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ANTIOXOTREMORINE ACTION OF PROPRANOLOL

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1 The influence of propranolol on the neuromuscular, tremor-producing and muscarinic effects of oxotremorine was examined.

2 In the isolated rat phrenic nerve-diaphragm preparation the neuromuscular blocking effect of oxotremorine was inhibited by propranolol in a dose-dependent manner.

3 Propranolol intensified the paralytic effect of tubocurarine in the rat diaphragm and prevented antagonism of tubocurarine paralysis by tetraethylammonium.

4 Propranolol was devoid of any curare-like effect in the isolated rectus abdominis muscle of the frog.

5 Vasodepressor responses to oxotremorine in rats and spasmogenic responses to oxotremorine in guinea-pig ileum were not antagonized by propranolol.

6 A dose-dependent antagonism of oxotremorine-induced tremor in mice was observed after pretreatment with propranolol and it is suggested that this effect is due to an antagonism of a presynaptic effect of oxotremorine at skeletal neuromuscular junctions.

Introduction

Propranolol diminishes tremor in patients with Parkinson's disease (Owen & Marsden, 1965; Marsden, Meadows, Lange & Watson, 1967) and inhibits oxotremorine-induced tremor in mice and rats (Jacobi, 1957; Agarwal & Bose, 1967; Cox & Potkonjak, 1970). Some other central effects of oxotremorine, including its analgesic and hypothermic effects are not affected by propranolol (Hermansen, 1968) and it has therefore been suggested that the anti-tremor action of propranolol is mediated peripherally (Marsden *et al.*, 1967). However, the effect does not appear to be due to blockade of β-adrenoceptors (Agarwal & Bose, 1967).

Oxotremorine is known to affect skeletal neuromuscular sites (Csillik, 1964; Elmqvist & McIssac, 1967; Ganguly & Chaudhuri, 1970) and produces a nicotinic vasopressor response in atropinized rats (Ganguly & Saha, 1972) and this paper presents evidence for a neuromuscular site of action for the anti-tremor effect of propranolol on oxotremorine-induced tremor.

Methods

Isolated phrenic nerve-diaphragm preparations were prepared from albino rats (150 to 200 g) of either sex, according to the method of Bülbring (1946). The preparations were suspended in a 40 ml bath containing physiological solution of the following composition (g/l): NaCl 8, KCl 0.2, $CaCl_2$ 0.2, $NaHCO_3$ 1, dextrose 2, NaH_2PO_4 0.05, $MgCl_2$ 0.01 bubbled with oxygen at 31°C. Muscle contractions were recorded with a simple straw lever of minimum load giving 8-fold magnification. The phrenic nerve was stimulated at 0.2 to 0.3 Hz with supramaximal rectangular pulses of 0.2 ms duration.

The rectus abdominis muscle of the frog was prepared in the usual way using a 10 ml bath containing frog-Ringer solution (g/l): NaCl 6.0, KCl 0.075, $CaCl_2$ 0.1, $NaHCO_3$ 1.0 bubbled with air.

Blood pressure effects were examined in male albino rats (150 to 230 g) anaesthetized with urethane (1.4 g/kg s.c.). After inserting cannulae into the left carotid artery and the left femoral vein, heparin was administered (100 u i.v.) and the animal was artificially ventilated. Blood pressure was recorded using a mercury manometer.

Guinea-pig isolated ileum was set up in Tyrode solution (g/l): NaCl 8, KCl 0.2, $NaHCO_3$ 1, dextrose 1, NaH_2PO_4 0.05, $MgCl_2$ 0.01 at 37°C.

Ten minutes after intraperitoneal injection of oxotremorine (250 mg/kg), tremor was assessed in groups of 10 male mice (20–25 g) by visual scoring and by using an objective scoring device. Propranolol (1, 2 and 4 mg/kg i.p.) was given to some groups 1 h before

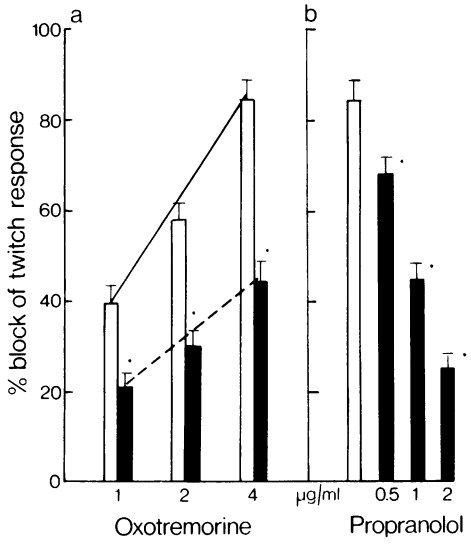

Figure 1 The percentage blockade of the twitch response of stimulated rat diaphragm caused by oxotremorine alone (open columns) and oxotremorine together with propranolol (closed columns). In (a) the concentration of propranolol was 1 μg/ml and that of oxotremorine was varied. In (b) the concentration of oxotremorine was 4 μg/ml and that of propranolol was varied. Vertical lines represent s.e. * indicates a significant difference in the oxotremorine effect caused by addition of propranolol ($P < 0.05$).

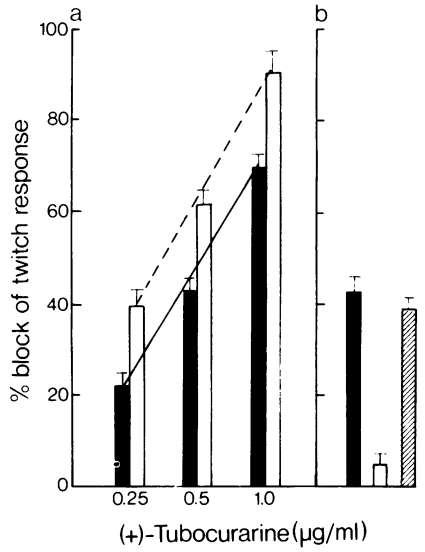

Figure 2 The percentage blockade of the twitch response of indirectly stimulated rat diaphragm caused in (a) by (+)-tubocurarine (0.25, 0.5 and 0.1 μg/ml) alone (closed columns) and after 5 min incubation with 1 μg/ml propranolol (open columns) and in (b) by (+)-tubocurarine (0.1 μg/ml) alone (closed columns) and after 5 min incubation with 50 μg/ml TEA (open column) or 50 μg/ml TEA and 1 μg/ml propranolol (hatched column). Vertical lines represent s.e.

oxotremorine. Scores were assessed visually by the method of Spencer (1965); no tremor = 0, moderate or intermittent tremor = 1.0 and pronounced continuous tremor = 2.0. A permanent record of tremor was obtained by a modification of the method described by Ahmed & Taylor (1959). A perforated plastic soap box (114 mm × 63 mm × 37 mm) was suspended by a stainless steel wire from an iron stand. A thin steel nozzle (length = 25 mm), permanently fixed to one side of the plastic box, was attached to a piezoelectric transducer connected to the preamplifying channel (model R 67z) of a Gallileo 3-channel ink-writing polygraph to obtain a record of tremor. The plastic box, stand and transducer were placed in a rectangular glass case to reduce environmental disturbance. Mice were acclimatized by placing them individually in the box for 10 to 15 min before each experiment. Recordings were made before and 10 min after injecting oxotremorine.

The following drugs were used: oxotremorine sesquifumarate (Aldrich), (±)-propranolol hydrochloride (I.C.I.), (+)-tubocurarine chloride (Sigma), acetylcholine chloride (E. Merck), tetraethylammonium bromide (Fluka).

Results

Isolated rat phrenic nerve-diaphragm

Twitch responses of indirectly stimulated rat diaphragm preparation were unaffected within 15 min of adding propranolol alone at concentrations of up to 5 μg/ml but prior incubation with propranolol for 5 min markedly reduced the neuromuscular blocking effect of oxotremorine (Figure 1).

In another series of experiments, interactions of propranolol with (+)-tubocurarine and tetraethylammonium (TEA) were studied. TEA is known to antagonize the paralytic effect of (+)-tubocurarine by increasing the release of acetylcholine at neuromuscular junctions (Bowman, Hemsworth & Rand, 1962; Collier & Exley, 1963). Prior incubation with propranolol for 5 min intensified the paralytic effect of tubocurarine (Figure 2). Previous incubation with TEA (50 to 100 μg/ml) for 5 min significantly reduced the effect of (+)-tubocurarine (0.5 μg/ml) and this antagonistic effect of TEA was almost lost in the presence of propranolol (1 μg/ml; Figure 2). A typical experiment is illustrated in Figure 3.

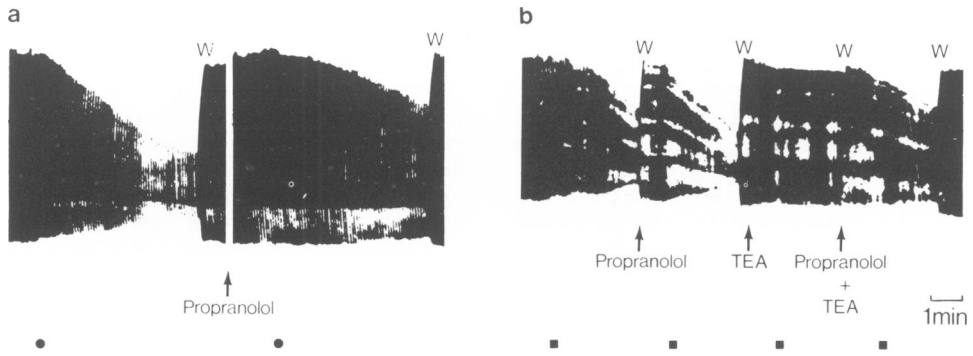

Figure 3 Tracings of twitch responses of indirectly stimulated rat diaphragm. (a) The effect of 2 μg/ml oxotremorine (●) alone and 5 min after incubating the diaphragm with propranolol (1 μg/ml). (b) The effect of 0.5 μg/ml (+)-tubocurarine (■) alone and at 5 min after incubation with propranolol (1 μg/ml), TEA (100 μg/ml) or both. w = wash.

Frog isolated rectus abdominis

The influence of propranolol on postsynaptic skeletal muscle receptors was examined by using the frog isolated rectus abdominis preparation. Propranolol, up to a concentration of 5 μg/ml, failed to modify the responses to exogenous acetylcholine (4 experiments). Moreover, the inhibitory effect of (+)-tubocurarine on responses to exogenous acetylcholine remained unaffected in the presence of propranolol (5 experiments).

Muscarinic responses to oxotremorine

Vasodepressor responses to oxotremorine were not significantly affected when repeated 30 min after propranolol (2 mg/kg i.v.; 5 experiments). Similarly the vasodepressor responses to acetylcholine were unchanged after giving propranolol (5 experiments).

Spasmogenic responses of guinea-pig isolated ileum to oxotremorine (0.01 μg/ml) and acetylcholine (0.01 μg/ml) were not altered in the presence of propranolol (1 μg/ml; 4 experiments).

Tremor responses to oxotremorine

Pretreatment with propranolol (1, 2 and 4 mg/kg i.p.) markedly reduced oxotremorine-tremor in mice. In the studies reported in Figure 4 tremor was assessed visually but confirmation was obtained using objective measurement (see **Methods**).

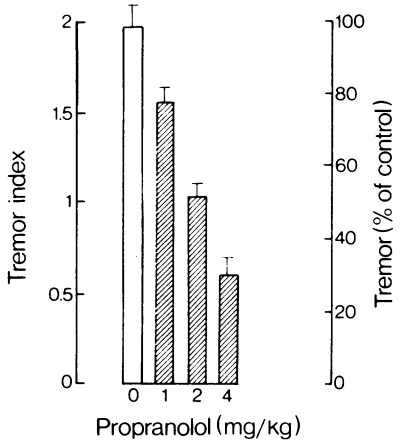

Figure 4 The tremor index of mice given oxotremorine (250 μg/kg i.p.): open columns, controls; hatched columns, at 1 h after propranolol (1, 2 and 4 mg/kg i.p.). Mean score for groups of 10 mice and the percentage of control values. Vertical lines represent s.e.

Discussion

The present experiments confirm the anti-tremor action of propranolol reported by others (Jacobi, 1957; Agarwal & Bose, 1967; Cox & Potkonjac, 1970). The β-adrenoceptor blocking property of propranolol is thought not likely to contribute to its anti-tremor effect since blockade of β-adrenoceptors by (+)-N-isopropyl-*p*-nitrophenylethanolamine is ineffective against tremor induced by tremorine in mice (Agarwal & Bose, 1967).

The ability of propranolol to protect significantly against the skeletal myoneural effects of oxotremorine on the rat isolated diaphragm presented here appears to be attributable to a presynaptic action. The

mechanism could be diminished transmitter output since propranolol increased the paralytic effect of (+)-tubocurarine and reduced the antagonistic action of TEA on (+)-tubocurarine paralysis. That the effect is presynaptic is also indicated by the failure of propranolol to antagonize responses to acetylcholine of frog rectus abdominis muscle.

In studies of some known anti-Parkinson drugs, Ahmed & Marshall (1962) found a close parallelism between anti-tremorine and anti-acetylcholine effects. That the mechanism by which propranolol inhibits oxotremorine-tremor is unlikely to be due to blockade of peripheral muscarinic receptors is supported by the present experiments using rat blood pressure and guinea-pig ileum.

It is not possible to exclude a central site of action of the anti-tremor effect of propranolol although Hermansen (1968) found that propranolol did not affect oxotremorine-induced analgesia and hypothermia in mice. Cox & Potkonjak (1970) postulated that oxotremorine-tremor was mediated through peripheral propranolol-sensitive receptors perhaps of the same kind as those which on stimulation enhance physiological tremor (Marsden *et al.*, 1967). The present results indicate that such peripheral propranolol-sensitive receptors may be located at the skeletal myoneural junction.

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