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CURARE-LIKE EFFECT OF PROPRANOLOL ON RAT EXTRAOCULAR MUSCLES

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1 The effects were studied of the β-adrenoceptor blocking drug, (±)-propranolol and its (+)- and (−)-isomers on contractility of rat isolated diaphragm and inferior rectus muscles.

2 Propranolol (10^{-5} M) did not modify the resting tension nor the electrically-induced twitch contraction in diaphragm and extraocular muscles, nor the tonic tension evoked in the latter by high K concentrations.

3 In inferior rectus preparations (±)-propranolol (5×10^{-6} M) reduced significantly tensions evoked by succinylcholine (SCh). Cumulative dose-response curves to SCh were shifted to the right in a nearly parallel manner by (+)- and (−)-propranolol which were about equiactive.

4 It is concluded that the depressant action of propranolol is independent of its adrenoceptor blocking potency and it is not due to an alteration of the contraction mechanism. Propranolol appears to depress SCh-evoked tensions through a curare-like effect on cholinoceptors.

Introduction

Mammalian extraocular muscles (EOM) contain, in addition to singly innervated or twitch fibres, a population of multiply innervated fibres which are absent in limb muscles (Hess & Pilar, 1963; Matyushkin, 1964). Multiply innervated fibres respond to depolarizations caused by cholinomimetic agents or by elevated K concentrations with sustained tensions *in vivo* and *in vitro* (Duke Elder & Duke Elder, 1931; Lincoff, Breinin & De Voe, 1957; Eakins & Katz, 1966; Chiarandini, 1976).

Studying the contractile responses evoked in cat EOM by intravascular injections of succinylcholine (SCh), Eakins & Katz (1966) found that pronethanol, a β-adrenoceptor blocker, had a long lasting depressant action on SCh-evoked tensions. In addition they observed that adrenaline exerted a weak stimulating effect on EOM. Based on these findings they suggested that an adrenergic mechanism may be involved in the response of EOM to SCh. The present study was designed to investigate *in vitro* if the β-adrenoceptor blocker, propranolol, which structurally is closely related to pronethanol, also has a blocking effect on SCh tensions and to attempt to establish its mode of action. It was found that both (+)- and (−)-propranolol in relatively low concentrations reduced tensions induced by SCh in the isolated inferior rectus muscle of the rat. The basis of this depressant action seems to involve competitive blockade at the cholinoceptors.

Methods

Inferior rectus and diaphragm muscles were dissected from Wistar rats weighing between 125 to 175 g under sodium thiamylal or ether anaesthesia, according to the procedure described previously (Chiarandini, 1976).

To reduce diffusional delay most of the belly region of the inferior rectus was removed, leaving only the global layer of the muscle. Diaphragm muscles were split into strips along the length of their fibres. The diaphragm strips (measuring about 0.5 × 2 × 10 mm) or the inferior rectus muscles were mounted horizontally in a chamber. Tension was recorded with an isometric strain gauge, connected to a rectilinear pen recorder. The length of the preparation was adjusted for optimal twitch tension. Oxygenated control and test solutions at room temperature (20 to 24°C) were changed rapidly and flowed continuously through the chamber at a rate of 2–3 ml/min. Muscles were stimulated directly with a pair of platinum electrodes by applying at a low frequency (0.05 to 0.2 Hz) square pulses with a duration of 1 ms and a voltage sufficient to produce maximal responses. The electrodes were placed along the muscle length or at one end.

The muscles were bathed with a control saline containing (mM): NaCl 136, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 11 and imidazole sulphate 5. To this solution different drugs were added to the desired concentration from the following stock solutions made up in control saline: 10^{-3} M succinylcholine chloride, 10^{-3}

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M (±)-propranolol (Sigma Chemical Co., St Louis, Mo), 10^{-3} M (+)- or (−)-propranolol (Ayerst Laboratories, Inc., New York, N.Y.), and 10^{-4} M (+)-tubocurarine (Chemicals Procurement Laboratories, Inc., College Point, N.Y.). Solutions with an increased potassium concentration, [K], were prepared by raising [K] and keeping constant the [K] × [Cl] product as described previously (Chiarandini, 1976). The pH of the solutions was 7.35.

Cumulative dose-response curves

These were obtained according to the following protocol. Initially, two control tensions were evoked by applying to the muscle 2×10^{-6} M SCh with a rinse in control saline for about 20 min between applications and the average value recorded. Twenty min later concentrations of SCh of 2×10^{-7}, 5×10^{-7}, 10^{-6}, 2×10^{-6}, 10^{-5}, 5×10^{-5}, or 10^{-4} M were applied successively without intervening rinses in drug-free solution until each increment in evoked tension reached a steady value. If no change in the baseline was detected 7 to 10 min after applying a given [SCh], that concentration was considered to be subthreshold. Usually, each cumulative curve was completed in 10 to 15 min. The muscle was then returned to control saline and 45 to 60 min later its responsiveness to 2×10^{-6} M SCh was tested again. If the last and the initial control tensions differed by more than 15% the experiment was discontinued. In nearly all the accepted experiments the difference between the two tensions was less than 5%. After further washing for 20 min, electrical stimulation was started and several min later the blocking agent was added. About 10 min later stimulation was interrupted and solutions containing both the antagonist and increasing [SCh], sometimes up to 1×10^{-3} M, were applied successively.

Tension responses are expressed as a percentage of the maximal response to SCh, which ranged between 0.9 and 1.5 g. ED_{50} values were obtained from individual dose-response curves by graphical interpolation over the linear portion of the curve. The changes in tension and ED_{50} values observed after various treatments are given in the text as a percentage change for paired data, calculated according to the relation:

$$\frac{\text{value after treatment} - \text{control value}}{\text{control value}} \times 100\%.$$

Results are given as mean ± s.e. mean.

Results

Effect of (±)-propranolol on twitch contractions

The effect of propranolol on twitches evoked by direct electrical stimulation was examined in seven inferior rectus and six diaphragm muscles. After twitches were recorded in control saline for 5 to 15 min, propranolol (5×10^{-6} to 1×10^{-4} M) was added for about 15 min without interrupting the stimulation. Concentrations of propranolol larger than 10^{-5} M produced a gradual decline of the twitch amplitude which was fully reversible by washing. A typical experiment on inferior rectus is shown in Figure 1. Similar results were obtained in diaphragm muscles.

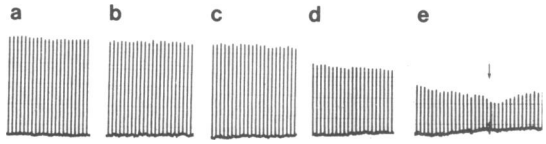

Figure 1 Effects of different concentrations of (±)-propranolol on inferior rectus muscles. After recording twitches in control saline (a) this muscle was exposed to increasing concentrations of propranolol, each for about 15 min, as follows: (b) 5×10^{-6} M; (c) 10^{-5} M; (d) 5×10^{-5} M and (e) 10^{-4} M. Arrow indicates return to control saline. Records shown begin 6 to 7 min after changing the saline. Frequency of stimulation: 0.05 Hz; initial twitch amplitude: 0.45 g.

Effect on K-induced tensions

When inferior rectus muscles are exposed to a saline containing 20 mM K or more they develop a maintained tension which has been attributed to the contraction of multiply innervated or tonic fibres (Chiarandini, 1976). The effect of (±)-propranolol on this tension was explored in this series of experiments.

Inferior rectus muscles were exposed two or three times at 20 min intervals to saline containing 20 or 25 mM K. This elevated [K] induces tensions of 20 to 40% of the maximal K-induced tension (Chiarandini, 1976), an example of which is shown in Figure 2a. After the last increase in tension the muscle was bathed in normal saline for 20 min before it was exposed to a saline containing 10^{-5} M propranolol. In no case did the addition of propranolol induce tension. When the same elevated [K] together with propranolol was applied to the muscle, a tension (Figure 2b) similar to the control (Figure 2a) was obtained. In eight muscles the tension was 0.45 ± 0.03 g in the absence of propranolol and 0.43 ± 0.05 g in its presence. A concentration of 20 mM K was used for 4 muscles and 25 mM for the other 4 muscles. The mean difference between the two tensions: −6.2 ± 4.2% was not significant for paired data ($P > 0.1$). However, the same exposure to propranolol reduced considerably the tension evoked by 2×10^{-6} M SCh. Figures 2c and d demonstrates this depressant action in the same muscle in which the drug did not modify K-evoked tensions (Figure 2a and b).

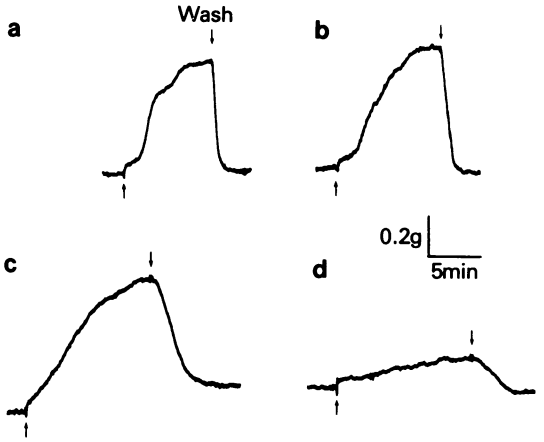

Figure 2 Effects of 5×10^{-6} M (±)-propranolol on K- and succinylcholine (SCh)-evoked tensions in inferior rectus muscle. (a) Control tension elicited with saline containing 20 mM K. (b) The muscle was exposed for 10 min to propranolol before applying saline with 20 mM K and propranolol. The tension was not modified by the drug. (c) Response evoked in the same muscle by 2×10^{-6} M SCh about 1 h later. (d) Response to the same [SCh] after exposing the muscle to propranolol for 9 min. In this case the tension was markedly reduced.

Effects of propranolol isomers on succinylcholine-induced tensions in inferior rectus muscles

In preliminary experiments, (±)-propranolol (5×10^{-6} M) reduced tensions evoked by 2×10^{-6} M SCh without affecting responses to 5×10^{-5} M SCh; in 4 muscles the tension was reduced from 0.44 ± 0.10 g to 0.24 ± 0.08 g, a change of −51.2 ± 6.7%, which is statistically significant for paired data ($P < 0.005$). In contrast, in the same muscles the tension evoked by 5×10^{-5} M SCh was 0.69 ± 0.19 g in the absence of propranolol and 0.67 ± 0.19 g in its presence, a change that is not significant for paired data ($P > 0.1$). The depressant action of propranolol was rather long lasting in that after about 10 min exposure about 45 min washing was necessary to observe a complete recovery of responsiveness to SCh.

The effect of propranolol was further examined by studying the effects of its (+)- and (−)-isomers on cumulative dose-response curves for SCh. The range of propranolol concentrations useful for these experiments was relatively narrow since concentrations lower than 5.0×10^{-6} M exerted a depressant action on SCh tensions only occasionally and doses larger than 10^{-5} M consistently reduced twitch contractions suggesting a local anaesthetic action (Figure 1). Therefore the experiments were limited to a study of the effect of each isomer at concentrations of 5.0 and 7.5×10^{-6} M. In none of these experiments did (+)- or (−)-propranolol reduce the twitch contractions.

Figure 3 shows the effects of (+)-propranolol on dose-response curves to SCh. It can be seen that (+)-propranolol shifted to the right in approximately a parallel manner the control dose-response relationship, and that the effect was concentration-dependent.

The changes of SCh responses were analyzed statistically for paired data (Table 1). At a concentration of 5×10^{-6} M (+)-propranolol reduced significantly the tensions evoked by 10^{-6} M and 2×10^{-6} M SCh, while at 7.5×10^{-6} M it depressed significantly the responses to all [SCh] from 5×10^{-7} to 1×10^{-4} M. When, in the presence of (+)-propranolol, [SCh] was increased to 10^{-3} M (not shown in Figure 3), the response increased further to 88.1 ± 3.7% of maximal (in two muscles to 92 and 103%), which represents a significant increment of 6.7 ± 2.2% over the tension evoked by 10^{-4} M SCh ($P < 0.05$ for paired data).

Table 1 Effects of (+)-propranolol on succinylcholine-induced (SCh) tensions

	(+)-*Propranolol* 5×10^{-6} M	(+)-*Propranolol* 7.5×10^{-6} M
[*SCh*] (M)	% *change of tension amplitude*	
2×10^{-7}	−23.0 ± 27.1 (6)	−19.0 ± 72.4 (5)
5×10^{-7}	−57.5 ± 55.0 (6)	1.0 ± 9.3 (5)
10^{-6}	−47.0 ± 13.4* (9)	−88.2 ± 5.5*** (6)
2×10^{-6}	−53.0 ± 8.3*** (10)	−78.5 ± 7.9*** (6)
10^{-5}	−4.0 ± 2.2 (10)	−25.2 ± 5.4* (6)
5×10^{-5}	−5.1 ± 3.2 (8)	−19.2 ± 3.4** (6)
10^{-4}	−3.1 ± 1.5 (10)	−17.7 ± 3.5** (6)

Significant for paired data: * ($P < 0.01$), ** ($P < 0.005$), *** ($P < 0.001$).
In parentheses, number of muscles.

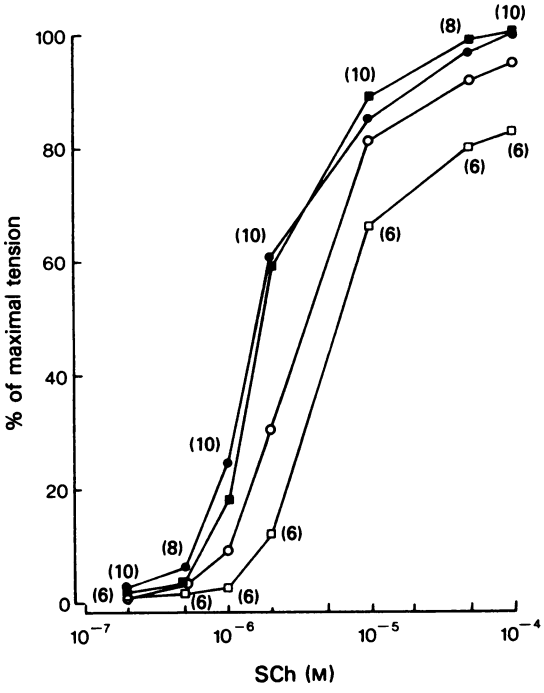

Figure 3 Effect of (+)-propranolol on the cumulative dose-response curve to succinylcholine (SCh). Abscissa scale: concentration of SCh plotted logarithmically. Ordinate scale: tension expressed as percentage of maximum tension evoked by 10^{-4} M SCh in control saline. Each point represents the mean response for the number of muscles indicated in parentheses. Control and treated with 5×10^{-6} *M* (+)-propranolol: (●) and (○). Control and treated with 7.5×10^{-6}M (+)-propranolol: (■) and (□).

The ED_{50} was increased from a control value of $1.75 \pm 0.24 \times 10^{-6}$ M to $3.82 \pm 0.42 \times 10^{-6}$ M by 5×10^{-6} M (+)-propranolol and from $1.84 \pm 0.18 \times 10^{-6}$ M to $6.93 \pm 1.08 \times 10^{-6}$ M by 7.5×10^{-6} M (+)-propranolol. For paired data these changes correspond, respectively, to significant increments of $129 \pm 22\%$ and $273 \pm 37\%$ ($P < 0.001$).

The effect of (−)-propranolol was studied in comparable groups of muscles: 5×10^{-6} M (−)-propranolol produced little shift of the curve (Figure 4) but the analysis of paired data demonstrated that responses to 10^{-6} M and 2×10^{-6} M SCh were significantly reduced (Table 2). Following exposure to 7.5×10^{-6} M (−)-propranolol the whole curve was shifted to the right and the responses evoked by all [SCh] from 2×10^{-7} to 1×10^{-4} M were reduced significantly (Figure 4 and Table 2). When [SCh] was increased to 10^{-3} M (not shown in Figure 4) the response increased further to $82.3 \pm 2.2\%$ of maximal (in one muscle to 94.5%), an increase of $8.5 \pm 2.6\%$ over the tension recorded with 10^{-4} M SCh, which is statistically significant for paired data ($P < 0.02$).

The ED_{50} was increased from $1.91 \pm 0.28 \times 10^{-6}$ M to $2.45 \pm 0.31 \times 10^{-6}$ M by the lower concentration of (−)-propranolol and from $2.56 \pm 0.38 \times 10^{-6}$ M to $10.89 \pm 2.88 \times 10^{-6}$ M by the higher concentration. For paired data these changes represent, respectively, significant increments of $33 \pm 9.0\%$ and $302 \pm 43\%$ ($P < 0.01$ and $P < 0.001$).

The possibility that the depression of SCh-induced tensions observed after adding propranolol isomers was due to a reduction of the sensitivity of muscle fibres to SCh brought about by the previous exposure to various high [SCh] was investigated in 5 muscles. Dose-response curves to SCh were obtained following a protocol identical to that of the experiments above except that the exposure to propranolol was omitted.

Table 2 Effects of (−)-propranolol on succinylcholine-induced (SCh) tensions

	(−)-*Propranolol* 5×10^{-6} M	(−)-*Propranolol* 7.5×10^{-6} M
[*SCh*] (M)	*% change of tension amplitude*	
2×10^{-7}	2.64 ± 27.2 (5)	−91.6 ± 7.3** (6)
5×10^{-7}	41.7 ± 77.0 (7)	−93.3 ± 6.1** (5)
10^{-6}	−40.0 ± 8.8* (7)	−88.4 ± 7.0** (6)
2×10^{-6}	−20.5 ± 4.9* (9)	−78.7 ± 6.4** (6)
10^{-5}	4.1 ± 3.8 (10)	−31.9 ± 5.1* (6)
5×10^{-5}	3.0 ± 3.2 (10)	−25.7 ± 2.8** (6)
10^{-4}	1.1 ± 3.5 (10)	−24.3 ± 2.2** (6)

Significant for paired data: * ($P < 0.005$), ** ($P < 0.001$).
In parentheses number of muscles.

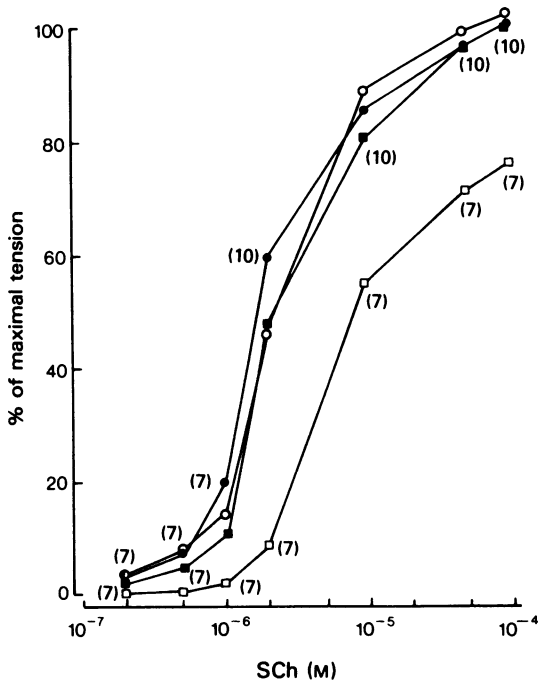

Figure 4 Effect of (−)-propranolol on the cumulative dose-response curve to succinylcholine (SCh). Abscissa scale: concentration of SCh plotted logarithmically. Ordinate scale: tension expressed as percentage of maximum tension evoked by 10^{-4} M SCh. Each point represents the mean response for the number of muscles indicated in parentheses. Control and treated with 5×10^{-6} M (−)-propranolol: (●) and (○). Control and treated with 7.5×10^{-6} M (−)-propranolol: (■) and (□).

The two curves thus obtained were very similar and the difference between the amplitude of the tensions for all [SCh] was not statistically significant for paired data. The ED_{50} values were $2.25 \pm 1.02 \times 10^{-6}$ M for the first and $2.29 \pm 1.20 \times 10^{-6}$ M for the second run; the difference for paired data was $3.7 \pm 4.5\%$ ($P > 0.4$).

Effects of (+)-tubocurarine

The blockade by propranolol was compared with that of the known cholinoceptor blocker (+)-tubocurarine (3×10^{-7} and 1×10^{-6} M). Both concentrations of the drug shifted to the right the dose-response curve to SCh (Figure 5), and reduced the responses to [SCh] lower than 10^{-5} M (Table 3). The ED_{50} values were increased from $1.64 \pm 0.32 \times 10^{-6}$ M to $3.55 \pm 0.37 \times 10^{-6}$ M by the lower concentration and from $1.57 \pm 0.14 \times 10^{-6}$ M to $4.62 \pm 0.37 \times 10^{-6}$ M by the higher concentration of (+)-tubocurarine. These changes represent, for paired data, increments of $143 \pm 31\%$ and $199 \pm 70\%$ which are statistically significant ($P < 0.01$ and $P < 0.05$).

Discussion

Propranolol blocks β-adrenoceptors (Black, Duncan & Shanks, 1965) and also has a substantial local anaesthetic activity (Morales-Aguilerá & Vaughan Williams, 1965). Neither of these pharmacological actions appears to be involved in the reduction of SCh-evoked tensions here described. The possibility that this reduction is due to a blockade of the adrenergic mechanism suggested by Eakins & Katz (1966) to modulate SCh responses in extraocular muscles can be ruled out because both isomers of the drug were

Table 3 Effects of (+)-tubocurarine on succinylcholine (SCh)-induced tensions

	(+)-*Tubocurarine* 3×10^{-7} M	(+)-*Tubocurarine* 10^{-6} M
[SCh] (M)	*% change of tension amplitude*	
2×10^{-7}	−72.4 ± 27.6 (5)	−100 ** (6)
5×10^{-7}	−74.4 ± 13.1* (5)	−80.3 ± 5.7** (6)
10^{-6}	−72.6 ± 10.5** (7)	−79.7 ± 8.4** (6)
2×10^{-6}	−55.1 ± 4.8** (7)	−75.8 ± 11.1* (6)
10^{-5}	−4.6 ± 5.1 (7)	−5.8 ± 3.5 (6)
5×10^{-5}	−2.1 ± 3.7 (7)	−7.2 ± 3.2 (6)
10^{-4}	−2.9 ± 3.7 (7)	−5.0 ± 3.2 (6)

Significant for paired data: *($P < 0.01$), **($P < 0.005$), ***($P < 0.001$).
In parentheses, number of muscles.

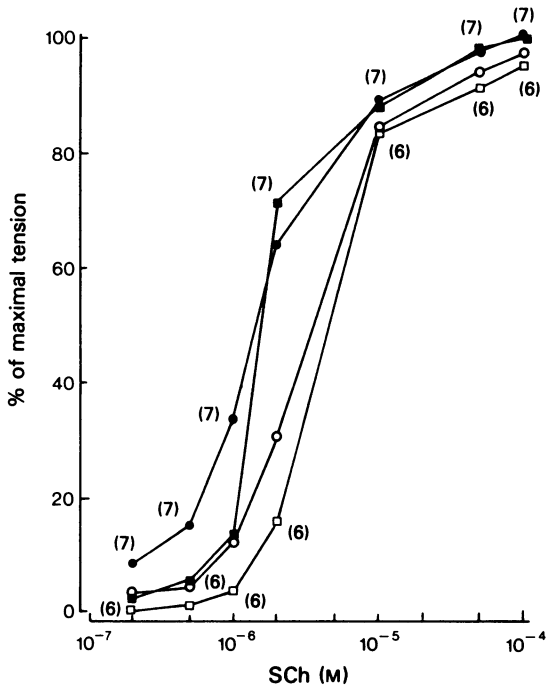

Figure 5 Effect of (+)-tubocurarine on the cumulative dose-response curve to succinylcholine (SCh). Abscissa scale: concentration of SCh plotted logarithmically. Ordinate scale: tension expressed as percentage of maximum tension evoked by 10^{-4} M SCh. Each point represents the mean response for the number of muscles indicated in parentheses. Control and treated with 3×10^{-7} M (+)-tubocurarine: (●) and (○). Control and treated with 10^{-6} M (+)-tubocurarine: (■) and (□).

similarly effective whereas the blockade of β-adrenoceptors exhibits stereospecificity such that the potency of the (−)-isomer is about one hundred times that of the (+)-isomer (Howe & Shanks, 1966; Barrett & Cullum, 1968).

The integrity of electrically-induced twitch contractions and K-induced tensions after exposure of the muscles to propranolol concentrations that depress SCh responses indicates that, at these concentrations, the drug was not exerting local anaesthetic activity (Morales-Aguilerá & Vaughan Williams, 1965; Barrett & Cullum, 1968) and did not affect impulse propagation or the various processes in muscle contraction caused by these stimuli.

The blocking effect of propranolol does not involve a depolarizing action of the type exhibited by SCh and methonium compounds (Paton & Zaimis, 1952) because the application of propranolol was not followed by tension development. Moreover, measurements of resting potential have shown that 6.8×10^{-5} M propranolol, a concentration about ten times larger than those used here, did not depolarize skeletal muscle fibres (Larsen & Teräväinen, 1978).

On the basis of the above considerations and of the observation that the depressant action could be overcome by increasing [SCh] and involved an approximately parallel shift to the right of the dose-response curves to SCh, it is postulated that propranolol acts on cholinoceptors probably as a competitive blocker, in micromolar concentrations.

Propranolol depresses postsynaptically neuromuscular transmission in frog and rat (Werman & Wislicki, 1971; Larsen & Teräväinen, 1978). The concentration of drug used in the latter experiments was 6.8×10^{-5} M, much higher than that used here and it is reasonable to assume that the synaptic depression involved not only blockade of cholinoceptors but also a local anaesthetic effect. Procaine, which has a local anaesthetic potency about half that of propranolol, is as effective as curare in reducing acetylcholine depolarizations in frog endplate (Del Castillo & Katz, 1957; Morales-Aguilerá & Vaughan Williams, 1965).

The depressant action of propranolol on SCh-evoked tensions in rat inferior rectus muscle is qualitatively comparable to that of (+)-tubocurarine although the latter is about 10 times more potent according to the changes in ED_{50} values.

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