Central injection of captopril inhibits the blood pressure response to intracerebroventricular choline

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Abstract

In the present study, we investigated the involvement of the brain renin-angiotensin system in the effects of central cholinergic stimulation on blood pressure in conscious, freely moving normotensive rats. In the first step, we determined the effects of intracerebroventricular (icv) choline (50, 100 and 150 µg) on blood pressure. Choline increased blood pressure in a dose-dependent manner. In order to investigate the effects of brain renin-angiotensin system blockade on blood pressure increase induced by choline (150 µg, icv), an angiotensin-converting enzyme inhibitor, captopril (25 and 50 µg, icv), was administered 3 min before choline. Twenty-five µg captopril did not block the pressor effect of choline, while 50 µg captopril blocked it significantly. Our results suggest that the central renin-angiotensin system may participate in the increase in blood pressure induced by icv choline in normotensive rats.

Introduction

Central cholinergic neurons and cholinergic receptors are involved in cardiovascular regulation (1-6). It has been shown that intracerebroventricularly (icv) injected choline increases blood pressure and decreases heart rate in conscious, freely moving, normotensive rats (2,4,6). In a number of studies, it has been reported that the effects of cholinomimetics on the cardiovascular system are mediated by central muscarinic (1,5) or both nicotinic and muscarinic receptor activation (6), which results in the activation of the sympathoadrenergic system (5,6). The vasopressinergic system also mediates the effects of choline on blood pressure. Centrally injected acetylcholine and several other cholinergic agents have been shown to increase vasopressin (VP) secretion (6-8).

Another system involved in blood pressure regulation is the renin-angiotensin system (9-11). All elements of the renin-angiotensin system have been identified in brain tissue (12-15). Central angiotensin II (Ang II) increases arterial blood pressure, stimulates drinking behavior, increases VP and corticotropin secretion and inhibits renin secretion (12,16-21). The effect of central Ang II on heart rate is controversial. Investigators have found that centrally injected Ang II decreases, increases or does not affect heart rate (18,22-27). The central effects of Ang II on blood pressure are mediated by an in-

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crease in sympathetic tone and depend on the integrity of central catecholaminergic neurons (9,10).

Arslan et al. (6) and Ulus et al. (8) have reported an increase in blood pressure and a decrease in plasma renin activity following icv choline injection. It has been concluded that the peripheral renin-angiotensin system does not participate in the effect of central cholinergic stimulation on blood pressure. Others have observed an increase in plasma renin activity due to intravenous (iv) injection of physostigmine, an indirect cholinomimetic agent (28). Since centrally administered cholinomimetics inhibit plasma renin activity by increasing VP secretion, it is not surprising that iv physostigmine does not have the same effect.

The central relationship between the angiotensinergic and cholinergic systems in cardiovascular regulation is not fully understood. Therefore, the objective of the present study was to determine whether the increase in blood pressure due to central cholinergic stimulation would be changed by inhibition of the central renin-angiotensin system, using captopril, a specific and potent inhibitor of Ang II formation.

Material and Methods

Male Sprague-Dawley rats (Experimental Animals Breeding and Research Centre, Uludag University Medical Faculty, Bursa, Turkey) weighing 250-300 g were used in this study. Rats were housed 4-6 to a cage with food and water available ad libitum. The colony room was maintained at 20-24°C with a 12-h light-dark cycle.

The surgical and experimental protocols used were approved by the Animal Care and Use Committee of Uludag University.

Under ether anesthesia, rats were implanted through the right femoral artery with PE 50 tubing filled with heparinized saline (100 U/ml). For icv injections, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1-1.5 mm posterior to the bregma on the right side. Through this hole, a 10 mm length of 20 gauge stainless steel hypodermic tubing was directed toward the right lateral ventricle. The cannula was lowered perpendicularly 4-4.5 mm below the surface of the skull and fixed to the skull with acrylic cement.

Following the surgical procedure, rats were placed in individual cages and allowed to recover from anesthesia for at least 4 h. During the recovery period, the animals showed no evidence of pain.

Following recovery, the arterial line was connected to a volumetric pressure transducer (Statham P23) and blood pressure was recorded continuously on a polygraph (Grass 7D model). Systolic blood pressure (mmHg) was recorded throughout the experiment. Control baseline recordings were obtained for 15 min before the drug injections.

In the first step, choline (50, 100 or 150 µg/10 µl) or saline (10 µl) was injected icv, in order to observe the effect of icv choline on blood pressure. In the second step, we investigated the involvement of the central renin-angiotensin system in the action of choline on blood pressure. For this purpose, rats were injected icv with the angiotensin-converting enzyme inhibitor captopril (25 or 50 µg/10 µl) or saline (10 µl) 3 min before 150 µg icv choline (the dose of choline to which a long-lasting blood pressure response was obtained).

At the end of the experiments, 5 µl of a methylene blue solution was injected into the cerebral ventricle through the cannula to confirm the placement of the inner end of the cannula. After decapitation, the brains were removed and sections were observed macroscopically to ascertain whether the cannula had been correctly placed into the lateral cerebral ventricle.

Choline chloride and captopril were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in saline. All doses of drugs refer to the free base in 10 µl.
Blood pressure response to central choline and captopril

Results

Effect of icv choline on blood pressure

In the saline-treated control group, no significant difference was found in the systolic blood pressure before and after saline injection (Figure 1). Also, 50 µg choline did not produce a significant increase in blood pressure, except for an initial significant rise (P<0.05), which disappeared after a few minutes. In contrast, 100 µg and 150 µg choline induced an immediate and marked increase in blood pressure (P<0.01 and P<0.001, respectively). Ten minutes after 100 µg choline injection, the increase in blood pressure was no longer significant compared to pretreatment values. On the other hand, 150 µg choline induced a more significant and long-lasting increase in blood pressure. Mean systolic blood pressure was 114 ± 2.5 mmHg before 150 µg choline injection and significantly increased to 130 ± 1.2 mmHg within the second minute after the injection (P<0.001), followed by a value of 133 ± 1.4 mmHg 5 min later (P<0.001) and 136 ± 1.7 mmHg 10 min later (P<0.001). The blood pressure values were still significantly higher (129 ± 1.3 mmHg) during the 15th minute (P<0.001). The values obtained 20 and 25 min after the injection did not differ significantly from pretreatment values.

Role of icv captopril in the action of icv choline on blood pressure

The angiotensin-converting enzyme inhibitor captopril given icv at a dose of 25 µg did not affect blood pressure significantly (Figure 2). With 50 µg icv captopril a decrease in blood pressure was observed, which was significant by the 10th minute after injection (P<0.05) and was followed by significantly lower values 15, 20 and 25 min after injection compared to pretreatment values (P<0.001).

Data are reported as means ± SEM. Data were analyzed statistically by analysis of variance, with the level of significance set at P<0.05.

Figure 1. Effect of intracerebroventricular injection of choline on blood pressure in normotensive rats. Choline was injected at three doses (50, 100, and 150 µg/10 µl) (time “0”), following baseline blood pressure recordings (time “-5”). Data points indicate the mean ± SEM for 7 rats. *P<0.05, **P<0.01 and +P<0.001 compared to pretreatment values (ANOVA).

Figure 2. Effect of intracerebroventricular injection of captopril on blood pressure in normotensive rats. Captopril was injected at doses of 25 or 50 µg/10 µl (time “0”), following baseline blood pressure recordings (time “-5”). Values represent the mean ± SEM for 7 rats. *P<0.05 and **P<0.001 compared to pretreatment values (ANOVA).
Previous injection of 25 µg captopril (3 min) did not block the pressor response induced by 150 µg choline (Figure 3), with no difference compared to the saline + 150 µg choline group. On the other hand, 50 µg captopril significantly inhibited the blood pressure rise induced by 150 µg choline (P<0.05 and P<0.001) 2 and 5 min after choline injection. Since 50 µg captopril per se decreases blood pressure 10 min after injection, we analyzed the effect of captopril on choline-induced increase in blood pressure only during the first 10-min period. Indeed, the effect of choline on blood pressure was maximum throughout this corresponding period, decreasing slightly thereafter.

**Discussion**

Our results demonstrate that icv injected choline increases blood pressure in conscious, freely moving, normotensive rats. The effect of choline was dose- and time-dependent under normotensive conditions, in agreement with previous studies. Previous studies have shown that centrally administered choline increases blood pressure in both normotensive and hypotensive rats. The pressor effect of choline on normotensive rats is lower and shorter (5 min) than that in hemorrhage-induced hypotensive rats (2,4,8). The pressor effects of choline and other cholinomimetics on the cardiovascular system are associated with the sympathoadrenergic system and VP, as proven by the increase in both catecholamines and VP following central choline injection (1,6,8).

Centrally administered Ang II also increases blood pressure and this effect is especially important in hypovolemic conditions (12,15,16,19,29-31). Intracerebroventricular injections of renin-angiotensin system antagonists decrease blood pressure in spontaneously hypertensive and normotensive rats (9,17,19,20,32,33). We also confirmed that captopril, an angiotensin-converting enzyme inhibitor, decreases blood pressure in a dose-dependent manner.

In the present study, we tried to determine the participation of the brain renin-angiotensin system in the blood pressure response to central choline injections. Our results showed that the rise in blood pressure induced by choline was significantly reduced by prior central injection of 50 µg captopril. We did not observe a similar effect with 25 µg captopril, which suggests that captopril dose-dependently inhibits the effect of centrally administered choline on blood pressure. The attenuation of the blood pressure response to icv choline did not seem to be related to the blood pressure-lowering effect of captopril, since captopril becomes significantly effective at the 10th minute following injection, as shown in Figure 2. Therefore we may interpret our data to indicate a central interaction of the renin-angiotensin system and the cholinergic system.

There are a number of reports concerning the relationship between these two systems but the results are contradictory. Moore and Drexler (29) have pointed out that iv atropine sulfate and icv hemicholinium signifi-

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Figure 3. Effect of intracerebroventricular captopril (25 or 50 µg/10 µl) on blood pressure response to central choline (150 µg) injection. Captopril was injected 3 min before choline. Time “0” represents the point of choline injection. Values represent the mean ± SEM for 7 rats. *P<0.05 and **P<0.001 compared to the saline + 150 µg choline group (ANOVA).
cantly inhibit the blood pressure response to *icv* Ang II. They have also reported that methyl atropine, which does not cross the blood-brain barrier, does not significantly inhibit the effect of *icv* Ang II on blood pressure and concluded that the inhibitory action of atropine itself might be centrally mediated.

Nicoletta et al. (22) have also shown that *icv* hemicholinium and *icv* atropine reduce the blood pressure increase induced by *icv* Ang II. Hemicholinium did not affect the Ang II-induced bradycardia, while high doses of atropine significantly reduced it. On the other hand, inhibition of the brain renin-angiotensin system by *icv* captopril (25 µg and 50 µg) has been reported not to change the blood pressure and heart rate response to central physostigmine injection. The latter results are not consistent with our findings, since the brain renin-angiotensin system has not been considered to participate in the cardiovascular effects induced by either systemic or central cholinergic stimulation. The discrepancy between our results and those of Nicoletta et al. may be due to the different cholinergic system agonist and the different dosage used in the experiments, or to the different time of captopril injection.

In contrast, recent data have provided evidence supported by the present results. Saad et al. (34) have reported that central administration of losartan, an AT<sub>1</sub>-receptor blocker, and ramipril, a converting enzyme inhibitor, effectively blocks the pressor response induced by *icv* Ang II and *icv* carbachol. These investigators concluded that the pressor effects of Ang II and carbachol involve the central angiotensin system and AT<sub>1</sub>-receptor activation. In contrast, Hoffman and Phillips (35) suggested that angiotensin and carbachol act upon independent receptors in the brain to produce blood pressure and drinking responses, but at some point they share common central effector pathways. In fact, both systems involve the activation of vasopressinergic and sympathoadrenergic systems and this may account for their similar effects on blood pressure. We also suggest a participation of the central angiotensinergic system, at some point, in the action of the cholinergic system, since inhibition of Ang II formation dose-dependently blocked the blood pressure-increasing effect of choline.

In conclusion, our results suggest a role for the endogenous brain renin-angiotensin system in the blood pressure increase in response to *icv* choline in normotensive rats.

**References**