J. Pharm. Pharmacol. 1979, 31: 164–167

Received August 25, 1978

Pentobarbitone and skeletal muscle contractions: on the interaction with the effect elicited by the β-adrenoceptor agonist, terbutaline

EVA HOLMBERG AND BERTIL WALDECK*

AB Draco†, Research and Development Laboratories, Fack, S-221 01 Lund, Sweden

The soleus, a slow-contracting muscle, and the extensor digitorum longus (EDL), a fast-contracting muscle from guinea-pig were prepared for isometric recording in vitro. Subtetanic contractions were evoked by transmural field-stimulation. Pentobarbitone increased the force of contraction in both muscles. In the soleus it shifted the stimulation frequency-response curve to the left. Terbutaline caused a decrease in the force of subtetanic contractions of the soleus, an effect which was dependent on the stimulation frequency. In the presence of pentobarbitone, the stimulation frequency had to be lowered by about 2 Hz in order to maintain the optimum response to terbutaline. The EDL responded to terbutaline with an increased force of contraction. In this case the stimulation frequency was less critical and the effects were the same in the presence and in the absence of pentobarbitone. Experiments with α-chloralose yielded results similar to those obtained with pentobarbitone.

In slow-contracting skeletal muscle sympathomimetics cause decreases in both twitch tension and twitch duration, resulting in a decrease in the force and the degree of fusion of subtetanic contractions. In fast-contracting muscles the effects are the opposite (see Bowman & Nott 1969). These effects have mostly been studied in vivo in animals anaesthetized either with pentobarbitone (Olsson 1974; Apperly & Levy 1975) or with a mixture of α-chloralose and pentobarbitone (Bowman & Nott 1970; Bohmer & Raper 1976).

Among its diverse pharmacological effects, pentobarbitone increases the twitch height and prolongs the active state in skeletal muscle contractions (Sirnes 1954; Gjone 1956; Foulks et al 1973). Moreover, the duration of the action potential of the skeletal muscle fibre is lengthened by pentobarbitone; chloralose has a similar effect (Thesleff 1956). All these effects occur at anaesthetic concentrations. It seemed important therefore, to see if pentobarbitone influences the effects elicited by sympathomimetics on skeletal muscle contractions.

In the present study the actions of and the interaction between terbutaline (a β_2-adrenoceptor agonist: Bergman et al 1969; Persson & Olsson 1970) and pentobarbitone have been studied in vitro on directly evoked, subtetanic contractions of the slow-contracting soleus muscle, and the fast-contracting muscle the extensor digitorum longus (EDL) from the guinea-pig. The sympathomimetic-induced effects on these preparations have been documented previously (Waldeck 1977; Holmberg & Waldeck 1977). Some experiments were also performed with α-chloralose.

* Correspondence.
† Subsidiary to AB Astra, Sweden.

MATERIALS AND METHODS

Male guinea-pigs, about 200 g were anaesthetized with pentobarbitone sodium (50–60 mg kg^{-1}, i.p.). The soleus and extensor digitorum longus (EDL) muscles, were dissected out, and mounted in organ baths (39 ml) containing oxygenated Krebs solution maintained at 37 °C.

Subtetanic contractions were evoked by transmural field stimulation: supramaximal pulses of 0·5 ms duration were delivered at a frequency of about 10 Hz for 1·5 s to the soleus and at a frequency of 40 Hz for 150 ms to the EDL every 15 s. The contractions were recorded isometrically (for details see Waldeck 1976). Each experiment started with the addition of a dose of terbutaline greater than that necessary to produce a maximal response (2·3 μM). The subsequent effects were calculated as percentages of the maximum response to this concentration. In some experiments the force of contraction was studied as a function of the stimulation frequency. These measurements always proceeded from lower to higher frequencies: two trains of stimuli were given at every 2 Hz interval.

Statistical analysis was by analysis of variance and Student's *t*-test.

Drugs used were: terbutaline sulphate (Draco); pentobarbitone sodium (ACO), α-chloralose

(Merck). Solutions were made up in 0·9% w/v NaCl containing 0·1 mg ml^{-1} ascorbic acid. The substances were added in a volume of about 0·1 ml. α-Chloralose, because of its poor solubility, was made up in Krebs solution at the concentration desired. This solution was prewarmed and substituted for the normal Krebs solution.

RESULTS

Actions and interactions of pentobarbitone and terbutaline on subtetanic contractions of skeletal muscles

Pentobarbitone was added cumulatively to preparations of soleus and EDL muscles respectively. The drug caused a dose-dependent increase in the force of subtetanic contractions in both muscles up to a concentration of 0·33 mM (Fig. 1). This increase originated from both an increased twitch tension and an increased degree of fusion as was evident from recordings at a high chart speed. In the soleus, however, increased fusion appeared to be the major factor. From 0·33 to 1·0 mM the force of the EDL contractions continued to increase ($P < 0·01$) while the reinforcement of the soleus muscle contractions appeared to be optimum at 0·33 mM.

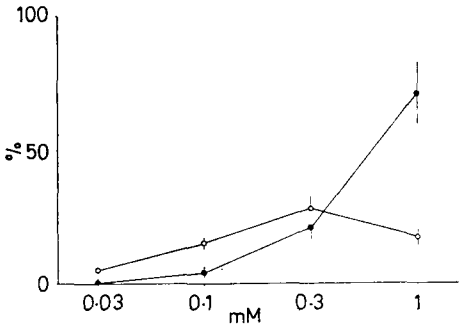

FIG. 1. Effect of pentobarbitone on subtetanic contractions of the soleus muscle (○—○) and the extensor digitorum longus (●—●) from the guinea-pig in vitro. The contractions were induced by transmural field stimulation every 15 s for 1·5 and 0·15 s respectively. The means ± s.e. of 4 experiments are shown. Ordinate: % increase in force of contraction. Abscissa: concentration of pentobarbitone.

In the next experiment the effect of terbutaline (0·4 μM) was determined in the presence and in the absence of pentobarbitone. The results are in Table 1. The effects of pentobarbitone per se were similar to those observed in the previous experiment. In the soleus muscle the depression of the subtetanic contractions caused by terbutaline was diminished in the presence of 0·33 μM pentobarbitone ($P < 0·001$). After rinsing and recovery the muscle responded normally to terbutaline. The effect of terbutaline on the EDL was not significantly changed by 0·33 mM pentobarbitone.

Table 1. Actions and interactions of pentobarbitone and terbutaline on contractions of skeletal muscles from guinea-pig in vitro. Subtetanic contractions were induced by transmural field stimulation every 15 s for 1·5 and 0·15 s respectively in soleus and extensor digitorum longus (EDL). Shown are the means ± s.e. with the number of experiments in parentheses.

Muscle	Pentobarb. mM	Force of contraction, increase %	Effect of terbutaline, 0·4 μM, % max. resp.
Soleus	0	—	72 ± 4 (9)
	0·03	5 ± 2 (4)	64 ± 12 (4)
	0·10	12 ± 4 (5)	60 ± 11 (5)
	0·33	24 ± 8 (8)	34 ± 6 (8)
	0*	—	69 ± 3 (6)
EDL	0	—	66 ± 8 (5)
	0·33	19 ± 5 (5)	58 ± 14 (5)

* After contact with pentobarbitone and subsequent rinsing and recovery.

Effects of pentobarbitone and terbutaline on subtetanic contractions of skeletal muscles as a function of the frequency of stimulation

The force of subtetanic contractions of the soleus muscle was measured at various frequencies of stimulation in the presence or absence of either pentobarbitone (0·33 mM) or terbutaline (2·3 μM). The results have been expressed as multiples of the twitch tension. In the absence of drugs the contractions of the soleus muscle started to fuse at about 8 Hz. For the next 10 Hz there was a frequency-dependent increase in the force of the subtetanic contractions indicating a gradually increasing fusion (Fig. 2).

In the presence of pentobarbitone, fusion started earlier and at 8 Hz the force of contraction was 40–50% higher as compared with the untreated muscle ($P < 0·05$). At higher frequencies the difference became less apparent or absent. Contrary to pentobarbitone, terbutaline shifted the frequency-response curve to the right. At 12 Hz there was an optimum depression of the soleus contractions of about 30% ($P < 0·001$). When the stimulus frequency was increased to 20 Hz, an effect of terbutaline was no longer visible.

In another series of experiments the frequency of stimulation required for maximum depression of the

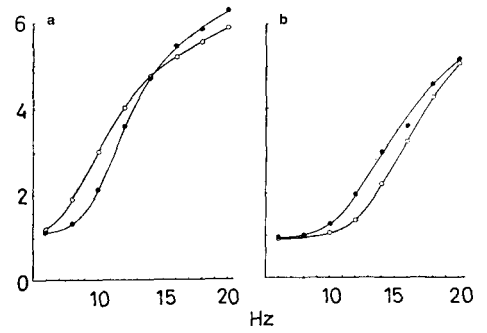

FIG. 2. Effects of pentobarbitone (a) and terbutaline on subtetanic contractions of the isolated extensor muscle from the guinea-pig. Contractions were induced by transmural field stimulation every 15 s for 1·5 s. The means of 3 experiments in the absence (●—●) and in the presence (○—○) of drug are shown. Ordinate: force of contraction expressed as multiples of the twitch tension at the start of the experiment. Abscissa: stimulus frequency.

soleus muscle contractions by terbutaline (2·3 μM) was estimated. The mean ± s.e. of 6 determinations was 12·2 ± 0·2 Hz in the absence and 10·3 ± 0·1 Hz in the presence of pentobarbitone (0·33 mM), the difference being statistically significant ($P < 0.001$). There was no change in the magnitude of the maximum depression obtained.

The frequency-response relation for subtetanic contractions was studied also on the isolated EDL. In contrast to the condition for the soleus, the contractions of the EDL started to fuse at about 30 Hz. Also, the frequency-response curve was less steep for EDL than for soleus (cf. Fig. 2 with Fig. 3).

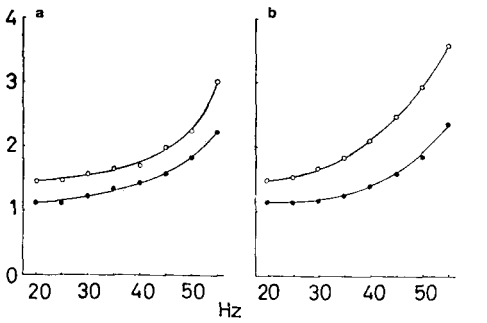

FIG. 3. Effects of pentobarbitone (a) and terbutaline (b) on subtetanic contractions of the isolated extensor digitorum longus from the guinea-pig. Contractions were induced by transmural field stimulation every 15 s for 0·15 s. The means of 3 experiments, in the absence (●—●) and in the presence (○—○) of drug are shown. Ordinate: force of contraction expressed as multiples of the twitch tension at the start of the experiment. Abscissa: stimulus frequency.

In the presence of pentobarbitone (0·33 mM) the force of contractions increased by about 20% ($P < 0.05$ at 30 and 35 Hz). Terbutaline (2·3 μM) increased the force of contraction by 20–30% ($P < 0.05$–$P < 0.01$). There was no sharp effect optimum of the frequency-response curves as was the case for the soleus.

Effect of α-chloralose on subtetanic contractions of skeletal muscles

The effect of α-chloralose on subtetanic contractions of the soleus muscle was measured at three different frequencies of stimulation and at three different drug concentrations. α-Chloralose caused a dose-dependent increase in the force of contraction (Fig. 4a). The increase observed after 3 mM α-chloralose in all three experiments was more pronounced when measured at 9 Hz than it was at 12 Hz. When measured at 15 Hz, the effect of α-chloralose was, if anything, further diminished.

A similar experiment was made with the EDL. In this experiment α-chloralose also caused a dose-dependent increase in the force of contraction (Fig. 4b). The effect was, however, independent of the stimulus frequency used (25, 35 or 45 Hz).

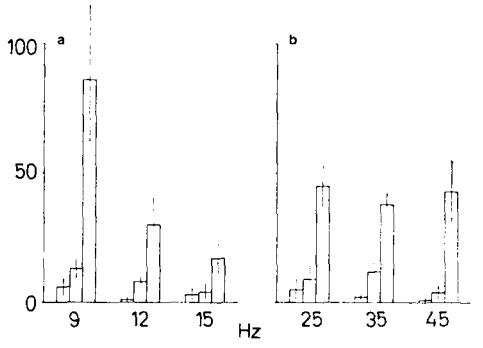

FIG. 4. Effect of α-chloralose on subtetanic contractions of the isolated soleus muscle (a) and the extensor digitorum longus (b) from the guinea-pig. Measurements were made at three different concentrations (0·3, 1 and 3 mM, from the left to the right in each block of bars) and at various stimulus frenquencies. The means ± s.e. of 3 experiments are shown. Ordinate: % increase in the force of contraction.

DISCUSSION

Pentobarbitone increased the force of subtetanic contractions in both EDL and soleus. These effects were reversible and therefore an influence of any pentobarbitone remaining from the anaesthesia in our muscle preparations may be ruled out. In the presence of pentobarbitone (0·33 mM) the depressive effect of terbutaline on the soleus muscle contrac-

tions seemed to be diminished (Table 1). No such interaction was observed for the increase in force of contraction elicited by terbutaline on the EDL.

The use of subtetanic contractions to reveal changes in the contraction pattern of skeletal muscles as proposed by Bowman & Zaimis (1958) provides a most sensitive means of detecting effects of inter alia catecholamines on the soleus muscle (Bowman & Nott 1970). The magnitude of the effect recorded in this way is dependent on a variety of factors such as the resting tension, the stimulation frequency and the temperature. However, the optimal stimulation frequency for demonstrating the effect of sympathomimetic amines on the soleus muscle is always that on the steepest part of the tension-frequency curve (Bowman & Nott 1970). Under our experimental conditions this point is reached when the ratio between the force of the subtetanic contraction and the single twitch is about 2·5 : 1 (Fig. 2, cf. also Waldeck 1976).

In the present experiments pentobarbitone, in contrast to terbutaline, shifted the tension frequency curve for the soleus to the left by about 2 Hz. If the frequency of stimulation is optimized under normal conditions, i.e. in the absence of drugs, the response to terbutaline in the presence of pentobarbitone will then be underestimated. This is illustrated by the data presented in Table 1. Moreover, since the frequency of stimulation was optimized to detect a decrease in tension (the standard condition in our experiments with terbutaline), the effect of pentobarbitone on the soleus shown in Fig. 1 and Table 1 may have been underestimated.

Our experiments further show that if the frequency of stimulation is optimized in the presence of pentobarbitone, the maximum depression of the soleus muscle by terbutaline remains unchanged. Thus it seems unlikely that pentobarbitone may seriously interfere with in vivo measurement of sympathomimetic-induced effects in animals anaesthetized with this agent.

The tension frequency curve for EDL was less steep than that for the soleus and the choice of frequency for subtetanic stimulation does not seem to be critical (Fig. 3). Consequently pentobarbitone did not appreciably change the effect elicited by terbutaline (Table 1).

The experiments with α-chloralose yielded results similar to those with pentobarbitone. Thus there was a dose-dependent increase in the force of contraction in both muscles. This increase was dependent on the frequency of stimulation in the soleus but not in the EDL.

As yet knowledge about the mechanism underlying the changes in contraction pattern of skeletal muscles induced by barbiturates and by β-adrenoceptor agonists is incomplete. Changes in ionic conductance (Thesleff 1956; Foulks et al 1973) and in Ca^{2+} transport (Carsten & Mommaerts 1964; Dransfeld et al 1969) appear to be involved in the effects of barbiturates and a cAMP mediated effect on Ca^{2+}-uptake into the sarcoplasmic reticulum has been suggested for the effects elicited by β-adrenoceptor agonists (Bowman & Nott 1969; 1974). These facts have to be considered when planning experiments on skeletal muscles in anaesthetized animals notably in studies on mechanisms of action.

REFERENCES

Apperly, G. H., Levy, G. P. (1975) Br. J. Pharmacol. 54: 260–261 P

Bergman, J. Persson, H., Wetterlin, K. (1969) Experientia 25: 899

Bohmer, K., Raper, C. (1976) Arch. Int. Pharmacodyn. Ther. 221: 60–65

Bowman, W. C., Nott, M. W. (1969) Pharm. Rev. 21: 27–72

Bowman, W. C., Nott, M. W. (1970) Br. J. Pharmacol. 38: 37–49

Bowman, W. C., Nott, M. W. (1974) Clin. Exp. Pharmacol. Physiol. 1: 309–323

Bowman, W. C., Zaimis, E. J. (1958) J. Physiol. (London) 144: 92–107

Carsten, M. E., Mommaerts, W. F. H. M. (1964) J. Gen. Physiol. 48: 183–197

Dransfeld, H., Greeff, K., Schorn, A., Ting, B. T. (1969) Biochem. Pharmacol. 18: 1335–1345

Foulks, J. G., Perry, F. A., Sanders, H. D., Washio, H. (1973) Can. J. Physiol. Pharmacol. 51: 68–78

Gjone, E. (1956) Acta Pharmacol. Toxicol. 12: 1–10

Holmberg, E., Waldeck, B. (1977) Naunyn-Schmiedebergs Arch. Pharmacol. 301: 109–113

Olsson, O. A. T. (1974) Acta Pharmacol. Toxicol. 34: 106–114

Persson, H., Olsson, T. (1970) Acta Med. Scand. Suppl. 512: 11–19

Sirnes, T. B. (1954) Acta Pharmacol. Toxicol. 10: suppl. 1

Thesleff, S. (1956) Acta Physiol. Scand. 37: 335–349

Waldeck, B. (1976) J. Pharm. Pharmacol. 28: 434–436

Waldeck, B. (1977) Ibid. 29: 550–554