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Analysis of the β-receptor mediated effect on slow-contracting skeletal muscle *in vitro*

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Subtetanic contractions of the guinea-pig isolated soleus, a slow-contracting skeletal muscle, were evoked by transmural field-stimulation. Isoprenaline caused a dose-dependent depression of the contractions. This effect was inhibited by propranolol and H 35/25 (1-(*p*-tolyl-2-isopropylamino-1-propanol) but not by practolol. Similar results were obtained for terbutaline. Tazolol and H 80/62 (1-isopropylamino-3-(*p*-hydroxyphenoxy)-2-propanol HCl), selective β_1-agonists, had no effect *per se* but inhibited the effect of terbutaline. Adrenaline, noradrenaline, and dopamine all caused a dose-dependent decrease in the force of the soleus contractions, their potencies being in that order. Tyramine did not appreciably affect the contractions nor did it inhibit the effect of terbutaline. Pretreatment with reserpine, if anything, increased the response to terbutaline. It is concluded, in conformity with previous *in vivo* studies, that the adrenergic receptor mediating the effect on the soleus muscle contractions is of the β_2-type. Indirect sympathomimetic effects do not contribute to the responses observed on the isolated soleus muscle.

Sympathomimetics produce a decrease in tension and in the duration of maximum twitches of slow-contracting skeletal muscles, resulting in reduced tension and degree of fusion of subtetanic contractions. This effect has been extensively studied *in vivo* on the cat soleus, a muscle rich in slow-contracting fibres (see Bowman & Nott, 1969).

Further *in vivo* studies on cat (Bowman & Nott, 1970; Apperly, Daly & Levy, 1976) and on guinea-pig (Apperly & Levy, 1975; Bohmer & Raper, 1976) showed, by the use of selective agonists and antagonists, that the effect of sympathomimetics on the soleus muscle is mediated via β_2-receptors, according to the current subdivision of the β-adrenoceptor.

These observations were confirmed and extended in the present study by the use of a recently developed *in vitro* preparation of the guinea-pig soleus (Waldeck, 1976; cf. also Tashiro, 1973). Moreover, the possible contribution of indirect sympathomimetic effects on the isolated soleus muscle was evaluated by the use of tyramine and reserpine.

METHODS

Male guinea-pigs, about 200 g, were anaesthetized with pentobarbitone sodium. The soleus muscle was dissected free and mounted in an organ bath containing oxygenated Krebs solution at 37°. Subtetanic contractions were evoked by transmural field stimulation: supramaximum pulses of 0·5 ms duration were delivered at a frequency of about 10 Hz for 1·5 s every 15 s. The contractions were recorded isometrically (for details see Waldeck, 1976).

Dose-response curves were obtained by adding single doses in increasing order, separated by rinsing and recovery periods. Unless otherwise stated, the experiments started with the addition of terbutaline, 2·5 μM, eliciting a maximum depression of the soleus contractions (cf. Waldeck, 1976). The subsequent effects were calculated in % of this response. Statistical evaluation of the results was made using Student's *t*-test.

The drugs used and their sources were: terbutaline sulphate (Draco), (±)-isoprenaline HCl, (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, dopamine HCl and tyramine HCl (Sigma), propranolol and practolol (ICI), H 35/25 (1-(*p*-tolyl-2-isopropylamino-1-propanol), H 80/62 (KWD 2033, 1-isopropylamino-3-(*p*-hydroxyphenoxy)-2-propanol HCl) and H 110/38 (tazolol) (Hässle), reserpine (Ciba), pentobarbitone sodium (ACO). In general solutions were made up in saline containing 0·1 mg ml^{-1} ascorbic acid.

RESULTS

Inhibition of the effect of isoprenaline by β-antagonists

Six soleus muscles were exposed to six increasing concentrations of isoprenaline up to 0·4 μM. In half of the experiments, propranolol (0·03 μM) was added to the bath 10–20 min before the last three doses of isoprenaline. The remaining three muscles served as controls. At the end of the experiment, practolol (3 μM) was added to the control and propranolol (0·3 μM) to the other muscles 10–20 min before the addition of isoprenaline (0·4 μM). The effects were

calculated in per cent of the depression caused by isoprenaline (0·1 μM) given at the beginning of the experiment.

Isoprenaline caused a dose-dependent reduction in the force of the subtetanic contractions of the soleus (Fig. 1). When propranolol (0·03 μM) was added to the bath beforehand, the dose-response curve for isoprenaline was moved to the right ($P < 0.001$). Propranolol (0·3 μM) further inhibited the response to isoprenaline ($P < 0.005$). In contrast, practolol (3 μM) was devoid of effect.

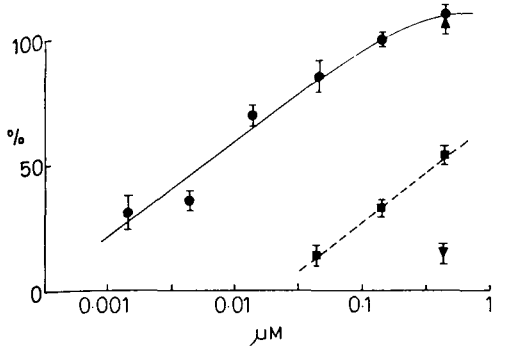

FIG. 1. Inhibition by propranolol, but not by practolol, of the effect of (±)-isoprenaline (μM) on subtetanic contractions of the guinea-pig soleus muscle *in vitro*. Shown are the mean ± s.e. of 3 experiments. The first three points on the control curve are based on 6 experiments. ● Control; ■ propranolol 0·03 μM, ▼ propranolol 0·3 μM, ▲ practolol 3 μM. Ordinate—Depression in % of maximum response.

In the next experiment, increasing doses of H 35/25 were added to the bath 10–20 min before a maximum dose of isoprenaline (yielding a concentration of 0·4 μM) (Fig. 2). H 35/25 caused a dose dependent inhibition of the effect of isoprenaline. The inhibition was almost complete at 40 μM H 35/25.

Inhibition of the effect of terbutaline by β-antagonists

In this experiment, the concentration of the agonist, terbutaline, was kept constant. Each muscle was exposed first three times to terbutaline alone and then three times with either inhibitor added 10–20 min before terbutaline. The variation between muscles was small and therefore data from different muscles have been treated statistically as one sample.

Terbutaline (0·3 μM) produced a depression of the subtetanic contractions of the soleus by about 80% of the maximum response (Table 1). This effect was largely inhibited when propranolol (0·03 μM) or H 35/25 (1 μM) had been added to the bath beforehand. Practolol (3 μM) had no effect in this respect.

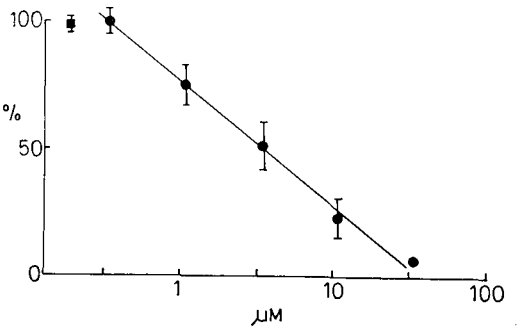

FIG. 2. Inhibition by H 35/25 (μM) of the effect of isoprenaline on subtetanic contractions of the guinea-pig soleus muscle *in vitro*. Shown are the mean ± s.e. of 5 experiments. Isoprenaline, 0·4 μM, alone ■, and in the presence ● of H 35/25. Ordinate—Depression in % of maximum response.

Effects of tazolol and H 80/62

Two sets of experiments were performed with tazolol and H 80/62. In the first set, increasing doses of the compounds, separated by rinsing and recovery, were added to the bath. In the second set, dose-response curves were established for terbutaline alone and in the presence of either tazolol or H 80/62 added 10–20 min beforehand.

Table 1. *Inhibition by β-antagonists of the effect of terbutaline on subtetanic contractions of the guinea-pig soleus muscle* in vitro. The data are expressed as % of the depression elicited by a maximum dose of terbutaline. Shown are the mean ± s.e. with the number of experiments in parentheses.

Inhibitor	Terbutaline, 0·3 μM (% of maximum response)	% inhibition
None	79 ± 3·3 (6)	
Propranolol, 0·03 μM	10 ± 0·8 (6)	87 ($P < 0.001$)
None	91 ± 2·5 (6)	
Practolol, 3 μM	87 ± 2·0 (6)	4 (N.S.)
None	82 ± 3·1 (9)	
H 35/25, 1 μM	15 ± 2·1 (9)	82 ($P < 0.001$)

Tazolol and H 80/62 in concentrations from 0·1 to 100 μM had no significant effect on the subtetanic contractions of the soleus muscle (Table 2). However, when given before terbutaline, they inhibited the depression of the contractions produced by this agonist (Fig. 3). The response to 0·3 μM terbutaline was inhibited by 50% ($P < 0.001$) in the presence of

Table 2. *Effect of tazolol and H 80/62 on subtetanic contractions of the guinea-pig soleus muscle* in vitro. The data are expressed as % of the depression elicited by a maximum dose of terbutaline. Shown are the mean ± s.e. with the number of experiments in parentheses.

	Concentration, μM			
Drug	0·1	1	10	100
Tazolol	7 ± 7 (4)*	4 ± 2 (4)	0 ± 0 (4)	10 ± 4 (4)
H 80/62	0 ± 0 (2)	0 ± 0 (2)	0 ± 0 (2)	3 ± 9 (2)

* Reversed response i.e. increased force of contraction.

1 μM tazolol. The inhibition induced by 1 μM H 80/62, if anything, was stronger. The inhibitory effect of both tazolol and H 80/62 was reversible, although tazolol appeared to be more readily washed out.

Effects of adrenaline, noradrenaline and dopamine

The catecholamines were added to the muscle bath in increasing doses. Adrenaline, noradrenaline, and dopamine caused a dose-dependent decrease in the

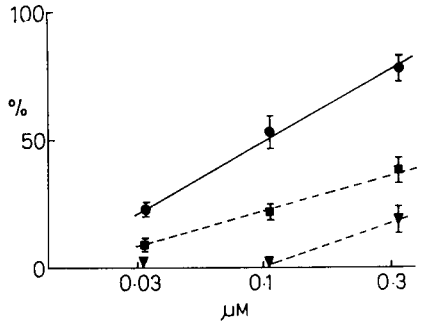

FIG. 3. Inhibition by tazolol and H 80/62 of the effect of terbutaline (μM) on subtetanic contractions of the guinea-pig soleus muscle *in vitro*. Shown are the mean ± s.e. ● Control (n = 7), ■ tazolol 1 μM (n = 5), ▼ H 80/62 1 μM (n = 2). Ordinate—Depression in % of maximum response.

force of subtetanic contractions of the soleus muscle (Fig. 4). The dose-response curves were close to parallel and the rank order of potency was adrenaline > noradrenaline > dopamine ($P < 0{\cdot}001$). The concentration (± s.e.) required to produce 50% of the maximum effect elicited by 2·5 μM terbutaline was for adrenaline $1{\cdot}3 \pm 0{\cdot}28 \times 10^{-8}$, for noradrenaline $9{\cdot}5 \pm 2{\cdot}9 \times 10^{-7}$, and for dopamine $7{\cdot}0 \pm 1{\cdot}0 \times 10^{-5}$ M. The number of experiments were 4, 4, and 3, respectively.

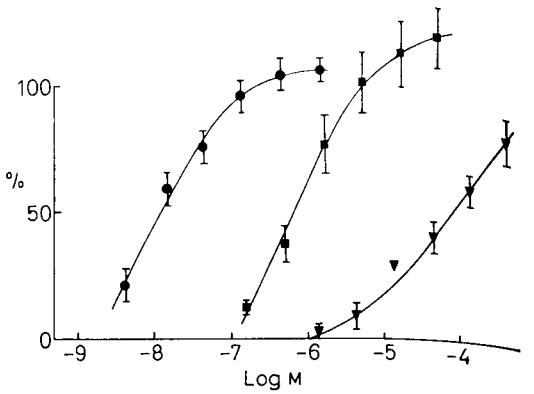

FIG. 4. Effect of catecholamines on subtetanic contractions of the guinea-pig soleus muscle *in vitro*. Shown are the mean ± s.e. ● Adrenaline (n = 4), ■ noradrenaline (n = 4), ▼ dopamine (n = 3). Ordinate—Depression in % of maximum response.

Effects of tyramine and reserpine

Six muscle preparations were exposed 5 times to a constant dose, 0·3 μM, of terbutaline. Ten or fifteen min before the second and fourth challenge, tyramine at 80 or 260 μM was added. The order of addition of the high and low dose of tyramine was reversed in half of the experiments in order to reveal possible tachyphylaxis.

Terbutaline (0·3 μM) caused a depression of the soleus muscle contractions which were reduced by 60–70% of the maximum response (Table 3). Tyramine (80 μM) had no effect *per se* and did not change the response to terbutaline. At 260 μM concentration, tyramine depressed the soleus muscle contractions by about 20%. In the presence of this high concentration of tyramine, the muscle responded normally to terbutaline. The effect of the high

Table 3. *Effect of tyramine, alone and together with terbutaline, on subtetanic contractions of the guinea-pig soleus muscle* in vitro. The data are expressed as % of the depression elicited by a maximum dose of terbutaline. Shown are the mean ± s.e. of 6 experiments.

Tyramine μM	Terbutaline 0·3 μM	Tyramine	Tyramine + terbutaline 0·3 μM
0	66 ± 5	—	—
80	—	2 ± 4	63 ± 9
0	67 ± 6	—	—
260	—	19 ± 2	71 ± 7
0	59 ± 6	—	—

dose of tyramine appeared to be unspecific, as it was not blocked by 0·3 μM propranolol (data not shown).

In some experiments guinea-pigs were pretreated with reserpine for 3 days (5 + 3 + 3 mg kg^{-1}). The animals were taken for experiment 24 h after the last injection. The depression of the soleus muscle contractions produced by 0·3 μM terbutaline was 93 ± 6% (mean ± s.e. of 4 experiments). In 4 control experiments, the effect of the same concentration of terbutaline was 73 ± 7%.

DISCUSSION

In conformity with previous *in vivo* studies (see introduction) the present data indicate that the depression of the subtetanic contractions of the soleus muscle produced by sympathomimetics is mediated via adrenoceptors of the β_2-type. Thus the effect of isoprenaline, an unselective β-agonist, was inhibited by propranolol, an unselective antagonist, and H 35/25, a β_2-selective antagonist (Levy & Wilkenfeld, 1969), but not by practolol, a β_1-selective (Dunlop & Shanks, 1968) antagonist. Similar results were obtained for the β_2-selective (Bergman, Persson & Wetterlin, 1969; Persson & Olsson, 1970) agonist, terbutaline.

The β_2-specific character of the adrenoceptor of the soleus muscle is further supported by the lack of effects of the β_1-selective agonists tazolol (Roszkowski, Strosberg & others, 1972; Sörenby, 1975) and H 80/62 (Carlsson, Persson, Dahlöf & Hedberg, unpublished) and the fact that adrenaline was about 50 times more potent than noradrenaline in depressing the soleus contractions (cf. Bowman & Zaimis, 1958).

In clinical studies, propranolol blocked the skeletal muscle tremor produced by adrenaline and isoprenaline (Marsden, Foley & others, 1967) but practolol was less effective (Thiringer & Svedmyr, 1976; cf. also Larsson & Svedmyr, 1977). Noradrenaline does not seem to cause tremor in therapeutic doses (Marsden & others, 1967).

This agreement between *in vivo* and *in vitro* data, on one hand, and studies in man, on the other, supports the view that the tremor observed during treatment of patients with sympathomimetic bronchodilators is due to stimulation of adrenoceptors of the β_2-type in slow contracting skeletal muscle and that the soleus is a suitable organ for the study of this effect (see Bowman & Nott, 1969, 1970).

Tazolol and H 80/62, although without effect *per se*, inhibited the response of the soleus muscle to terbutaline. Previous studies have shown that, in the guinea-pig isolated trachea and heart and in the dog *in vivo*, tazolol shows stimulating as well as blocking properties on β-receptors (Sörenby, 1975; Strosberg, 1976). This demonstrates the subtle balance between agonism and antagonism, so often seen among drugs acting at the β-adrenoceptor (cf. Ariëns, Simonis & van Rossum, 1964).

Dopamine was about 5000 times less potent than adrenaline in depressing the soleus contractions. This is consistent with the very weak β-agonistic effects of dopamine previously observed (see Goldberg, 1972). A recent *in vivo* study (Ferko, 1976) shows that dopamine in very high doses, intra-arterially, causes a depression of indirectly evoked twitches in the cat anterior tibialis muscle. This effect was blocked by dopamine antagonists but not by propranolol. A similar effect of dopamine at high concentrations on the soleus cannot be ruled out at present.

The experiments with the indirectly acting sympathomimetic, tyramine, and with the animals that had their endogenous catecholamine stores depleted by reserpine (Carlsson, 1965) show that indirect effects mediated via endogenous catecholamines are practically absent in the soleus preparation. Thus no precautions have to be taken to eliminate this source of error in the evaluation of sympathomimetics in this organ. Moreover, even a high concentration of tyramine did not significantly interfere with the response to terbutaline. This indicates a very low affinity of tyramine for the receptors mediating the depression of the soleus contractions.

The mechanism behind the effects observed in the present study is not clear. It has been suggested, mainly on indirect evidence, that the sympathomimetic induced effect on the soleus muscle is a cyclic AMP mediated effect on the Ca^{2+} transport mechanism in the sarcoplasmic reticulum (Bowman & Nott, 1969, 1974). In view of the harmony between *in vivo* and *in vitro* data, the soleus preparation *in vitro* might prove useful in a further analysis of this mechanism.

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