± S.E.M. for eight rats. Difference from controls as in Fig. 1.

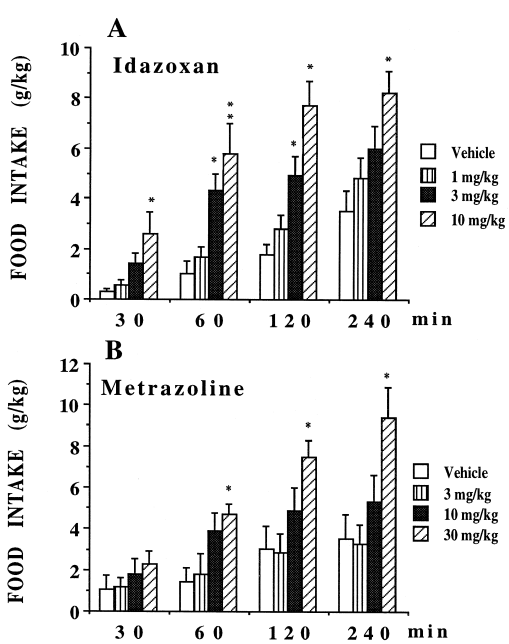

Fig. 5. Cumulative food intake in 4 h after i.p. injection of idazoxan, 1–10 mg/kg (Panel A) or metrazoline, 3–30 mg/kg (Panel B) or vehicle in freely feeding rats. Data are means ± S.E.M. for six rats. Difference from controls as in Fig. 1.

zoxan (Fig. 5A) evoked a statistically significant increase in food intake at the doses of 3 and 10 mg/kg, while metrazoline (Fig. 5B) induced a statistically significant effect at 30 mg/kg. Thus, the orexigenic potency of the two compounds was higher following i.p. injection than following s.c. injection.

3.4. Effect of central injections of idazoxan or metrazoline on food intake in freely feeding rats

Neither drug produced a significant increase in food intake following injection into either the lateral ventricle at doses of 1, 10 or 100 μg/rat (Fig. 6A), or the third (Fig. 6B) or fourth ventricle at doses of 1, 10 or 50 μg/rat (Fig. 6C).

The treated rats showed no visible modification of their behaviour; only in response to 100 μg/rat in the lateral cerebroventricle did the rats appear slightly sedated.

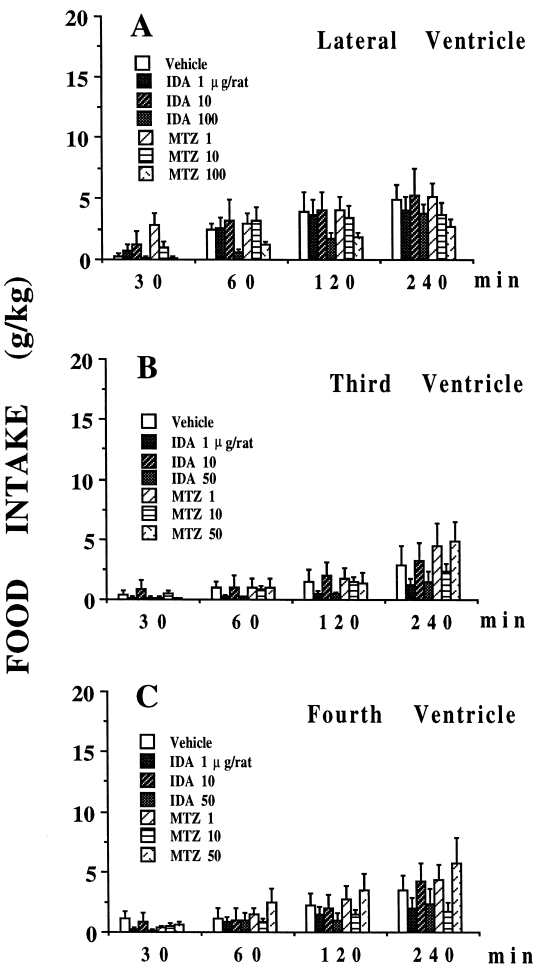

Fig. 6. Cumulative 4-h food intake in freely feeding rats after injection into the lateral (Panel A), third (Panel B) or fourth (Panel C) ventricle of different doses of idazoxan (IDA) or metrazoline (MTZ), or of vehicle. Data are means ± S.E.M. for three (lateral ventricle), six (third ventricle) or seven (fourth ventricle) rats. Difference from controls was not statistically significant.

4. Discussion

The present results confirm those of previous studies showing that peripheral administration of idazoxan stimulates food and water intake in freely feeding rats during the light phase (Sleight et al., 1988; Jackson et al., 1991). In the present study, a dipsogenic effect was evoked by the s.c. injection of idazoxan, 1 and 10 mg/kg, that did not stimulate feeding; thus, idazoxan-induceddrinking was not secondary to increased feeding. A higher dose, 30 mg/kg, induced a lower water intake, but a pronounced hyperphagic effect. Idazoxan exhibits high affinity for imidazoline I_2 binding sites, but it is also a potent antagonist at α_2-adrenoceptors (Michel et al., 1989). In this regard, the results obtained with idazoxan, 1 mg/kg, and with the selective α_2-adrenoceptor antagonist RX821002 (which failed to stimulate feeding, but significantly increased drinking) are in accordance with the hypothesis that the dipsogenic effect of idazoxan is due to antagosnism at α_2-adrenoceptors, while the hyperphagic effect is mediated by imidazoline I_2 sites (Jackson and Nutt, 1996; Jackson et al., 1991).

Interestingly metrazoline and benazoline, that exhibit low affinity for α_2-adrenoceptors (Table 1), did not induce drinking, as did idazoxan. Increased water intake after s.c. metrazoline injection was observed concomitantly with increased feeding, but was not induced by metrazoline when food was not available. These findings support the idea that metrazoline-induced water intake is not the expression of a primary dipsogenic effect, but represents food-associated drinking. These findings are in keeping with the hypothesis that antagonism at α_2-adrenoceptors is responsible for the dipsogenic effect of idazoxan.

Metrazoline, which shows the highest affinity for imidazoline I_2 binding sites among the compounds tested, evoked a marked and reproducible hyperphagic effect. The effect was slightly less potent than that of idazoxan, but of similar intensity. Benazoline significantly increased feeding, but its effect was less pronounced than that of metrazoline; tracizoline and *o*-nitro-tracizoline were ineffective. Thus, the relative orexigenic potency of the novel compounds is essentially in keeping with their affinity at imidazoline I_2 sites (Table 1). However, it is noteworthy that their orexigenic potency in comparison to that of idazoxan is not related to affinity for these sites (Table 1). Metrazoline proved to be less potent than idazoxan to stimulate food intake, although it exhibited a higher affinity than idazoxan for imidazoline I_2 binding sites; benazoline induced a modest hyperphagic effect and tracizoline and *o*-nitro-tracizoline were inactive at doses higher than those of idazoxan, even though their affinity for imidazoline I_2 binding sites is similar to that of idazoxan. Such a discrepancy was also reported for other compounds. Thus, RX801077 (2-(2-benzofuranyl)-2-imidazoline) and RX821029 (2-(1,3-benzodioxanyl)-2-imidazoline) have a higher affinity than idazoxan for imidazoline I_2 binding

sites (their K_i are, respectively, about 7 and 5 times lower than that of idazoxan), but are about 10 times less potent than idazoxan to stimulate feeding (Jackson and Nutt, 1996). These observations raise the question of whether receptors different from the imidazoline I_2 receptors might contribute to the orexigenic activity of idazoxan.

In this regard, it is unlikely that imidazoline I_1 binding sites, for which most of the tested compounds show some affinity (Table 1), could play any role. Idazoxan shows low affinity for these sites, but it exhibits a potent orexigenic action. Benazoline and tracizoline show a higher affinity than idazoxan for imidazoline I_1 binding sites, but tracizoline was inactive and benazoline was less active.

Idazoxan is also a potent antagonist at α_2-adrenoceptors. However, antagonism at α_2-adrenoceptors is apparently not a determinant per se of orexigenic activity (the selective α_2-adrenoceptor antagonists RX811059 or RX821002 have negligible effects on food intake). Moreover, when the selective α_2-adrenoceptor antagonist, RX821002, was coadministered with metrazoline by s.c. injection, it did not increase the orexigenic effect of metrazoline (Polidori, personal communication). Thus, antagonism at α_2-adrenoceptors apparently does not account for the higher hyperphagic potency of idazoxan.

Idazoxan has been reported to act as an agonist at brain 5-HT_{1A} autoreceptors (Llado et al., 1996); thus, its orexigenic effect might be at least in part due to disinhibition of feeding mechanisms from the inhibitory control of serotonin (Dourish et al., 1985). However, results obtained with the selective 5-HT_{1A} receptor antagonist, WAY100135, or by desensitization of 5-HT_{1A} autoreceptors argue against the involvement of central 5-HT_{1A} autoreceptors in the hyperphagic effect of idazoxan (Hartley et al., 1994; Jackson and Nutt, 1996). Moreover, the results of the present study, suggesting a peripheral site of action (see below) are not consistent with the involvement of central 5-HT_{1A} receptors or of central α_1- or α_2-adrenoceptors (Wellman et al., 1993) in the effect of idazoxan.

It might be speculated that different pharmacokinetics of the compounds could account for their different orexigenic activity. On the other hand, the small differences in liposolubility of the novel compounds and the finding that the site of action is not in the central nervous system make it unlikely that their different orexigenic activity may be influenced by different availability/pharmacokinetics.

High densities of imidazoline I_2 binding sites have been found in the hypothalamus and the area postrema (Mallard et al., 1992; French, 1995; MacKinnon et al., 1995), which are known to be involved in feeding control in rats (Bernstein et al., 1985; Sugrue, 1987). However, when idazoxan or metrazoline was injected into the third or fourth ventricle (which are contiguous to the hypothalamus and area postrema) or in the lateral ventricle, they did not evoke hyperphagia. These findings suggest a peripheral, rather than central site of action for the orexigenic effect of ligands at imidazoline I_2 binding sites.

In this regard, compounds with an imidazoline moiety have been reported to stimulate insulin secretion (Morgan et al., 1995); idazoxan has been reported to produce hypoglycemia (Grupp et al., 1997), and hypoglycemia can stimulate feeding (Orosco and Nicolaidis, 1996). However, results of preliminary experiments with rats (Polidori, personal communication) have shown that serum glucose levels 30 min after s.c. idazoxan, 30 mg/kg, or metrazoline, 50 mg/kg, were not significantly different from those of the controls. Imidazoline I_2 binding sites in peripheral tissues have been found in the liver (Tesson et al., 1991), in adipocytes (Langin and Lafontan, 1989; Langin et al., 1990), in the rat and human stomach (Molderings et al., 1998). All these peripheral structures may represent interesting sites of action for the orexigenic effect of imidazoline I_2 ligands.

The s.c. doses of idazoxan that induced hyperphagia in the present study were higher than those reported to be effective by i.p. injection (Jackson et al., 1991). Moreover, our experiments showed a more potent hyperphagic action of idazoxan and metrazoline following i.p. than following s.c. injection. This finding raises the question of whether the higher orexigenic potency following i.p. injection may be related to a site of action easily accessible from the peritoneal cavity or to more rapid absorption following this route of administration.

In conclusion, the results of the present study showed that the novel selective ligands for imidazoline I_2 binding sites, metrazoline and benazoline, increase food intake in rats, thus further supporting the idea of involvement of imidazoline I_2 receptors in the control of feeding. Moreover, our results suggest that the site of action for the orexigenic effect of idazoxan and metrazoline is not in the central nervous system, but maybe peripherally. The finding that idazoxan elicits a more potent hyperphagic effect than do metrazoline and benazoline, although its affinity for imidazoline I_2 binding sites is lower than that of metrazoline and similar to that of benazoline, raises the question of whether its hyperphagic effect might also be due to interaction with other receptors.

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