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Actions of some sympathomimetic bronchodilator and beta-adrenoceptor blocking drugs on contractions of the cat soleus muscle

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Summary

1. (−)-Isoprenaline, salbutamol, orciprenaline and quinterenol injected intravenously decreased the tension and degree of fusion of incomplete tetanic contractions of the soleus muscle of the anaesthetized cat.

2. Under the most sensitive conditions, the smallest effective dose of (−)-isoprenaline was of the order of 0·01 μg/kg intravenously. Salbutamol was usually 6–10 times, orciprenaline 20–30 times and quinterenol about 35 times less potent than isoprenaline. The effects of salbutamol were about 1·6 times, of orciprenaline about 1·8 times and of quinterenol more than 20 times as long lasting as those of (−)-isoprenaline.

3. The effects of the sympathomimetic amines were blocked by propranolol, H56/28, H35/25 and butoxamine but not by ICI 50172. The combined results with agonists and antagonists indicate that the receptors involved can be classified as of the β_2 type.

4. The effect of the amines on the cat soleus muscle appears to be analogous to that causing enhancement of physiological tremor in man, which suggests that skeletal muscle tremor may be an occasional unwanted side effect of the use of these bronchodilators.

Introduction

In contrast to their action on fast-contracting muscles, adrenaline and isoprenaline produce a decrease in the tension and in the duration of maximal twitches of slow-contracting mammalian skeletal muscles such as the cat soleus. As a result, the tension and degree of fusion of incomplete tetanic contractions of the muscle are markedly reduced (Bowman & Zaimis, 1955, 1958). This effect, which is mediated via β-adrenoceptors, is a consequence of a direct action on muscle contractility ; it is independent of concomitant vascular changes and probably arises through an increase in the rate of decline of the active state of the stimulated muscle (Bowman & Zaimis, 1958 ; Bowman, Goldberg & Raper, 1962 ; Bowman & Raper, 1962 ; Jurna & Rummel, 1962 ; for a review, see Bowman & Nott, 1969). The same effect is produced in the slow motor units present in human muscles (Marsden & Meadows, 1968). Bowman & Zaimis (1958) suggested that the ability of adrenaline to decrease fusion in slow motor units probably accounts for the enhancement of physiological tremor that is produced by adrenaline infusions (Barcroft & Swan, 1953 ; Marsden,

Foley, Owen & McAllister, 1967), and which occurs in patients with phaeochromocytoma (Pickering, 1955).

The effect on the cat soleus muscle is one of the most pronounced effects of β-receptor stimulants, so it was of interest to test the ability of some of the newer sympathomimetic bronchodilator drugs to produce this effect, and at the same time to study the effects of some β-adrenoceptor blocking drugs.

Methods

Adult cats of either sex were anaesthetized with a mixture of chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg) injected intravenously or intraperitoneally. The cat was laid prone on the operating table and a hind limb was rigidly clamped in a horizontal position by means of drills through the femur and the tibia and fibula. A skin incision was made from the level of the Achilles tendon to the popliteal space where the sciatic nerve was severed. The soleus muscle was separated from neighbouring muscles, the gastrocnemius-plantaris muscle was retracted, and small shielded bipolar stimulating electrodes were placed on the soleus branch of the nerve close to the muscle. The tendon of insertion of the soleus muscle was cut and attached to a Grass (model FT 10C) strain gauge, and the skin flaps were raised to form a deep pool which was filled with warm mineral oil (heavy liquid paraffin, B.P.). A small heating device was placed under the surface of the oil and muscle temperature was maintained at 36°–38° C. Muscle temperature and intraperitoneal temperature were recorded by means of small thermocouples (Grant Instruments, Cambridge). The soleus nerve was stimulated with rectangular pulses of 100 μs duration and of about twice the strength necessary to evoke a maximal muscle twitch. When incomplete tetanic contractions were elicited, two Tektronix stimulators were used, one to trigger the other, so that tetani of the required frequency and of 0·5 or 1 s duration were evoked automatically every 10 s. The strain gauge was coupled to a Beckmann (curvilinear) or Devices (rectilinear) twin channel recorder in order to obtain a continuous record, and to both beams of a Nihon Kohden VC 7A cathode ray oscilloscope. One beam of the oscilloscope was used to spread out the contractions so that their shapes could be observed in detail. The other was adjusted to high gain so that small changes in the applied resting tension on the muscle could be made and detected. Blood pressure was recorded from a common carotid artery by means of a Bell & Howell pressure transducer coupled to the other channel of the pen recorder. Drugs were injected intravenously through a cannula in a brachial vein. The trachea was cannulated but the cat was allowed to breathe spontaneously in all experiments.

The drugs used were (−)-isoprenaline bitartrate (Wyeth), orciprenaline base (Boehringer-Ingelheim), salbutamol (Allen & Hanbury), quinterenol dihydrochloride (Pfizer), (±)-propranolol hydrochloride (Imperial Chemical Industries), 4-(2-hydroxy-3-isopropylamino-propoxy) acetanilide (ICI 50172, Imperial Chemical Industries), butoxamine hydrochloride (Burroughs Wellcome), 1-(4′-methylphenyl)-2-isopropylamino-propanol hydrochloride (H35/25, Hässle), 1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol hydrochloride (H56/28, Aptin, Hässle), and methixene hydrochloride (Tremonil, Wander). Stock solutions were freshly prepared either in distilled water or in dilute HCl and were diluted with 0·9% w/v NaCl solution immediately before injection. The doses quoted refer to the bases.

Results

The effects of increasing the frequency of stimulation on twitches of the soleus muscle are qualitatively similar to the effects of catecholamines; both treatments decrease the twitch tension and, at the same time, increase the rate of relaxation (Bowman *et al.*, 1962). However, the effects of catecholamines, particularly on the rate of relaxation of the twitch, always exceeded the changes due to the negative staircase effect itself. Small doses of isoprenaline (below 0·25 μg/kg intravenously) often increased the rate of relaxation of the twitch without decreasing peak tension, and in a few cats out of hundreds studied over several years, peak twitch tension was unaffected by any dose, but rate of relaxation was always markedly increased.

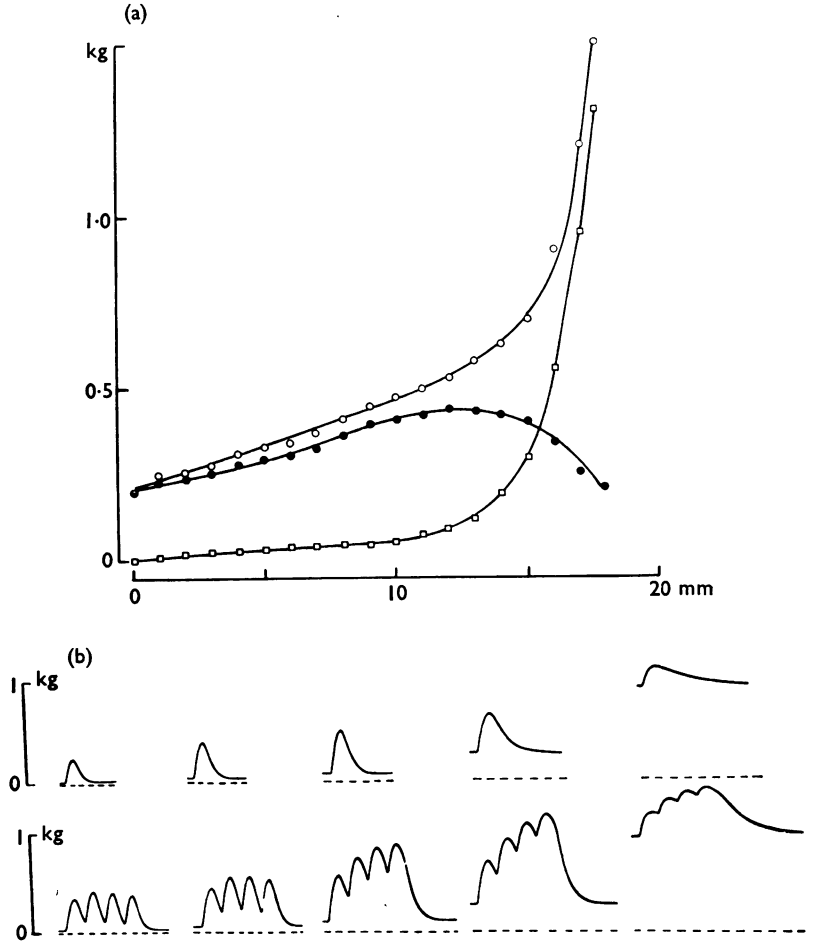

FIG. 1. a, Cat 3·2 kg. Tension-length curves for indirectly evoked maximal twitches of the soleus muscle, progressively lengthened from an arbitrary zero. —□—, shows the tension-length curve for the resting muscle; —○— shows that of the active muscle stimulated through its motor nerve once every min; and —●—, the difference between the other two curves, shows the twitch tension developed on stimulation. Note that the maximal evoked twitch tension was produced when the muscle was lengthened by 12 mm, which was equivalent to an applied tension of 86 g. b, Cat 3·3 kg. Representative twitches and incomplete tetanic contractions (8 Hz for 0·5 s) evoked at different resting tensions. Optimal evoked twitch tension occurred at a resting tension of 100 g (third response shown), but optimal evoked incomplete tetanic tension occurred at a resting tension of 250 g (fourth response shown).

When incomplete tetanic contractions of the soleus muscle are evoked instead of twitches, the more important effect of catecholamines is that on rate of relaxation. The decrease in fusion which results from the increase in the rate of relaxation of the unit of contraction gives rise to a pronounced reduction in overall tension, and such contractions provide the most sensitive means of detecting the effects of catecholamines. For this reason, in most of the present experiments, incomplete tetanic contractions of 1 s duration were evoked every 10 s by one of a range of stimulation frequencies. Under the most sensitive conditions, the smallest effective dose of isoprenaline necessary to depress incomplete tetanic contractions of the soleus muscle was found to be of the order of 0·01 μg/kg given as a single intravenous injection. Any dose big enough to affect the general arterial blood pressure produced a pronounced effect on the soleus muscle, and the effect on the soleus muscle always long out-lasted the effect on blood pressure, as illustrated in Figs. 4 and 6.

The optimal stimulation frequency for demonstrating the effect of catecholamines lies within the range 5–15 s (Bowman & Zaimis, 1958). In the present experiments, the precise optimal frequency within this range differed in different cats and in different experimental conditions, and to some extent was also dependent on the dose of catecholamine used.

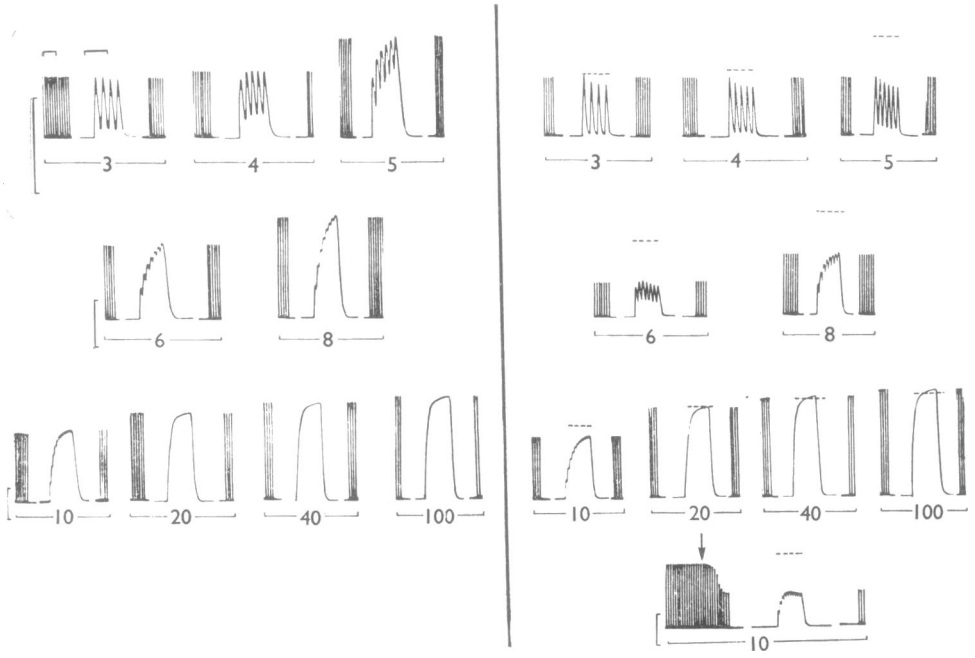

FIG. 2. Cat 2·2 kg. Effects of isoprenaline on contractions of the soleus muscle evoked at different frequencies. Control contractions are on the left of the vertical line; contractions evoked during intravenous infusion of isoprenaline are on the right. The number below each group denotes the frequency of stimulation used to evoke the contractions. Each group includes a contraction recorded on fast moving paper. The gain was reduced as the tension of the contractions increased. Tension calibrations on the left are all equivalent to 0·25 kg. Time calibrations at the top left are 1 min and 1 s for the slow and fast paper speeds respectively. The dotted lines above the contractions on the right show the tension of the control contractions before isoprenaline. The rate of infusion was 0·25 μg/kg per min except for the bottom contraction on the right, for which it was increased to 1 μg/kg per min at the arrow. The applied resting tension in this experiment was 150 g.

The type of clonic contractions most affected by and most sensitive to the action of catecholamines was that (for example, 5 or 6 Hz on the left of Fig. 2) in which a considerable degree of fusion was present and in which the second and subsequent stimuli of each train added further increments of tension to the first. Because of the negative staircase effect that occurs in the soleus muscle, low frequencies of stimulation may produce a contraction in which, although some degree of fusion is present, the successive units of tension within a single clonic contraction either do not exceed the first (for example, the first in Fig. 2), or are smaller than the first. The peak tension of such contractions does not differ from that of a twitch, and the effect of catecholamines on their tension does not therefore differ from that on twitch tension. The type of contraction that would be most affected by and most sensitive to the action of catecholamines could therefore be forecast from its appearance when spread out on the oscilloscope or on fast-moving paper, and in experiments to compare the relative potency of different drugs, the frequency of stimulation to be used was selected in this way. The optimal stimulation frequency always lay on the steepest part of the tension frequency curve and was, in fact, that frequency at which the smallest reduction in frequency caused the greatest reduction in tension (Fig. 3).

The frequency of stimulation necessary to produce fusion of responses is obviously lower, the longer the duration of the single twitch, and this differs to some extent in different animals under the same recording conditions, but also markedly in the same animal according to the resting tension applied to the muscle (Fulton, 1925,

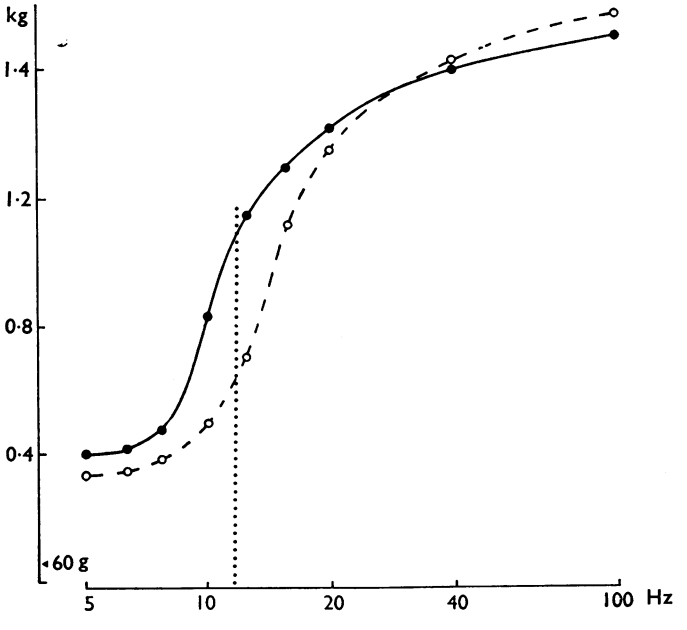

FIG. 3. Cat 2·3 kg. Tension/log frequency curve for a soleus muscle determined in an experiment similar to that illustrated in Fig. 2. The continuous line is the control curve; the broken line shows the changes in tension produced by a continuous intravenous infusion of isoprenaline (1 μg/kg per min). The vertical dotted line indicates the frequency of stimulation at which the contractions were most affected. Resting tension in this experiment was 60 g.

1926; Buller, Eccles & Eccles, 1960). Figure 1a illustrates tension-length curves for indirectly evoked maximal twitches of a soleus muscle. Evoked twitch tension was greatest at resting lengths equivalent to an applied tension of 60–100 g. In the experiment of Fig. 1a, the greatest twitch tension was evoked when the muscle length was increased by 12 mm from an arbitrary zero, and this was equivalent to a resting tension of 86 g. Twitch duration always continued to increase as the muscle was stretched beyond that giving the optimal twitch tension and up to the maximum lengthening applied in any experiment (equivalent to 1·25 kg). This is illustrated in the upper records of Fig. 1b. In this experiment, the optimal evoked twitch tension (the middle one shown) was produced at a resting tension of 100 g. Twitch duration was further prolonged, however, by increase in resting tension even when, as in the last twitch on the right, the applied resting tension was such that the evoked twitch tension was much reduced. The lower records of Fig. 1b show that as twitch duration was increased, so also was the degree of fusion of incomplete tetanic contractions. Consequently, the tension of such contractions continued to increase as the muscle was stretched beyond the point giving the optimal twitch tension. In Fig. 1b, optimal sub-tetanic tension was produced at a resting tension of 250 g (fourth contraction shown). Subsequently, the tension of incomplete tetanic contractions diminished when the degree of stretching was such that the pronounced fall off in the tension of the unit responses was sufficient to counteract the increase in fusion. This is illustrated by the last twitch and sub-tetanic contraction in

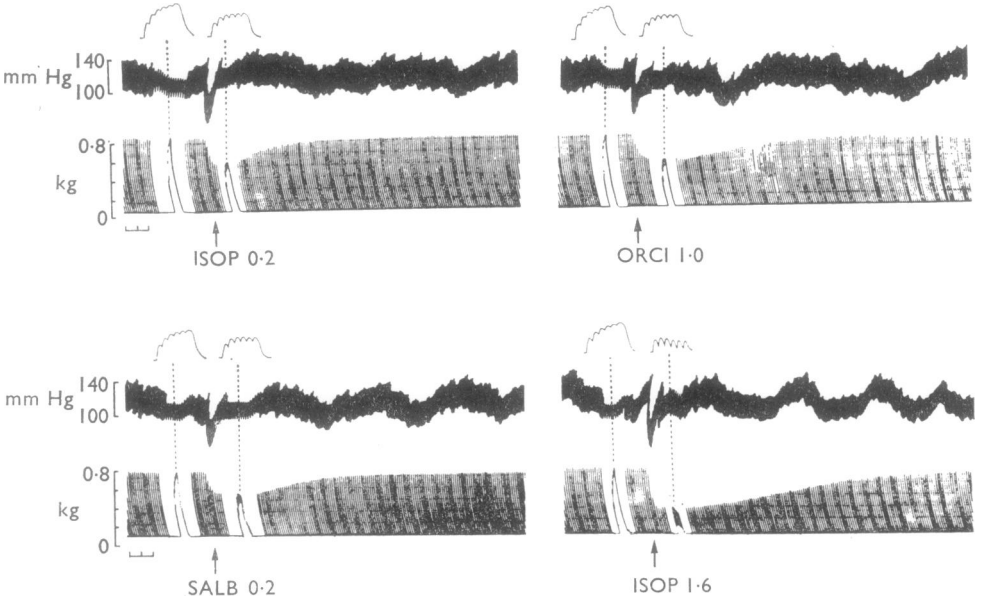

FIG. 4. Cat 2·2 kg. Part of an experiment to determine the relative potencies of isoprenaline (ISOP), orciprenaline (ORCI) and salbutamol (SALB). Contractions of a soleus muscle were evoked by stimulating the motor nerve at a frequency of 5 Hz for 1 s every 10 s. These were recorded simultaneously on a pen recorder and on a cathode ray oscilloscope. The pen recording includes two contractions recorded on fast moving paper in each panel, and the oscilloscope records of these two contractions are also shown connected by the dotted lines. The middle record in each panel is of general arterial blood pressure. The numbers denote the doses of the sympathomimetic amines in μg/kg given as single intravenous injections. This cat was unusually insensitive to isoprenaline. 30 min elapsed between doses. Time calibration for the slow paper speed is in min.

Fig. 1b. Increases in twitch duration are more important, the lower the frequency of stimulation used to produce the incomplete tetani. Consequently, as frequency of stimulation was increased the optimal resting tension diminished towards that giving optimal twitch tension.

Isoprenaline was similarly effective whatever the recording conditions used, the only difference being that the longer the duration of the twitch, the lower was the optimal stimulation frequency for demonstrating its effects on incomplete tetanic contractions.

Figure 2 illustrates parts of an experiment in which clonic or tetanic contractions of 1 s duration were evoked every 10 s at frequencies of stimulation ranging from 3 to 100 Hz. These patterns of stimulation were applied both before and during infusions of isoprenaline. With the relatively high resting tension applied (150 g), onset of fusion occurred at a frequency of 3 Hz and the maximum decrease in evoked tension (55%) produced by isoprenaline 0·25 μg/kg per min occurred with a stimulation frequency of 6 Hz. The lowest right part of Fig. 2 illustrates that the range of frequencies affected also depends to some extent on the dose of isoprenaline. When the rate of infusion was increased to 1 μg/kg per min, contractions evoked at a frequency of 10 Hz were further depressed. This concentration produced the maximal effect at all frequencies. Its effects at frequencies

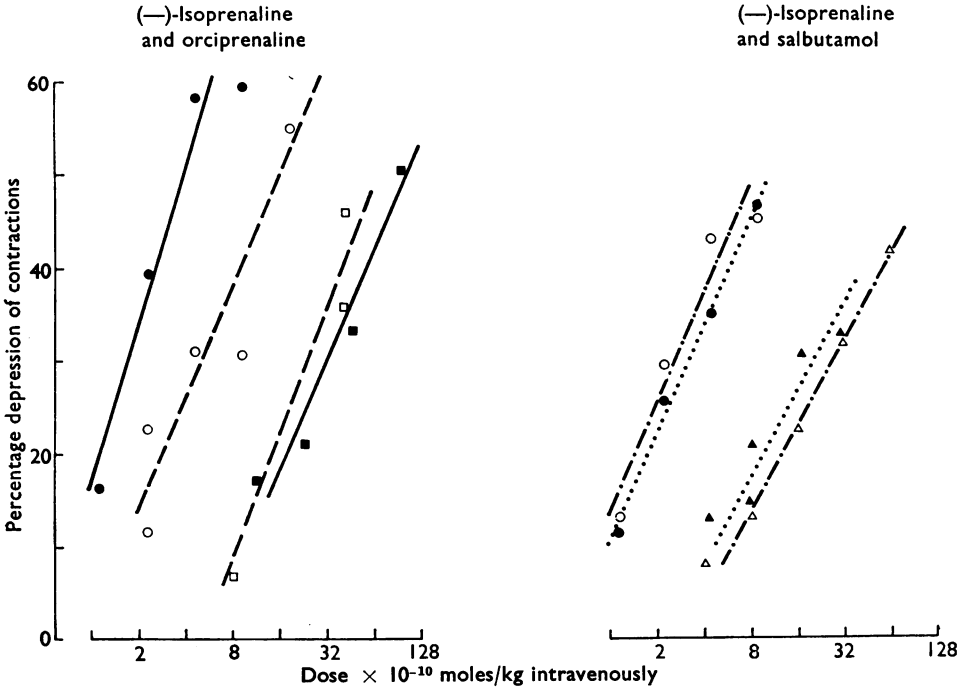

FIG. 5. Log dose/response lines from four experiments similar to that illustrated in Fig. 4 to compare the potencies of orciprenaline and salbutamol with that of (−)-isoprenaline in depressing incomplete tetanic contractions of soleus muscles. The ordinate is the percentage depression of the tension of contraction. The abscissae should be multiplied by 10^{-10} to give the doses in moles/kg. ● and ○, isoprenaline; ■ and □, orciprenaline; ▲ and △, salbutamol. The points for each different experiment are joined by a different type of line.

up to 5 Hz did not differ much from those of the lower dose, however, because this had already decreased fusion almost to the extent that the contractions had become a series of twitches.

Figure 3 is a graphical representation of the whole of a similar experiment and shows the effects of an infusion of isoprenaline (1 μg/kg per min) sufficiently large to produce the maximal depression at all affected frequencies. In this experiment the resting tension (60 g) was that giving the optimal twitch tension. Onset of fusion occurred at about 7 Hz and the maximal decrease in evoked tension (43%) produced by isoprenaline occurred with stimulation frequencies of about 12 c/s.

Isoprenaline no longer depressed the contractions in any dose at frequencies of stimulation above 20 to 30 Hz, depending on the recording conditions (Figs. 2 and 3). At frequencies above 40 Hz, tetanic tension was usually slightly increased (by 1–3%) as illustrated in Figs. 2 and 3. This trivial increase in tetanic tension was not studied further. It may have been a consequence of the vasodilator action of isoprenaline and improved muscle blood flow, or of an action on fast-contracting fibres within the muscle that had become fatigued as a result of the repeated stimulation.

In experiments to compare the relative potencies of the catecholamines, the applied resting tension was always adjusted to lie within the range 30–100 g and the optimal stimulation frequency was selected by inspection of the records before drug administration. This frequency was applied for 1 s every 10 s and this pattern of stimulation was maintained throughout the experiment. Three or four different doses of isoprenaline and of salbutamol or of orciprenaline were then injected alternately at intervals of 30 min, and the percentage depression of the peak tension produced was measured. Salbutamol and orciprenaline were each studied in four cats, isoprenaline being used for comparison in all of them, and at the end of each experiment a single dose of the remaining compound was injected. With these three drugs, onset of action occurred in 20 to 40 s and reached its peak in 100 to 200 s after injection. Figure 4 illustrates part of an experiment to compare the potency of isoprenaline with that of orciprenaline and salbutamol, and Fig. 5 shows log dose/response lines from four other experiments. The rank order of potency was always isoprenaline>salbutamol>orciprenaline. In seven experiments in which relative potencies were determined, the equivalent doses on a molar basis, giving isoprenaline the value of 1, were in the ratios: salbutamol 6–10, and orciprenaline 20–30. In one further experiment, illustrated in Fig. 4, the cat was less sensitive than usual to isoprenaline but not to salbutamol or orciprenaline. In this experiment salbutamol was almost equipotent with isoprenaline, while orciprenaline was about 4 times less potent on a molar basis.

Quinterenol differed from the other compounds in its slow onset of action (4–5 min after injection), in its slow development of effect (10–15 min to the maximum effect) and in its very prolonged duration of action (Fig. 6b). A similar slow onset and prolonged duration has been described for its action on the bronchi (Scriabine, Moore, Iorio, Goldman, McShane & Booker, 1968). Because of pronounced cumulative effects it could not be compared with isoprenaline at more than one dose level. Three different doses of isoprenaline were injected at intervals of 30 min and these were followed, 30 min later, by a single submaximal dose of quinterenol, the effects of which outlasted the experiment. On this basis, in three experiments, quinterenol was approximately 35 times less potent than isoprenaline on a molar basis.

The duration of effect of isoprenaline from injection to full recovery after a dose just big enough to produce the maximal depression (0·5–1 μg/kg intravenously) was 15–20 min. When comparing the relative durations of action of the compounds, a dose of each that gave the same submaximal decrease in tension was injected, the recording being made on very slowly moving paper. Figure 6a illustrates part of an experiment to compare isoprenaline, orciprenaline and salbutamol in this way.

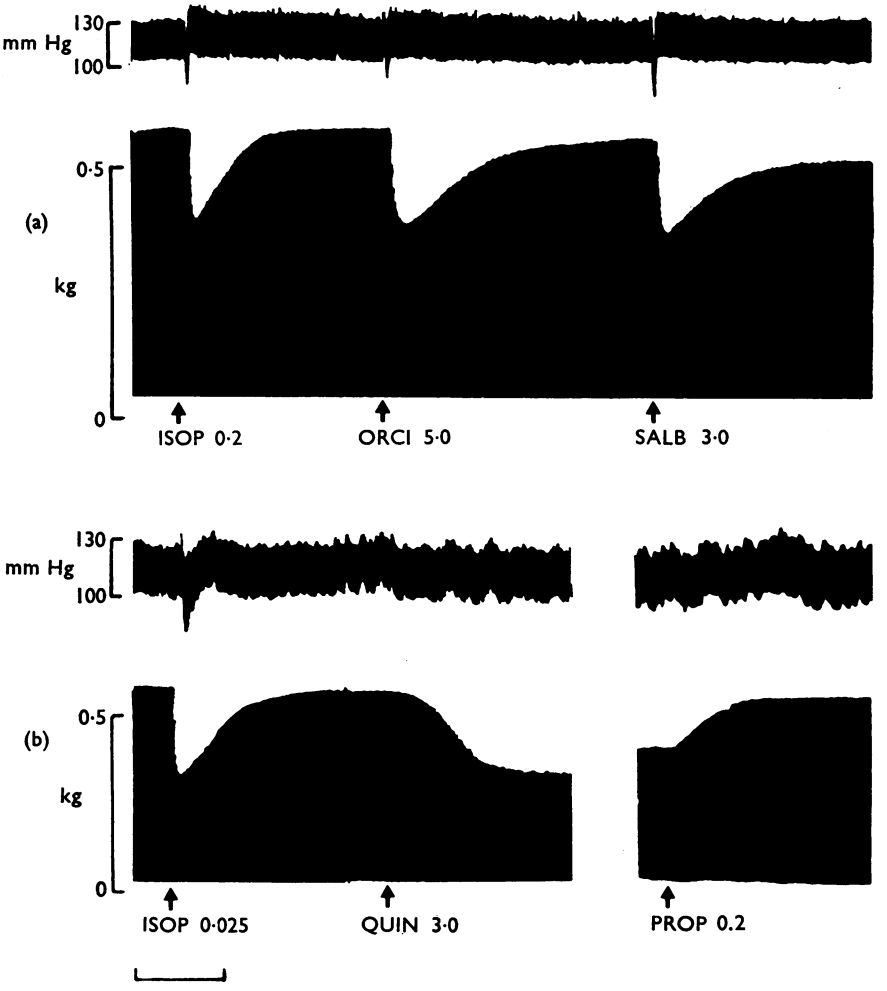

FIG. 6. a, Cat 3·0 kg. Part of an experiment to compare the durations of action of isoprenaline (ISOP), orciprenaline (ORCI) and salbutamol (SALB). The experimental arrangement was similar to that for the experiment illustrated in Fig. 4 except that no oscilloscope records are included, and a very slow paper speed was used throughout. In this experiment contractions were evoked at a frequency of 10 Hz for 1 s every 10 s, the paper speed being too slow to separate them. The numbers denote the doses of the sympathomimetic amines in μg/kg given as single intravenous injections. These doses produced equivalent submaximal depressions of contractions. b, Cat 2·1 kg. Part of an experiment similar to that in (a). At ISOP, isoprenaline 0·025 μg/kg; at QUIN, quinterenol 3·0 μg/kg, and at PROP, propranolol 0·2 mg/kg. The gap in the response to quinterenol corresponds to 120 min. Note that very little recovery from its depressant effect had occurred during this time. Propranolol then restored the contractions to the control level. Time calibration (bottom left) for a and b, 10 min.

Duration of action was measured as the time from onset to half recovery. On this basis, equivalent doses of salbutamol, orciprenaline and quinterenol produced, respectively, effects about 1·6 times, about 1·8 times, and more than 20 times as long lasting as those produced by isoprenaline.

Propranolol or H56/28 in doses of 0·2 mg/kg and higher completely prevented the effects of isoprenaline, orciprenaline and salbutamol subsequently injected in doses at least four times as big as those previously necessary to produce the maximal depression. The same dose of propranolol injected during the prolonged effect of quinterenol restored the contractions to normal (Fig. 6b). Butoxamine was somewhat variable in its effectiveness, but in doses ranging from 2·5 to 8 mg/kg in different experiments it blocked the effects of isoprenaline. The larger doses of butoxamine slightly augmented the control contractions. H35/25, in doses of 5 mg/kg, itself produced an effect like that of isoprenaline except that at this dose level it was less potent than isoprenaline 0·25 μg/kg. During this effect of H35/25, isoprenaline (0·25 μg/kg) did not produce any further depression, and its action was still much reduced when injected after the depression produced by H35/25 had worn off. The blocking action of H35/25 was relatively weak, however, because slightly larger doses of isoprenaline (0·5–1·0 μg/kg) were effective in its presence.

ICI 50172 in doses lower than 8 mg/kg was without effect by itself and did not antagonize the effect of isoprenaline. In doses of 8 mg/kg, ICI 50172 only slightly reduced the isoprenaline effect.

Methixene in doses up to 2 mg/kg was without effect by itself and did not modify the action of isoprenaline.

Antagonists effective against isoprenaline-induced effects on the soleus muscle also blocked the vasodepressor action, and those that did not block the effects on the soleus also left the effect on blood pressure unchanged.

Discussion

On the basis of the relative specificity of different agonists and antagonists, it has been proposed that there are two types of β-adrenoceptor (Lands & Brown, 1964; Arnold, McAuliff, Luduena, Brown & Lands, 1966; Lands, Arnold, McAuliff, Luduena & Brown, 1967; Lands, Luduena & Buzzo, 1967; Arnold & Selberis, 1968). These have been designated β_1 which regulate effects on the heart, lipolysis and intestine, and β_2 which regulate bronchodilatation, vasodilatation, effects on the uterus and muscle glycogenolysis. Lands, Arnold, McAuliff, Luduena & Brown (1967) tested the effects of a series of sympathomimetic amines for their ability to increase the twitches of the rat diaphragm depressed by excess KCl. On this basis they were able to designate the adrenoceptors involved as of the β_2 type.

Isoprenaline and the antagonists, propranolol and H56/28, are non-specific in their reaction with β-adrenoceptors (Åblad, Brogård & Ek, 1967; Dunlop & Shanks, 1968) and, as expected, these antagonists prevented the effects of the agonists on the soleus muscle. However, salbutamol (Cullum, Farmer, Jack & Levy, 1969) and orciprenaline (Engelhardt, Hoefke & Wick, 1961) and the antagonists butoxamine (Levy, 1966; Burns, Salvador & Lemberger, 1967) and H35/25 (Levy & Wilkenfeld, 1969), in so far as they have been tested, show more specificity for tissues said to possess β_2 adrenoceptors. In doses comparable with those found effective in other β_2 tissues by other workers, these agonists depressed the soleus contractions, and the

antagonists prevented this effect. In contrast, the antagonist ICI 50172, which shows affinity for β-adrenoceptors in the heart and in adipose tissue (β_1-adrenoceptors) but little affinity for receptors in blood vessels or the trachea (β_2-adrenoceptors) (Barrett, Crowther, Dunlop, Shanks & Smith, 1968 ; Dunlop & Shanks, 1968), had little effect in blocking the action of isoprenaline on the soleus muscle. These results therefore indicate that the soleus muscle, like the rat diaphragm, can be grouped with those tissues said to possess β_2-adrenoceptors. A further characteristic of such tissues is that noradrenaline has relatively less affinity for their β-adrenoceptors than for those in the other group. The soleus muscle is 20–50 times less sensitive to noradrenaline than to adrenaline or isoprenaline (Bowman & Zaimis, 1958 ; Bowman & Raper, 1967), and this also accords with its belonging to the group of tissues said to contain β_2-adrenoceptors. In unpublished experiments with sulbutamol and orciprenaline, Brittain and his coworkers (personal communication) have reached a similar conclusion.

Quinterenol directly stimulates β-adrenoceptors in the airways as judged by its effectiveness both *in vivo* and *in vitro*. Whereas it affects the heart *in vivo*, however, it has no action on isolated hearts, suggesting that its actions on cardiac receptors (β_1) may be indirect and possibly due to a metabolite (Scriabine *et al.*, 1968). The present experiments gave no information as to whether the action on the soleus muscle was direct or indirect, but the soleus muscle seems to match the bronchi in its sensitivity, and the action of quinterenol on the soleus muscle is probably direct.

Although the compound H35/25 blocked the action of isoprenaline on the soleus muscle, it was relatively weak in this effect, the block being easily overcome by a small increase in the dose of isoprenaline. This observation is similar to that of Parratt & Wadsworth (personal communication), who found H35/25 to be only weakly active in blocking the effects of isoprenaline on cat blood pressure ; the blood vessels are also among the tissues said to contain β_2-adrenoceptors.

It seems likely that the effect of sympathomimetics in decreasing fusion in the slow-contracting slow motor units of human muscles is responsible for the tremor produced by these drugs. The range of frequencies of stimulation of the cat soleus muscle that are most sensitive to this action are within the physiological range of frequencies for this muscle (Denny-Brown, 1929), and the cat soleus may therefore provide a useful test for forecasting the possibility of tremor being produced in man.

(−)-Isoprenaline is about twice as potent as the racemic compound in its action in the cat soleus muscle (Bowman & Raper, 1967), and taking into account that the laevo isomer of isoprenaline was used in the present experiments, the effective doses and relative potencies of the sympathomimetic amines on the soleus muscle by intravenous injection were similar to those published by other workers in relation to their actions on the bronchi (Cullum *et al.*, 1969). The synthesis of potent sympathomimetic bronchodilators with relatively little action on the heart is a step forward in the therapy of asthma, but since the bronchi and slow-contracting skeletal muscle fibres apparently possess the same type of β-adrenoceptors it may prove impossible, other than by route of administration, to separate the actions on these tissues, and the systemic administration of sympathomimetic bronchodilators may, therefore, carry with it the possibility of skeletal muscle tremor as an unwanted side effect.

Propranolol prevents the increase in Parkinsonian tremor produced by adrenaline (Marsden *et al.*, 1967) and in a controlled clinical trial, oral propranolol relieved spontaneous tremor in a proportion of Parkinsonian patients (Owen & Marsden,

1965). Methixene is an anti-Parkinson drug which, according to some authors, is especially active against tremor (Hartmann-Von Monakow, 1960a, b ; Birkmayer & Danielczyk, 1960 ; Steinbrecher, 1961 ; Sharkey, 1964 ; Clarke, Hay & Vas, 1966), although others have been unable to substantiate this claim (Ekdawi & Fowke, 1966 ; Norris & Vas, 1967). It was included in the present study because of mistaken reports in the literature that it possesses β-adrenoceptor blocking activity. In doses up to 2 mg/kg, which are in excess of those effective against tremor in man (Clarke *et al.*, 1966), methixene was without effect on the soleus muscle or on its response to isoprenaline. Nor did it modify the vasodepressor action of isoprenaline. It is therefore concluded that whatever the mechanism of the anti-tremor action of methixene in Parkinsonism, it does not involve peripheral β-adrenoceptor blocking activity.

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