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Chloroethylclonidine is a partial α_1-adrenoceptor agonist in aorta and caudal arteries of the Wistar Kyoto rat

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Abstract

The interaction between chloroethylclonidine (*N*-β-chloroethyl-*N*-methylamino-methyl-clonidine) and α_1-adrenoceptors mediating contraction in Wistar Kyoto rat arteries was examined. In caudal (α_{1A}-adrenoceptors) and aorta (α_{1D}-adrenoceptors) arteries, chloroethylclonidine (10^{-4} M) elicited contraction with different time frames (maximal effect within 4 min in the caudal artery and at 30–45 min in aorta). Phentolamine (10^{-6} M) completely prevented chloroethylclonidine-induced contraction in aorta, but partially did so in the caudal artery. Rauwolscine (10^{-7} M) partially prevented the chloroethylclonidine contractile effect in both arteries. Chloroethylclonidine attenuated the contractile effect of low concentrations of norepinephrine, however, maximal contraction was observed at catecholamine concentrations above 10^{-7} M in the caudal artery and 10^{-6} M in the aorta. It is concluded that chloroethylclonidine interacts with caudal α_{1A}-adrenoceptors as an irreversible partial agonist, inducing vascular contraction probably due to Ca^{2+} mobilisation, and with aorta α_{1D}-adrenoceptors as a partial agonist, inducing slow-onset muscular contraction.

Keywords: α_1-Adrenoceptor; Chloroethylclonidine; Phentolamine; Vascular contraction; Wistar Kyoto rat

1. Introduction

Chloroethylclonidine (*N*-β-chloroethyl-*N*-methylamino-methyl-clonidine) was originally described by Leclerc et al. (1980) as an irreversible agonist that produces phentolamine-sensitive contraction in rat aorta and an increase in blood pressure in pithed rats, suggesting that chloroethylclonidine activates α-adrenoceptors. Later, Muramatsu et al. (1990) described an irreversible chloroethylclonidine-induced contraction in rat aorta, supporting the finding of Leclerc et al. (1980); however, most of the studies do not report this chloroethylclonidine-elicited contraction. Other reports have addressed chloroethylclonidine as: (i) a selective α_{1B}-adrenoceptor irreversible antagonist, that has been used to differentiate among α_1-adrenoceptor subtypes (Minneman, 1988; Han et al., 1990), (ii) an antagonist that irreversibly inactivates α_{1B}-, α_{2A}- and α_{2C}-adrenoceptors, without inactivation of the α_{1A}- and α_{2B}-subtypes (Michel et al., 1993), (iii) an irreversible agonist at α_2-adrenoceptors (Bultmann and Starke, 1993; Nunes and Guimaraes, 1993; Docherty and O'Rourke, 1997) and (iv) a partial α_{1A}-adrenoceptor agonist in transfected cells (Villalobos-Molina et al., 1997). These controversial reports prompted us to study the possibility that chloroethylclonidine could stimulate rather than inactivate α_1-adrenoceptors. We now report on the effect of chloroethylclonidine in Wistar Kyoto rat caudal artery and aorta, which predominantly express the α_{1A}- and α_{1D}-adrenoceptors, respectively (Villalobos-Molina and Ibarra, 1996).

2. Methods

2.1. Determination of isometric tension changes

Male normotensive Wistar Kyoto rats, 3 months of age, were reared in our animal facilities under controlled light/dark conditions and fed ad libitum. The detailed procedure has been described elsewhere (Villalobos-Molina and Ibarra, 1996). In brief, arterial rings (4–5 mm in length) denuded of endothelium were placed in a 10-ml chamber filled with Krebs solution at 37°C, pH 7.4 and bubbled with 95% O_2:5% CO_2. Arteries were subjected to

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an initial optimal tension of 3 g for aorta or 2 g for caudal arteries. The tissue was challenged with norepinephrine (10^{-7} M) in the presence of propranolol (10^{-7} M) and rauwolscine (10^{-7} M), to block β and α_2-adrenoceptors, respectively and washed every 30 min for 2 h. Then, reproducible cumulative concentration–response curves for norepinephrine (10^{-9} to 10^{-5} M) were obtained for each artery. In order to avoid fatigue of the arterial preparation, a 60-min recovery period was allowed between norepinephrine curves. Chloroethylclonidine (10^{-4} M) was present for 45 min and then extensively washed out, prior to norepinephrine addition.

In receptor protection experiments, after the first norepinephrine curve, tissues were in contact with phentolamine (10^{-6} M), or rauwolscine (10^{-7} M) for 15 min before, and during chloroethylclonidine incubation. After washout, experiments were carried out as described above.

2.2. Drugs

(−)-Norepinephrine and (±)-propranolol were obtained from Sigma (St. Louis, MO, USA), CEC, phentolamine and rauwolscine were from Research Biochemicals Int. (Natick, MA, USA).

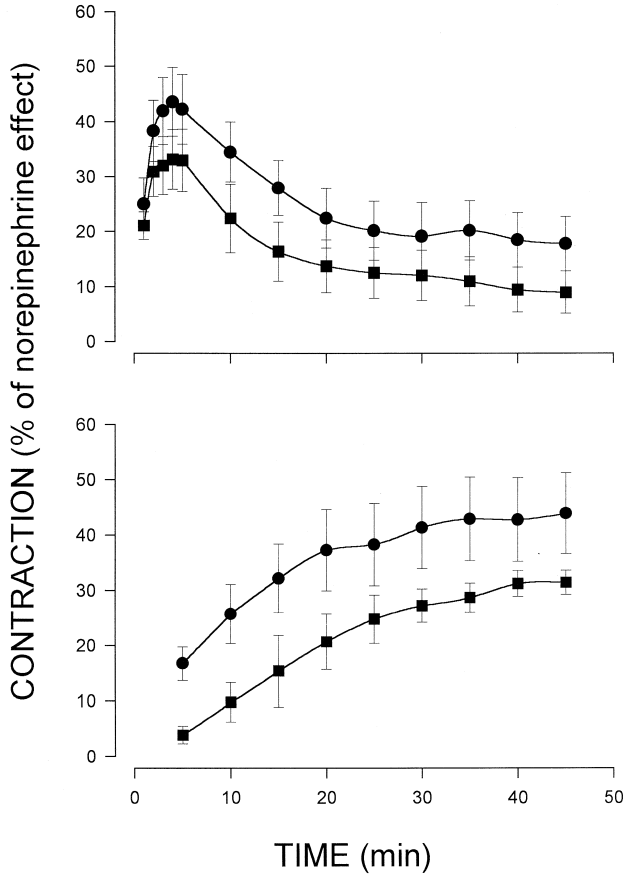

Fig. 1. Time–course of effects of chloroethylclonidine (10^{-4} M: ●) and of rauwolscine (10^{-7} M: ■) prior to chloroethylclonidine on contraction of isolated arteries of the Wistar Kyoto rat. Arteries were treated as described in Section 2: (top panel) caudal and (bottom panel) aorta arteries. Results represent the means ± S.E.M. of four to six different experiments.

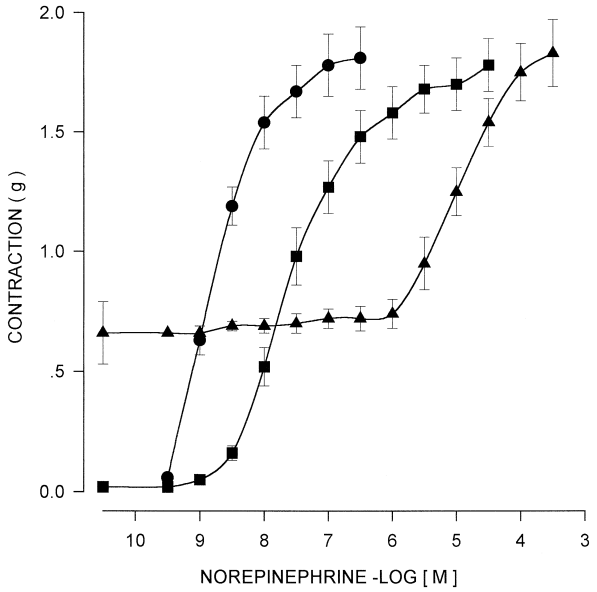

Fig. 2. Effects of chloroethylclonidine, phentolamine prior to chloroethylclonidine, or vehicle on norepinephrine-induced contractions in aorta of the Wistar Kyoto rat. Arteries were treated as described in Section 2 with vehicle (●), chloroethylclonidine (10^{-4} M) (▲) or phentolamine (10^{-6} M) before chloroethylclonidine (10^{-4} M) (■). Results are expressed in grams for easy comparison of the responses to the agents and represent the means ± S.E.M. of three to four experiments.

3. Results

Under our experimental conditions chloroethylclonidine induced a rapid (within 4 min), partially sustained contraction of caudal artery (≈ 43% of norepinephrine effect, $n = 6$), that persisted after extensive washout (Fig. 1, top panel). Chloroethylclonidine also induced contraction of aorta (≈ 39% of norepinephrine effect, $n = 6$) but with a completely different time frame (30–45 min to reach maximal contraction), this contraction also persisted after washout (Fig. 1, bottom panel). Rauwolscine (an α_2-adrenoceptor antagonist, at 10^{-7} M) partially antagonised chloroethylclonidine-induced contraction in both arteries (Fig. 1). The blocking action of rauwolscine did not modify the pattern of chloroethylclonidine-elicited contraction in the arteries, suggesting an interaction between chloroethylclonidine and the α_2-adrenoceptors present in these vessels.

Fig. 2 shows the concentration–response curves to norepinephrine in aorta. As observed, chloroethylclonidine elicited a contraction of approximately 40% of the maximal and markedly shifted to the right the norepinephrine-induced response in the aorta (Fig. 2); however, the maximal contractile effect was attained at 3.1×10^{-4} M norepinephrine (EC_{50}, 10^{-5} M vs. 2×10^{-9} M in the control).

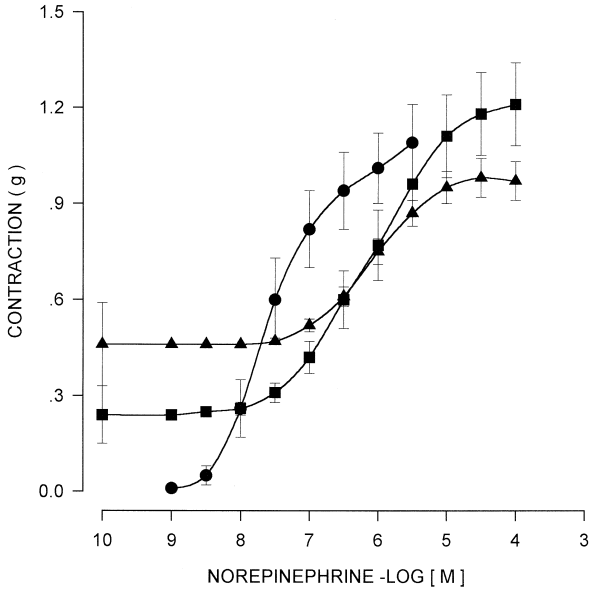

Fig. 3. Effects of chloroethylclonidine, phentolamine prior to chloroethylclonidine or vehicle in norepinephrine-induced contractions in caudal artery of the Wistar Kyoto rat. Arteries were treated as described in Section 2 with vehicle (●), chloroethylclonidine (10^{-4} M) (▲) or phentolamine (10^{-6} M) prior to chloroethylclonidine (10^{-4} M) (■). Results are expressed in grams for easy comparison of the responses to the agents and represent the means ± S.E.M. of three to four experiments.

Preincubation with phentolamine (10^{-6} M) protected the α_{1D}-adrenoceptors present in aorta from activation by chloroethylclonidine. Nevertheless, there was a shift to the right of the norepinephrine-induced contraction (EC_{50}, 4×10^{-8} M), resembling the rightward displacement observed for phentolamine alone (not shown), however, maximal contraction was reached (Fig. 2).

Fig. 3 shows the norepinephrine-induced contraction of caudal artery (EC_{50}, 4.1×10^{-8} M). Chloroethylclonidine elicited a contraction approximately 40% of the maximal and shifted to the right the norepinephrine-induced effect. However, the maximal contraction was reached at 3.1×10^{-5} M of the catecholamine (similar results were obtained when methoxamine was used as the agonist, not shown). Incubation of the arteries with the α_1-adrenoceptor antagonist, phentolamine (10^{-6} M), partially prevented both chloroethylclonidine-induced contraction and the rightward shift in the concentration–response curve for norepinephrine, suggesting that both effects were mediated through α_{1A}-adrenoceptors present in this vessel (Fig. 3).

4. Discussion

The effects of chloroethylclonidine on α-adrenoceptor function have been mainly interpreted as indicative of its ability to alkylate and inactivate the α_1-subtypes (i.e., it is an irreversible antagonist), with the following order of adrenoceptor sensitivity: $\alpha_{1B} > \alpha_{1D} \gg \alpha_{1A}$ (Perez et al., 1994), or being an irreversible agonist of the α_2-adrenoceptors (Bultmann and Starke, 1993; Nunes and Guimaraes, 1993; Piascik et al., 1995; Docherty and O'Rourke, 1997; O'Rourke et al., 1997). Our experiments consistently showed chloroethylclonidine-induced vascular reactivity in both caudal and aorta arteries of the Wistar Kyoto rat. This is consistent with previous reports (Leclerc et al., 1980; Muramatsu et al., 1990); however, other authors have not observed this effect in the aorta (Tian et al., 1990; Oriowo and Ruffolo, 1992; O'Rourke et al., 1995), or the caudal arteries (Piascik et al., 1995) of other rat strains. These findings suggest that the Wistar Kyoto rat arteries could have a larger number of α_1-adrenoceptors and, more importantly, that chloroethylclonidine occupies and activates these receptors.

Chloroethylclonidine-induced contraction in aorta showed two very interesting aspects, (a) after chloroethylclonidine washout, lower norepinephrine concentrations were not able to increase contraction further and (b) norepinephrine concentrations higher than 1 μM increased contraction to reach the control values. Our interpretation of these results is the following: chloroethylclonidine alkylates and activates the α_{1D}-adrenoceptor population which is responsible for contraction in this artery (Villalobos-Molina and Ibarra, 1996); for this reason norepinephrine, a selective α_{1D}-adrenoceptor agonist (Minneman et al., 1994; Perez et al., 1994), is not able to elicit further contraction at the concentrations where the catecholamine is interacting with α_{1D}-adrenoceptors (compare the control curve and that after chloroethylclonidine, Fig. 2). However, norepinephrine contracts the aorta at concentrations that activate the α_{1A}-adrenoceptors (see the control curve in the caudal artery, Fig. 3); this interpretation implies that α_{1D}-adrenoceptors are responsible for contraction in this artery, but when they are occupied/alkylated, then α_{1A}-adrenoceptors, present in the vessel (Piascik et al., 1994), could take over the function.

On the other hand, we reported previously that chloroethylclonidine behaved as a partial agonist at the α_{1A}-adrenoceptors, a property to be expected for an imidazoline (Tian et al., 1990), i.e., it is able to stimulate Ca^{2+} mobilisation in α_{1a}-adrenoceptor transfected cells (Villalobos-Molina et al., 1997), within a time frame comparable to that of the chloroethylclonidine-induced contraction in the rat caudal artery (present data). This suggests that chloroethylclonidine is activating the α_{1A}-adrenoceptors present in this artery (Villalobos-Molina and Ibarra, 1996). The fact that phentolamine prevented chloroethylclonidine effects in both models supports the suggested partial agonism of the alkylating agent.

The chloroethylclonidine-induced contraction in aorta and caudal arteries was partially blocked by rauwolscine, indicating the presence of α_2-adrenoceptors and their involvement in chloroethylclonidine-induced contraction in these arteries (Ruffolo et al., 1981; Piascik et al., 1995). However, our results suggest a minor role for these recep-

tors in the actions of chloroethylclonidine, in contrast with other reports where interpretation of the results is clearly oriented to a major role of α_2-adrenoceptors in chloroethylclonidine actions (Docherty and O'Rourke, 1997; O'Rourke et al., 1995, 1997). On the other hand, the chloroethylclonidine-elicited contraction of aorta had a much slower time frame than that in the caudal artery. This marked difference in time course of the effects could have several causes. It is possible that α_{1D}-adrenoceptors (aorta) are occupied/alkylated at a slower rate than receptors of the α_{1A}-subtype (caudal). It is also possible that chloroethylclonidine may activate different signaling effector pathways when it interacts with these receptor subtypes. Very recently, it has been suggested that agonists may interact with different sites in a receptor and that such interactions could be specific for activating specific G protein/effector pathways (Perez et al., 1996). It is clear that activation of the α_{1A}-adrenoceptor of the caudal artery by chloroethylclonidine is coupled to a rapid contraction onset that is consistent with a 'phasic' Ca^{2+} mobilisation. This possibility is consistent with what we observed in rat-1 fibroblasts transfected with this receptor subtype (Villalobos-Molina et al., 1997). The slow-onset contraction of aorta, induced by chloroethylclonidine, much resembles the slow-onset 'tonic' contraction induced by phorbol esters in rat and rabbit aorta (Danthuluri and Deth, 1984; Villalobos-Molina et al., 1990; Bazan et al., 1995), putatively due to protein kinase C activation. The absence of effect of chloroethylclonidine on cytosolic $[Ca^{2+}]$ in rat-1 fibroblasts expressing α_{1b}- or α_{1d}-adrenoceptors (Villalobos-Molina et al., 1997), indicates clearly that the intracellular ion is not affected by the action of the alkylating agent on these receptors, but the possibility remains open that other signaling devices could be activated.

Our results indicate that Wistar Kyoto rat arteries do contract in response to chloroethylclonidine, through a partial agonism at α_{1A}- (caudal) and at α_{1D}-adrenoceptors (aorta). Future experiments should provide an insight into chloroethylclonidine-α_1-adrenoceptor interactions, among them Ca^{2+} mobilisation, protein kinase C activation and increased number of receptor population.

Acknowledgements

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