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# Clonidine and Phenylephrine Injected Into the Lateral Preoptic Area Reduce Water Intake in Dehydrated Rats

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CALLERA, J. C., L. A. A. CAMARGO, L. A. DE LUCA, JR., J. V. MENANI, A. RENZI AND W. A. SAAD. *Clonidine and phenylephrine injected into the lateral preoptic area reduce water intake in dehydrated rats.* PHARMACOL BIOCHEM BEHAV **46**(1) 39–43, 1993. – In the present study, we investigated the effect of phenylephrine and clonidine ($\alpha_1$- and $\alpha_2$-adrenoceptor agonists, respectively) injected into the lateral preoptic area (LPOA) on the water intake induced by water deprivation in rats. In addition, the effects of prior injections of prazosin and yohimbine ($\alpha_1$- and $\alpha_2$-adrenoceptor antagonists, respectively) into the LPOA on the antidipsogenic action of phenylephrine and clonidine were investigated. After 30 h of water deprivation, the water intake of rats in a control experiment (saline injection) was 10.5 ± 0.8 ml/h. Injection of clonidine (5, 10, 20, and 40 nmol) into the LPOA reduced water intake to 6.3 ± 0.9, 4.9 ± 0.8, 3.6 ± 1.0, and 2.2 ± 0.7 ml/h, respectively. Similar reductions occurred after injection of 80 and 160 nmol phenylephrine into the LPOA (6.2 ± 1.6 and 4.8 ± 1.3 ml/h, respectively). Pretreatment with prazosin (40 nmol) abolished the antidipsogenic action of an 80-nmol dose of phenylephrine (11.3 ± 1.1 ml/h) and reduced the effect of a 20-nmol dose of clonidine (7.4 ± 1.4 ml/h). Yohimbine (20, 40, and 80 nmol), previously injected, produced no significant changes in the effects of either phenylephrine or clonidine. The present results show that phenylephrine and clonidine injected into the LPOA induce an antidipsogenic effect in water-deprived rat. They also suggest an involvement of $\alpha_1$-adrenoceptors in this effect. A possible participation of imidazole receptors in the effect of clonidine should also be taken into account.

Water intake  Preoptic area  α-Adrenoceptors  Thirst  Clonidine

SEVERAL studies have shown the importance of central catecholaminergic systems in the control of food and water intake (5,10–16,18,21–24,26). It has been described that either central or peripheral administration of clonidine (an $\alpha_2$-adrenoceptor agonist) impairs the dipsogenic response to peripheral administration of isoproterenol, hypertonic saline, and angiotensin II (ANGII), as well as to ICV ANGII and carbachol (13–16,21). Prior treatment with yohimbine (an $\alpha_2$-adrenoceptor antagonist) blocked the antidipsogenic action of ICV clonidine, suggesting an involvement of central $\alpha_2$-adrenoceptors in this effect (15). Recent studies from our laboratory have shown that not only clonidine but also phenylephrine (an $\alpha_1$-adrenoceptor agonist) injected into the lateral hypothalamus (LH) reduces the water intake induced by ANGII (10).

In the present study, we investigated the effect of clonidine and phenylephrine injected into the lateral preoptic area (LPOA) on the water intake induced by 30 h of water deprivation in rats. In addition, the effects of pretreatment in the same area with prazosin (an $\alpha_1$-adrenoceptor antagonist) and yohimbine on the antidipsogenic action of clonidine and phenylephrine were also studied. The LPOA is one of the more rostral structures of the rat brain involved in control of water intake (3). The existence of cholinergic and adrenergic pathways in the LPOA related to control of water intake has also been demonstrated (6,19).

## METHOD

### *Animals*

A total of 120 male Holtzman rats weighing 240–280 g were housed in individual metabolic cages, with free access to Purina chow and tapwater.

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*Brain Surgery*

Rats were maintained under ether anesthesia and restrained in a stereotaxic apparatus (Krieg model). A longitudinal incision was made on the skin of the animal's head. The SC tissue was pulled back and the skull was drilled with a spherical drill. A stainless steel cannula (14 × 0.7 mm o.d.) was introduced into the right LPOA. The coordinates for approaching the LPOA obtained from the Konig and Klippel atlas (20) were the following: 1.4 mm rostral to bregma, 1.6 mm lateral to midline, and 7.0 mm below the dura mater. The cannula was fixed to the skull with microscrews and acrylic resin. A prophylactic dose (60,000 IU) of antibiotic (Pentabiotico Fontoura Wyeth) was injected after surgery.

*Drug Injection*

Drug solutions in 0.15 M NaCl were injected into the LPOA by means of a Hamilton microsyringe (Hamilton Co., Reno, NV) (10 μl). The microsyringe was connected with PE-10 polyethylene tubing (25 cm) to a needle (0.3 mm o.d.), which was introduced into the brain through the implanted cannulae. The needle for injection into the LPOA was 2 mm longer than the guide cannula. The volume of injection was always 1 μl injected over a period of 30–60 s.

*Drugs*

L-phenylephrine HCl (Sigma Chemical Co., St. Louis, MO), clonidine HCl (Boehringer-Ingelheim, Ridgefield, CT), prazosin HCl (Pfizer, New York, NY), yohimbine HCl (Sigma). Saline (0.15 M NaCl) was used for control injections.

*Histology*

After the experiments, animals were deeply anesthetized with either and perfused through the heart with 10% formalin. The brain was removed and stored in 10% formalin for at least 1 week. The brain was then frozen and transverse sections (20–30 μm were stained with hematoxylin & eosin for examination by light microscopy. Only the results of rats (94 animals) whose LPOA was reached by the injection were used. Figure 1 shows the site of injection into LPOA.

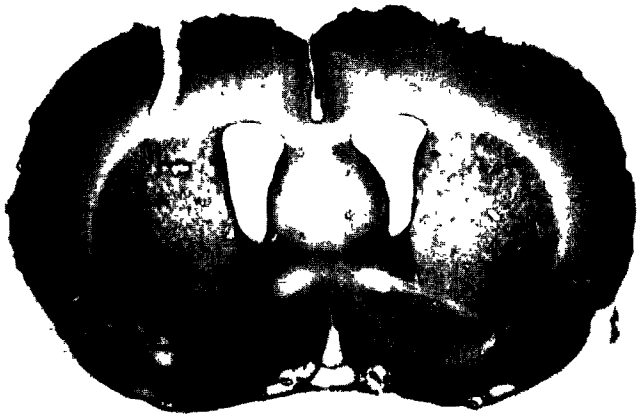

FIG. 1. Photomicrograph showing the site of injection (arrow) into the lateral preoptic area.

*Statistical Analysis*

The results are reported as mean ± SEM. The one-way analysis of variance and Student–Newmans–Keul's test were used for testing the significance. Values were considered statistically significant when $p < 0.05$.

*Experimental Protocol*

*Water ingestion.* Five days after brain surgery, animals were submitted to water, but not food, deprivation for a period of 30 h. After this period, rats were injected, into the LPOA, with an α-adrenoceptor agonist (either clonidine or phenylephrine) and water was offered 20 min later. The α-adrenoceptor antagonists (either prazosin or yohimbine), when used, were injected into the LPOA 20 min before injection of either clonidine or phenylephrine. The volume of water intake was measured during 1 h after injection of the α-adrenoceptor agonist, using graduated tubes adapted with metal spouts for drinking. No solid food was offered during the experiments. Each rat was submitted to five experimental sessions using different drugs or different doses of the same drug in each experimental session. The interval between two experimental sessions was 3 days. The following injections were performed into the LPOA: control (0.15 M NaCl); 0.15 M NaCl + clonidine (2.5, 5, 10, 20, and 40 nmol); 0.15 M NaCl + phenylephrine (20, 40, 80, and 160 nmol); prazosin (10, 20, and 40 nmol) + clonidine (20 nmol); prazosin (10, 20, and 40 nmol) + phenylephrine (80 nmol); yohimbine (20, 40, and 80 nmol) + clonidine (20 nmol); yohimbine (20, 40 and 80 nmol) + phenylephrine (80 nmol).

## RESULTS

*Water Intake After Injection of Either Clonidine or Phenylephrine Into the LPOA of Water-Deprived Rats*

The water intake observed after 30 h of water deprivation in the control experiment (injection of 0.15 M NaCl into the LPOA) was 10.5 ± 0.8 ml over a period of 1 h. Injection of clonidine (5, 10, 20, and 40 nmol) into the LPOA reduced water intake to 6.3 ± 0.9, 4.9 ± 0.8, 3.6 ± 1.0, and 2.2 ± 0.7 ml/h, respectively (Fig. 2A). The 2.5-nmol dose of clonidine produced no change in water intake (8.2 ± 1.4 ml/h).

Phenylephrine (80 and 160 nmol) injected into the LPOA also reduced water intake to 6.2 ± 1.6 and 4.8 ± 1.3 ml/h, respectively (Fig. 2B). The doses of 20 and 40 nmol phenylephrine produced no significant change (7.6 ± 1.5 and 6.5 ± 1.5 ml/h).

In some rats with incorrect cannulae placement, it was possible to observe that injections of clonidine and phenylephrine into the medial preoptic area or lateral hypothalamus produced results similar to that observed with injections of these drugs into the LPOA.

*Effect of Pretreatment With Either Prazosin or Yohimbine on the Antidipsogenic Action of Phenylephrine and Clonidine*

Injections of prazosin (10, 20, and 40 nmol) into the LPOA blocked the antidipsogenic action of phenylephrine (80 nmol) into the same area (Fig. 3A). The 40-nmol dose of prazosin, but not 10 and 20 nmol, also reduced the antidipsogenic effect of 20 nmol clonidine (Fig. 3B).

Yohimbine (40 and 80 nmol) pretreatment produced a small alteration in the antidipsogenic action of phenylephrine

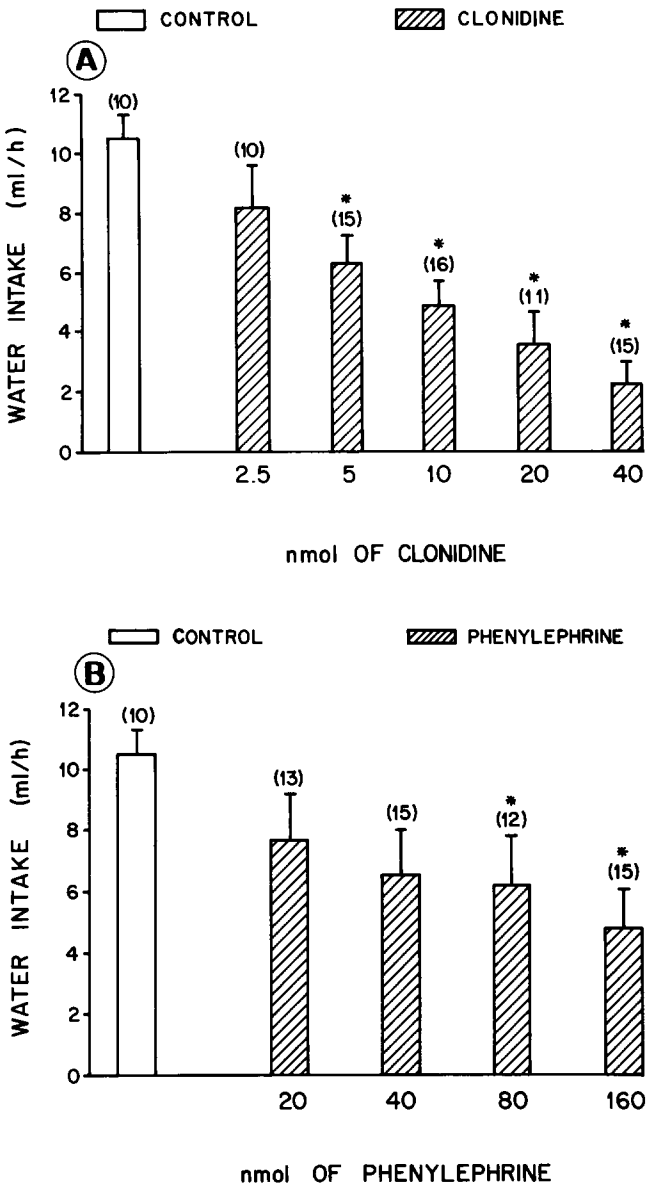


FIG. 2. Water intake after 30 h of water deprivation in control (0.15 M NaCl) and after injection of: (A) clonidine or (B) phenylephrine into the lateral preoptic are. The results are represented as means ± SEM. The number of animals is indicated at the top of each column. *Significant differences ($p < 0.05$) as compared with the control group.

(80 nmol) but no change in the antidipsogenic effect of clonidine (Fig. 4).

## DISCUSSION

Phenylephrine and clonidine injected into the LPOA reduced water intake of dehydrated rats. Pretreatment with prazosin, in all doses used, abolished the antidipsogenic effect of phenylephrine, and the highest dose also reduced the effect of clonidine. Yohimbine produced small changes in the effect of phenylephrine but no change in the effect of clonidine.

Several studies have shown that central catecholamines are involved in the control of water intake (11,17,18,22-24). An important role of central catecholaminergic pathways in the dipsogenic response to ANGII is suggested (2,12,19,25). On the other hand, central clonidine, noradrenaline, and other adrenergic drugs have also been shown to reduce water intake induced by ANGII, hypertonic saline, central cholinergic activation, and water deprivation (10,14-16,18,21-24).

Jones (19) observed that bilateral injections of either phentolamine (a nonspecific $\alpha$-adrenoceptor antagonist) or prazosin, but not yohimbine, into the rostral hypothalamus, including the LPOA, depressed the pressor and drinking responses induced by ICV ANGII in rats, suggesting an involvement of

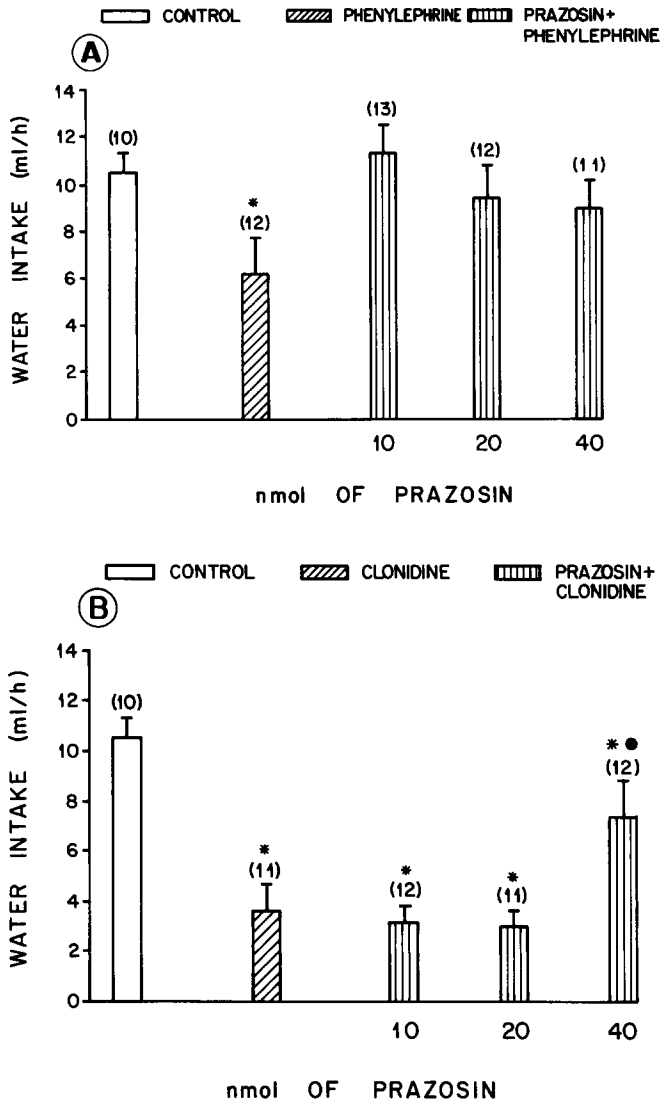


FIG. 3. Water intake after 30 h of water deprivation in controls (0.15 M NaCl) and after injection of : (A) prazosin + phenylephrine (80 nmol) and (B) prazosin pl clonidine (20 nmol) into the lateral preoptic are. The results are represented as means ± SEM. The number of animals is indicated at the top of each column. *Significant differences ($p < 0.05$) compared with the control group; •significant difference ($p < 0.05$) compared with the clonidine-alone group.

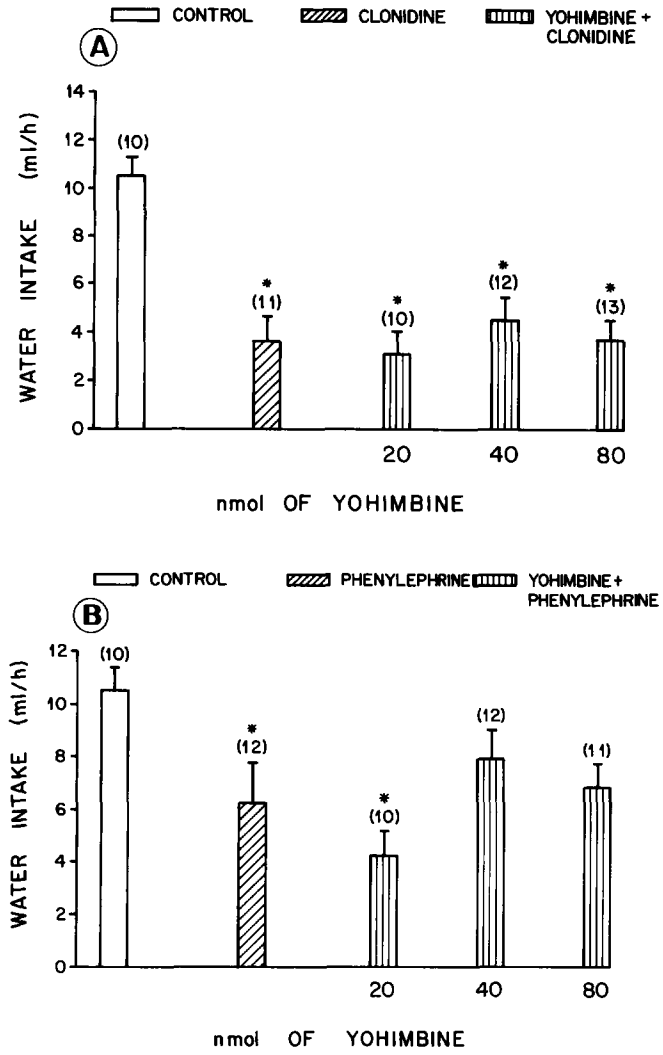


FIG. 4. Water intake after 30 h of water deprivation in controls (0.15 M NaCl) and after injection of: (A) yohimbine + clonidine (20 nmol) and (B) yohimbine + phenylephrine (80 nmol) into the lateral preoptic area. The results are represented as means ± SEM. *Significant differences ($p < 0.05$) compared with the control group.

$\alpha_1$-adrenoceptors of this area in the response to central ANGII. The present results show that adrenergic activation of the LPOA induces an antidipsogenic effect in water-deprived rats. Together, the results of Jones (19) and ours suggest that, despite their excitatory effect, the adrenergic pathways of LPOA could also be involved in a central inhibitory system for water intake. An involvement of $\alpha_1$-adrenoceptors of the LPOA in the antidipsogenic response induced by clonidine and phenylephrine is proposed. In the case of clonidine, although it is considered mainly an $\alpha_2$-adrenoceptor agonist it may also stimulate $\alpha_1$-adrenoceptors (1,27). Because the antidipsogenic effect of clonidine was only partially reduced by prazosin and no significant change was produced by yohimbine, the involvement of other types of receptors in the response to clonidine could also be suggested. Contrary to the present results, Fregly et al. (16) reported that ICV clonidine and phenylephrine act through $\alpha_2$-adrenoceptors to impair the dipsogenic response induced by ICV ANGII. These differences are probably related to the sites of injections. Fregly et al. (16) studied the role of the periventricular adrenoceptors, whereas the present results were obtained with injections in an area far from the brain ventricular system. In addition, the present results suggest that the antidipsogenic effect of central clonidine is not exclusively mediated by $\alpha_2$-adrenoceptor activation. The central action of clonidine may be mediated, at least in part, by putative imidazole receptors (4,8,9). The existence of imidazole binding sites in the rat ventrolateral medulla has recently been established (8). The endogenous substance that acts at the imidazole sites is unknown, but a potential candidate is the clonidine-displacing substance (CDS) (9). Thus, the inhibitory effect of clonidine injected into LPOA on water ingestion could be due to its action on $\alpha_1$-adrenoceptors and imidazole receptors.

In summary the present results show an antidipsogenic action of phenylephrine and clonidine injected into the LPOA in water-deprived rats. An involvement of $\alpha_1$-adrenoceptors in this effect is suggested, and imidazole receptors could also participate in explaining the effects of clonidine.

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