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THE EFFECTS OF THREE β-ADRENOCEPTOR BLOCKING DRUGS ON ISOLATED PREPARATIONS OF SKELETAL AND CARDIAC MUSCLE

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1 The effects of propranolol, oxprenolol and practolol on the isometric twitch responses to electrical stimulation of isolated diaphragm muscles from the rat and of isolated papillary muscles from the rabbit are described.

2 Depression of the twitch responses of the diaphragm muscle was produced by propranolol (20 μg/ml), by oxprenolol (100 μg/ml) and by practolol (500 μg/ml).

3 Depression of the twitch responses of the papillary muscles was produced by propranolol (20 μg/ml) by oxprenolol (100 μg/ml) and by practolol (200 μg/ml).

4 No increase of twitch tension was produced by oxprenolol or practolol on either tissue.

5 It is concluded that propranolol, oxprenolol and practolol produce negative inotropic actions on isolated cardiac muscle by a mechanism unrelated to blockade of β-adrenoceptors and which occurs at doses which are well in excess of those doses required to produce β-blockade.

Introduction

Results from experiments in animals and man have shown that β-adrenoceptor blocking drugs produce a decrease in heart rate and a depression of the force of contraction of the heart (e.g. see reviews by Fitzgerald, 1969; Dollery, Paterson & Conolly, 1969), but controversy exists as to whether the decrease in the force of contraction is a result of blockade of sympathetic nerves to the heart, a direct depression of the myocardium, or both (e.g. Nayler, Chipperfield & Lowe, 1969). In a previous investigation on the acutely denervated dog heart (Harry, Kappagoda, Linden & Snow, 1973) it was shown that propranolol does not produce direct depression of the myocardium of the dog in doses usually used to antagonize the action of isoprenaline or sympathetic nerve stimulation on the heart (Ledsome, Linden & Norman, 1965) but that depression was evident if the dose exceeded 1-2 mg/kg given intravenously. To support this important conclusion about the action of propranolol, it should be possible to demonstrate that the β-adrenoceptor blocking drug does not depress the force of contraction of isolated cardiac muscle, which is not influenced by any sympathomimetic agent. However, investigations with isolated cardiac muscle preparations (e.g. Levy & Richards, 1966; Blinks, 1967; Nayler *et al.*, 1969; Meier, 1970) have produced conflicting results. Consequently we have reinvestigated the action of three β-adrenoceptor blocking drugs, propranolol, oxprenolol and practolol on isolated cardiac muscle, and also on isolated skeletal muscle because we felt that any depressant action of these drugs affecting the muscle contractile mechanism should affect cardiac and skeletal muscle in the same way.

A preliminary report of the results has been communicated to the British Pharmacological Society (Harry, Linden & Snow, 1972).

Methods

Removal of tissues

Rat diaphragm muscles. Rats were stunned by a blow on the neck and bled. The whole diaphragm muscle was quickly dissected out and placed in ice cold Krebs solution. A small triangular shaped slip was then cut from the diaphragm, the base being muscle and the apex a portion of the central tendon. The base of the muscle strip was attached to the holder which was also used as one electrode; the other electrode was a fine piece of stainless steel wire tied into the apex of the triangular slip.

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Two strips were taken from one diaphragm muscle, and set up identically in two adjacent organ baths.

Rabbit papillary muscles. Rabbits were killed by a blow on the back of the neck. The chest was opened and the whole heart removed and placed in ice cold Krebs solution. The wall of the right ventricle was separated from the heart by cutting through the wall close to and along the line of the interventricular septum commencing at the pulmonary valve. This dissection exposes the papillary muscles in the wall of the right ventricle attached to the cusps of the tricuspid valve. A ligature was placed around the base and another around the tendon of the papillary muscle, and the muscle was removed and set up in a holder. Two papillary muscles were taken from one rabbit heart and set up identically in two adjacent organ baths.

Organ bath apparatus

The organ bath contained 5 ml of Krebs solution maintained at a temperature of 32.5°C and bubbled with 95% O_2 and 5% CO_2; the pH of the Krebs solution was between 7.35-7.4. The Krebs solution contained (+)-tubocurare 10 μg/l to ensure direct stimulation of the rat diaphragm muscle and to abolish effects of neuromuscular transmission. (+)-Tubocurare was also added to the Krebs solution bathing the papillary muscles to make these experiments comparable with those involving skeletal muscle. The muscles were stimulated with square wave impulses of supramaximal voltage delivered by a Grass stimulator (Grass Instruments, Quincy, Mass., U.S.A.) at a rate of 0.5 Hz for the papillary muscle preparations and 0.05 Hz for the diaphragm preparations.

The tensions produced by the muscles were recorded with strain gauges (Grass Instruments, Quincy, Mass., U.S.A.) the signals from which, after amplification by carrier amplifiers (S.E. Laboratories, Feltham, Middlesex), were recorded on a u.v. recorder (Model 2100, S.E. Laboratories).

Experimental procedures

After the tissues had been set up a period of 30 min was allowed before any measurements were made. A resting tension was then selected which produced the maximum twitch tension response to electrical stimulation; this resting tension was maintained constant throughout the experiment. The bath fluid was then changed twice and after a further 10 min period, records were taken for about 1 minute. The bath fluid was changed once more and after a further 10 min a set of control records were taken; twitch tensions were expressed as a percentage of this control tension. The bath fluid was changed again and the β-adrenoceptor blocking drug added to the bath fluid, left for 10 min and another record taken. The bath fluid was again changed on two occasions, separated by 1 min and the next drug concentration was added to the bath fluid and the procedure repeated. The same cycle was used for each concentration of the drug. In control preparations, the same procedure as described above was followed except that a volume of Krebs solution equal to that used for addition of the drug to the test preparations, was added to the bath fluid after the second change of bath fluid.

The drugs used were (±)-propranolol hydrochloride and (±)-practolol (I.C.I. Pharmaceutical Division, Macclesfield, Cheshire), (±)-oxprenolol hydrochloride (CIBA Laboratories Ltd, Shoreham, Sussex), and (+)-tubocurare (May & Baker Ltd, Dagenham). All drugs were dissolved in Krebs solution, and the concentrations are expressed as the salt.

Results were analysed statistically by Student's unpaired single tailed *t* test.

Results

Effects of β-adrenoceptor blocking drugs on the diaphragm

The resting tension of the diaphragm slips to which propranolol was added was 1.25 g (mean; range 0.8-1.7), to which oxprenolol was added was 1.05 g (mean; range 0.8-1.5), to which practolol was added was 0.91 g (mean; range 0.8-1.0) and to which Krebs solution only was added was 1.1 g (mean; range 0.65-1.4).

A typical record of the effects produced by these β-adrenoceptor blocking drugs on the isometric twitch of the rat diaphragm muscle is shown in Figure 1. This shows two records of isometric twitch tensions from two strips of diaphragm from the same rat; one of these was exposed to oxprenolol and the other was the control preparation. The figure shows that no depression of the twitch response occurred until a concentration of 100 μg/ml of oxprenolol was reached. There is no change in the twitch response of the control diaphragm strip to which Krebs solution was added over the same period. Results with all the β-adrenoceptor antagonists used on the diaphragm preparations were similar to those shown in Fig. 1 and a summary of these results is seen in Figure 2. This figure only shows the mean results of all the experiments, but a statistically

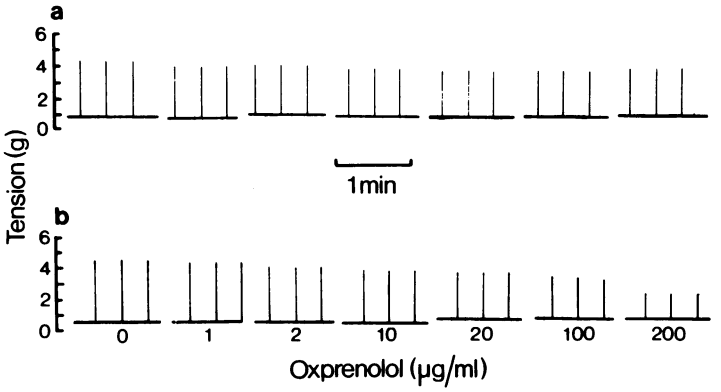

Fig. 1 The effects of increasing concentrations of oxprenolol on the twitch response to electrical stimulation of a slip of diaphragm muscle from one rat. (a) Was obtained from the control muscle to which Krebs solution alone was added and (b) from a second slip from the same diaphragm to which oxprenolol in Krebs solution was added. Each recording was obtained after the muscles had been exposed to the drug (at the concentrations shown) or to Krebs solution for 10 minutes.

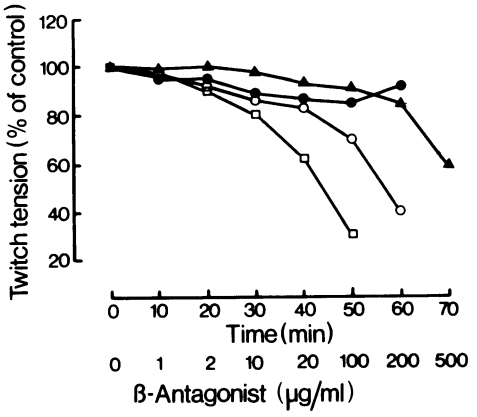

Fig. 2 The effects of propranolol (□; $n = 5$), oxprenolol (○; $n = 4$), practolol (▲; $n = 5$) and Krebs solution (●; control, $n = 7$) on the twitch response of the rat diaphragm muscle. Each point is the mean value from all the experiments performed. Horizontal scale, above, shows time for the control muscles and below, shows concentration of drug for the muscles exposed to drug.

significant depression of those muscles treated with the drugs compared with those not treated with the drug occurred at a concentration of propranolol of 20 μg/ml ($P = 0.0125$), at a concentration of oxprenolol of 100 μg/ml ($0.025 > P > 0.0125$) and at a concentration of practolol of about 500 μg/ml.

Effects of β-adrenoceptor blocking drugs on the papillary muscle

The resting tension on the papillary muscles to which propranolol was added was 1.00 g (mean; range 0.6-1.27), to which oxprenolol was added was 0.98 g (mean; range 0.65-1.4), to which practolol was added was 0.76 g (mean; range 0.65-0.9) and to which Krebs solution was added was 0.94 g (mean; range 0.84-1.3).

A typical record of effects of the β-adrenoceptor blocking drugs on the isometric twitch response of the papillary muscle is seen in Figure 3. This shows two records obtained from two papillary muscles taken from the same rabbit heart. There was no depression of the twitch response of the muscle which was exposed to propranolol until a concentration of 20 μg/ml was reached, whilst there was no depression of the twitch response of the control papillary muscle to which Krebs solution was added.

A summary of all the results obtained with the β-adrenoceptor blocking drugs on the papillary muscle is shown in Figure 4. This figure shows only the mean results of all the experiments but a statistically significant depression of those papillary muscles treated with drugs compared with those not subjected to drug occurred with a concentration of propranolol of 20 μg/ml ($0.025 > P > 0.0125$), with a concentration of oxprenolol of 100 μg/ml ($0.0025 > P > 0.0005$) and with a concentration of practolol of 200 μg/ml ($0.05 > P > 0.025$).

A comparison of the effects of propranolol, oxprenolol and practolol on the papillary muscle is

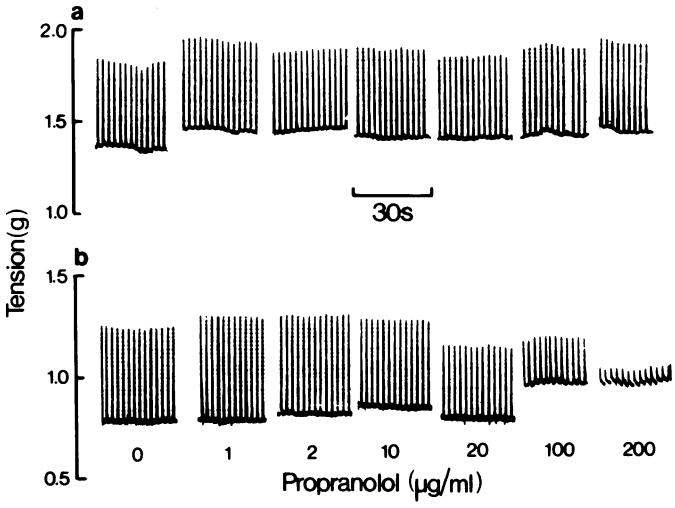

Fig. 3 The effect of increasing concentrations of propranolol on the twitch response to electrical stimulation of papillary muscle from one rabbit. (a) Was obtained from the control papillary muscle and (b) from a second papillary muscle from the same heart to which propranolol was added. Each recording was obtained after the muscles had been exposed to drug (at the concentrations shown) or to Krebs solution for 10 minutes.

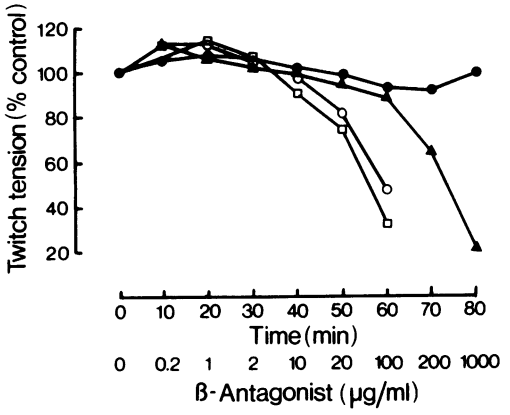

Fig. 4 The effects of propranolol (□; $n = 5$), oxprenolol (○; $n = 5$), practolol (▲; $n = 6$) and Krebs solution (●; control, $n = 10$) on the twitch responses of the rabbit papillary muscles to electrical stimulation. Each point is the mean value obtained from all the experiments performed. Horizontal scale, above, shows time for the control preparations, and below, shows concentration of drug, for the preparations exposed to drug.

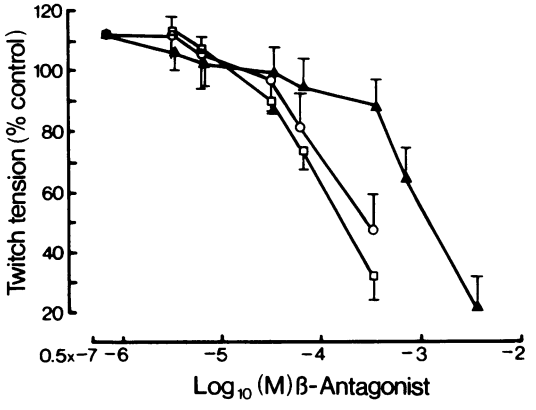

Fig. 5 The relationship between the twitch tensions of the papillary muscles from the rabbit and log molar concentrations of propranolol (□; $n = 5$) oxprenolol (○; $n = 5$) and practolol (▲; $n = 6$). Each point is the mean value (vertical bars show s.e. mean) of all the experiments performed.

shown in Fig. 5; there is no significant difference between propranolol and oxprenolol in producing depression of the myocardium, but depression with practolol occurs at higher concentrations than with oxprenolol and propranolol.

Intrinsic sympathomimetic effects

There was no evidence to show that oxprenolol or practolol produced an increase in the twitch responses of either the diaphragm or papillary muscle to electrical stimulation.

Discussion

The effects produced by the three β-adrenoceptor blocking drugs on both the isolated skeletal and isolated papillary muscles are similar. No depression of either tissue occurred with propranolol until the concentration in the bath fluid was 20 μg/ml. Depression of the diaphragm and papillary muscles was produced by oxprenolol at 100 μg/ml. For practolol depression of the diaphragm muscle was produced by 500 μg/ml and of the papillary muscle by 200 μg/ml. At higher concentrations further depression of the twitch responses of each tissue was produced by all three drugs. It is unlikely that this depressant action of these β-adrenoceptor blocking drugs is related to β-blockade of the effects of endogenous sympathomimetic amines present in the cardiac muscle because it occurred equally in those experiments involving skeletal muscle. Skeletal muscle is not innervated by nerves of the sympathetic nervous system and although large amounts of sympathomimetic drugs can increase the twitch tension of skeletal muscle fibres, there is no evidence to suggest that sympathomimetic amines are involved in the twitch response to electrical stimulation of the rat diaphragm preparation (see review by Bowman & Nott, 1969). Hence blockade of β-receptors by β-adrenoceptor antagonists should not affect the twitch responses of the diaphragm preparation. Further, the contractile mechanisms of both skeletal and cardiac muscles are probably similar (Katz, 1970) so that any drug affecting contractile mechanisms by a non-specific action would be likely to affect both skeletal and cardiac muscle; the result obtained in this investigation. This depressant action of the three drugs on both sets of muscles may be related to the non-specific membrane effect which each of these compounds is known to possess (e.g. Davis, 1970).

In our experiments the concentrations of the three β-adrenoceptor blocking drugs required to produce non-specific effects are well in excess of the concentrations required to produce blockade of β-adrenoceptors in isolated cardiac preparations. Propranolol is able to block the inotropic effects of isoprenaline on the guinea-pig atria at concentrations of 10^{-8} M upwards (Blinks, 1967; Meier, 1970); similar concentrations of oxprenolol are required to antagonize the inotropic actions of isoprenaline on guinea-pig isolated atria (Meier, 1970), while the concentration of practolol required to block the inotropic action of isoprenaline on the same tissue is in the region of 10^{-6} M (Wale, 1970). Nayler *et al.*, 1969 showed that propranolol (0.65×10^{-6} M) and oxprenolol (0.32×10^{-6} M) antagonized the positive inotropic effect of a single dose of isoprenaline (2.5 μg/l) in human isolated atria. The $ED_{50} \pm$ s.e. mean for propranolol against the inotropic action of isoprenaline on the rabbit isolated papillary muscle is 0.014 ± 0.031 μg/ml (Åblad, Brogard & Ek, 1967). These concentrations of β-adrenoceptor blocking drugs required to antagonize the actions of isoprenaline on isolated tissues are roughly 1000 times less than those required to produce non-specific depression of either the rabbit papillary muscle or the rat diaphragm as demonstrated in our experiments. We thus concluded from these experiments that propranolol, oxprenolol and practolol do not produce depression of isolated cardiac or skeletal muscle when used as β-blocking agents.

The results presented here are in agreement with the results of Blinks (1967) and Meier (1970). However, they do not agree with the results of Levy & Richards (1966) or with those of Nayler *et al.* (1969) and Nayler (1972). The results of Levy and Richards show that over the time course of their experiments, the twitch response of the isolated atria declined. This observation may indicate that their tissues were hypoxic and so the effect of propranolol in producing more depression at the concentration of 10^{-5} M may reflect the effect of this compound on dying atria rather than in viable atria.

The results of Nayler *et al.* (1969) were obtained from papillary muscles of dog and man with a diameter of 2 mm. It is probable that such isolated cardiac muscle preparations stimulated 37 times/min and at a temperature of 37°C are hypoxic. Evidence is available which suggests that at 30-32°C the maximum diameter of papillary muscle which will sustain adequate oxygenation is about 1 mm, e.g. histological changes representing cell death were found in the centre of cat papillary muscles with a diameter greater than 1 mm after several hours of contracting in an organ bath (Blinks & Koch-Weser, 1963). Coleman (1971) has shown, using cat papillary muscles, that a group of these muscles with a diameter greater than 1.1 mm utilize more oxygen when stretched passively, whereas muscles thinner than this did not utilize more oxygen. The inference from these experiments must be that muscles with a diameter greater than 1.1 mm are not adequately oxygenated until they are made thinner by stretching. Workers concerned with energetics of isolated cardiac muscle use papillary muscles which are consistently less than 1.1 mm in diameter, e.g. Gibbs, Mommaerts & Ricchiuti (1967); Gibbs & Gibson (1970). Estimation of the diameter of papillary muscles from the rabbit in

our laboratory, based upon weight and length of the muscles when stretched, showed that the maximum diameter would be 1.0 mm. In subsequent publications, Nayler (1972) and Nayler & Chang (1973) used thinner isolated muscle preparations which were stimulated at a lower rate, but still at a higher temperature of 35°C. These experiments produced different results for oxprenolol from those published previously (Nayler *et al.*, 1969), but still showed that propranolol produced depression of the twitch response to electrical stimulation in doses which would be considered as β-adrenoceptor blocking doses. This finding is in conflict with those reported here where both propranolol and oxprenolol did not depress the force of contraction of isolated papillary muscle until concentrations about 1000 times those required for adequate blockade of β-adrenoceptors were used.

Oxprenolol and practolol, two β-adrenoceptor blocking drugs known to produce direct sympathomimetic activity, did not increase the twitch tension response of either cardiac or skeletal muscle preparations. This is in agreement with the results of Blinks (1967), and Wale (1970). Meier (1970) found a small but significant increase in twitch tension with oxprenolol using the cat papillary muscle preparation. However, he was not using maximal twitch responses and further the cat papillary muscles to which no drug (control preparations) was added were not investigated. In the same experiments, propranolol can be seen from the results also to have produced a small increase in twitch tension. Some increase in twitch tension was noticed in our experiments in control papillary muscle preparations so the results for oxprenolol seen by Meier (1970) and ascribed to a stimulant action may be a non-specific effect and may merely reflect the normal time course of the twitch tension in this type of preparation.

In conclusion the results from this investigation confirm the conclusion we reached from the experiments on the denervated dog heart preparation (Harry *et al.*, 1973) that propranolol does not have a negative inotropic action (direct depression of the myocardium) on the heart in doses that will antagonize the action of isoprenaline or sympathetic nerves on the heart. Doses well in excess of this amount in the dog and on isolated cardiac muscle will cause direct depression of the myocardium.

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