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Anterior hypothalamic β-adrenergic activity in the maintenance of hypertension in aortic coarctated rats

Christian Höcht*, Javier A.W. Opezzo, Carlos A. Taira

Department of Pharmacology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Junín 956, (C1113AAD) Buenos Aires, Argentina

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Abstract

The aim of this work was to demonstrate an alteration of the anterior hypothalamic catecholaminergic system in aortic coarctated (ACo) rats by the perfusion of β-adrenergic antagonist and the microinfusion of β-adrenergic agonist. Wistar urethane-chloralose anesthetized rats were used. The carotid artery was cannulated for blood pressure recording and changes in blood pressure were measured. A concentric microdialysis probe was inserted in the anterior hypothalamus. Metoprolol (a β_1-adrenoceptor antagonist) perfusion (6 $\mu g\,ml^{-1}$) reduced the mean arterial pressure (MAP) in the ACo rats but not in sham operated (SO) animals. The anterior hypothalamic infusion of non-specific β-adrenergic agonist isoproterenol induced a dose-dependent decrease of blood pressure in both experimental groups, but the depressor response was significantly lower in ACo rats. The pretreatment with atenolol, a selective β_1-adrenoceptor antagonist, increased the depressor effect of isoproterenol in ACo rats, but not in SO rats. On the other hand, the hypotensive action of isoproterenol was significantly diminished after the administration of non-specific β-adrenoceptor antagonist propranolol in SO and ACo rats. The anterior hypothalamic infusion of clenbuterol, a selective β_2-adrenergic agonist, induced a dose-dependent decrease of blood pressure in both experimental groups. The depressor response to clenbuterol (1 nmol) was significantly lower in ACo rats than in SO rats.

In summary, this study provides the evidence that there is a β_1-adrenergic compromise in anaesthetized ACo rats and this compromise may be involved in the maintenance of hypertension. On the other hand, this study also suggests the existence of pressor β_1-adrenoceptors in the anterior hypothalamic area of ACo rats but not in SO rats. We also found a diminished depressor β_2-adrenergic activity in ACo rats.
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Keywords: Aortic coarctation; Reverse microdialysis; Anterior hypothalamic area; Metoprolol; Blood pressure; Isoproterenol; Clenbuterol

1. Introduction

Most forms of experimental hypertension are associated with a wide of functional changes in the hypothalamus [1]. The hypertensive state appears to be due to an increase in medullar pressor activity due to suppression from above of medullar activity [2,3]. The medulla's excitatory center is under the influence of the hypothalamus, the midbrain, a medullar inhibitor center slightly more caudal than the excitatory center, and the *nucleus tractus solitarius* [2,4].

The anterior hypothalamic area and the associated preoptic nuclei are primarily depressor regions due to an inhibition of the sympathetic nervous system and activation of vagal output to the heart [5]. It was also suggested that the anterior hypothalamic area mediates cardiovascular responses of the animal to external challenge but does not play a role in constant control of arterial pressure. So, it was demonstrated [6,7] that the anterior hypothalamus mediates baroreflex responses.

Microinfusion studies with adrenaline, noradrenaline, or clonidine [8–10] in anesthetized rats have demonstrated a depressor role for the anterior hypothalamic area. A depressor increase in catecholamine activity in the anterior hypothalamus and a depressor increase in the posterior hypothalamic nuclei were described in spontaneously hypertensive (SH) rats [1].

On the other hand, Zawoiski [11] found that isoproterenol instillation in the hypothalamic–thalamic region produced an immediate depressor response. However, the cardiovascular effect of β-adrenergic agonist in the anterior hypothalamic area has not been well studied. It was also found β-adrenergic receptor in the anterior hypothalamic area by inmunocytochemistry [12]. However, the level of binding

* Corresponding author. Tel.: +54-11-4964-8265; fax: +54-11-4508-3645.
E-mail address: chocht@ffyb.uba.ar (C. Höcht).

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density of β_1 and β_2-adrenoceptor in the anterior hypothalamic area is low [12].

In aortic coarctated (ACo) rats, the hypertensive state is attributed mainly to the activation of the renin–angiotensin system, to the effects of vasopressin and to the homeostasis of sodium [13–16]. There would also be a compromise of central regulatory mechanisms as the cardiovascular baroreceptor reflex [17,18]. In this experimental model of hypertension, it was not studied a functional alteration of the different hypothalamic systems like the catecholaminergic system. In a previous work [19], we have demonstrated that the perfusion of atenolol in the posterior hypothalamus induced a decrease of blood pressure in ACo rats.

The aim of this work was to demonstrate an alteration of the anterior hypothalamic catecholaminergic system in ACo rats by the perfusion of metoprolol. On the other hand, in order to clarify the alterations observed in ACo animals, we also studied a possible alteration of the β_1 and β_2-adrenoceptor activity in the anterior hypothalamic area by microinfusion of β-adrenergic agonists.

2. Materials and methods

2.1. Experimental procedure

Male Wistar rats (250–300 g) were used. The aortic coarctation was carried out according to the method described by Rojo-Ortega and Genest [20] in rats anesthetized with ether. The technique consists on the bond of the aorta artery between the two renal arteries. Control rats were sham operated (SO). The experiments were carried out 7 days after the corresponding operation. On the day of the experiment, rats were anesthetized with a mix of chloralose (50 mg kg^{-1} i.p.) and urethane (500 mg kg^{-1} i.p.).

A carotid artery was cannuled and connected to a Statham Gould P23ID pressure transducer coupled to a Grass 79D polygraph. Mean arterial pressure was calculated according to the formula: diastolic pressure + (systolic pressure − diastolic pressure)/3. Heart rate was calculated by counting blood pressure waves.

A concentric microdialysis probe was inserted in the anterior hypothalamus (A/P −1.7 mm, L/M 0.6 mm, V/D 9.5 mm, from the bregma [21]). The microdialysis probe was perfused with a solution consisted of 147 mM NaCl, 2.4 mM $CaCl_2$, 4.0 mM KCl, pH 7.3 pumped at a rate of 1 μl min^{-1} during the equilibration period. Afterwards the microdialysis probe was perfused with a Ringer solution containing metoprolol (6 μg ml^{-1}) or with Ringer solution for 2 h. Changes in blood pressure were monitored.

In second experiment, a 32-gauge stainless steel needle was inserted into the anterior hypothalamus. After a 30-min stabilization period, rats were injected with 1 μl of isoproterenol (0.1, 1 and 10 nmol) and changes in blood pressure were measured. After recovery of basal blood pressure values, atenolol (40 nmol) or propranolol (40 nmol) were injected into the anterior hypothalamus. Then isoproterenol (1 and 10 nmol) was injected.

In third experiment, clenbuterol (0.1, 1 and 10 nmol) was injected in the anterior hypothalamus and changes in blood pressure were measured.

At the end of experiments, the position of probes was verified by the corresponding histological studies.

2.2. Statistics

Normal distribution of the variables of the study was verified using the Kolmogorov–Smirnov (K–S) test. Data are expressed as mean ± S.E.M. Statistical analysis was performed by a paired Student's "*t*" test [22] or by two-way ANOVA with post-hoc tests performed using a Bonferroni test. The statistical tests were performed using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Cardiovascular effects of metoprolol perfusion in anterior hypothalamus

Basal values of mean arterial pressure (MAP) and heart rate were 86 ± 5 mmHg ($n = 20$) and 356 ± 10 bpm in SO anesthetized rats and 109 ± 6 mmHg ($P < 0.05$ versus SO rats) and 365 ± 12 bpm ($n = 20$) in the ACo anesthetized rats.

Changes in blood pressure during metoprolol or Ringer solution perfusion in anterior hypothalamus of SO and ACo rats are shown in Fig. 1. Metoprolol perfusion reduced the MAP in the ACo rats but not in SO animals. In ACo rats ($n = 6$) the fall of MAP was −12 ± 1 mmHg after 30 min of metoprolol perfusion.

3.2. Cardiovascular effects of isoproterenol infusion in anterior hypothalamus

The infusion of isoproterenol (0.1, 1 and 10 nmol) in anterior hypothalamic area induces a dose-dependent decrease of blood pressure in both experimental groups (Fig. 2). The depressor response to isoproterenol (10 nmol) was significantly lower in ACo rats (−5.2 ± 1.5 mmHg, $n = 10$; $P <$ 0.05 versus SO rats) than in SO rats (−15.9 ± 2.3 mmHg, $n = 10$).

The pretreatment with a selective β_1-adrenergic receptor antagonist atenolol (40 nmol) induced a significative increase of the depressor effect of isoproterenol (1 and 10 nmol) in ACo rats (Fig. 3). However, the pretreatment with atenolol did not modify the hypotensive effect of isoproterenol in SO rats (Fig. 3).

On the other hand, the hypotensive action of isoproterenol was significantly diminished with the administration of propranolol (40 nmol) in SO and ACo (Fig. 3) rats.

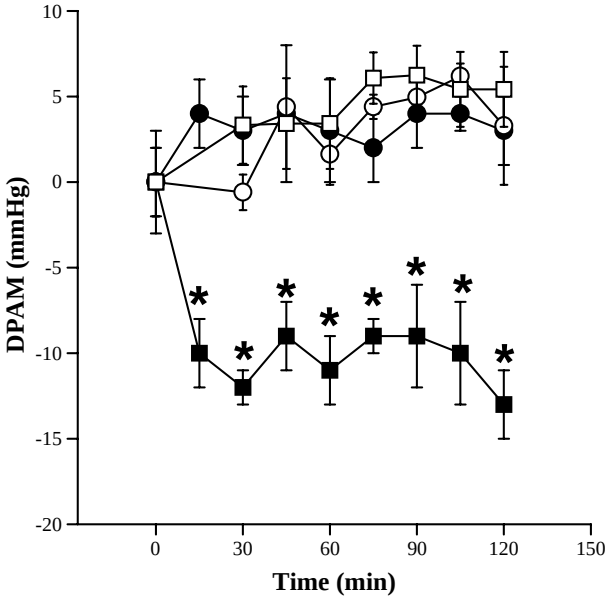

Fig. 1. Time course of the change of mean arterial pressure (ΔMAP), during metoprolol perfusion (filled symbols) and Ringer solution perfusion (empty symbols) in the anterior hypothalamic area of sham operated (SO, circles) and aortic coarctated (ACo, squares) rats. Each point shows the mean ± S.E.M. of six animals. $^{*}P < 0.05$ vs. SO rats.

3.3. Cardiovascular effects of clenbuterol infusion in anterior hypothalamus

The infusion of clenbuterol (0.1, 1 and 10 nmol), a selective β_2-adrenergic agonist, in anterior hypothalamic area induces a dose-dependent decrease of blood pressure in both experimental groups (Fig. 4). The depressor response to clenbuterol (1 nmol) was significantly lower in ACo rats (-5.0 ± 1.0 mmHg, $n = 5$) than in SO rats (-10.3 ± 2.0 mmHg, $n = 5$).

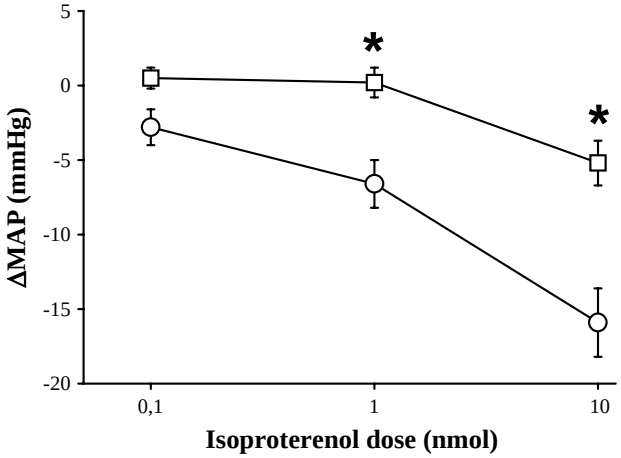

Fig. 2. Change of mean arterial pressure (ΔMAP), after injection of isoproterenol (0.1, 1 and 10 nmol) in the anterior hypothalamic area of sham operated (SO, circles) and aortic coarctated (ACo, squares) rats. Each point shows the mean ± S.E.M. of 10 animals. $^{*}P < 0.05$ vs. SO rats.

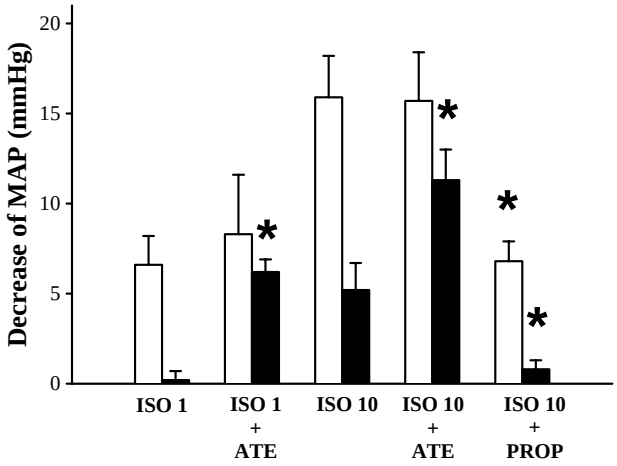

Fig. 3. Decrease of mean arterial pressure (MAP), after injection of isoproterenol (ISO; 1 and 10 nmol) in the anterior hypothalamic area of sham operated (SO) rats (empty bars) and aortic coarctated (ACo) rats (filled bars) after no pretreatment, pretreatment with atenolol (ATE, 40 nmol) and pretreatment with propranolol (PROP; 40 nmol). Each bar shows the mean ± S.E.M. of six animals. $^{*}P < 0.05$ vs. isoproterenol.

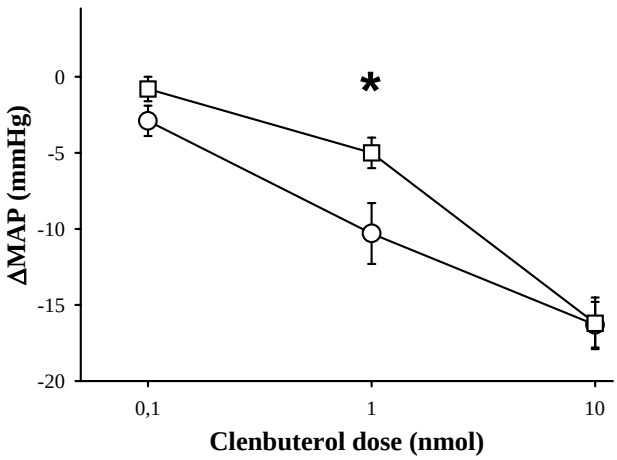

Fig. 4. Change of mean arterial pressure (ΔMAP), after injection of clenbuterol (0.1, 1 and 10 nmol) in the anterior hypothalamic area of sham operated (SO, circles) and aortic coarctated (ACo, squares) rats. Each point shows the mean ± S.E.M. of five animals. $^{*}P < 0.05$ vs. SO rats.

4. Conclusions

In the present study, the perfusion of the β_1-adrenoceptor antagonist metoprolol into the anterior hypothalamic area caused a depressor response in anesthetized ACo rats but not in SO animals. In the anterior hypothalamic area of ACo rats, there would be an involvement of β_1-adrenoceptors in the maintenance of blood pressure. This compromise could be in part responsible for the maintenance of hypertension in ACo rats. On the other hand, we found a decrease depressor response to isoproterenol in ACo rats. The diminished hypotensive response could be explained to the existence of pressor β_1-adrenoceptors in the ACo rats.

The basal MAP values of ACo anesthetized rats were significantly higher than those of SO anesthetized rats, but these are not hypertensive values. It is well known that the anesthesia can modify blood arterial pressure and can also modify the cardiovascular response to drugs [23]. In a previous work, we have observed hypertensive MAP basal values in conscious ACo rats [24]. In the present work, the used technique and the employed anaesthetic drugs possible could cause a depressor effect in blood arterial pressure of both experimental groups.

In the present study we have observed during the metoprolol perfusion of the anterior hypothalamic area a depressor effect on blood arterial pressure in both experimental groups.

We used the reverse microdialysis technique for the perfusion of metoprolol. An application of this technique is the evaluation of neural regulation of cardiovascular function in the rat. This technique provides information on the actual concentration of the drug that has been delivered to the tissue [25]. In a previous work we have determined (data not shown), that after an intravenous administration of metoprolol ($3\,mg\,kg^{-1}$), the metoprolol concentration in hypothalamic dialysate was about $60\,ng\,ml^{-1}$. Considering that our microdialysis probe has an in vivo recovery of 8%, we have calculated the metoprolol perfusion concentration ($6\,\mu g\,ml^{-1}$). This perfusate concentration should be sufficient to obtain levels of metoprolol in the surrounding area of the probe similar to that obtained when it was administered intravenously ($3\,mg\,kg^{-1}$). However, metoprolol levels in other central areas should be significantly lower than those near the probe. Although in this experiment we cannot exclude that the effect of the drug is exercised on other areas of the central nervous system, it is pausible that the cardiovascular effect is due to its action on the anterior hypothalamic area, because these areas were perfused with a small amount of metoprolol.

Zawoiski [11] found that isoproterenol instillation in the hypothalamic–thalamic region produced an immediate depressor response. He also found the existence of β-adrenoceptor in the anterior hypothalamic area by inmunocytochemistry or by binding studies [12]. However, the cardiovascular effect of β-adrenergic agonist after injection in the anterior hypothalamic area has not been studied.

Many authors have demonstrated a central hypotensive action of β-adrenergic antagonist. Pearson et al. [26] have determined that the i.c.v. administration of atenolol induced a fall of blood pressure in normotensive animals. Zawoiski [11] has also observed a central antihypertensive activity of propranolol in SH rats. In a previous work [19], we have demonstrated that the perfusion of atenolol in the posterior hypothalamus induced a decrease of blood pressure in ACo rats.

We have observed a depressor response to metoprolol perfusion only in ACo rats, suggesting a compromise of hypothalamic β_1-adrenoceptors of ACo rats but not in SO rats in the maintenance of blood pressure.

To confirm the existence of pressor β_1-adrenoceptor in the anterior hypothalamic area of ACo rats we studied the effect on blood pressure of the β-adrenergic agonist hypothalamic infusion. Therefore, we studied the cardiovascular response to isoproterenol (non-specific β-adrenergic agonist) infusion and the alteration of this response by the pretreatment with a selective β_1-adrenergic antagonist (atenolol) or a non-specific β-adrenergic antagonist (propranolol). On the other hand, we also studied the cardiovascular effect of the injection of clenbuterol, a selective β_2-adrenergic agonist.

Isoproterenol infusion into the anterior hypothalamic area also induces a dose-dependent fall of blood pressure in both experimental groups. However, the hypotensive effect of all experimental dose of isoproterenol was smaller in ACo rats than in SO rats. Therefore, we studied the effect of β-adrenergic blockade on the hypotensive action of isoproterenol.

We studied the effect of preadministration of propranolol on the hypotensive action of isoproterenol. In both experimental groups, the preadministration of non-specific β-adrenoceptor antagonist propranolol reduced the depressor effect of isoproterenol and this result suggests the β-adrenoceptor participation in the effect of the agonist. On the other hand, in SO rats, the atenolol β_1-adrenergic blockade did not modify the depressor effect of isoproterenol, suggesting that the β_1-adrenoceptors in the anterior hypothalamus of normotensive rats are not involved in the effect of isoproterenol. Therefore, it is possible that the depressor effect of isoproterenol is mediated by the β_2-adrenoceptors in the normotensive rats. In the ACo rats, the previous administration of atenolol induces an increase in the hypotensive action of isoproterenol. So, these findings suggest the existence of pressor β_1-receptor activity in the anterior hypothalamus of ACo rats and that the hypotensive effect of isoproterenol is mediated by β_2-adrenoceptors.

The infusion of clenbuterol in the anterior hypothalamic area induces a dose-dependent descent of blood pressure in both experimental groups. These results also suggest the existence of depressor β_2-adrenoceptors in the anterior hypothalamus of ACo and SO rats. However, the hypotensive effect of intermediate dose of clenbuterol was smaller in ACo rats than in SO rats suggesting a possible diminished hypotensive activity of hypothalamic β_2-adrenoceptors in coarctated animals.

In summary, this study provides the evidence of a pressor β_1-adrenoceptor mechanism in the anterior hypothalamic area of ACo rats but not in SO rats. This β_1-adrenoceptor mechanism may be involved in the maintenance of hypertensive state in ACo rats. Moreover, this study also provides the evidence of a vascular depressor mechanism mediated by β_2-adrenoceptor in the anterior hypothalamus of normotensive control rats and in ACo animals. The results also suggest a diminished depressor β_2-adrenergic activity in ACo rats.

Therefore, aortic coarctation reveals the existence of pressor β_1-adrenergic receptors in the anterior hypothalamic area

of rats and this pressor mechanism opposes the depressor response of the β_2-adrenoceptors stimulation in ACo rats.

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