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Opioidergic Receptors in the Arcuate Nucleus Are Not Involved in the Cardiovascular Effects of Clonidine

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BARBER, D. A. AND R. L. TACKETT. *Opioidergic receptors in the arcuate nucleus are not involved in the cardiovascular effects of clonidine.* PHARMACOL BIOCHEM BEHAV 49(3) 579-582 1994.—The arcuate nucleus is the bed nucleus for the pro-opiomelancortin system of the brain with important connections with other nuclei involved in cardiovascular function. Clonidine has been reported to produce its cardiovascular effects through an interaction with opioid and α_2-adrenergic receptors. The present study examined the arcuate nucleus as a site of action of clonidine. Male spontaneously hypertensive rats were anesthetized with pentobarbital and were instrumented for the measurement of blood pressure and heart rate. Cannulae were placed either through the cisterna magna (IC) or in the arcuate nucleus. Administration of clonidine (0.03-3.75 μg, IC) produced a dose-dependent hypotension and bradycardia. Pretreatment with naloxone (30 μg, IC) prior to clonidine administration resulted in a significant attenuation of both the clonidine-induced hypotension and bradycardia. In contrast, administration of naloxone (100 ng) into the arcuate nucleus prior to the central administration of clonidine did not alter the cardiovascular effects of clonidine. These results support the role of central opioidergic receptors in the cardiovascular effects of clonidine but do not support the arcuate nucleus as the site of action.

Clonidine Arcuate nucleus Opiates α_2 Agonists

CENTRALLY acting antihypertensive drugs have been demonstrated to lower blood pressure by interacting with α_2-adrenergic receptors in the brain, which decreases sympathetic nervous system activity (9). The reduction in sympathetic neural activity and subsequent reduction in blood pressure may be, in part, also associated with the release of β-endorphin. In addition to the interaction of clonidine with central catecholaminergic receptors, several studies have provided evidence for an interaction with central opioidergic receptors. Farsang et al. (4,5) demonstrated that the hypotension and bradycardia associated with clonidine and α-methyldopa were attenuated by pretreatment with the opiate antagonists, naloxone and naltrexone. Kunos et al. (10) demonstrated that clonidine increased the release of β-endorphin from brain stem slices of spontaneously hypertensive rats.

The hypothalamic arcuate nucleus is recognized as the bed nucleus for the pro-opiomelanocortin system in the brain with important connections with other nuclei associated with cardiovascular function (1). Mastrianni et al. (13) recently demonstrated the existence of a β-endorphin pathway projecting from the arcuate nucleus to the ipsilateral dorsal vagal complex, which is associated with depressor and bradycardiac responses. These data, as well as a previous report by Mosqueda-Garcia et al. (14), have led to the postulation that the arcuate nucleus may be a potential site of action for clonidine (11). The present study examines whether opioid receptor mechanisms in the arcuate nucleus participate in the cardiovascular effects of clonidine.

METHOD

Male spontaneously hypertensive rats (16 weeks old, 326 ± 6 g) were anesthetized with pentobarbital sodium (65 mg/kg, IP). The femoral artery was cannulated and connected to a Statham pressure transducer for blood pressure recording. Heart rate was determined from the pulse rate of the blood pressure tracing. All parameters were recorded on a Grass Model 7 polygraph. The rats were then mounted in a stereo-

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taxic apparatus with the head tilted and stabilized at a 55° angle. To expose the cisterna magna for IC administrations, an incision between the rhomboid and trapezoidal muscles was made and the muscle retracted. A cannula was lowered through the leptomeninges and correct positioning was verified by the efflux of cerebrospinal fluid. For injection into the arcuate nucleus, a cannula was positioned with the following stereotaxic coordinates: A/P −3.8 mm to bregma, 0.4 mm lateral to midline, and dorsoventral 9.7 mm to dura (15). All injections were administered in a final volume of 5 μl for IC and 100 nl for the arcuate.

At the conclusion of each experiment, 100 nl of dye was injected through the arcuate cannula. The brains were fixed with an intracardiac infusion of normal saline followed by phosphate buffered 10% formalin. After overnight storage in the 10% formalin solution, the brain was dehydrated in a 30% sucrose solution. Frozen coronal sections (50 μm) were stained with neutral red and injection sites were verified with light microscopy.

Clonidine HCl, sodium pentobarbital, and naloxone were obtained from Sigma Chemical Co. (St. Louis, MO) and all drugs were dissolved in normal saline.

A one-way ANOVA was utilized to determine the existence of significant differences between means. A Fischer's least significant difference post hoc test was performed on the means to deterermine where significant differences occurred. All data are presented as the mean ± SEM A value of $p < 0.05$ was taken to indicate statistical significance.

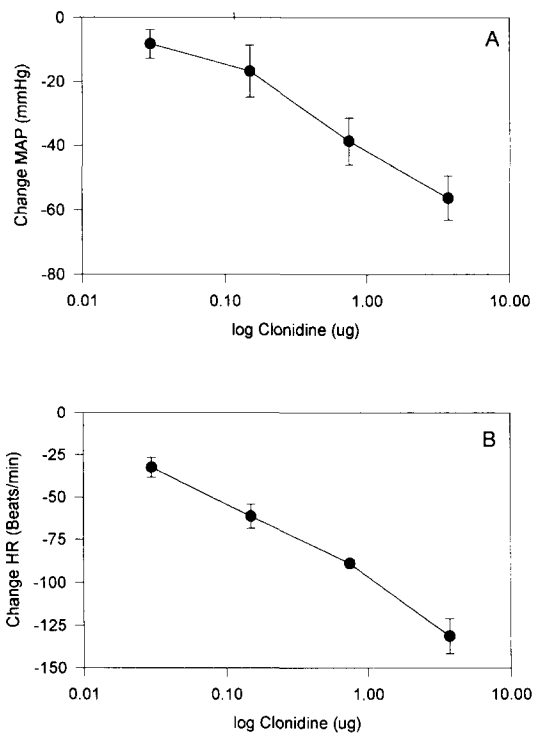

FIG. 1. Effect of IC-administered clonidine on MAP (A) and HR (B) in the pentobarbital-anesthetized SHR. Increasing doses of clonidine (0.03, 0.12, 0.60, and 3.15 μg) were administered every 30 min to yield cumulative doses of 0.03, 0.15, 0.75, and 3.75

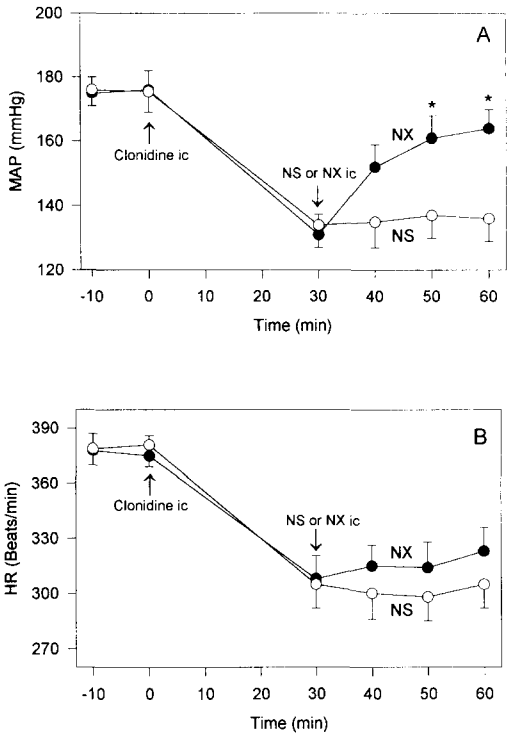

FIG. 2. Naloxone-mediated reversal of clonidine hypotension but not bradycardia in the pentobarbital-anesthetized SHR. Clonidine (0.75 μg) was administered IC at time zero. Thirty minutes later, either saline (NS, ○, $n = 4$) or 30 μg naloxone (NX, ●, $n = 5$) was administered IC. MAP (A) and HR (B) were followed for the next 30 min. *$p < 0.05$.

RESULTS

Baseline mean arterial pressure (MAP) and heart rate (HR) values of the anesthetized rats were 176 ± 9 mmHg and 378 ± 13 bpm, respectively. Increasing doses of clonidine (0.03, 0.12, 0.60, and 3.15 μg) were administered IC every 30 min to yield cumulative doses of 0.03, 0.15, 0.75, and 3.75 μg. Clonidine administered in this matter produced a dose-dependent reduction in MAP with a maximal decrease of 56 ± 7 mmHg (Fig. 1A). The maximal decrease in heart rate was 131 ± 10 bpm (Fig. 1B). These maximal responses occurred at 30 min after the cumulative administration of 3.75 μg of clonidine. From the above dose-response curve, we chose a dose of 0.75 μg for the remainder of the experiments. This dose produced a maximal decrease of MAP of 38 ± 7 mmHg and a reduction of heart rate of 88 ± 3 bpm. The maximal responses to 0.75 μg of clonidine occurred at 30 min and remained stable for at least 2 h postclonidine administration. To ascertain the role of opiate receptors in the cardiovascular effects of clonidine, the opiate antagonist, naloxone (30 μg, IC), was administered 30 min after the administration of clonidine (0.75 μg, IC). Naloxone reversed the clonidine-induced hypotension. Thirty minutes after the administration of naloxone, MAP had returned to 12 ± 5 mmHg below baseline, which represented a 73% reversal of the clonidine-induced hypotension (Fig. 2A). Intracisternal injection of an equivalent volume of vehicle (normal saline) did not affect the clonidine-induced hypotension (Fig. 2A).

Clonidine-induced bradycardia was not reversed by IC naloxone administration. Thirty minutes following naloxone administration, heart rate was reduced 61 ± 10 bpm, which was not significantly different from the saline control (75 ± 12 bpm below baseline). These results are depicted in Fig. 2B.

To evaluate the role of arcuate opioidergic receptors in clonidine-induced cardiovascular responses, naloxone (100 ng) was injected into the arcuate nucleus (Fig. 4) prior to the administration of clonidine (0.75 μg, IC). Microinjection of naloxone or an equivalent volume of normal saline into the arcuate nucleus did not affect MAP or HR. Rats pretreated with vehicle demonstrated a decrease in MAP of 39 ± 4 mmHg and a decrease in HR of 74 ± 6 bpm in response to IC-administered clonidine (Fig. 3A,B). Similarly, rats in which naloxone was microinjected into the arcuate nucleus demonstrated a decrease in MAP of 41 ± 7 mmHg and a reduction in HR of 71 ± 11 bpm in response to clonidine (Fig. 3A,B).

DISCUSSION

The results of the present study confirm a role for central opioidergic receptors in the hypotensive response of clonidine and support the interaction between catecholaminergic and opioidergic systems. However, our study does not support the role of opioidergic receptors for clonidine-induced bradycardia. Furthermore, although centrally administered naloxone blocked the clonidine-induced hypotension, this action does not appear to be mediated at the level of the arcuate nucleus.

Several studies have implicated an interaction of centrally acting hypotensive drugs with catecholaminergic and opioidergic systems. Farsang et al. (4) demonstrated that naloxone or naltrexone pretreatment attenuated the hypotension and bradycardia associated with clonidine or methyldopa. This interaction with opioidergic systems was not due to a nonspecific effect of the opiate antagonists because clonidine and naloxone did not cross-react with each other's binding sites in the brain (5), but rather appears to involve the release of an endogenous opiate through the activation of α-adrenergic receptors (4). In this context, Kunos et al. (10) have demonstrated that clonidine increased the release of β-endorphin from the brain stem slices of spontaneously hypertensive rats. Studies with other agents also support the involvement of opioidergic receptors in the hypotensive response of central α_2 agonist. BHT 933, an α_2 agonist with greater specificity than clonidine, was recently shown to produce hypotension in normotensive dogs that was blocked by pretreatment with naloxone (18). We also demonstrated that β blocking antagonists are capable of lowering blood pressure by interacting with central α_2-adrenergic and opioidergic receptors (7,12,19).

The site of action of clonidine probably involves multiple sites, which have been postulated to include the nucleus tractus solitarius (6) and the ventrolateral medulla (17). The arcuate nucleus has been suggested as a possible site of action of clonidine (13,14). Several factors would support the possibility of the arcuate as a potential site of action, especially with regard to the opioidergic component that has been demonstrated with centrally acting hypotensive drugs. The arcuate nucleus is recognized as the bed nucleus for pro-opiomelanocortin-containing neurons and has multiple opioidergic projections that integrate several cardiovascular regulatory centers in the brain (8). Mastrianni et al. (13) demonstrated the existence of a cardioregulatory opioidergic pathway that originates in the arcuate nucleus and terminates in the nucleus tractus solitarius. Additionally, clonidine has been shown to act in the arcuate nucleus to affect growth hormone secretion

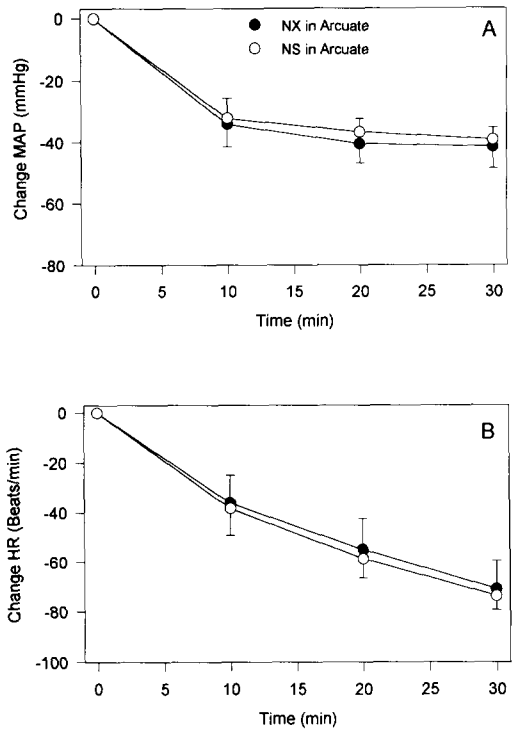

FIG. 3. Microinjection of either saline (NS, ○, n = 4) or 100 ng naloxone (NX, ●, n = 5) into the hypothalamic arcuate nucleus of SHRs followed by the IC administration of 0.75 μg clonidine. Compared to the saline control, naloxone in the arcuate did not attenuate clonidine-mediated decreases in MAP (A) or HR (B).

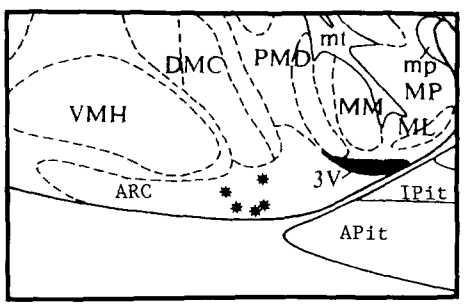

FIG. 4. Sagittal section of the hypothalamus at 0.4 mm lateral from midline illustrating the approximate anatomical location of the five arcuate injections (*) verified by dye injection at the conclusion of each experiment (15). Left and right illustration borders are −1.8 mm and −5.4 mm from bregma, respectively. Structures illustrated: arcuate hypothalamic nucleus (ARC), ventromedial hypothalamic nucleus (VMH), dorsomedial hypothalamic nucleus (DMC), premammillary nucleus-dorsal (PMD), mammillothalamic tract (mt), mammillary peduncle (mp), medial mammillary nucleus-posterior (MP), medial mammillary nucleus-medial (MM), medial mammillary nucleus-lateral (ML), third ventricle (3V), intermediate lobe pituitary (IPit), anterior lobe pituitary (APit).

(2). Despite these studies, which would support the arcuate as a site of action of clonidine, the results of our study do not support this hypothesis. Microinjection of naloxone into the arcuate prior to clonidine administration failed to affect the cardiovascular effects of clonidine in the spontaneously hypertensive rat, which has been suggested to have a highly active opioidergic component in the modulation of cardiovascular function (4,5,11). However, the arcuate nucleus is a long bed and, although in this study injections were midposterior (Fig. 4), it is possible that more peripheral regions of the arcuate are involved. It is important to note that an opioidergic component was operable in our study because IC naloxone reversed clonidine-induced hypotension. An alternative site for the opiodergic component of clonidine-induced hypotension may be the rostral ventrolateral medulla, which has been shown to be a site of catecholaminergic and opioidergic for clonidine (3) as well as other centrally acting hypotensive drugs (16,20).

In summary, the results of the present study support the role of opioidergic receptors in the hypotensive response of clonidine. However, our results do not support the arcuate nucleus as a site of action for clonidine.

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