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Adrenergic neuron blocking properties of (±)-propranolol and (+)-propranolol

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The action of (±)-propranolol and (+)-propranolol on the electrical stimulation of adrenergic nerves to smooth muscle has been studied in the isolated ear artery from rabbits and the isolated vas deferens preparation from rats. Both drugs exhibited an adrenergic neuron blocking action at a pre-junctional site at concentrations ranging from 4·6 to 14 μg/ml. At lower concentrations the effects were variable and more often potentiation of the responses was observed. The responses to added noradrenaline were uniformly potentiated. The effect was related to local anaesthetic activity and not considered to be a specific adrenergic neuron blocking effect as occurs with guanethidine or bretylium.

The observed hypotensive effects of propranolol in man (Prichard & Gillam, 1969; Zacharias & Cowan, 1970) cannot, at present, be accounted for by a convincing pharmacological explanation. Possible mechanisms involving a reduction in cardiac output (Frohlich, Tarazi & others, 1968) or a re-setting of baro-receptors (Prichard & Gillam, 1969) have yet to be substantiated experimentally. For this reason, the investigations of Day, Owen & Warren (1968) comparing the pre-synaptic adrenergic neuron blocking actions of guanethidine and propranolol were of special interest. They demonstrated that guanethidine was three times more potent than propranolol on the rat vas deferens preparation and that the two drugs were equipotent in the rabbit isolated ear artery preparation. (The doses recommended for the treatment of hypertension are 30–60 mg daily for guanethidine and 240–300 mg daily for propranolol.)

In addition to its specific adrenergic β-receptor blocking properties (Black, Crowther & others, 1964), propranolol also possesses a powerful local anaesthetic action equivalent to that of lignocaine (Morales-Aguilera & Vaughan Williams, 1965). A comparison of the isomers of propranolol showed that the (−)-isomer was at least 100 times more active than the (+)-isomer in antagonizing β-receptor stimulation whereas the isomers were indistinguishable in terms of local anaesthetic effects (Barrett & Cullum, 1968). It was important, therefore, to confirm the findings concerning adrenergic neuron blockade and to define the properties of (+)-propranolol in this respect.

EXPERIMENTAL

The technique for the rabbit isolated ear artery preparation was as described by De la Lande & Rand (1965). The rat isolated vas deferens was studied by the same technique as Day & others (1968) with the exception that the duration of the

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stimulus was 1 ms, the frequency 2–10 pulses/s and stimulation maintained for 5 s, repeated at 3 min intervals.

The drugs used were propranolol hydrochloride, (+)-propranolol, practolol (ICI) and guanethidine sulphate (Ciba). All concentrations are expressed in terms of the base.

RESULTS

Rabbit isolated ear artery preparation. At a concentration of 10 μg/ml propranolol produced a highly significant decrease (91% ± 4%; mean ± s.e.; n = 6) in the pressor responses to electrical stimulation. The onset of inhibitory action was always within 10 min and recovery was rapid following washing out of the drug. Similar results were obtained with (+)-propranolol at 10 μg/ml, the mean inhibition being 63 ± 16% (n = 5), a value which was not significantly different, statistically, from that obtained with the racemic compound. In the presence of both drugs the response to extraluminal noradrenaline (0·1–0·4 μg/ml) was always potentiated (Fig. 1).

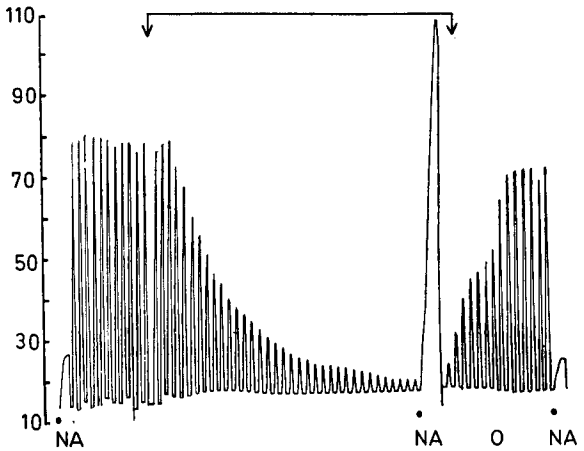

FIG. 1. Responses of rabbit isolated ear artery to electrical stimulation (10 pulses/s for 15s every 2 min) and to noradrenaline (0·1 μg/ml) in the absence and presence (between the arrows) of (+)-propranolol (10 μg/ml).

At lower concentrations (0·1–5 μg/ml) propranolol did not exert a consistent effect on the responses to electrical stimulation. From a total of 22 preparations, inhibition was only observed five times whereas potentiation of the response was present on 13 occasions and no effect on four. The effects were not clearly dose related since both 0·1 and 5 μg/ml produced potentiation and at 3 μg/ml four preparations were inhibited and two potentiated. At these concentrations the response to noradrenaline was potentiated in most preparations. Comparable experiments with guanethidine (0·5–1·0 μg/ml) demonstrated a 75–100% inhibition of the responses to electrical stimulation (Fig. 2). From these experiments it was calculated that guanethidine was 6–20 times more potent than propranolol or (+)-propranolol in producing adrenergic neuron blockade.

Rat isolated vas deferens preparation. In preliminary experiments it was found that in this preparation also the effects of propranolol were substantially less than

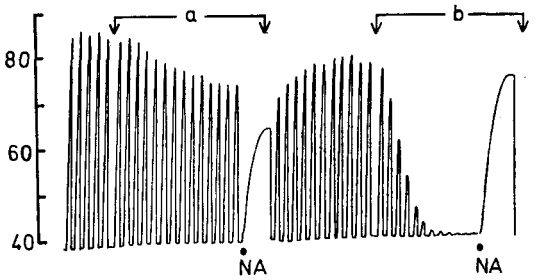

FIG. 2. Responses of rabbit isolated ear artery to electrical stimulation (10 pulses/s for 15 s every 2 min) and noradrenaline (0·2 μg/ml) in the presence and absence of (a) (±)-propranolol (3 μg/ml) and (b) guanethidine (1 μg/ml).

those of guanethidine (Fig. 3). In the presence of propranolol the response to noradrenaline (0·5 μg/ml) was clearly potentiated. Raising the concentration of propranolol produced a dose-dependent inhibition of the responses to electrical stimulation. The onset of blockade was within 5 min but recovery was variable with the exception of the highest concentration when it was uniformly rapid (Fig. 4). The effects of (+)-propranolol were quantitatively and qualitatively similar. From experiments in 15 preparations the mean concentration of propranolol required to produce a 50% inhibition of the contractions was 14 μg/ml. At the lower concentration of both propranolol and its (+)-isomer a modest potentiation of the response to electrical stimulation was observed on two occasions for each drug. From

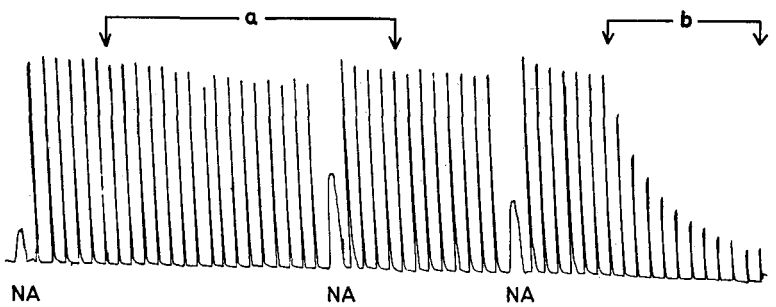

FIG. 3. Responses of rat isolated vas deferens to electrical stimulation (5 pulses/s for 5 s every 3 min) and noradrenaline (0·5 μg/ml) in the presence and absence of (a) (±)-propranolol (4 μg/ml) and (b) guanethidine (1 μg/ml).

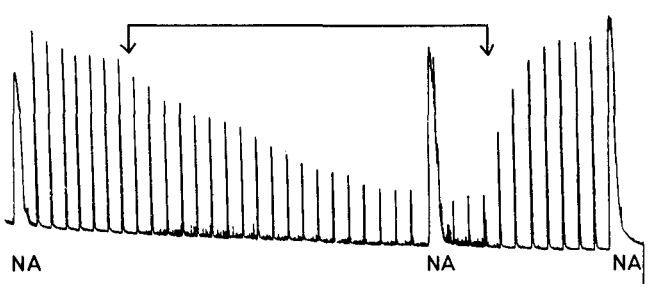

FIG. 4. Response of rat isolated vas deferens to electrical stimulation (5 pulses/s for 5 s every 3 min) and noradrenaline (0·5 μg/ml) in the presence and absence of (±)-propranolol (20 μg/ml).

these experiments it was calculated that guanethidine was 20 times more potent than propranolol. Practolol (50 μg/ml) did not significantly alter the responses of the vas deferens to electrical stimulation.

DISCUSSION

Contractions of rat isolated vas deferens or rabbit isolated ear artery preparations are inhibited by (±)-propranolol and (+)-propranolol when stimulation is electrical via nervous tissue but not when due to added noradrenaline. Such a pre-junctional blocking action is also showed by xylocholine, bretylium and guanethidine. As occurs with these three agents, the responses to exogenous noradrenaline are potentiated by propranolol. It has been suggested by Day & others (1968) that propranolol possesses a potent blocking action on adrenergic sympathetic neurons in isolated smooth muscle comparable to that of guanethidine with the exception that unlike the blockade produced by bretylium or guanethidine, reversal by amphetamine (Day, 1962) is not apparent in the case of propranolol. Three important differences emerged between the results of the present experiments and those of Day & others (1968). First, the potency of propranolol in our hands was 6–20 times less than that of guanethidine whereas results with the latter substance were similar to those of Day & others (1968) and many other workers. Second, the duration of blockade following washing out of the drug was short and not prolonged. Third, at concentrations below those necessary to produce blockade, potentiation of responses to electrical stimulation was frequently seen with both (±)- and (+)-propranolol. The concentrations of propranolol required to produce a 50% decrease in electrically stimulated contractions ranged from 4·6 to 14·0 μg/ml whereas the concentration found necessary to produce a 50% block of conduction in frog isolated sciatic nerves was about 20 μg/ml (Barrett & Cullum, 1968). There was therefore no great difference in the concentrations required to produce evidence of adrenergic neuron blockade or conduction block in motor nerves.

The effective concentrations of propranolol in isolated smooth muscle preparations are similar to those needed for xylocholine or bretylium. These substances are potent local anaesthetics (Boyd, Chang & Rand, 1960; Morales-Aguilera & Vaughan Williams, 1963) but in the cases of xylocholine (Bain, 1960), bretylium (Boyd & others, 1960) and guanethidine (Bein, 1960) there are powerful arguments for dissociating this property from adrenergic neuron blocking activity. No such evidence exists for propranolol and indeed the failure of a dose of 15 mg/kg intravenously to affect post-ganglionic stimulation of the cat nictitating membrane (Raper & Wale, 1969) argues strongly against the relevance of these observations *in vitro* to the situation *in vivo*.

The release of noradrenaline from isolated nerve granules is blocked by propranolol at 3×10^{-4}M (77·5 μg/ml) (Euler & Lishajko, 1966). Similarly, uptake of noradrenaline either by isolated nerve granules (Euler & Lishajko, 1966) or by isolated rabbit hearts (Foo, Jowett & Stafford, 1968) is also inhibited by similar concentrations of propranolol. In contrast, practolol had no effect in the present experiments or those of Foo & others (1966) and was found by Papp & Vaughan Williams (1969) to possess 1/100 the activity of propranolol as a local anaesthetic. Inhibition of noradrenaline uptake may not be irrelevant in man since the elevated excretion of noradrenaline during exposure to a sauna bath (Huikko, Jouppila & Kärki, 1966) is

enhanced by pretreatment with propranolol (10 mg) whilst elevation of plasma free fatty acids is inhibited (Arvela & Huikko, 1969). Furthermore, the potentiation of electrically stimulated contractions observed in the present experiments at lower concentrations of propranolol and the potentiation of exogenous noradrenaline would also be compatible with a decrease in the uptake of released noradrenaline.

The fact that the (+)-isomer of propranolol exerts a similar local anaesthetic (Barrett & Cullum, 1968) and adrenergic neuron blocking properties argues against the relevance of β-adrenoreceptor blocking activity in the present context. However, a non-specific axonal blockade could well account for the previously unexplained inhibition of vagal slowing following the sudden release of carotid occlusion after larger (3 mg/kg) doses of propranolol when smaller doses (0·25 mg/kg) are sufficient to inhibit the associated reflex tachycardia (Ledsome, Linden & Norman, 1965).

In conclusion it cannot be proved that the non-specific adrenergic neuron blocking activity of propranolol is not contributing to the hypotensive action of this drug in man but this effect could well augment the effects of β-blockade on cardiac output. The absence of any clear-cut orthostatic hypotension in patients receiving propranolol (Prichard & Gillam, 1969) does, however, militate against this speculation.

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