

Effects of central imidazolinergic and α_2 -adrenergic activation on water intake

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Abstract

Non-adrenergic ligands that bind to imidazoline receptors (I-R), a selective ligand that binds to α_2 -adrenoceptors (α_2 -AR) and mixed ligands that bind to both receptors were tested for their action on water intake behavior of 24-h water-deprived rats. All drugs were injected into the third cerebral ventricle. Except for agmatine (80 nmol), mixed ligands binding to I-R/ α_2 -AR such as guanabenz (40 nmol) and UK 14304 (20 nmol) inhibited water intake by 65% and up to 95%, respectively. The selective non-imidazoline α_2 -AR agonist, α -methyl-noradrenaline, produced inhibition of water intake similar to that obtained with guanabenz, but at higher doses (80 nmol). The non-adrenergic I-R ligands histamine (160 nmol, mixed histaminergic and imidazoline ligand) and imidazole-4-acetic acid (80 nmol, imidazoline ligand) did not alter water intake. The results show that selective, non-imidazoline α_2 -AR activation suppresses water intake, and suggest that the action on imidazoline sites by non-adrenergic ligands is not sufficient to inhibit water intake.

Key words

- Imidazolines
- Agmatine
- UK 14304
- Guanabenz
- α -Methylnoradrenaline
- Thirst
- Dehydration

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Introduction

Clonidine, an antihypertensive drug that binds to α_2 -adrenoceptors (α_2 -AR), has been known for many years to be a drug that acts in the brain to inhibit water intake (for a review, see Ref. 1). Early experiments suggested that this effect of clonidine depends on the central activation of α_2 -AR since it is inhibited by yohimbine, an α_2 -AR antagonist (2,3). However, some experiments have shown that yohimbine does not fully antagonize the antidipsogenic effect of clonidine (3). A possible explanation for this result is that, in addition to binding to α_2 -AR, cloni-

dine also binds to imidazoline receptors (I-R) (4). The inhibition of water intake through activation of α_2 -AR has been recently confirmed in part by the antagonism of moxonidine, a mixed I₁-R/ α_2 -AR ligand, by the selective α_2 -AR antagonist RX 821002 (5). However, moxonidine is more selective for I₁-R than for α_2 -AR (6), and RX 821002, although considered a selective α_2 -AR antagonist, also has an imidazoline structure (7), thereby binding occasionally to imidazoline sites (8). This opens the possibility that imidazoline activation, in addition to α_2 -AR activation, also produces inhibition of water intake. An approach to test this hypo-

thesis is to screen the effects of drugs that not only are selective for one receptor, but, in this particular case, that also differ in terms of presence or absence of the imidazoline ring.

Imidazole-4-acetic acid (IAA) is an interesting imidazole ligand to imidazoline receptors, on the one hand because it does not bind to adrenoceptors and on the other hand, because it is produced endogenously as a metabolite of another imidazole, histamine (9). Histamine in turn also binds to I-R (9).

Another endogenous ligand to I-R, but also to α_2 -AR, is agmatine (decarboxylated arginine) (10). Mixed I-R/ α_2 -AR ligands of interest (7) like guanabenz (GBZ), a guanidine, and UK 14304, an imidazoline, inhibit sodium intake (11). Imidazolidine mixed ligands to I₁-R/ α_2 -AR, such as clonidine and moxonidine, are known to inhibit both water and sodium intake (1-3,5,12).

The effect of a selective non-imidazoline α_2 -AR ligand has not been tested on the behavior of water intake. A useful tool for this task is the α_2 -AR agonist α -methyl-noradrenaline (mNOR), which is likely to have the same inhibitory effect on water intake as noradrenaline (nonselective α - and β -AR ligand) does (13). Thus, in the present study we tested the effects of intracerebroventricular (*icv*) injections of IAA, histamine, agmatine, GBZ, UK 14304 and mNOR on water intake by water-deprived rats.

Material and Methods

Animals

Male Holtzman rats weighing 260-300 g at the beginning of the experiments were housed individually in a room on a 12/12-h light-dark cycle beginning at 7:00 am. Standard Purina pellets and tap water were available *ad libitum* unless otherwise stated. All experiments began between 8:00 and 9:00 am at least three days after surgery, when

body weight and daily water intake were back to pre-surgery values.

Surgery

The animals were maintained under tribromoethanol (Aldrich, Milwaukee, WI, USA) (20 mg/100 g body weight) anesthesia throughout surgery. A stainless steel guide cannula (15 x 0.7 mm OD) was stereotaxically implanted into the third cerebral ventricle (3rdV). Coordinates were AP = 0.2 mm behind bregma, V = 7.6 mm from cranial surface, L = 1.2 mm from lambda, incisor bar = 2.5 mm below the interaural line, and cannula at 10° angle from the sagittal plane. The cannula was secured to the top of the skull with acrylic cement and fastened with two screws. Insertion of a close-fitting stylet kept the lumen free of debris and clots. A prophylactic pre-surgical dose of penicillin (30,000 IU) was given intramuscularly.

Intracerebral injections

Single-pulse intracranial injections were made after gently removing the animal from its cage, replacing the stylet with an injector that protruded 1.0 mm beyond the tip of the guide cannula and that was connected by PE-10 tubing to a 10- μ l syringe, and injecting a total volume of 1.0 μ l over a period of 20 s. Stylet and injector were always wiped with cotton soaked in 70% alcohol between injections. After the injection, the injector was removed and replaced with the stylet, and the animal was returned to its cage for observation of its behavior.

Drugs

Agmatine was purchased from Sigma, (St. Louis, MO, USA) and IAA, histamine, GBZ, UK 14304 and mNOR were purchased from RBI (Natick, MA, USA). The drugs were dissolved in 0.9% saline, but GBZ and

mNOR were dissolved in acidified (pH = 4.6) 0.9% saline and UK 14304 was dissolved in 2:1 water:propyleneglycol solution.

Histology

At the end of the experiments, the animals were deeply anesthetized with chloral hydrate (Merck, Rio de Janeiro, RJ, Brazil) (20 mg/100 g body weight) and perfused with 10% formalin through the left ventricle of the heart. After fixation in 10% formalin, the brains were cut with a cryo-cut microtome. Transverse sections (50 μ m) were analyzed by light microscopy to confirm the position of the cannula in the 3rdV.

Statistical analysis

Data are reported as means \pm SEM. Two-way (drug and time as factors) analysis of variance was used for comparisons between groups followed by the Student-Newman-Keuls test. The level of significance was set at $P < 0.05$ for all tests.

Water intake test

Water was removed from the cage and then only food remained available for 24 h. Then, food was removed prior to returning water to the animals. Agonists or vehicle was injected into the 3rdV 20 min before the burettes containing water became available to the animals. Water intake was measured for 2 h (at 0, 15, 30, 60 and 120 min) in the absence of food. Water and standard food were returned to the animals at the end of the water intake test. The experiments were separated by 3-4-day intervals.

Results

Injection of the selective α_2 -AR agonist mNOR and water intake in water-deprived rats

Icv injection of mNOR (10, 20, 40, 80

and 160 nmol) reduced water intake from 39 to 76% during the whole test, except at 15 min for 10 and 40 nmol (Figure 1). Lower doses (2.5 and 5 nmol) of mNOR had no effect on water intake.

Injection of the mixed I-R/ α_2 -AR ligands GBZ, UK 14304 and agmatine and water intake in water-deprived rats

Icv injection of GBZ reduced water intake from 30% (2.5 nmol) to 65% (5, 10, 20, 40 and 80 nmol) during the whole test (Figure 2A).

UK 14304 (5 and 10 nmol) reduced water intake by 43 and 70% from 15 to 60 min and had no effect at 120 min (Figure 2B). UK 14304 (20 and 40 nmol) reduced water intake by 90-95% from 15 to 30 min and by 60-80% from 60 to 120 min (Figure 2B).

Agmatine (80 nmol) did not alter water intake (Figure 2C).

Injection of the non-adrenergic I-R ligands IAA and histamine and water intake in water-deprived rats

Icv injection of either IAA (40 and 80 nmol) or histamine (160 nmol) did not alter water intake in water-deprived animals (Figure 3).

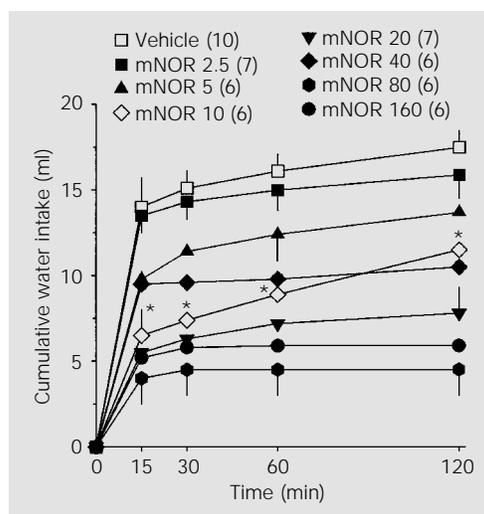
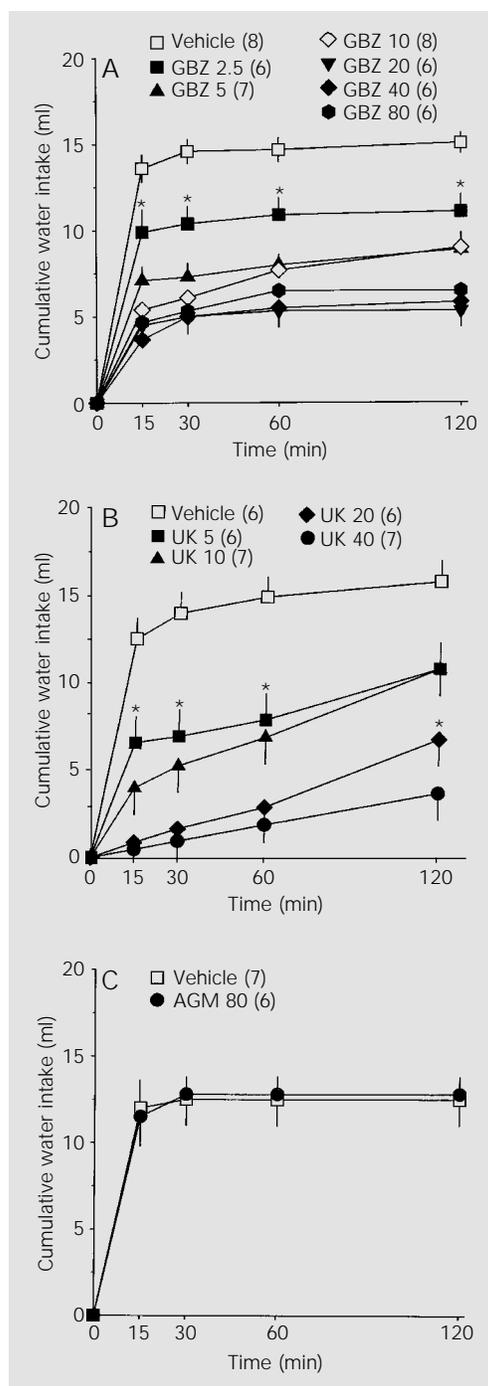


Figure 1. Cumulative water intake by 24-h water-deprived rats after intracerebroventricular injection of α -methylnoradrenaline (mNOR; 2.5, 5, 10, 20, 40, 80 and 160 nmol) or vehicle (open squares). The number of animals per group is given in parentheses. * $P < 0.05$ vs vehicle (Student-Newman-Keuls test).

Other behaviors

The highest dose (40 nmol) of UK 14304 produced motor deficits such as immobilization and prostration that lasted 30 min in 20% of the animals. This effect completely

Figure 2. Cumulative water intake by 24-h water-deprived rats after intracerebroventricular injection of: A, guanabenz (GBZ; 2.5, 5, 10, 20, 40 and 80 nmol); B, UK 14304 (UK; 5, 10, 20 and 40 nmol), and C, agmatine (AGM; 80 nmol) or vehicle (open squares). The number of animals per group is given in parentheses. * $P < 0.05$ vs vehicle (Student-Newman-Keuls test).



disappeared and the animals looked normal at lower doses. Neither GBZ nor mNOR produced any observable alteration in behavior other than inhibition of water intake.

Discussion

The mixed I-R/ α_2 -AR ligands GBZ and UK 14304, but not agmatine, inhibited water intake by water-deprived rats. A similar effect was obtained with α -mNOR, a selective α_2 -AR agonist. The non-adrenergic ligands to I-R, IAA and histamine, did not alter water intake.

GBZ is considered to be a selective α_2 -AR agonist, but it also binds to I₂-R (7). UK 14304 also has preference for I₂-R (7), but less affinity for α_2 -AR than GBZ (14). Agmatine, a ligand to α_2 -AR and to I₁-R, and a potential candidate as the endogenous clonidine-displacing substance (10), had no effect on water intake. This contrasts with the potent inhibitory effect that moxonidine and clonidine, also mixed ligands to α_2 -AR and to I₁-R, have centrally on water intake (1-3,5,12). Different from moxonidine and clonidine, agmatine has no hypotensive effect when injected centrally (15,16). It is still not known why it is hard to obtain an effect with agmatine.

IAA had no effect on water intake, suggesting that binding to I-R and not to α_2 -AR is not sufficient to inhibit water intake. This compound injected into the rostral ventrolateral medulla also causes hypotension attributed to activation of I-R (17). A possible involvement of IAA through GABA receptor binding (18,19) should have altered water intake (20), but such alteration did not occur in the present study. Histamine, a mixed histaminergic/imidazoline receptor ligand and a precursor of endogenous IAA (21), does not bind to α_2 -AR and also did not alter water intake. Failure to inhibit water intake by histamine can be attributed in part to its dipsogenic property expressed through activation of histaminergic receptors (22), but

no enhancement of water intake was observed in the present study.

The selective α_2 -AR ligand mNOR (14) inhibited water intake. This is the first demonstration that a non-imidazoline selective ligand to α_2 -AR inhibits water intake. The effect of mNOR on water intake is similar in potency to the effect of the endogenous ligand noradrenaline (13) and this suggests an inhibitory role for central α_2 -AR in the control of thirst. However, neither noradrenaline (13), mNOR or GBZ are able to fully inhibit water intake by water-deprived rats. This contrasts with the near complete inhibition of water intake by clonidine (13), moxonidine (5) and UK 14304 (present study).

The results of the present study are consistent with prior evidence for an inhibitory role of α_2 -AR in the control of water intake (2,3,5). However, a straightforward interpretation of the results favoring α_2 -AR on the one hand and ruling out I-R on the other is not yet possible. Consider, first, the complete central antagonism of moxonidine by RX 821002, a selective α_2 -AR antagonist (5). Nevertheless, RX 821002 is an imidazoline molecule which can also bind to I-R (8) and moxonidine is an imidazolidine selective for I-R compared to clonidine (6,14,17). Second, the inhibitory potency of the drugs on water intake seems to correlate with their molecular nature. Clonidine, moxonidine and UK 14304 are imidazolidines/imidazoline that bind to α_2 -AR and I-R and are the most potent inhibitors of water intake compared to the phenylethylamines noradrenaline, mNOR and phenylephrine (α_1 -AR agonist). To obtain the same amount of inhibition of water intake achieved with imidazolidines/imidazoline, it is necessary to increase by four- to eight- fold all the doses below the maximum dose of noradrenaline (13) and mNOR. In addition, full inhibition of water intake is not achieved with maximum doses of noradrenaline (13), mNOR and GBZ. It is noteworthy, however, that the minimum dose needed to obtain an effect with GBZ is four

times lower than the minimum dose needed to obtain an effect with mNOR. On the one hand, since GBZ binds weakly to I₁-R, the results favor the participation of α_2 -AR. On the other hand, the higher potency of GBZ compared to mNOR suggests that binding to I-R might help. In either case, GBZ did not exert full inhibition on water intake. Thus, some kind of interaction may occur between α_2 -AR and I-R, particularly in the imidazoline/imidazolidine domain, that is necessary for the full effect or potency of the agonists. Of course, this is not the only possible explanation and higher metabolic rates, for example, could also explain the need for higher doses of phenylethylamines to induce inhibition. Nevertheless, this hardly explains why the inhibition produced by these compounds never exceeds 70% in spite of the higher doses used.

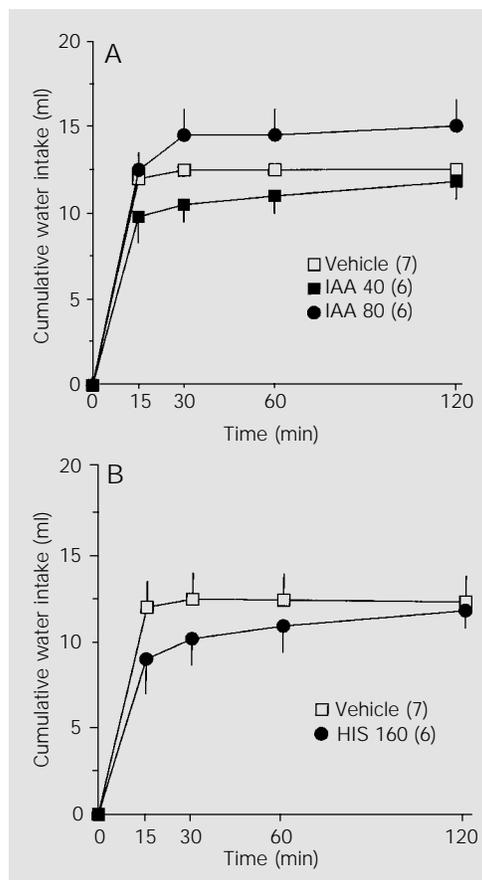


Figure 3. Cumulative water intake by 24-h water-deprived rats after intracerebroventricular injection of: A, imidazole-4-acetic acid (IAA; 40 and 80 nmol) and B, histamine (HIS; 160 nmol), or vehicle (open squares). The number of animals per group is given in parentheses.

Although evidence has pointed out distinct receptor populations (16), overlapping of α_2 -AR and I-R may also complicate the interpretation of the results. This is reflected by the ongoing controversy about the role of α_2 -AR and I-R as antihypertensive drugs (16,19,23,24). However, suggestions that an interaction between these receptors does occur are increasing (7) and our results are somehow consistent with them. Further data

on receptor characterization and cloning of imidazoline-binding sites (25) will also help to clarify possible interactions with α_2 -AR.

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