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The effect of propranolol on sympathetic nerve stimulation in isolated vasa deferentia

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(±)-Propranolol hydrochloride (0·5 mg kg^{-1} twice daily, subcutaneously, for 3 days or approximately 2·4 mg kg^{-1} daily, orally, for 21 days) failed to produce ptosis or to affect responses to transmural stimulation of isolated vasa deferentia removed from treated mice. In guinea-pig isolated vasa deferentia responses to transmural stimulation through parallel electrodes were reduced by propranolol (1 to 20 μg ml^{-1}); blockade was concentration dependent, fast to equilibrium (45 min), easily reversed by washing but not reversed by (+)-amphetamine sulphate (0·2 μg ml^{-1}). At lower concentrations (0·04 and 0·2 μg ml^{-1}), propranolol marginally potentiated responses to transmural stimulation. In contrast, guanethidine (0·2 μg ml^{-1}) produced a slow onset blockade which was completely reversed by (+)-amphetamine. The response to electrical stimulation through concentric ring electrodes was reduced by low concentrations of propranolol but this effect is ascribed to the known local anaesthetic actions of propranolol and no evidence of true adrenergic neuron blockade was found.

In addition to blocking β-adrenoceptors, propranolol can also produce local anaesthesia (Barrett & Cullum, 1968; Day, Owen & Warren, 1968) and, possibly, adrenergic neuron blockade. This latter effect has been demonstrated *in vivo* in the cat (Eliash & Weinstock, 1971; 1972) and *in vitro* in the guinea-pig isolated vas deferens (Mylecharane & Raper, 1970; 1973). More recently however, using a variety of techniques, Dawes & Faulkner (1975) were unable to detect adrenergic neuron blockade after administration of various doses of propranolol to anaesthetized cats and dogs and in view of this discrepancy we have re-examined the effects of propranolol on the response of vasa deferentia to electrical stimulation of their sympathetic nerves.

METHODS

Guinea-pigs and mice were killed by a blow on the head, the vasa deferentia were removed, placed in cold McEwen solution (McEwen, 1956), stripped of their mesenteric coat and set up in McEwen solution at 37° in an organ bath (28 ml) bubbled with 5% CO_2 in oxygen. A constant flow of fresh pre-heated McEwen solution (containing drugs when appropriate) was infused into the organ bath at 5 ml min^{-1}. Periods of electrical stimulation were applied at 5 min intervals usually through parallel platinum wire electrodes using a low output impedence stimulator delivering rectangular pulses of 0·5 ms duration and supramaximal voltage (35 V). In some experiments this type of stimulation (at 10 Hz for 15 s) was alternated with stimulation through bipolar ring electrodes as described by Mylecharane & Raper (1973). Changes in length of the tissues in response to electrical stimulation were recorded isotonically (load 200 mg).

In experiments on mouse vas deferens, after allowing the tissues to settle down for 10 min, electrical stimulation was applied for 5 s periods at frequencies of 50, 50, 1, 2,

5, 10, 20 and 50 Hz in that order. The amplification and load of the recording system were held constant throughout all experiments in a particular series and responses have been expressed in arbitrary units. (±)-Propranolol hydrochloride was administered to the mice using two dose regimes. One group received 0·5 mg kg^{-1} subcutaneously twice daily for three days (experiment on day 4), controls receiving equivalent injections of 0·9% NaCl. A second group was given free access to water containing sucrose (10 mg ml^{-1}) and (±)-propranolol hydrochloride (12 μg ml^{-1}) while the controls drank water containing sucrose alone; both the control and the treated groups were maintained under these conditions for 3 weeks. In all animals the width of the palpebral fissure was assessed periodically.

In experiments on guinea-pig vas deferens, tissues were allowed to settle down for 45 min during which time 15 s periods of 10 Hz stimulation were applied every 5 min. This type of stimulation was continued throughout the experiment except when frequency-response curves were determined. Frequency-response curves [consisting of periods of electrical stimulation of 30 s (1 Hz), 15 s (3 Hz), 15 s (10 Hz), 5 s (30 Hz) and 5 s (100 Hz)] were determined after the initial settling down period; 30, 115 and 200 min after changing the infusion solution to McEwen solution + drug; 20 min after changing to McEwen solution + drug + (+)-amphetamine sulphate (0·2 μg ml^{-1}); and 45 min after changing back to McEwen solution alone. All responses have been expressed as a percentage of the 10 Hz response in the initial frequency-response curve. All results are expressed as mean ± standard error and tests for statistical significance utilised Student's *t*-test.

Drugs used: (+)-amphetamine sulphate (BDH), guanethidine sulphate (Ciba), isoprenaline sulphate (BW), (±)-propranolol hydrochloride (ICI), sodium pentobarbitone (M & B), sucrose (BDH). All concentrations are expressed in terms of these salts.

RESULTS

Systemic administration of propranolol to mice. Frequency-response curves obtained on vasa deferentia from propranolol-treated animals were not significantly different from those obtained on controls at any frequency tested ($P > 0{\cdot}4$) either in those mice which had received propranolol by injection for 3 days or in those which had received oral propranolol for 3 weeks (Fig. 1). The latter animals were housed in one cage and it is therefore impossible to calculate the precise amount of propranolol consumed by each mouse. On average however, each mouse drank approximately 7·5 ml of fluid per day (containing 12 μg ml^{-1} (±)-propranolol hydrochloride); thus the average oral dose of propranolol was approximately 2·4 (mg kg^{-1}) day^{-1} over the 3-week period. This treatment would appear to be effective in blocking β-adrenoceptors since the resting heart rate [in animals anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) via the tail vein] was 463 ± 26 beats min^{-1} in control animals (mean ± standard error; n = 5) while in two animals given propranolol as above the resting heart rates were 377 and 307 beats min^{-1} respectively. When challenged with isoprenaline sulphate (50 ng kg^{-1} via the tail vein) control animals responded with a tachycardia of 62 ± 4 beats min^{-1} (mean ± standard error; n = 5) while the two animals which had received propranolol showed responses of 40 and 22 beats min^{-1} respectively. Neither of the treated groups exhibited any significant degree of ptosis at any time during treatment.

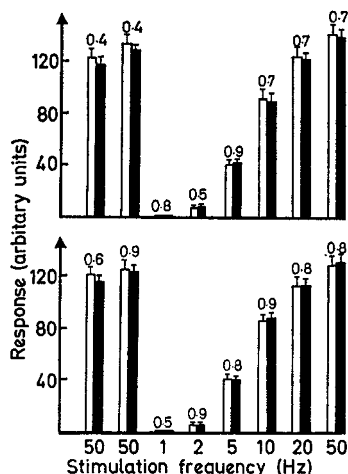

FIG. 1. (Upper) Showing lack of effect of systemic propranolol on frequency-response curves obtained under isotonic conditions from mouse isolated vas deferens preparations in McEwen solution at 37°. Electrical stimulation (rectangular pulses of 0·5 ms duration and 35 V) was applied every 5 min for 5 s. Open columns–controls (16 tissues). Solid columns – (±)-propranolol hydrochloride, (0·5 mg kg^{-1} subcutaneously twice daily for 3 days [16 tissues]). The bars show the standard error and the figures above each pair of columns show the degree of statistical significance (*P*) between means (Student's *t*-test).
(Lower) As above except that (±)-propranolol was administered in drinking water for 3 weeks; approximate dose 2·4 (mg kg^{-1}) day^{-1}; (12 tissues).

Guinea-pig isolated vas deferens. The results for the 10 Hz response obtained in each frequency-response curve are presented in Fig. 2—data obtained at other frequencies has been omitted from this paper but the conclusions which could be drawn from this data are substantially the same as those drawn from the 10 Hz data.

At a concentration of 20 $\mu g\ ml^{-1}$ (±)-propranolol produced complete blockade of the response to 10 Hz transmural stimulation within 45 min, the blockade was not reversed by (+)-amphetamine (0·2 $\mu g\ ml^{-1}$) but was easily and completely reversed by washing with drug-free McEwen solution for 45 min. At concentrations of 10 and 5 $\mu g\ ml^{-1}$, propranolol produced a partial blockade within 45 min which did not deepen appreciably even though the tissues were exposed to these concentrations of propranolol for a further 170 min. (+)-Amphetamine sulphate failed to reverse this partial blockade and, in the case of the 10 $\mu g\ ml^{-1}$ concentration, produced a significant deepening of the blockade. Washing the tissues with drug-free McEwen solution returned responses to control levels. At concentrations of 1·0, 0·2 and 0·04 $\mu g\ ml^{-1}$, propranolol produced no marked degree of blockade at any time during the 215 min for which the tissues were exposed to these solutions. Indeed, at the two lower concentrations some potentiation of the response was seen as compared with control tissues in which propranolol was omitted. (+)-Amphetamine sulphate marginally potentiated the response to transmural stimulation in tissue which had been exposed to any of these three lower concentrations of propranolol as it did in control tissues.

In contrast to the effects observed with propranolol, guanethidine (0·2 $\mu g\ ml^{-1}$) produced a blockade of the response to transmural stimulation which deepened steadily over the period the tissues were exposed to the drug and had not reached equilibrium even after 215 min. The application of (+)-amphetamine through the infusion solution reversed this blockade completely and returned responses to control levels (Fig. 2).

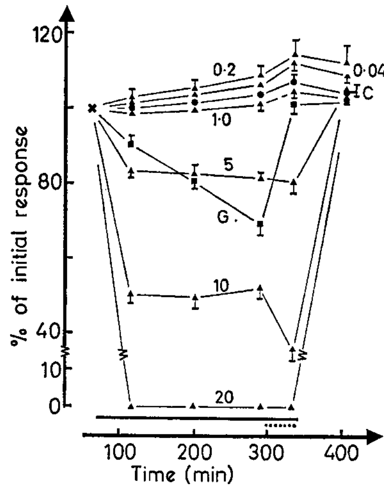

FIG. 2. Transmurally stimulated guinea-pig vas deferens (15 s of 10 Hz, 0·5 ms, 35 V pulses every 5 min–see text). Showing response to electrical stimulation (as % of initial response) during exposure to (±)-propranolol hydrochloride (▲; at various concentrations see numbers (μg ml^{-1}) on figure), guanethidine sulphate (■; 0·2 μg ml^{-1}) and in the absence of either drug (●; Control, C). The horizontal axis shows the time elapsed from the beginning of the experiment and the long horizontal bar shows the presence of propranolol or guanethidine. The short broken horizontal bar shows the presence of (+)-amphetamine sulphate (0·2 μg ml^{-1}). Points are means (plus or minus standard errors where convienent). Numbers of tissues contributing to each point were: propranolol (concentration, number), 20,6; 10,12; 5,12; 1,12; 0·2,12; 0·04,8; control, 12; guanethidine, 8.

When tissues were stimulated alternately through parallel platinum wire electrodes (transmural stimulation) or through a bipolar concentric ring electrode, propranolol (100 ng ml^{-1}) produced little effect on the response to transmural stimulation. The response to stimulation through the concentric ring electrode was however attenuated by about 25% (Fig. 3).

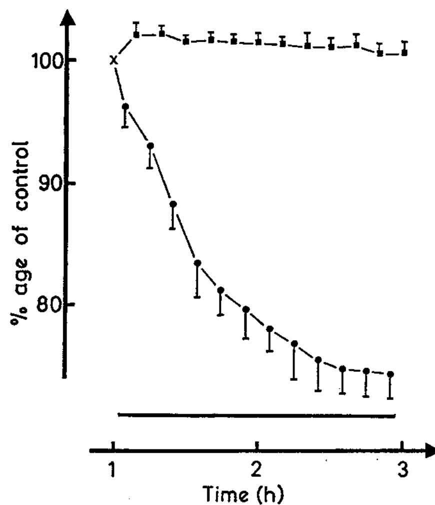

FIG. 3. Effect of propranolol (100 ng ml^{-1} during the horizontal bar) on the response of guinea-pig vas deferens to electrical stimulation (10 Hz; 15 s every 5 min) applied alternately through either parallel platinum wire electrodes (0·5 ms pulse duration; ■) or through concentric ring electrodes (1·0 ms pulse duration; ●). The vertical axis shows the percentage of the response before propranolol was applied. Each point is the mean of 5 tissues and the bars shows the standard error.

DISCUSSION

The possible adrenergic neuron blocking action of propranolol is difficult to reverse by washing alone (Mylecharane & Raper, 1973) and it would be expected therefore that this type of action would still be evident in isolated tissues removed from animals which had been treated systemically with doses of propranolol capable of producing adrenergic neuron blockade. Nevertheless, the response of mouse isolated vasa deferentia to transmural stimulation was unimpaired by either of the dose schedules employed in these experiments. Furthermore, the animals did not exhibit ptosis as would be expected if adrenergic neuron blockade were present. It is possible however, that species difference, high circulating concentrations of catecholamine (Eliash & Weinstock, 1972) or incorrect dose levels of propranolol could account for our inability to demonstrate adrenergic neuron blockade.

A more rigorous test of the ability of propranolol to produce adrenergic neuron blockade was undertaken in the experiments on guinea-pig vas deferens utilising a wide range of concentrations and a prolonged contact time. Initial control experiments showed that tissues deteriorated during this prolonged contact time unless the bath fluid was changed periodically. A constant flow of fresh McEwan solution (containing drugs when appropriate) was therefore infused into the bath and no deterioration was noted when this procedure was carried out. Indeed, the response of control tissues to transmural stimulation increased slightly as the experiment progressed.

Concentrations of propranolol of 1 to 20 μg ml^{-1} reduced the response to transmural stimulation: the blockade was concentration dependent, quick to equilibrium (45 min), easily reversed by washing but not reversed by (+)-amphetamine. With the 10 μg ml^{-1} concentration, (+)-amphetamine actually deepened the blockade and this effect has been reported before with local anaesthetic agents (Bentley, 1965). In fact, all of the characteristics of the blockade by propranolol are typical of a local anaesthetic type of action and are very different from the blockade produced by guanethidine which was very slow in onset and easily and completely reversed by (+)-amphetamine. Since propranolol is known to produce inhibition of axonal conduction in frog sciatic nerve at a concentration of 20 μg ml^{-1} (Barrett & Cullum, 1968) we would ascribe the blockade produced by propranolol at these concentrations to a local anaesthetic type of action.

At concentrations below 1 μg ml^{-1} no blockade of the response to transmural stimulation was observed. Indeed, a slight potentiation was evident which could be due to blockade of noradrenaline uptake by propranolol (Foo, Jowett & Stafford, 1968) or, more probably at these concentrations, to blockade of inhibitory β-adrenoceptors which are known to be present in the vas deferens (Large, 1965; Ganguly & Bhattacharya, 1970). Although (+)-amphetamine further potentiated the response to transmural stimulation in the presence of these low concentrations of propranolol this effect was no more marked than that seen with control tissues.

In agreement with Dawes & Faulkner (1975) therefore we can find no evidence of an adrenergic neuron blocking action of propranolol and our results at the lower concentrations are at variance with those of Mylecharane & Raper (1973) and Eliash & Weinstock (1972). Both these groups of workers employed stimulation methods (concentric ring electrodes) which involve considerable axonal conduction of nerve action potentials whereas transmural stimulation with parallel wire electrodes excites terminal axons throughout the tissue. In view of the established local anaesthetic action of propranolol on nerve axons this difference in technique may be critical.

Alternate stimulation through both types of electrode assembly showed that at a low concentration propranolol does not reduce the effectiveness of transmural stimulation but is effective in inhibiting responses to stimulation through concentric ring electrodes. Since we have demonstrated that the response to transmural stimulation is susceptible to the classical adrenergic neuron blocking agent, guanethidine, we would suggest that the blockade of the response to concentric ring electrode stimulation is more likely to be accounted for in terms of the known local anaesthetic action of propranolol and the greater component of axonal conduction involved in this type of stimulation.

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