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Effects of clenbuterol treatment on the responses to vasodilators in urethane-anaesthetized rats

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Abstract—Seven or 14 days of treatment with the β_2-adrenoceptor agonist clenbuterol, 0·3 mg kg^{-1}, s.c., twice daily, increased the basal mean blood pressure in normotensive urethane-anaesthetized rats. The elevated pressure values were maintained until 48 h after the end of the 14 day treatment. Clenbuterol treatment decreased the vasodilatory responses to the β-adrenoceptor agonist isoprenaline and adenosine, agents which act through an increase in intracellular cyclic AMP. Decreased responses were maintained until 48 h after a 14 day treatment with clenbuterol. On the other hand, its administration to rats for 14 days did not modify the vasodilator responses to acetylcholine or sodium nitroprusside, two agents that exert their effects by enhancing cyclic GMP. The increase in mean blood pressure in urethane-anaesthetized rats after clenbuterol treatment may be a consequence of a reduced vasodilator β_2-adrenoceptor-mediated response to circulating catecholamines.

Activation of vascular β-adrenoceptors leads to relaxation of smooth muscle, and this response may involve the stimulation of adenylate cyclase and cyclic (c)AMP production (see reviews of Hardman 1984; Bülbring & Tomita 1987). Vasodilator agents, such as adenosine, may also exert responses through an increase in intracellular cAMP levels (Stiles 1986) or by enhancing cyclic (c)GMP levels. This latter effect may depend on the presence and integrity of vascular endothelium (e.g. via acetylcholine) or may be independent of endothelium (e.g. via nitroderivatives) (Peach et al 1985).

Continuous exposure to a β-adrenoceptor agonist results in a loss of the β-adrenoceptor-mediated responsiveness of the exposed tissue or cell (Harden 1983). The possible mechanisms involved in this desensitization phenomenon may be 'down regulation' of the β-adrenoceptor (Strader et al 1984), 'uncoupling' of the β-adrenoceptor and the adenylate cyclase (Harden et al 1979), or alterations in mechanisms that lead to cAMP production (Tsujimoto & Hoffman 1985).

We have investigated the effects of prolonged in-vivo administration to rats of the selective β_2-adrenoceptor agonist, clenbuterol (Engelhardt 1976; Cohen et al 1982) on the hypotensive responses induced by vasodilatory agents which are thought to act by increasing cAMP or cGMP levels. This treatment induces desensitization of β-adrenoceptors (β_2-type) in certain tissues such as the rat cerebellum (Ordway et al 1987; Vos et al 1987). We decided to analyse the nature and time-course of desensitization and reversal of desensitization of the responses to the vasodilators by using the urethane-anaesthetized rat as an in-vivo model.

Methods

Normotensive Wistar rats, 170–230 g, were anaesthetized with urethane 1.2 g kg^{-1} (i.p.). The trachea was cannulated and the arterial blood pressure was measured from a carotid cannula with a Statham P23-AC transducer recorded on a Model 7 Grass polygraph. A femoral vein was also cannulated for systemic drug administration. After 30 min equilibration, vasodilatory drugs were administered through the venous catheter in increasing doses, in a volume of 0·1 mL/100 g. Succeeding doses were given when the effect of the preceding dose had finished and the cardiovascular parameters had returned to basal values. Mean blood pressure (MBP) was calculated according to the formula: 1/3 (systolic pressure − diastolic pressure) + diastolic pressure.

Clenbuterol was dissolved in 0·9% NaCl (saline) and administered daily (0.3 mg kg^{-1}, s.c., twice a day) for 1, 7 or 14 consecutive days before the day of the experiment. The experiments were carried out 24, 48 or 72 h after the last dose of the drug. All the drugs were dissolved in saline. The doses are expressed in terms of the salt. Statistical evaluation was performed using Student's t-test.

The drugs used were: clenbuterol hydrochloride (generously donated by Dupomar Lab., Argentina), (±)-isoprenaline hydrochloride (dl-isoproterenol hydrochloride Winthrop, USA), adenosine, sodium nitroprusside and acetylcholine chloride (Sigma, USA) and urethane (Cient. Central, Argentina).

Results

Normotensive urethane-anaesthetized rats treated with clenbuterol for 7 or 14 days before the day of the experiment showed a significant increase in the basal mean blood pressure (MBP). The pressor effect observed was maintained until 48 h after cessation of treatment. Seventy-two hours later the MBP returned to control values. Fourteen days treatment with saline did not modify MBP (Table 1).

The vasodilation induced by isoprenaline was reduced by 7 or 14 days of clenbuterol treatment. The decreased response to isoprenaline obtained with 14 days of clenbuterol treatment, was observed up to 48 h after the end of the drug administration and the vasodilatory response was completely restored after 72 h (Fig. 1). Saline-treated rats did not show changes in isoprenaline-induced vasodilation (data not shown).

Table 1. Effect of clenbuterol treatment on the basal mean blood pressure (MBP) of normotensive anaesthetized rats.

Clenbuterol Treatment (days)	n	Basal MBP (mmHg)
—	18	90·2 ± 1·6
1	28	92·8 ± 1·3
7	10	102·1 ± 4·4*
14	20	117·1 ± 2·5**
14 (48 h later)	8	103·3 ± 3·9**
14 (78 h later)	10	86·7 ± 3·4

Clenbuterol treatment was administered daily for 1, 7 or 14 consecutive days (0·3 mg kg^{-1}, s.c., twice a day). Normally the experiments were carried out 24 h after the end of treatment. n = number of experiments. Basal MBP of rats injected with saline solution for 14 days was 93·34 ± 7·23 mmHg (n = 8).
* $P < 0.005$, ** $P < 0.001$.

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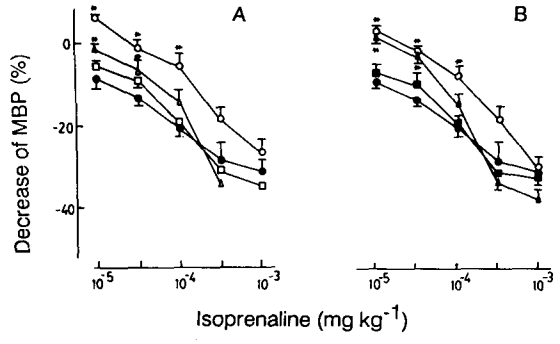

FIG. 1. Effect of clenbuterol treatment on isoprenaline dose-response curves. Decrease in mean blood pressure (MBP) was expressed as a percentage of the basal values. A: ● saline, □ 1, △ 7 or ○ 14 days of clenbuterol treatment. The experiments were carried out 24 h after the treatment. B: ● saline, ○ 24, ▲ 48 or ■ 72 h after 14 days of treatment. The mean ± s.e.m. of 4–9 experiments is shown. * $P < 0.05$.

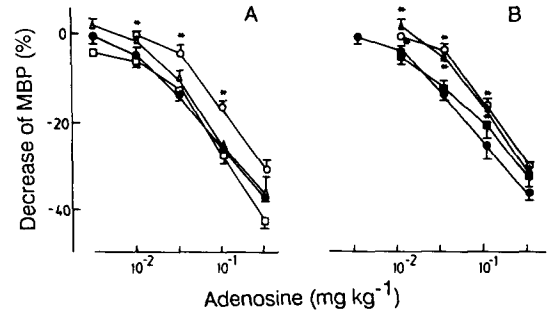

FIG. 2. Effect of clenbuterol treatment on adenosine dose-response curves. Key—same as in Fig. 1.

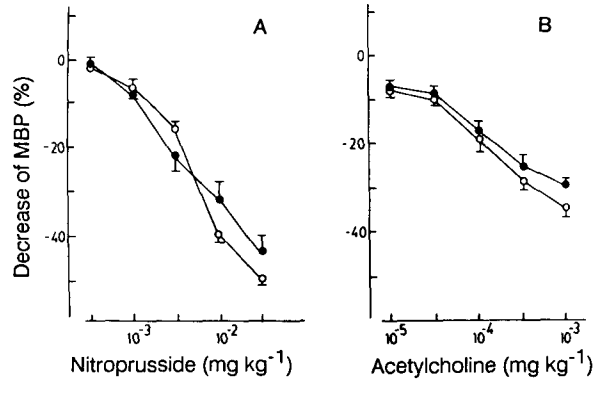

FIG. 3. Effect of clenbuterol treatment on dose-response curves to sodium nitroprusside (A) and acetylcholine (B). Decrease in mean blood pressure (MBP) was expressed as a percentage of the basal values. Rats received clenbuterol (○) or saline (●) for 14 days, and the experiments were carried out 24 h after the end of the treatment. The mean of 3–6 experiments is shown.

After 14 days of clenbuterol treatment the vasodilatory response to adenosine was also reduced. This effect was maintained for up to 48 h after treatment (Fig. 2).

Clenbuterol treatment for 14 days did not modify the vasodilatory response induced by acetylcholine or sodium nitroprusside (Fig. 3).

Discussion

The present study shows that after prolonged administration (7 or 14 days) of the β-adrenoceptor agonist clenbuterol, there is a desensitization of the isoprenaline-induced hypotensive effect in urethane-anaesthetized rats. This effect was maintained for 48 h after the end of the treatment. It has been reported that prolonged treatment with β-adrenoceptor agonists reduced β-adrenoceptor-mediated vasodilation, in vascular beds in in-vitro experiments (Tsujimoto & Hoffman 1985; Hayes et al 1986).

In the rat vasculature, β-adrenoceptors may be mainly of the β_2-subtype (Wilffert et al 1982). It has been demonstrated that in phenoxybenzamine- treated rats, adrenaline was 80 times more potent than noradrenaline in producing a hypotensive effect (Himori et al 1984). We have also previously shown that the selective β_2-adrenoceptor antagonist ICI 118551 antagonized isoprenaline-induced vasodilation without affecting its tachycardic effect (Kazanietz et al 1989). Furthermore, clenbuterol treatment (14 days) did not modify isoprenaline-induced tachycardia in urethane-anaesthetized rats (Kazanietz et al 1986) or in conscious rats (Gutkind et al 1989), or in isolated rat atria (Kazanietz & Enero 1989), thus confirming the β_2-nature of the vascular β-adrenoceptor and the selectivity of clenbuterol for these receptors.

Seven or 14 days of treatment with clenbuterol increased the basal MBP in normotensive urethane-anaesthetized rats. This hypertensive effect was maintained for 48 h after the end of the treatment. It was shown that urethane activates sympathetic outflow from the central nervous system to peripheral tissues (Maggi & Meli 1986), thus increasing catecholamine secretion from adrenal medulla (Armstrong et al 1982). We have previously observed high levels of circulating catecholamines (mainly adrenaline) in urethane- but not pentobarbitone- anaesthetized rats, and this may represent activation of vascular β_2-adrenoceptors (Kazanietz et al 1989). In consequence, a reduction in β_2-adrenoceptor-mediated vasodilation by clenbuterol treatment may result in an increase in MBP in urethane-anaesthetized rats. These data are in agreement with previous results showing a hypertensive response under a stress situation in clenbuterol-treated rats (Gutkind et al 1989). Therefore, as was demonstrated by Himori et al (1984) and Himori & Ishimori (1988), circulating adrenaline may play an important role in determining the normal peripheral vascular tone through activation of β_2-adrenoceptors in certain animal species, such as the rat, but not in other species including dogs and guinea-pigs.

Prolonged treatment with clenbuterol also reduced adenosine-induced hypotension in urethane-anaesthetized rats. Adenosine may exert its hypotensive effect mainly by a direct effect on smooth muscle, and this action may be mediated by activation of A_2-receptors coupled to adenylate cyclase (in analogy with β_2-adrenoceptors). Adenosine also induced a bradycardic response which was not affected by the treatment (data not shown). The time-courses of the desensitization and reversal of desensitization were similar to those of isoprenaline, suggesting a common site altered as a consequence of the treatment. Tsujimoto & Hoffman (1985) have shown that after prolonged treatment of rats with adrenaline, there was a desensitization of β-adrenoceptor-mediated vasodilation in mesenteric arteries by alterations in mechanisms distal to cAMP production. In contrast, in rat aorta the desensitization process may be related only to changes at the level of the β-adrenoceptor (Hayes et al 1986). Taken together, these results suggest important variations in the regulation of β-adrenoceptor-mediated vasodilation in different vascular beds.

Fourteen days' treatment with clenbuterol did not affect acetylcholine or sodium nitroprusside-induced hypotension in urethane-anaesthetized rats, suggesting that the treatment did not affect the vasodilatory mechanisms mediated by cGMP as a

second messenger (whether endothelium dependent or not). Similar results were shown in mesenteric arteries of adrenaline-treated rats (Tsujimoto & Hoffman 1985).

In conclusion, these results indicate that prolonged treatment with clenbuterol modifies the vascular relaxation induced by agents which act through a cAMP pathway, without affecting the relaxant effect produced through an increase in cGMP levels. Moreover, we have recently demonstrated that this treatment reduced isoprenaline-induced vasodilation and cAMP production in aortic rings (Kazanietz & Enero 1990), confirming the desensitization of β-adrenoceptor-mediated responses in the rat vasculature after clenbuterol treatment. Our results could not permit us to make conclusions with respect to the possible site altered by the treatment, but it is possible that the lesion may be distal to cAMP production (a common site involved in β-adrenoceptor and adenosine-mediated vasodilation). The reduced β-adrenoceptor-mediated vasodilation may alter the peripheral vascular tone particularly in situations where catecholamine levels are substantially elevated, such as in the urethane-anaesthetized rat.

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