Short communications

Comparative partial agonist activity of β-adrenoceptor antagonists

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Inotropic dose-response curves were constructed for a series of β-adrenoceptor antagonists, based on the inotropic responses of isolated dog trabecular muscles stimulated to contract at a regular rate. Propranolol exerted only a negative inotropic effect but KO1366, LB46, oxprenolol and practolol all evoked a positive inotropic response, whether or not catecholamine-depleted muscles were used. The order of potency for this positive inotropic activity was KO1366>LB46> oxprenolol>practolol. The duration of the positive inotropic response to the antagonists was more prolonged than that due to isoprenaline. Propranolol significantly reduced the positive inotropic response to KO1366, LB46, oxprenolol and practolol.

Recently Barrett & Carter (1970) described the partial agonist potency of a series of β-adrenoceptor antagonists, in terms of their chronotropic effect in anaesthetized rats. Under these conditions the partial β-agonist potency of LB46>practolol>INPEA>oxprenolol>pronethalol> alprenolol>ICI45,761 (KO592), an order which differs from that described for their potency as β-adrenoceptor antagonists (Barrett & Carter, 1970). Chronotropic responses in intact animals are often difficult to interpret, because of reflex changes in response to alterations in blood pressure. In the following experiments the relative partial agonist potencies of a similar, but smaller series of β-adrenoceptor antagonists has been established ; the positive inotropic responses of isolated dog trabecular muscles were used as a criterion of partial agonist activity.

Methods.—Paired trabecular muscles of cross sectional area<1·2 mm² and approximately 0·5 cm long were excised from small (4–6 kg) adult mongrel dogs and suspended isometrically in 40 ml aerated (95% O_2+ 5% CO_2) modified Tyrode solution (Nayler, Stone, Carson, McInnes, Mack & Lowe, 1969), at 35° C. In each experiment one muscle served as the control and the other as the test organ. Stimulation was effected with d.c. pulses 20% greater than threshold, delivered from a Tektronix square wave generator at a frequency of 0·2 Hz. Tension developed during contraction was detected and displayed as previously described (Nayler *et al.*, 1969). Each preparation was equilibrated in Tyrode solution for 120 min before drugs were added, and the resting tension applied at the end of the equilibration period (4·8 ± 0·8 gm/mm²) retained throughout the experiment. Cumulative doses of drugs were not used, and to avoid tachyphylaxis each preparation received only one dose of the particular drug being studied. Catecholamine-depleted preparations were obtained by injecting the dogs daily with 0·3 mg/kg reserpine intraperitoneally for three days before use.

Isoprenaline was used as the sulphate, and practolol, propranolol, oxprenolol, LB46 (1-4-(2-hydroxy-3-iso propylamino-propoxy)-indole) and KO1366 (*O*-2-hydroxy-3-(tertbutylamino) propoxybenzonitrile (Nayler & Tay, 1972) as the (±) form of the hydrochloride salts. Dilutions were prepared in modified Tyrode solution immediately before use, and added in aliquots of 0·1 ml.

Results. — Isolated trabecular muscles which were stimulated to contract regularly at 12 beats/min developed 5·62 ± 0·38 gm/mm² (mean ± S.E.M., 24 experiments) peak tension during contraction. Provided that drugs were not added and the Tyrode solution was continuously aerated the peak tension developed during contraction did not change significantly ($P<0·8$) during a 2 h period. Experiments therefore were limited to 2 hours.

Addition of isoprenaline to the Tyrode solution bathing control (not catecholamine-depleted) muscles resulted as shown by the appropriate dose-response curve in Fig. 1A, in a marked dose-dependent increase in peak tension developed during contractions. The similar addition of either KO1366, LB46, oxprenolol or practolol likewise resulted in an increase in the tension developed during contraction, but as shown in Fig. 1A, the responses were smaller and the dose-response curves flatter than those obtained for isoprenaline. Over the dose range studied, propranolol produced only a negative inotropic effect (Fig. 1A).

To ensure that the positive inotropic effect exerted by these compounds was not due to the release of endogenously stored catecholamines the experiments were repeated with catecholamine-depleted preparations. Fluorimetric assay showed that the reserpine-pretreatment significantly reduced ($P<0.001$) the endogenous catecholamine levels in trabecular muscles from control levels of 0.69 ± 0.16 μg/g wet wt (mean $\pm$ S.E.M., 12 experiments) to 0.015 ± 0.020 μg/g wet wt (mean $\pm$ S.E.M., 12 experiments). The dose-response curves obtained for the inotropic effect of isoprenaline, LB46, KO1366, oxprenolol, practolol and propranolol on these catecholamine-depleted trabecular muscles are shown in Fig. 1B and reveal the same order of potency as that described (Fig. 1A) for control non-catecholamine depleted preparations. The positive inotropic effect exerted by these β-antagonists on both control (non-catecholamine-depleted) and catecholamine-depleted muscles was signi-

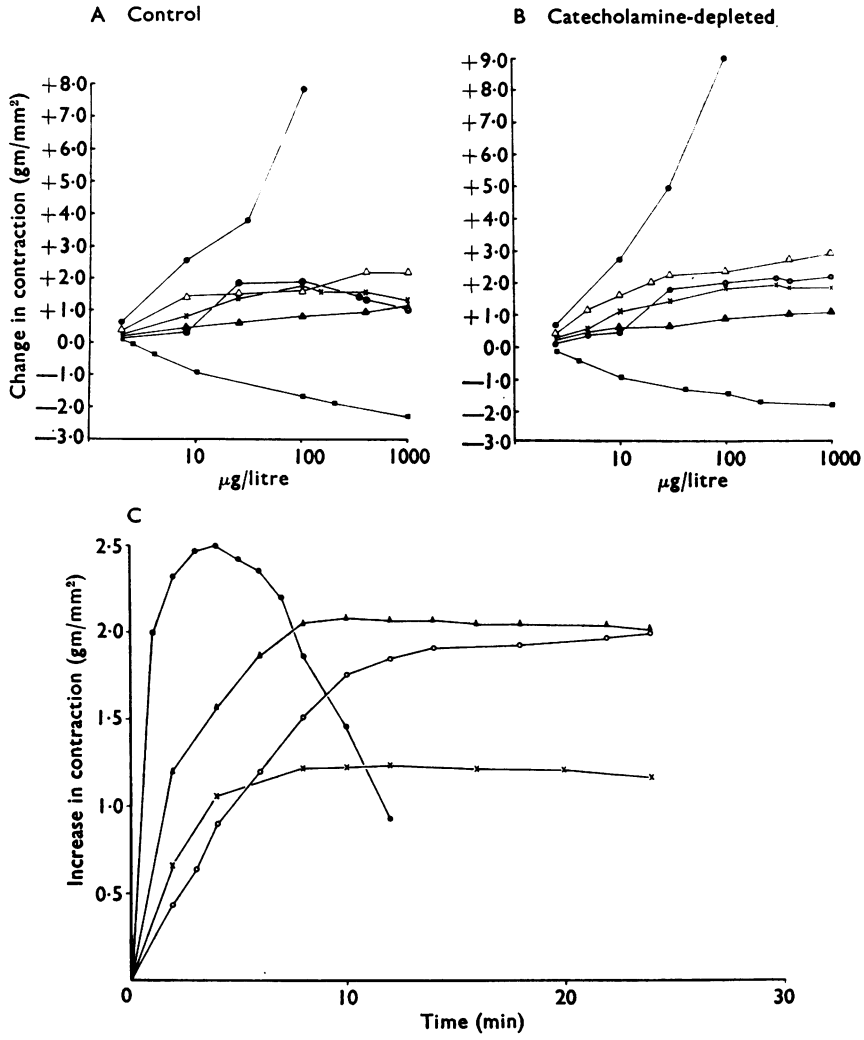

FIG. 1A-C. Effect of isoprenaline, KO1366, LB46, oxprenolol, practolol and propranolol on tension produced during isometric contraction by (A) control (non-catecholamine depleted) and (B) catecholamine-depleted dog trabecular muscles. Fig. 1C compares the duration of the positive inotropic response evoked in catecholamine-depleted trabecular muscles by 8 μg/l. isoprenaline, 8 μg/l. KO1366, 40 μg/l. LB46 and 40 μg/l. oxprenolol. Each point represents the mean of six experiments on trabecular muscles from different dogs. ●——●, Isoprenaline; △——△, KO1366; ○——○, LB46; ×——×, oxprenolol; ▲——▲, practolol; ■——■, propranolol.

ficantly reduced ($P<0.001$) and often abolished if, 20–25 min earlier, propranolol had been added to provide a final concentration of 0·5 mg/l. in the Tyrode solution bathing the muscles.

The dose-response curves shown in Fig. 1A and Fig. 1B relate to the maximum inotropic responses obtained at the indicated dose levels. Figure 1C shows that the positive inotropic effect of isoprenaline on catecholamine-depleted muscles develops more rapidly and is maintained for only a short period of time relative to that for which the positive inotropic effect of LB46, KO1366 and oxprenolol persists.

Discussion.—These results show that the positive inotropic effect exerted by some β-adrenoceptor antagonists can be used to establish dose-response curves for their partial β-agonist properties. Dose-response curves previously have been constructed for these agonist properties by the use of chronotropic responses in intact rats as an index of agonist activity (Barrett & Carter, 1970). Because it is difficult to determine whether relatively small changes in peak tension developed by *in situ* heart muscle reflect a direct inotropic response, or alternatively, a secondary response to some other change within the cardiovascular system—for example an altered end-diastolic volume, the present experiments were performed under *in vitro* and not *in vivo* conditions. Catecholamine-depleted preparations were required, so that the noradrenaline which is released from endogenous storage sites as a direct result of electrical stimulation (Blinks, 1967) would not influence the tension developed before the appropriate antagonist was added.

In catecholamine-depleted preparations the partial agonist activity detected for KO1366>oxprenolol and LB46>practolol. This order of potency differs from that described by Barrett & Carter (1970) who found the activity of practolol>oxprenolol. Whether this difference in order of agonist potency reflects a difference between the β-adrenoceptors which mediate chronotropic and those mediating inotropic changes is not known. Both the present study and that of Barrett & Carter failed to reveal any significant agonist activity for propranolol, and in both studies the duration for which the agonist activity of isoprenaline persisted was less than that observed for the partial agonist activity of the various β-antagonists used.

Although the dose-response curves established here for the positive inotropic effect of KO1366, LB46, oxprenolol and practolol are flatter than those obtained for isoprenaline they clearly demonstrate that these drugs exert a positive inotropic effect, the magnitude of which is dose-dependent over part of the range studied. The reason why the curves for KO1366, LB46, oxprenolol and practolol flatten out at the upper end of the selected dose range is not known. Possibly it reflects a balance between agonist and antagonist activity. The β-blocking potency of LB46>oxprenolol>propranolol>practolol, whilst that of KO1366, established by constructing dose-response curves for its effect on the inotropic and chronotropic responses to both isoprenaline and stellate ganglion stimulation, exceeds that of either propranolol or practolol.

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