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Effects of α_1-adrenoceptors and muscarinic cholinoceptors on water intake in rats

Shahrbanoo Oryan[a], Maryam Eidi[b,*], Akram Eidi[c], Behrooz Kohanrooz[a]

[a] *Department of Biology, Science Faculty, Teacher Training University, Tehran, Iran*
[b] *Department of Biology, Varamin Campus, Islamic Azad University, P.O. Box: 16535-617, Tehran, Iran*
[c] *Department of Biology, Science and Research Campus, Islamic Azad University, Tehran, Iran*

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Abstract

The present study investigated the effects of α_1-adrenoceptors and muscarinic cholinoceptors on water intake in adult male rats. Intracerebroventricular (i.c.v.) injections were carried out in all experiments after 24-h deprivation of water. After deprivation, the volume of consumed water was measured for 1 h. Administration of pilocarpine, a muscarinic cholinoceptor agonist (0.5–1 μg/rat), and prazosin, the α_1-adrenoceptors antagonist (2 μg/rat), increased, while scopolamine, a muscarinic cholinoceptor antagonist (5–10 μg/rat), and phenylephrine, an α_1-adrenoceptor agonist (30 μg/rat), decreased water intake in rats. The activation of muscarinic cholinoceptors by pilocarpine attenuated the inhibitory effect induced by phenylephrine. Blockade of muscarinic cholinoceptors did not change the phenylephrine-induced response. Pretreatment with prazosin decreased the pilocarpine-induced response. However, pharmacological blockade of muscarinic cholinoceptors by scopolamine decreased the prazosin-induced effect on water intake. It is concluded that muscarinic cholinoceptors and α_1-adrenoceptors may interact on water intake.

Keywords: Water intake; Drinking; α_1-Adrenoceptor; Muscarine cholinoceptor; (Rat)

1. Introduction

Water intake is controlled by excitatory and inhibitory mechanisms that are still fully unclear, as shown by several reviews (Fitzsimons, 1989; Franci, 1994; McCann et al., 1989, 1994; Phillips, 1987). Noradrenaline acting on the central nervous system controls ingestive behavior. This neurotransmitter has an inhibitory action on water intake (Grossman, 1962; Leibowitz, 1980). How specific the inhibitory action of noradrenaline on fluid intake remains a question to be answered. The contrast between the induction of food intake and the inhibition of water intake produced by noradrenaline injected centrally (Grossman, 1962; Leibowitz, 1980) suggests that inhibition is specific for water intake (Yada et al., 1997). Therefore, the participation of central adrenoceptors in the inhibition of water intake has been proposed (De Luca and Menani, 1997).

Furthermore, cholinoceptor antagonist administration in the lateral hypothalamus reduces water intake (Sciorelli et al., 1972), whereas cholinergic agonists promote drinking (Sciorelli et al., 1972; Grossman, 1960). The detection of choline acetyltranferase-like immunoreactive structures also shows the existence of cholinergic elements in structures around the hypothalamic area (Rao et al., 1987) that have usually been associated with eating or drinking behaviors (Grossman, 1960).

To study the roles of and interactions between α_1-adrenoceptors and muscarinic cholinoceptors as they affect the regulation of water intake, the present experiments compared the effects of i.c.v.-administered α_1-adrenoceptors and muscarinic cholinoceptor agonists and antagonists alone or in combination on water intake in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (200–250 g) were placed in individual cages with ad libitum access to food and water available through a stainless steel spout attached to a

* Corresponding author. Tel.: +98-21-772448; fax: +98-21-2705836.
E-mail address: eidi@iauvaramin.ac.ir (M. Eidi).

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graduated glass cylinder mounted on the wall of the cage. The rats were kept at a controlled temperature (22–24 °C) with a 12-h light/12-h dark cycle and relative air humidity (40–60%).

2.2. Cannula guide implantation

The rats were anesthetized with ketamine–xylazine (100 mg/kg ketamine–5 mg/kg xylazine) and placed in a David Kopt apparatus. The skull was leveled between bregma and lambda. A stainless steel 21-gauge guide cannula (0.8 mm) was implanted into the lateral cerebral ventricle using coordinates from the atlas of Paxinos and Watson (1986) at least 5–7 days before testing. The coordinates used were 0.8 mm posterior to the bregma, 1.6 mm lateral to the midline and 3.4 mm below the top of the skull. The cannula was fixed to the skull using one screw and dental acrylic. A stylet was inserted into the cannula to keep it patent prior to injections.

2.3. Intracerebroventricular (i.c.v.) injections

The rats were gently restrained by hand, the stylet was withdrawn from the guide cannula and a 27-gauge injection needle (projecting 0.5 mm beyond the tip of the implanted guide cannula) was inserted. The injection needle was attached to a 5-μl Hamilton syringe by a polyethylene tube. The injection solutions were administered in a total volume of 2 μl. The injection needle was left in the guide cannula for an additional 30 s after the injection to facilitate diffusion of the drugs.

2.4. Drugs

The drugs included phenylephrine dihydrochloride, an α_1-adrenoceptor agonist, prazosin, an α_1-adrenoceptor antagonist (Sigma, Poole, UK), pilocarpine, a muscarinic cholinoceptor agonist (Sigma, St. Louis, MO) and scopolamine *N*-butylbromide, a muscarinic cholinoceptor antagonist, (Boehringer Ingelheim, Germany). All the drugs were dissolved in saline. The drugs were used (i.c.v.) in a volume of 2 μl/rat.

2.5. Experimental procedure

The experiments were performed in conscious freely moving rats 5–7 days after brain surgery. All rats were deprived of water overnight, for 24 h, before each test day. After 24-h water deprivation, the drugs were injected (i.c.v.) and water bottles were returned to the cages. All rats received two injections. Either a control saline injection followed 20 min later by injection of a drug, or one drug followed 20 min later by another to determine the effect of the first drug on the response to the second. In the control group, saline was injected 20 min before a second administration of saline. Immediately, after drug administration, water intake was recorded for 1 h by reading from the graduated glass cylinder mounted on the wall of the cages. The proposal was established and approved by the Research and Animal Ethical Committees of Teacher Training University, Tehran, Iran.

2.6. Data analysis

Data are reported as the means ± S.E.M. Analysis of variance (ANOVA) was used to test statistical significance. Differences were considered significant at $P < 0.05$.

2.7. Histology

At the end of the experiments, all rats were given 2 μl of methylene blue in a lateral ventricle, and were then deeply anaesthetized with ether and perfused transcardically with a phosphate-buffered saline solution (pH = 7.4). The brains were removed and placed in formaldehyde (4%). After 3 days, the brains were sliced into 60-μm slices. Data from rats with incorrect placement were excluded from the analysis.

3. Results

Fig. 1 shows the effect of phenylephrine alone or in combination with pilocarpine on water intake. Two-way ANOVA indicated that phenylephrine (30 μg/rat) interacted with pilocarpine (0.5 and 1 μg/rat) [pilocarpine, $F(2,42) = 41.77$, $P < 0.0001$; phenylephrine, $F(1,42) = 85.33$, $P < 0.0001$; pilocarpine × phenylephrine, $F(2,42) = 3.65$, $P < 0.05$]. Further analysis by one-way ANOVA showed that injections of pilocarpine increased water intake, while phenylephrine decreased this response. Activation of muscarinic cholinoceptors by pilocarpine attenuated the inhibitory effect induced by phenylephrine.

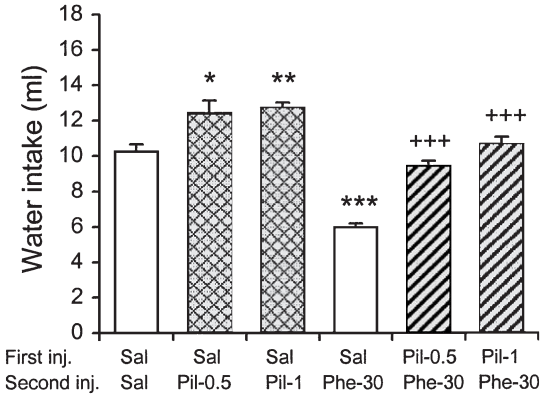

Fig. 1. Effect of phenylephrine alone or in combination with pilocarpine on water intake. Rats were injected (i.c.v.) with either saline or different doses of pilocarpine (0.5 and 1 μg/rat). Phenylephrine (30 μg/rat) was administered 20 min after injections of pilocarpine and water intake was measured for 1 h. Columns represent the means ± S.E.M. for eight rats. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ different from saline group. +++$P < 0.001$ different from phenylephrine control group.

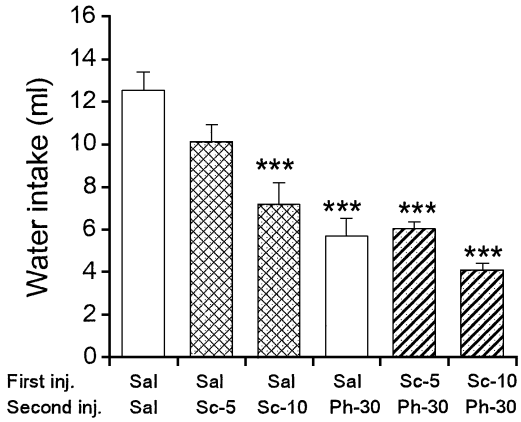

Fig. 2. Effect of phenylephrine alone or in combination with scopolamine on water intake. Rats were injected (i.c.v.) with either saline or different doses of scopolamine (5 and 10 μg/rat). Phenylephrine (30 μg/rat) was administered 20 min after injection of scopolamine and water intake was measured for 1 h. Columns represent the means ± S.E.M. for eight rats. ***$P<0.001$ different from saline group.

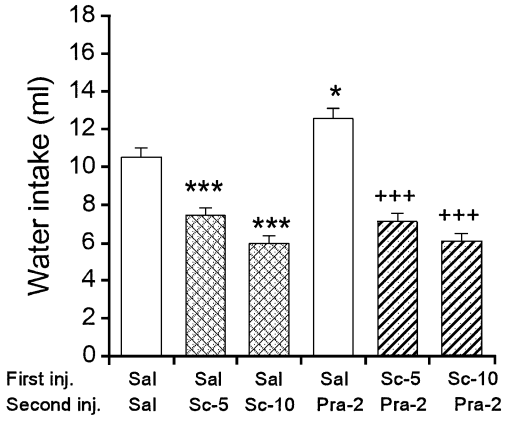

Fig. 4. Effect of scopolamine alone or in combination with prazosin on water intake. Rats were injected (i.c.v.) with either saline or different doses of scopolamine (5 and 10 μg/rat). Prazosin (2 μg/rat) was administered 20 min after injection of scopolamine and water intake was measured for 1 h. Columns represent the means ± S.E.M. for eight rats. *$P<0.05$, ***$P<0.001$ different from saline group. +++$P<0.001$ different from prazosin control group.

Fig. 2 shows the effect of phenylephrine alone or in combination with scopolamine on water intake. Two-way ANOVA indicated that the different doses of scopolamine (5 and 10 μg/rat) interacted with phenylephrine (30 μg/rat) [scopolamine, $F(2,42)=12.1$, $P<0.0001$; phenylephrine, $F(1,42)=61.5$, $P<0.0001$; scopolamine × phenylephrine, $F(2,42)=3.43$, $P<0.05$]. Further analysis by one-way ANOVA showed that both scopolamine and phenylephrine decreased water intake. Blockade of muscarinic cholinoceptors by scopolamine did not alter the inhibitory effect of α_1-adrenoceptors on water consumption.

Fig. 3 shows the effect of prazosin alone or in combination with pilocarpine on water intake. Two-way ANOVA indicated that the different doses of pilocarpine (0.5 and 1 μg/rat) interacted with prazosin (2 μg/rat) [pilocarpine, $F(2,42)=0.19$, $P>0.05$; prazosin, $F(1,42)=3.17$, $P>0.05$; pilocarpine × prazosin, $F(2,42)=13.89$, $P<0.0001$]. Further analysis by one-way ANOVA showed that both pilocarpine and prazosin increased water intake. Pretreatment with prazosin decreased the pilocarpine-induced response.

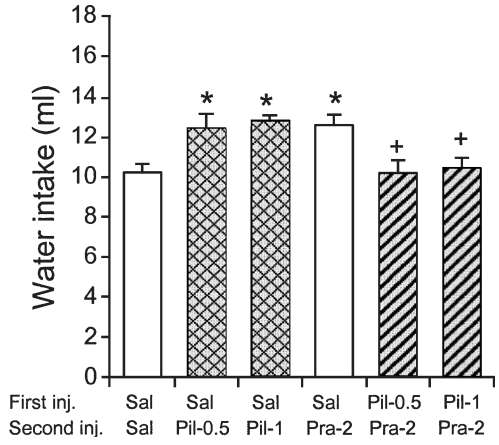

Fig. 3. Effect of prazosin alone or in combination with pilocarpine on water intake. Rats were injected (i.c.v.) with either saline or different doses of pilocapine (0.5 and 1 μg/rat). Prazosin (2 μg/rat) was administered 20 min after injections of pilocarpine and water intake was measured for 1 h. Columns represent the means ± S.E.M. for eight rats. *$P<0.05$ different from saline group. +$P<0.05$ different from prazosin control group.

Fig. 4 shows the effect of scopolamine alone or in combination with prazosin on water intake. Two-way ANOVA indicated that the different doses of scopolamine (5 and 10 μg/rat) interacted with prazosin (2 μg/rat) [scopolamine, $F(2,42)=91.24$, $P<0.0001$; prazosin, $F(1,42)=2.94$, $P<0.1$; scopolamine × prazosin, $F(2,42)=4.48$, $P<0.05$]. Further analysis by one-way ANOVA showed that scopolamine decreased, while prazosin increased water intake. Pharmacological blockade of muscarinic cholinoceptors with scopolamine decreased the prazosin-induced effect on water intake.

4. Discussion

Our results showed that i.c.v. administration of phenylephrine, an α_1-adrenoceptor agonist, decreased water intake in rats, while pharmacological blockade of α_1-adrenoceptors by prazosin increased this response.

Consistent with this, it has been reported that microinjection of phenylephrine into the lateral hypothalamus inhibited not only the angiotensin-II-induced dipsogenic effect, but also the drinking that follows water deprivation (Ferrari et al., 1991). Adrenergic agonists may have different effects on food and water intake, depending the dose of the specific agonists, route of administration and experimental conditions (Racotta et al., 1998). Other investigators have also suggested a role for central catecholaminergic pathways in the dipsogenic effects of angiotensin-II (Bastos et al., 1994; Fitzsimons and Setler, 1971, 1975; Gordon et al., 1979;

Jones, 1988). It has also been reported that the central actions of angiotensin-II are due, at least in part, to its interactions with neurotransmitters, especially catecholamines (Summers, 1992). It has also been suggested that adrenergic neurotransmitters in several hypothalamic areas may participate in angiotensin-II in full regulation of hydromineral fluid intake in a process that involves α_1-, α_2- and β-adrenoceptors (Bastos et al., 1994; Chan et al., 1991; Pereira da Silva et al., 1995).

The present results show that i.c.v. administration of pilocarpine, the muscarinic cholinoceptor agonist, increased, while pharmacological blockage of muscarinic receptors by scopolamine decreased water intake in rats.

Consistent with this, it was reported that drinking is triggered in non-deprived animals by activation of muscarinic receptors (Grossman, 1960; Sciorelli et al., 1972), whereas blockade of cholinoceptors in water-deprived animals results in drinking inhibition (Puig de Parada et al., 1997). Based on this evidence, it is tempting to speculate that the modifications of activity displayed by both neurochemical systems might well be related to the triggering and termination of this particular behavior (Puig de Parada et al., 1997). Water presentation when the organism is under the influence of water deprivation elicits activation of lateral hypothalamus cholinergic neurons, which must be one of the natural events contributing to the animal's drive to drink. The acetylcholine increase detected during the first 20 min after water presentation suggests such cholinergic activation. A reduction in cholinergic activity is apparently a consequence of water replenishment, and such modifications might be part of the neurophysiological events contributing to drinking termination (Puig de Parada et al., 1997). Thirst drive is partly regulated by the cholinergic stimulation of the lateral hypothalamus (Grossman, 1960) and perifornical regions (Fisher and Coury, 1962) induces drinking.

In the present experiment, effects of the interaction between α_1-adrenoceptors and muscarinic cholinoceptors on water intake were studied. The results show that the activation of muscarinic cholinoceptors by pilocarpine attenuated the inhibitory effect induced by phenylephrine. Blockade of muscarinic cholinoceptors did not change the phenylephrine-induced response. Pretreatment with prazosin decreased the pilocarpine-induced response. Pharmacological blockade of muscarinic cholinoceptors by scopolamine decreased the prazosin-induced effect on water intake. There is considerable evidence suggesting that cholinergic as well as noradrenogenic processes in the hypothalamus participate in the regulation of water intake. It is well known that cholinergic stimulation of the hypothalamus induces water intake (Grossman, 1960; Takahashi et al., 2001; Fisher and Coury, 1962; Levitt and Fisher, 1966). Previous experiments showed that cholinergic and adrenergic synapses located within the hypothalamus mediated the mechanisms which are involved in water and electrolyte balance (Bastos et al., 1994). It has been suggested that the effects on water intake are mediated by the hypothalamus via noradrenogenic axons that stimulate α-adrenoceptors on cholinergic interneurons.

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