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β_2-Adrenergic agonist effects of medroxalol and labetalol on rat and mouse uterine muscle

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Medroxalol and labetalol are antihypertensive agents possessing α- and β-adrenoceptor blocking properties (Dage et al 1981; Cheng et al 1980; Brittain & Levy 1976). However, it has been reported recently that labetalol had β_2-adrenergic agonist effects on the rat isolated uterus (Carey & Whalley 1979a, b) and guinea-pig tracheal strips (Carpenter 1981). A β_2-adrenergic agonist activity has also been demonstrated for medroxalol on the vascular bed of the gracilis muscle in anaesthetized dogs (Hsieh & Dage personal communication) and in guinea-pig isolated tracheal muscle stris (Spedding 1981).

In the present investigation, we have compared the β_2-adrenergic agonist effect of medroxalol with that of labetalol in potassium chloride (KCl)-contracted uterine muscle strips isolated from rats and mice.

Female Long Evans rats (200–300 g) and female CD-1 mice (20–40 g), supplied by Charles River, were used. The stage of the oestrus cycle was determined by microscopic examination of the vaginal smear each morning. Only those animals in natural oestrus were used in this investigation. The animals were killed by a blow to the head. Uterine horns were then dissected rapidly and placed in a Petri dish containing oxygenated Krebs-Henseleit solution, which had the following composition in mM: NaCl, 110; KCl, 4·8; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $NaHCO_3$, 25; dextrose, 11; and disodium ethylenediaminetetraacetic acid (Na_2EDTA), 0·027. One side of the rat uterine horn was cut open longitudinally and again cut longitudinally into two long strips; a piece of uterine muscle (5 × 30 mm), taken from the middle portion of the strip, was set up in an organ bath (37 °C) for recording isometric contractions using a Grass FTO3C transduducer and a Grass Model 7 polygraph. A similar strip of uterine muscle was prepared from the contralateral uterine horn. These two strips were used concurrently for medroxalol and labetalol. Both mouse uterine horns were cut longitudinally, yielding two strips of approximately 5 × 30 mm, and set up for the concurrent evaluation of medroxalol and labetalol as described above. Thus, four muscle strips (two from each animal) were set up in four 25 ml organ baths for measuring spontaneous and KCl-induced contractions and subsequent relaxation by medroxalol or labetalol. The uterine strips were loaded with an initial tension of 1 g.

As soon as the muscle strips were set up in the organ baths, spontaneous contractions developed. KCl (60 mM final bath concentration) was then injected into the organ bath to produce a sustained contracture. Medroxalol or labetalol was then injected into the organ bath in a cumulative manner (van Rossum 1963) to produce relaxation of the muscle strips. Following completion of the dose response experiment, papaverine (10^{-3} M) was injected into the organ bath to produce a maximum relaxation of the muscle. Muscle relaxation produced by medroxalol or labetalol was expressed as percent of the maximum relaxant effect produced by papaverine.

Medroxalol or labetalol was prepared as a 100 μmol ml^{-1} solution in dimethylacetamide solution. Subsequent dilutions were made with distilled water. Student's *t*-test (Steele & Torrie 1960) and analysis of variance for repeated measurements followed by Dunnett's procedure (Winer 1971) were used to assess significant differences between drug treated and control groups at the same dose level. A probability of less than 0·05 was considered to be statistically significant.

Most uterine muscle strips began to contract spontaneously immediately after being set up in the organ bath. The frequency was 1·3 ± 0·2 contractions min^{-1} for rats and 1·1 ± 0·1 for mice (mean ± s.e., n = 20). KCl produced a sustained contraction lasting longer than 3 h; developed tension was 2·93 ± 0·22 g (mean ± s.e., n = 20) in strips obtained from rats and 1·63 ± 0·17 g (mean ± s.e., n =20) in strips obtained from mice.

Medroxalol and labetalol produced dose-dependent relaxation of KCl-contracted uterine muscle strips isolated from rats and mice. The relaxation dose response curves appeared to be biphasic: a gradual relaxation phase which occurred between 10^{-8} and 10^{-5} M for both compounds in both species, and a steep relaxation phase which occurred between 10^{-5} and 10^{-4} M, also for both compounds and in both species. Medroxalol and labetalol produced only a partial relaxation of strips obtained from both species; the potencies of medroxalol and labetalol were similar in this regard: −log ED20's for medroxalol and labetalol in rat uterine strips were 6·76 ± 0·32 (n = 10) and 7·21 ± 0·23 (n = 9), respectively, and in mouse strips, 6·77 ± 0·33 (n = 10) and 7·02 = 0·29 (n = 10), respectively. Vehicles used for medroxalol and labetalol had no significant effect on the contractility of the uterine strips.

Preincubation of the uterine muscle strips with propranolol (10^{-7} M) shifted the medroxalol and labetalol dose response curves to the right. However, propranolol failed to antagonize the uterine muscle relaxation produced by high concentrations of medroxalol and labetalol (10^{-5} and 10^{-4} M). The uterine muscle relaxant effects of medroxalol and labetalol were also examined in the presence of

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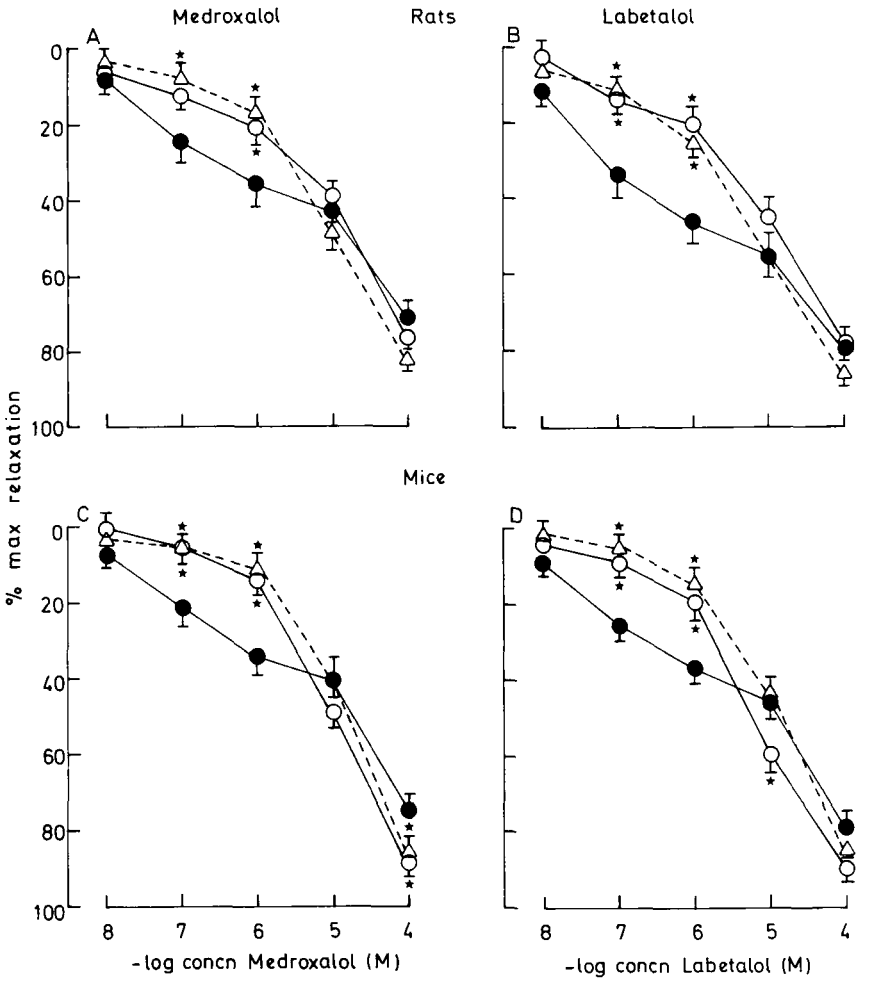

FIG. 1. Relaxation of KCl-contracted rat and mouse uterine muscle strips by medroxalol and labetalol: antagonism by propranolol (10^{-7} M) or propranolol (10^{-7} M) and phentolamine (10^{-6} M) pretreatment. Asterisks represent a statistically significant ($P < 0.05$) difference from control group.

propranolol (10^{-7} M) and phentolamine (10^{-6} M). This combination of propranolol and phentolamine did not produce any further antagonism than that observed with propranolol alone.

Mammalian uterine muscle possess both α- and β-adrenoceptors, the α-receptors are excitatory while the β-receptors are inhibitory (Alquist 1962). However, in the non-pregnant rat uterus, the response to noradrenaline, adrenaline and isoprenaline is inhibitory throughout the four stages of the natural oestrus cycle (Digges & Boyle 1979). The relaxation produced by the catecholamines is mediated by β_2-adrenoceptor activation (Lands et al 1967). In this investigation, medroxalol and labetalol produced a partial relaxation which was antagonized by propranolol. Thus, both medroxalol and labetalol are partial agonists for β_2-receptors in uterine muscle strips isolated from rats and mice. The results with labetalol were consistent with those reported by Carey & Whalley (1979a, b) in the rat uterus. The α-blocking activity of medroxalol and labetalol did not appear to contribute to the relaxation effect produced by these two compounds since the relaxation dose response curves were unaltered by the addition of phentolamine to the bathing solution.

Propranolol failed to antagonize the uterine muscle relaxation produced by high concentrations of medroxalol and labetalol (10^{-5} to 10^{-4} M). The lack of antagonism of labetalol by propranolol is also consistent with the report of Carey & Whalley (1979b). These results suggest that medroxalol and labetalol may relax the uterine muscle by an action other than β-adrenoceptor activation.

In summary, the relaxation dose response curves produced by medroxalol and labetalol appeared to be biphasic: an initial gradual relaxation phase which occurred between 10^{-8} and 10^{-5} M and a subsequent steep relaxation phase

which occurred between 10^{-5} and 10^{-4} M for both compounds. The gradual relaxation phase was antagonized by propranolol while the steep relaxation phase was not. It is concluded that medroxalol and labetalol have β_2-adrenoceptor agonist activity which relaxes uterine muscle, and an additional relaxant activity which is unrelated to β-adrenoceptor activation.

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Aminophylline-induced contractions of rabbit ear artery in high-K^+ Ca^{2+}-free medium

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The phasic plus tonic components of the bimodal response of rabbit ear artery to noradrenaline (NA) has been attributed to release of Ca^{2+} from cellular stores and mobilization of extracellular Ca^{2+} respectively (Bevan et al 1973; Steinsland et al 1973). Since methylxanthines produce contractures of both skeletal (Bianchi 1961; Endo 1975; Bianchi & Friedman 1979) and cardiac (Chapman & Leoty 1976; Matsumura & Narita 1980) muscle through Ca^{2+} release from sarcoplasmic reticulum (for a review see Fabiato & Fabiato 1977) it appeared worthwhile to determine the effect of aminophylline on rabbit ear artery under experimental conditions suitable for studying cellular Ca^{2+} mobilization-dependent contractions.

Methods

Male albino rabbits, 2·5–3 kg were anaesthetized with urethane (1·5 g kg^{-1} i.p.) and heparinized (1000 U.I. i.v.). A 3 cm segment of central ear artery was dissected free from adhering tissues, cannulated at both ends with polyethylene tubing and transferred to a 7 ml organ bath (at 37 °C) with a volume maintained constant by means of an overflow. The arterial segment was perfused intraluminally by means of De Saga 131900 six-channels peristaltic pump at a rate of 5 ml min^{-1} while extraluminal perfusion at a rate of 8 ml min^{-1} was obtained by means of a Mariotte bottle. Both intraluminal and extraluminal perfusion fluid were gassed with 95% O_2 and 5% CO_2 and heated at 37 °C. Changes in intraluminal perfusion pressure, recorded by means of a pressure transducer, were taken as an indirect measure of arterial contraction over the resting tone (20·4 ± 0·8 mmHg; n = 19). The artery was perfused intra and extraluminally with Krebs solution (mM) (NaCl 119, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $CaCl_2$ 2·5, glucose 11). After 1 h stabilization period the perfusion fluid was replaced with high-K^+ Ca^{2+} free-solution (NaCl 69, $NaHCO_3$ 25, KCl 54·7 $MgSO_4$ 1·5 KH_2PO_4 1·2 glucose 11 mM) which produced a rapid contraction followed by return to basal values. Five minutes later intraluminal perfusion fluid was substituted with high-K^+ CA^{2+}-free solution containing NA or aminophylline at the desired concentration which produced a contraction followed by a return to resting values. Preliminary experiments showed that a 25 min perfusion with Krebs solution provided comparable responses to subsequent challenge with NA or aminophylline. When testing the influence of aminophylline (10^{-2} M) on contractions produced by a supramaximal (5×10^{-6} M) dose of NA, the procedure was similar to that described above with the difference than 2 min before NA challenge the inner perfusion fluid was substituted with a high K^+-Ca^{2+}-free solution containing aminophylline.

Results

In high-K^+ Ca^{2+}-free medium both NA ($1 \times 10^{-8} - 5 \times 10^{-6}$ M) and aminophylline ($1 \times 10^{-3} - 5 \times 10^{-2}$ M) produced a dose-dependent transient contraction with maximal values of 68·7 ± 2·2 and 17·49 ± 0·5 mmHg respectively (Fig. 1). The ED50 values calculated according to Litchfield & Wilcoxon (1949) where $7{\cdot}97 \times 10^{-8}$ M ($3{\cdot}92 \times 10^{-8} - 1{\cdot}62 \times 10^{-7}$) and $3{\cdot}15 \times 10^{-3}$ M

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