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Chronic subcutaneous treatment with acebutolol: haemodynamic effects and metabolism in spontaneously hypertensive rats

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Chronic administration of acebutolol (15 mg kg^{-1} s.c. three times a week for five weeks, then 30 mg kg^{-1} for three weeks) did not lower blood pressure in 17 and 33 weeks-old spontaneously hypertensive rats (SHR). At the end of this treatment, the plasma concentrations of acebutolol and diacetolol were measured by HPLC. After 24 h, acebutolol was absent from plasma while diacetolol was lower after chronic treatment than after acute administration. Twenty-four hours after the last injection of acebutolol, both isoprenaline-induced tachycardia and vasodilatation were reduced. The vasomotor agents, noradrenaline, bradykinin and angiotensin, exhibited the same activity in control and treated SHR. These findings suggest that the lack of antihypertensive effect of acebutolol in SHR may be the result of a decrease in diacetolol formation together with blockade of β_2 vascular receptors.

The success of β-blocking drugs in preventing development of hypertension or in lowering blood pressure by the oral route in spontaneously hypertensive rats, has been variable (Weiss et al 1974; Levy 1976; Takeda & Bunag 1980). For example, acebutolol, in doses up to 100 and 1000 mg kg^{-1} day^{-1} chronically, did not influence the development of hypertension (Richer et al 1979, 1980). Although the oral route has been most commonly used, some successful attempts have been made with propranolol given parenterally (Smits et al 1980; Burkan & Leach 1981; Wexler & McMurtry 1981). The half-lives of elimination of acebutolol and propranolol are similar, 2·9 and 3·8 h, respectively (Ritschel 1980), and the main metabolite of acebutolol, diacetolol, has similar β-adrenoceptor antagonist properties (Thibonnier et al 1982) with a plasma half-life of about 12 h (Flouvat et al 1981), although acebutolol's β-blocking potency is reported to be one-third that of propranolol (Giudicelli 1984). We have investigated in 17 and 33 weeks-old spontaneously hypertensive rats (SHR) the effects of chronic treatment with acebutolol, given at a dose equipotent to that dose of propranolol used successfully by Wexler & McMurty (1981). We have measured the plasma concentration of the drug and its main metabolite, diacetolol, and the degree of β-blockade. In addition, we examined the reactivity of treated rats to vasomotor drugs.

Materials and methods

Male, spontaneously hypertensive rats (SHR) of the Okamoto strain were used (IFFA-CREDO breeding laboratories).

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Seventeen weeks-old rats (n = 11) and 33 weeks-old rats (n = 11) received acebutolol 15 mg kg^{-1} s.c., three times a week for five weeks, then 30 mg kg^{-1} s.c. for three weeks. In addition, 0·9% NaCl (saline) 1 ml kg^{-1} s.c. was injected in 17 weeks-old rats (n = 10) and 33 weeks-old rats (n = 10). During this time, body weight, systolic blood pressure and heart rate (Electrosphygmomanometer, Narco Biosystem) were measured weekly.

Acebutolol and metabolite determination. Acebutolol and its major metabolite, diacetolol, were assayed by HPLC as follows: 3 and 24 h after the last dose of acebutolol, 500 μl of blood was collected into heparinized tubes by retro-orbital puncture. Plasma was obtained by centrifugation and acebutolol and diacetolol were taken up by a mixture of $CHCl_3$ and 1 M NaOH (10 : 0·5 v/v). Before extraction, a known quantity of internal standard ((±)-1-(2-propyl-4-n-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane) was added to the plasma.

The high pressure liquid chromatograph was fitted with a Spheri-Sorb 5 ODS column. Mobile phase was a mixture of H_2O, methanol and triethylamine (500 : 500 : 0·5 v/v). Absorbance was measured at 254 nm. Under these experimental conditions, acebutolol and its acetyl metabolite, diacetolol, were detected.

Plasma concentrations of acebutolol and its metabolite were also measured in 17 and 33 weeks-old SHR after a single injection of acebutolol (30 mg kg^{-1} s.c.).

Cardiovascular studies. Twenty-four hours after the last acebutolol injection, rats were first lightly anaesthetized with ether, then with chloralose i.v. (50 mg kg^{-1}). Blood pressure and heart rate were recorded continuously (NARCO Physiograph MK III). Drugs were then administered intravenously at 7 min intervals, in the following order and dose: noradrenaline (NA) 2, bradykinin (BK) 1, angiotensin (AG) 2, and isoprenaline (IS) 0·4, 0·8 and 1·2 μg kg^{-1}.

Drugs. The drugs used were: acebutolol, diacetolol and (±)-1-(2-propyl-4-n-butyramidophenoxy)-2-hydroxy-3-isopropylaminopropane (Rhône-Poulenc, Paris, France), bradykinin lysine (B 6133, Sigma, St-Louis, MO, USA), angiotensin (Ciba-Geigy, Basle, Switzerland).

Results

Blood pressure and heart rate. In 17 weeks-old SHR, the blood pressure was 185 mm Hg before treatment and during the experiment it rose in both the saline and treated groups reaching significantly higher values after five weeks (205 mm Hg) (Fig. 1). Heart rates were about 400 beats min^{-1} before treatment and acebutolol caused a non-significant bradycardia (minimum: 370 beats min^{-1}).

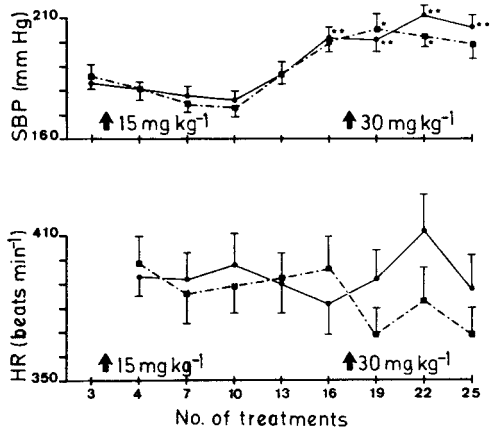

FIG. 1. Systolic blood pressure (SBP) and heart rate (HR) of 17 weeks-old SHR during chronic s.c. treatment with saline (●) or acebutolol (■) 15 mg kg^{-1} three times a week, then 30 mg kg^{-1} (means ± s.e.m.) ★ $P \leq 0.05$, ★★ $P \leq 0.01$ vs initial value (paired *t*-test).

In 33 weeks-old SHR, the blood pressure rose from 185 to 200 mm Hg at the end of the experiment and acebutolol did not modify this. Heart rate was not significantly changed by the treatment (Fig. 2).

There was no difference between the body weights of the treated and control animals during the study.

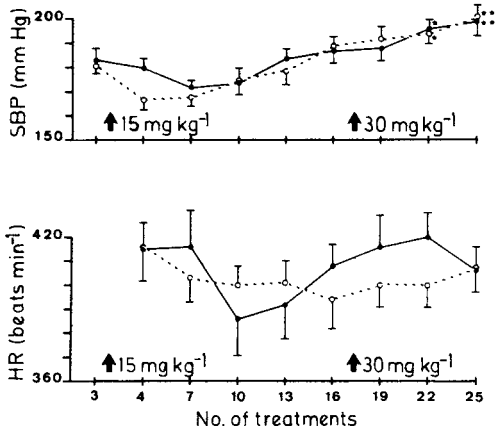

FIG. 2. Systolic blood pressure (SBP) and heart rate (HR) of 33 weeks-old SHR during chronic s.c. treatment with saline (●) or acebutolol (○) 15 mg kg^{-1} three times a week, then 30 mg kg^{-1} (means ± s.e.m.). ★ $P \leq 0.05$, ★★ $P \leq 0.01$ vs initial value (paired *t*-test).

Pharmacokinetic study. Three hours after the acute dose of acebutolol, its plasma concentration did not differ among 17 weeks-old rats (n = 10; 1·41 ± 0·09 μg ml^{-1}) and 33 weeks-old rats (n = 8; 1·48 ± 0·13 μg ml^{-1}).

Three hours after the last acebutolol dose in chronically treated rats, its plasma concentrations were not significantly different (17 weeks-old rats, n = 4; 1·53 ± 0·32 μg ml^{-1}; 33 weeks-old rats, n = 4; 1·12 ± 0·22 μg ml^{-1}).

Twenty-four hours after administration, there was no acebutolol detectable in plasma, but diacetolol was present: the acutely treated rats showed no significant difference (17 weeks-old rats, n = 11; 35·8 ± 8·5 ng ml^{-1} diacetolol; 33 weeks-old rats, n = 8; 16·7 ± 7·7 ng ml^{-1} diacetolol). Likewise, no significant difference was observed between the two groups of rats after chronic administration (17 weeks-old rats, n = 6; 11·8 ± 3·5 ng ml^{-1} diacetolol; 33 weeks-old rats, n = 5; 8·5 ± 2·9 ng ml^{-1} diacetolol).

In contrast, a significant difference ($P < 0.05$) was found in the diacetolol concentrations of 17 week-old rats acutely and chronically treated, being lower in the latter.

Haemodynamic study. In 17 week-old SHR, heart rate after chloralose anaesthesia reached 362 ± 4·6 in control and 364 ± 12·1 beats min^{-1} in treated rats. Mean blood pressure was respectively 182 ± 6·7 mm Hg and 178·5 ± 4·9 mm Hg in control and treated groups. Acebutolol also did not modify changes in mean blood pressure observed after noradrenaline, bradykinin and angiotensin (Fig. 3).

Whatever the dose of IS (0·4; 0·8; 1·2 μg kg^{-1}), in the control SHR group the maximal increases in heart rate to all doses of isoprenaline were similar (40 to 43 beats min^{-1}). In the treated group, tachycardia was always the same (30 beats min^{-1}) while there was significant difference between control and treated SHR after 1·2 μg kg^{-1} isoprenaline. Depressor responses to

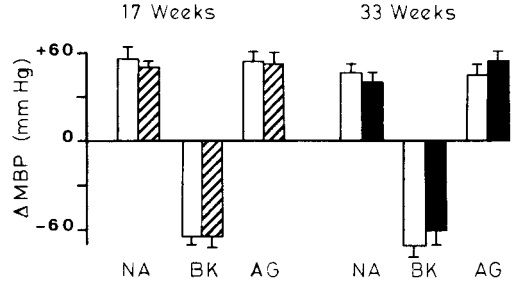

FIG. 3. Changes in mean blood pressure (MBP) induced by i.v. administrations of noradrenaline (NA) 2 μg kg^{-1}, bradykinin (BK) 1 μg kg^{-1} and angiotensin (AG) 2 μg kg^{-1}, in 17 and 33 weeks-old SHR. Vertical bars represent s.e.m. of the means. Open columns, controls; hatched columns, acebutolol administered to 17 weeks-old rats; solid columns, acebutolol to 33 weeks-old rats.

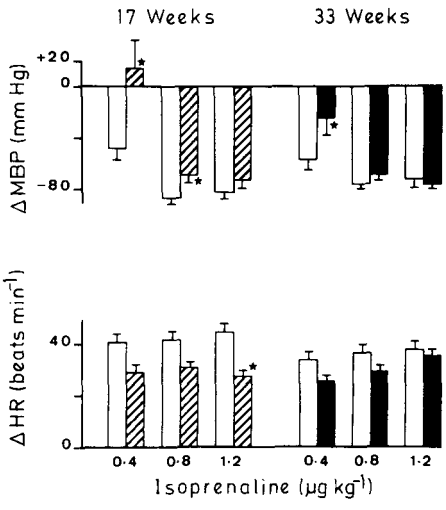

FIG. 4. Changes in mean blood pressure (MBP) and heart rate (HR) induced by i.v. administration of isoprenaline 0·4, 0·8 and 1·2 μg kg^{-1} in 17 and 33 weeks-old SHR. Vertical bars represent s.e.m. of the means. Open columns, control; hatched columns, acebutolol administered to 17 weeks-old rats; solid columns, acebutolol to 33 weeks-old rats. ★ $P \leq 0{\cdot}05$ vs control (same age).

isoprenaline were markedly attenuated by acebutolol, the 0·4 μg kg^{-1} dose was rendered ineffective while the 0·8 μg kg^{-1} effect was reduced (Fig. 4).

In 33 weeks-old SHR, heart rate was slightly lowered by chloralose, from 400 ± 10 to 364 ± 11 beats min^{-1} in control and from 403 ± 8 to 371 ± 10·7 beats min^{-1} in treated rats ($P < 0.05$ paired t-test). The mean blood pressure values were 192 ± 8·3 mm Hg in control and 188 ± 7 mm Hg in treated rats.

The vascular effects of noradrenaline, bradykinin and angiotensin were the same in control and treated rats (Fig. 3). Chronotropic responses to isoprenaline were the same in the two groups of SHR. The fall in blood pressure caused by the 0·4 μg kg^{-1} dose was reduced and the effects of higher doses were unchanged (Fig. 4).

Discussion

As by the oral route, chronic s.c. injection of acebutolol did not lower blood pressure in SHR even though this route would avoid the first pass metabolism (Meffin et al 1978; Ritschel 1980; Giudicelli 1984) and therefore antihypertensive activity would be expected with this treatment.

Plasma determinations showed that acebutolol did not accumulate after multiple doses and disappeared rapidly. Moreover, 24 hours after administration, the concentrations of diacetolol were lower after the chronic administration than after the single dose. This result differs from findings in man by Cuthbert & Collins (1975) and Gulaid et al (1981), where acebutolol and mainly diacetolol concentrations were higher during repeated dosing than after a single administration.

In 33 weeks-old SHR, acebutolol elimination seemed more rapid and the diacetolol concentration lower which is in agreement with the observation of a weaker β-blockade in older SHR.

The reduction in the heart-rate response to IS validate the dosage of acebutolol used. Inhibition of IS-induced vasodilatation was unexpected since acebutolol has been found to be cardioselective in the cat (Basil et al 1973; Maxwell & Collins 1974). Further studies could not demonstrate cardioselectivity relative to vascular receptors in the dog (Harms & Spoelstra 1978) and in man (Briant et al 1974). Moreover, the action of acebutolol on β_2-vascular receptors could account for the increase in peripheral vascular resistance which has been found after chronic treatment in the rat (Richer et al 1980).

Finally we studied the reactivity of treated rats to vasomotor agents. In rats of both age groups, the pressor responses to NAD and AG and the vasodilatation induced by BK were not modified by acebutolol. Consequently, lower diacetolol formation and blockade of β_2-vascular receptors are two factors possibly involved in the lack of antihypertensive effect of acebutolol in SHR.

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Does naloxone induce relaxation of guinea-pig airway smooth muscle?

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The effects of the opiate receptor antagonist naloxone were investigated on isolated preparations of guinea-pig trachealis contracted with either histamine, methacholine or KCl. The commercially available solution of naloxone (Narcan) induced concentration-dependent relaxation of the contracted airway preparations. In stark contrast, aqueous solutions of naloxone were without any significant relaxant effect. Aqueous solutions of the preservatives (methyl and propyl hydroxybenzoate) present in the vehicle used in the commercial formulation of naloxone mimicked exactly the relaxant effects induced by Narcan. Thus, naloxone does not directly induce relaxation of airway smooth muscle. The effects of Narcan can be solely attributed to the activity of the preservatives present in the vehicles. The mechanism underlying the bronchodilator activity of methyl and propyl hydroxybenzoate is unknown but is not related to receptor blockade or to alterations in the intracellular levels of cyclic AMP.

Opioids administered intravenously to experimental animals and man induce increases in airways resistance, laryngospasm and respiratory stimulation (Foldes et al 1966; Jennett et al 1968; Willette et al 1983). Furthermore, asthmatic attacks induced by a combination of chlorpropamide and ethanol in diabetic patients are mimicked by intravenous administration of enkephalin analogues and inhibited by the opiate receptor antagonist naloxone (Leslie et al 1980). It has been proposed, therefore, that in such diabetic patients the asthmatic episodes are mediated via endogenous peptides with opiate-like activity (Leslie et al 1980). Recently, however, it has been reported that the preservatives (methyl and propyl hydroxybenzoate) present in commercially available solutions of naloxone possess vasodilator activity (Brandt et al 1983; Crockard et al 1983). In the study of Leslie et al (1980) it is not clear whether the patients received the appropriate vehicle, in which commercial preparations of naloxone are formulated, as a control. Thus, in view of the reported smooth muscle relaxant activity of the preservatives, it is conceivable that the anti-asthmatic effect observed by Leslie et al (1980) was not entirely attributable to the opiate receptor-blocking activity of the naloxone.

The object of this study, therefore, was to investigate whether naloxone possesses any direct relaxant activity in isolated preparations of guinea-pig airway smooth muscle. A preliminary account of these findings has been reported to the British Pharmacological Society (Soulioti et al 1986).

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Methods

Male Dunkin-Hartley guinea pigs were killed by stunning and bleeding. Tracheae were rapidly excised from the animals, dissected free of extraneous connective tissue and then prepared as follows.

Tension studies. Spirally cut preparations of tracheae were suspended in Krebs-Henseleit solution (KHS) at 37 °C and bubbled with 95% O_2 and 5% CO_2. An initial stretching tension of 20 mN was applied to the tissues which were left for 60 min to equilibrate, during which time the bathing medium was changed three times. Changes in tension were recorded using isometric force-displacement transducers (FT03C; Grass Instruments, Quincy, Mass.) coupled to a Grass (model 7) curvilinear, ink-writing polygraph. The composition of the KHS used in these studies was as follows (in mM); NaCl 118; KCl 4·7; $MgSO_4$ 1·2; KH_2PO_4 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25 and glucose 11·7.

Following equilibration, the tissues were contracted with histamine (1×10^{-4} M) to gauge the normality and viability of the tissue. Following washout of the histamine and return to baseline tension, an interval of 30 min was allowed before addition of the same concentration of histamine. This time at the peak of the tonic (sustained) component of the contraction a cumulative concentration-effect curve was constructed using the relaxant solution under test (Narcan, naloxone hydrochloride or the vehicle present in Narcan). Control preparations which received saline in place of drug were run in parallel with those in which test solutions were studied. Drug-induced relaxations, corrected for any fall-off in tension recorded in the control preparations, were calculated as a percentage of the maximum relaxation achieved. Most control preparations exhibited little loss of tension over the 60 min test period. In several experiments KCl (9×10^{-2} M) or methacholine (1×10^{-6} M) was used as the contracting agent in place of histamine. In experiments using these two latter