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An *in vitro* method for the study of β-receptor mediated effects on slow contracting skeletal muscle

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The soleus muscle of a guinea-pig was dissected out under pentobarbitone anaesthesia and mounted on a holder in an organ bath containing Krebs solution. The tendon was attached to a force transducer and subtetanic contractions were evoked by electrical field stimulation (0·5 ms pulses at 10–12 Hz for 1·5 or 3 s every 22 s). The experiments were performed at 37°. Terbutaline, a selective agonist at β_2-adrenoceptors, reduced the force of subtetanic contractions in a dose-dependent manner, the EC50 being 0·2 μM. The reduction was due to a lessened degree of fusion. The results conform to previous *in vivo* studies.

Most previous studies on the effects of sympathomimetic amines on slow-contracting skeletal muscle have been made *in vivo*, using the soleus muscle from either cat (Bowman & Nott, 1970; Olsson, 1974) or guinea-pig (Apperly & Levy, 1975). Although this technique comes close to the physiological conditions, an *in vitro* system offers certain advantages: (*a*) the drug concentration at the target organ can be controlled, (*b*) drug metabolism is largely avoided, (*c*) indirect effects mediated via the central nervous system or hormones are eliminated, and (*d*) effects of changes in pH, temperature etc. can easily be studied.

The effect of isoprenaline on twitches evoked by direct, electrical stimulation of the guinea-pig soleus muscle has been studied *in vitro* (Tashiro, 1973). Isoprenaline caused a decrease in tension and in the duration of single twitches evoked by supramaximum pulses of less than 1 ms duration (cf. Bowman & Nott, 1969). However, these effects are relatively small and difficult to quantify.

When subtetanic contractions of the soleus muscle are evoked instead of single twitches, the decrease in twitch duration produced by isoprenaline results in a decrease in fusion and a pronounced reduction in overall tension, as has been demonstrated in the cat *in vivo* (Bowman & Nott, 1970). This pattern of stimulation has been adopted in the present study in order to quantify the effect of terbutaline, a selective stimulant of β_2-adrenoceptors (Bergman, Persson & Wetterlin, 1969; Persson & Olsson, 1970), on the guinea-pig soleus muscle *in vitro*.

MATERIALS AND METHODS

Male guinea-pigs, about 200 g, were anaesthetized with pentobarbitone sodium (50–60 mg kg^{-1}, i.p.). The soleus muscle was dissected free. Ligatures were placed on the tendon and on the head of the muscle *in situ*. It was then removed, immediately immersed in oxygenated Krebs solution, and mounted in an organ bath at room temperature (22–25°) with the head tied to a holder and the tendon attached to a Grass FT03 force transducer for isometric recording (Fig. 1). The basal tone was adjusted to 2 g. Subtetanic contractions were evoked by means of longitudinal field stimulation via two ring-shaped

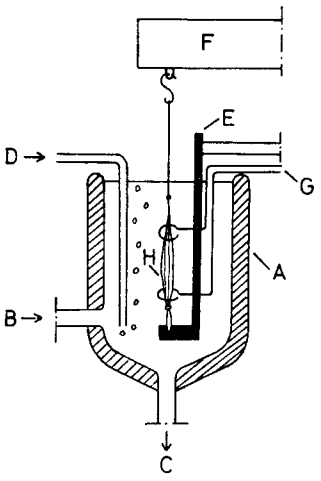

FIG. 1. Apparatus for *in vitro* measurement of contractions evoked by electrical field stimulation in the guinea-pig soleus muscle. A: temperature-controlled organ bath; B: prewarmed Krebs solution; C. waste; D: 5% CO_2 in oxygen; E: holder; F: force transducer connected to recorder; G: electrode wires, connected to stimulator; H: muscle surrounded, but not touched, by two stimulating electrodes.

(diam. 5 mm) electrodes, placed 20 mm apart, surrounding, but not touching, the muscle. Supramaximal pulses of 0·5 ms duration, at a frequency of 10 Hz, were delivered every 22 s for 1·5 or 3 s from a Grass S48 stimulator via an SIU5 stimulus isolation unit.

The contractions were recorded on a Grass Polygraph 7D recorder and, in some experiments, monitored on an oscilloscope.

When stable contractions were obtained, the temperature of the organ bath was slowly increased to 37° where it was kept constant with the aid of a thermostat. During the elevation of the temperature, the force of the subtetanic contractions first increased. After about 28°, it decreased due to a lessened degree of fusion and not to a reduction in twitch tension, as revealed by the oscilloscope. The frequency-response relation was tested for each muscle, and a frequency from the steepest part of the curve, usually about 12 Hz, was chosen for the subsequent experiment. This condition has proved optimum for the measurement of β-adrenoceptor-mediated effects on cat soleus muscle (Bowman & Nott, 1970) and was valid also in the present experiments.

Terbutaline, in various concentrations, was added to the bath (35 ml), and the response was calculated in per cent of the maximum effect elicited by 20 μg (yielding a concentration of 2·5 μM). After each challenge, the bath was rinsed three times with fresh, prewarmed Krebs solution, and the muscle was allowed to recover.

The Krebs solution contained (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·16; $NaHCO_3$, 25; KH_2PO_4, 1·18; D-glucose, 11·1. A stream of 5% CO_2 in oxygen was bubbled through the organ bath and the bulk of solution. Terbutaline sulphate in solution (Bricanyl, Draco, 0·5 mg ml^{-1}) was diluted in saline containing 0·1 mg ml^{-1} ascorbic acid.

RESULTS

Fig. 2 illustrates the effect of terbutaline on the guinea-pig soleus muscle *in vitro*. Under the experimental conditions used, the final force of contraction after each train of stimuli was 2–3 times that of the first twitch. Terbutaline caused a lessened degree of fusion resulting in a marked decrease in the force of a subtetanic contraction. In the example depicted, fusion was almost abolished. However, the height of the first twitch of each train remained unaffected. The onset of action was rapid and the effect was maximum 5 min after the addition of the drug. After rinsing, the muscle recovered and within 20 min the degree of fusion and the force of a subtetanic contraction had returned to the control levels. The effect of terbutaline was blocked by the β-selective antagonist propranolol.

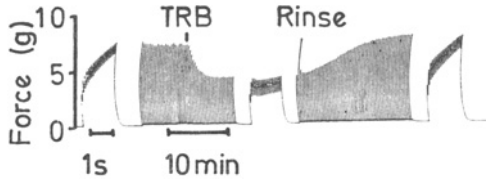

FIG. 2. Depression by terbutaline of subtetanic contractions evoked in the soleus muscle of guinea-pig *in vitro*. Electrical pulses, 0·5 ms, at 14 Hz were delivered every 22 s for 1·5 s. The shapes of the contractions recorded on fast-moving paper are shown before and after the addition of a supramaximum dose (20 μg) of terbutaline (TRB) and also after rinsing and recovery.

Two sets of experiments aimed at an analysis of the dose-response relation for terbutaline were performed. In the first, train duration was 3 s; in the second, 1·5 s. Each experiment started and ended with the addition of 20 μg (2·5 μM) terbutaline. All results were calculated as per cent of the response to the first dose. The difference in train duration did not significantly change the optimum frequency (11–12 Hz), the force of contraction (9–11 g), or the decrease in the force of contraction (about 35% reduction) caused by the first supramaximum dose of terbutaline (Table 1). The table also shows the scatter of the weight of the animals.

Table 1. *Effect of a supramaximum dose of terbutaline on the force of subtetanic contractions evoked by electrical field stimulation in guinea-pig soleus* in vitro. Pulses (0·5 ms) were delivered in trains every 22 s for 3 or 1·5 s. The means ± s.e. and the number of experiments are given. The table also gives statistical information on the size of the animals used.

Train duration	Optimum frequency, Hz	Force of contraction in g before terbutaline	Force of contraction % reduction after terbutaline	Body weight in g
3·0 s	11 ± 0·5 (6)	8·9 ± 1·6 (6)	38 ± 3·9 (6)	209 ± 13 (6)
1·5 s	12 ± 0·6 (7)	10·9 ± 1·2 (7)	32 ± 3·0 (7)	196 ± 15 (7)

The effect of terbutaline on the guinea-pig soleus muscle was dose-dependent (Fig. 3). A linear dose-response relation was obtained in both experiments ($P < 0·001$), the EC50 being 0·23 and 0·20 μM when train duration was 1·5 and 3 s, respectively. The difference was not statistically significant ($P > 0·5$). Maximum response to terbutaline appeared at about 1 μM.

DISCUSSION

The results obtained in the present study on the isolated guinea-pig soleus muscle conform qualitatively to results obtained from the cat *in vivo* (Bowman & Nott, 1970), a study in which (−)-isoprenaline was used as the β-agonist. Preliminary data

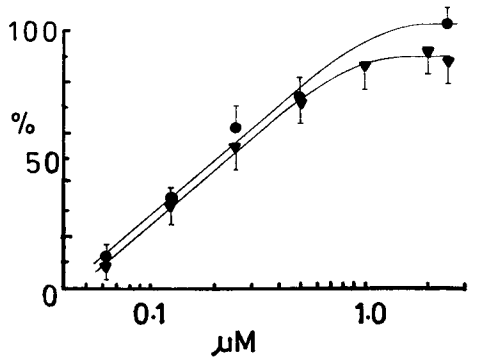

FIG. 3. Dose-response relation for the effect of terbutaline on the guinea-pig soleus muscle *in vitro*. The experiments are identical with those described in Table 1. The result is expressed in per cent of maximum response. The means ± s.e. of 6–7 experiments are given. Triangles refer to the experiments with train duration 1·5 s, closed circles to 3 s.

from our laboratory show that isoprenaline and salbutamol in the isolated guinea-pig soleus yield dose-response curves parallel with and moved to the left from that of terbutaline (unpublished). This corresponds to the *in vivo* situation for the guinea-pig (Apperly & Levy, 1975). The isolated soleus muscle of the guinea-pig is obviously suitable for the *in vitro* study of β-receptor mediated effects on slow-contracting skeletal muscle.

Although the preparation is fairly stable, a few precautions are necessary. The muscle is dissected out under anaesthesia and not *post mortem*, so that, vitally, the tissue will be at its best. Moreover, the muscle is allowed to stabilize at a low temperature (22°) before elevation to 37°. Thus the metabolism is kept at a reduced rate until the energy supply has become adequate (cf. below). The change in degree of fusion, observed when the temperature was increased, is probably due to an accelerated relaxation (cf. Tashiro, 1973). Some muscles that failed to respond to the electrical stimulation were activated and gained in force of contraction when pulse duration was increased to 5 ms. Pulse duration could then be reset to the normal 0·5 ms without appreciable loss in activity. Apparently the regular shifts between contraction and relaxation favour the energy supply to the muscle, possibly through facilitation of diffusion. But the ratio between train duration and train rate of the electrical stimulation does not appear to be critical, because identical dose response curves were obtained even when train duration was reduced to half.

In pilot experiments (+)-tubocurarine (5–50 μg ml^{-1}) was added to the organ bath, but without effect on the electrically induced contractions. This indicates direct stimulation of the muscle with little or no contribution of acetylcholine released from nerve endings during the experimental conditions used.

The reduction in force of subtetanic, isometric contractions, induced by a supramaximal dose of terbutaline, varied from muscle to muscle. In the present study, it ranged from 28 to 52%. However each muscle showed a dose-dependent response, and when results were calculated as per cent of the individual maximum response, EC50s were reproducible.

Dose-response curves can be obtained either by giving single doses, separated by rinsing and recovery periods, or by giving additive doses, i.e. cumulative curves. The first method was used in the present study. Although more time consuming than the cumulative technique, it gives more information about the onset and the duration of the drug action.

The method described here might not only be suitable for the study of β-receptor mediated effects on slow contracting skeletal muscle but could also be adopted for the *in vitro* study of other drug-induced effects.

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REFERENCES

APPERLY, G. H. & LEVY, G. P. (1975). *Br. J. Pharmac.*, **54**, 260P–261P.
BERGMAN, J. PERSSON, H. & WETTERLIN, K. (1969). *Experientia*, **25**, 899.
BOWMAN, W. C. & NOTT, M. W. (1969). *Pharmac. Rev.*, **21**, 27–72.
BOWMAN, W. C. & NOTT, M. W. (1970). *Br. J. Pharmac.*, **38**, 37–49.
OLSSON, O. A. T. (1974). *Acta pharmac. tox.*, **34**, 106–114.
PERSSON, H. & OLSSON, T. (1970). *Acta med. scand., Suppl.* **512**, 11–19.
TASHIRO, N. (1973). *Br. J. Pharmac.*, **48**, 121–131.