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THE EFFECT OF COMPOUND 48/80 ON MAMMALIAN SKELETAL MUSCLE

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Compound 48/80 was introduced by Baltzly, de Beer, Buck, and Webb (1949) as a potent long-acting vasodepressor substance. Feldberg and Paton (1951) and Paton (1951) established that most of the symptoms occurring after the injection of 48/80 into animals can be attributed to histamine which is liberated by this drug in large amounts. On the other hand, Dews, Wnuck, Fanelli, Light, Tornaben, Norton, Ellis, and de Beer (1953) reported that although the response of the isolated frog gastrocnemius muscle to acetylcholine decreased in the presence of 48/80 they did not observe a similar inhibition after applying histamine. Furthermore, during their study of the restoration of the histamine-content of tissues Feldberg and Talesnik (1953) treated rats with increasing amounts of 48/80 until the histamine-store of these animals was largely depleted; these rats still exhibited some muscular weakness following an injection of 48/80 at a time when symptoms of an "anaphylactoid" reaction no longer occurred. Recently Gertner (1955) demonstrated a ganglion-blocking action of this drug.

The present series of experiments was designed to investigate the effect of compound 48/80 upon the function of mammalian skeletal muscle. Most of the *in vivo* experiments were done on rabbits, for their vascular system is relatively resistant to this drug (Dews *et al.*, 1953). Moreover, it was reported by Schachter (1953) that 48/80 is not very effective in releasing histamine in rabbits.

METHODS

Experiments in vivo.—Rabbits (2.0–3.5 kg. body weight) were anaesthetized with urethane (0.6 g./kg. i.v.+0.7 g./kg. i.p.) supplemented if necessary with small amounts of ether during the operation. The sciatic nerve was dissected in the thigh and crushed or severed. The calcaneal tendon was sectioned at its attachment to the heel and the movements of the foot—representing the contractions of the tibial muscle—were recorded on a kymograph. The jugular vein, trachea, and carotid artery were cannulated to permit intravenous injections, the application of artificial respiration, and the registration of the arterial blood pressure with a mercury manometer.

Rats of 200–300 g. body weight were anaesthetized with sodium pentobarbitone (50 mg./kg. i.p.). The right gastrocnemius muscle was freed from the surrounding muscles and the calcaneal tendon was severed and connected with the writing lever. The sciatic nerve was exposed and cut. A cannula was inserted into the left iliac artery pointing towards the bifurcation of the aorta. The pressure in the carotid artery was recorded by means of a double membrane tambour carrying a lever.

In both rabbits and rats the sciatic nerve was stimulated through paired electrodes with single maximal square-wave impulses of 3 or 5 msec. duration at a frequency of 12/min. For direct muscular stimulation a single electrode was fixed on the muscle, and a copper plate between the abdominal wall and the skin served as an indifferent electrode. Contractions were recorded isotonically.

Isolated Phrenic Nerve-Diaphragm Preparation.—This was prepared as described by Bülbring (1946); the rats weighed 120–160 g. The 50 ml. bath was filled with Tyrode solution containing 0.2% glucose and 0.1% $NaHCO_3$. Fresh, warm Tyrode solution was supplied from the bottom; excess fluid was removed by a bent glass tube connected with a suction pump. When the fluid had to be changed, the bath was rinsed first with about 200 ml. Tyrode solution and then (after 2, 4, and 6 min.) with about 100 ml. The temperature was kept at 37° C. Oxygen with 5% CO_2 was used to aerate the bath. A spring-loaded lever recorded the contractions. Maximal electric impulses of 1 or 2 msec. duration were applied to the nerve at a rate of 6 or 12/min.; pulses of 5 or 10 msec. were necessary for direct stimulation.

All drugs were dissolved in or diluted with 0.9% NaCl in water. Compound 48/80 was used as the base, histamine as the hydrochloride, physostigmine as the sulphate, neostigmine as the methyl sulphate, mepyramine, acetylcholine, and (+)-tubocurarine as the chloride. When histamine hydrochloride was tested on an isolated diaphragm an equivalent amount of NaOH was added to the Tyrode solution to prevent *p*H changes.

RESULTS

Rabbits.—The contractions of the indirectly stimulated tibial muscle rapidly decreased after an intravenous injection of 48/80 (Fig. 1). A first dose of 3.3–5.0 mg. caused a depression of 50–95% which began almost immediately and was most profound 5–10 min. after the injection ; the muscle usually recovered in 30–70 min. A similar dose given after full recovery invariably had a much greater effect, sometimes causing complete paralysis for more than an hour, followed by a very slow recovery. After injection of 48/80 respiration became laborious, then shallow, and eventually stopped in 1–3 min.; apart from a slight transitory fall, the blood pressure was not altered by these doses.

Subsequently it was found that direct stimulation was still effective when indirect excitability was completely suppressed by 48/80 (Fig. 2). Furthermore, in animals completely paralysed by tubocurarine the contractions of the directly stimulated tibial muscle remained virtually constant for at least 10 min. after intravenous injection of 5, 10, or 20 mg. 48/80.

Histamine hydrochloride—up to 10 mg. intravenously—did not produce a paralysis like that after 48/80. Small amounts—up to about 0.5 mg. —had no visible influence. Larger doses—0.5 to 10 mg.—caused a severe drop in blood pressure and sometimes a depression of the tibial contractions ; but in contrast to the rather abrupt action of 48/80 the depression by massive amounts of histamine developed gradually. Indeed, in some experiments the maximal twitches gradually increased until the animal died (Fig. 3).

Although the intravenous injection of histamine did not produce an effect similar to that of 48/80, it was of interest to see whether antihistaminic drugs could prevent the action of the latter. This appeared not to be so, for after intravenous injection of 10 mg./kg. mepyramine hydrochloride 48/80 still paralysed the tibial muscle.

We next attempted to determine whether 48/80 acted by competition with acetylcholine or by depolarization. Some characteristics of the paralysis from 48/80 suggested a relation to that from tubocurarine—the inhibition of the single twitches was never preceded by a potentiation or by a spontaneous fasciculation ; the muscle did not maintain a tetanus with 40 pulses/sec. for 5 sec. applied to the sciatic nerve ; after such tetanizing stimulation the twitches were slightly larger than before (although this effect was not always so obvious as with tubocurarine) ; if tubocurarine was administered between two injections of 48/80, the second dose of 48/80 had a greater effect than the first. In many respects, therefore, 48/80 fulfils the requirements for true curariform action (Zaimis, 1954). Anticholinesterases, however, failed to antagonize the effect of 48/80. Physostigmine (1–2 mg.) or neostigmine (0.25–1.0 mg.), administered during partial or total paralysis caused by 48/80, did not significantly alleviate its effect. In control experiments identical doses of the two drugs readily relieved a comparable neuromuscular block by tubocurarine.

Rats.—In rats an intravascular injection of even small doses of 48/80 caused profound shock and so made it difficult to analyse the primary changes brought about by the drug itself in the muscle. Nevertheless, if rather large amounts were injected intra-arterially, an abrupt paralysis of the indirectly stimulated gastrocnemius muscle clearly preceded the fall of the arterial pressure (Fig. 4). In these experiments the impairment of muscular function obviously could not be secondary to circulatory failure ; it should be mentioned, however, that the paralysis did not always develop with equal rapidity. In a small series of six experiments, in which the doses varied from 1.0 to 3.0 mg., the degree of inhibition in the first minute ranged from 10% to 90%. These amounts were invariably lethal, death ensuing in 15–30 min.

Denervated Rat Muscle.—The right sciatic nerve of six rats was sectioned under ether anaesthesia and 10–13 days later we tested the sensitivity of the denervated gastrocnemius muscle to compound 48/80. The tone of the muscle did not change after intra-arterial injection of 0.1–6.0 mg. 48/80. If the muscle was stimulated directly when 48/80 was applied, the contractions remained constant for 90–120 sec., whereafter they decreased slowly —probably because of circulatory shock. As a control, acetylcholine (4–10 μg.) was injected repeatedly before 48/80 in three of the six rats ; the characteristic contracture of chronically denervated muscle was obtained.

Isolated Phrenic Nerve-Diaphragm Preparation. —Addition of 3–5 mg. 48/80 to the bath during regular stimulation of the phrenic nerve was followed by a gradual decrease of the contractions of the diaphragm. If the drug was washed out after a few minutes and a similar dose added when the muscle had recovered, the depression was more marked than at the first exposure. A dose that had scarcely any effect on a fresh diaphragm caused a rapid loss of contractility when applied for the third time (Fig. 5).

If 4 mg. or more of 48/80 was allowed to act upon a preparation for a prolonged period the

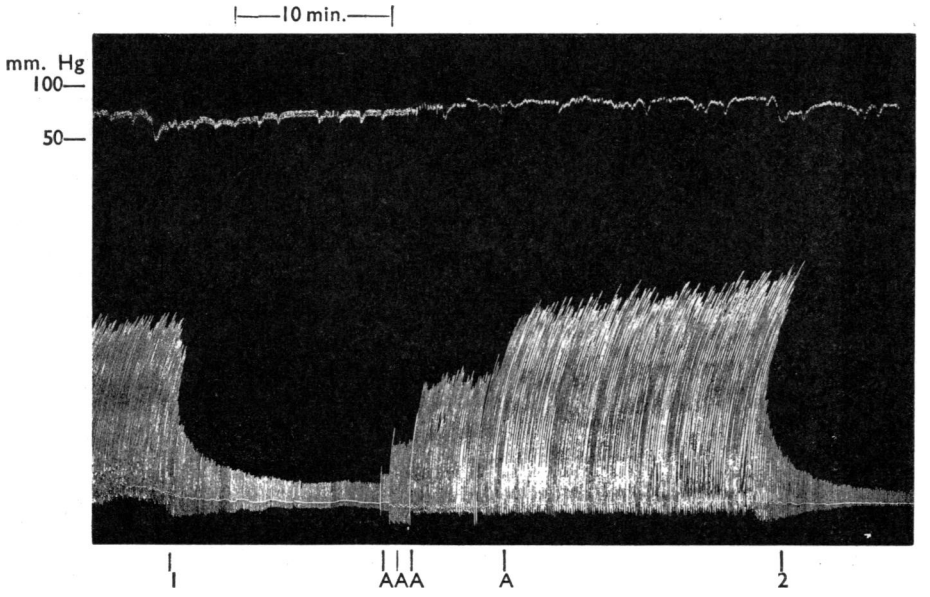

FIG. 1.—Rabbit, 2·2 kg. Upper tracing, pressure in the carotid artery; lower tracing, contractions of the tibial muscle. The sciatic nerve was stimulated with a frequency of 12 min. At 1 and 2 compound 48/80 was injected i.v. (5 and 2·5 mg., respectively). At A the kymograph was stopped. Total time between the two injections, 70 min.

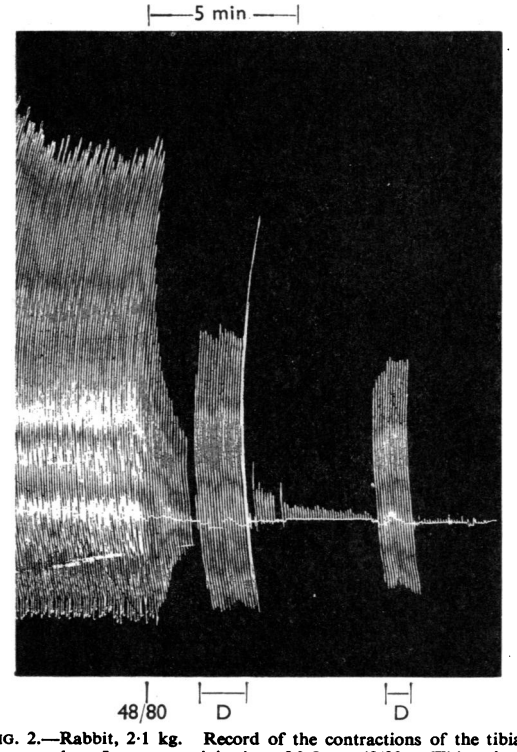

FIG. 2.—Rabbit, 2·1 kg. Record of the contractions of the tibial muscle. Intravenous injection of 3·5 mg. 48/80. (This animal had already been injected with 5 mg. about 80 min. earlier.) D, direct stimulation of the muscle.

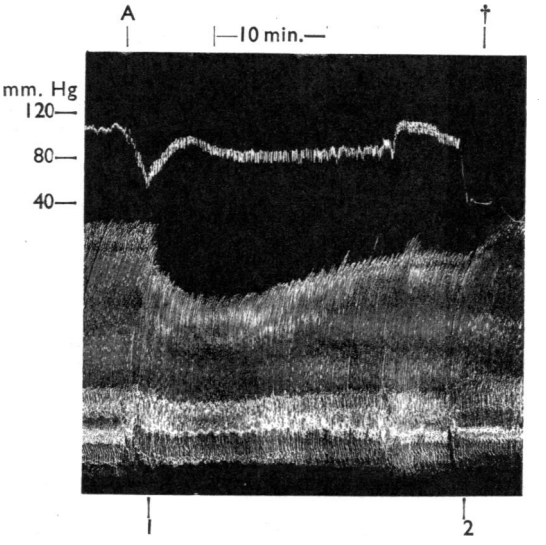

FIG. 3.—Rabbit, 3·3 kg. Records of blood pressure and contractions of the tibial muscle. 1, 4 mg. 48/80 i.v. 2, 0·5 mg. histamine-hydrochloride i.v. A, artificial respiration begins. †, death.

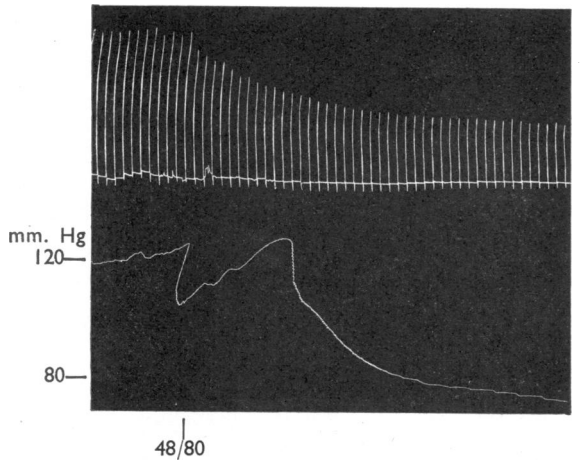

FIG. 4.—Rat 280 g. Upper tracing, contractions of the gastrocnemius muscle, frequency 12/min. Lower tracing, blood pressure. Intra-arterial injection of 3 mg. 48/80. The slight fall of the blood pressure at the moment of the injection was probably caused by traction on the iliac artery.

muscle eventually became refractory to nervous stimuli. The time necessary for such a complete inhibition varied from 3 to 30 min. and seemed to depend not only on the concentration of the drug but even more on the number of previous exposures. During a complete paralysis to indirect stimulation vigorous contractions could be obtained by direct stimulation, but, in contrast to the experiments on rabbits, these contractions tended to decrease if 48/80 was present in the bath for 5–15 min., especially when larger amounts (6–10 mg.) were used.

When 48/80 (4 mg.) was administered together with tubocurarine (40 μg.), the paralysis was more pronounced than with either drug alone.

Neostigmine neither prevented nor relieved the effect of 48/80. Fig. 6 illustrates the difference in the effect of neostigmine after tubocurarine and after 48/80. In three other experiments 2.5 μg. neostigmine was added 3 min. before 5 mg. 48/80, or 40 μg. neostigmine simultaneously with 4 mg. 48/80; after a further 3 min. the drugs were washed out and the muscle permitted to recover. Following this the same amount of 48/80 alone was added to the bath and kept there for 3 min.; then again the same dose of 48/80 together with neostigmine. In these tests too neostigmine failed to antagonize 48/80.

Histamine hydrochloride, in doses up to 20 mg. on three different preparations, did not have any effect upon the amplitude of the contractions of the diaphragm, stimulated by the phrenic nerve.

DISCUSSION

Our results seem to indicate that 48/80 in suitable doses causes a marked peripheral paralysis in rabbits and, under certain conditions, also in rats. The site of this action cannot be the muscle itself, because the contractions induced by direct stimulation are not affected to an appreciable extent at the concentrations used.

It may be assumed, for at least three reasons, that 48/80 itself and not liberated histamine is responsible for this effect: 1, administration of

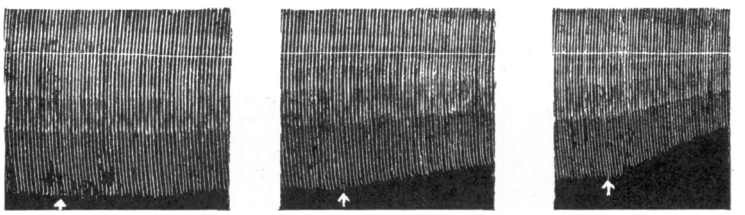

FIG. 5.—Isolated rat phrenic nerve-diaphragm preparation. Bath volume, 50 ml. Rate of stimulation, 12/min. Records taken from the same preparation. At each arrow administration of 2 mg. 48/80. Between the applications the drug has been washed out thoroughly.

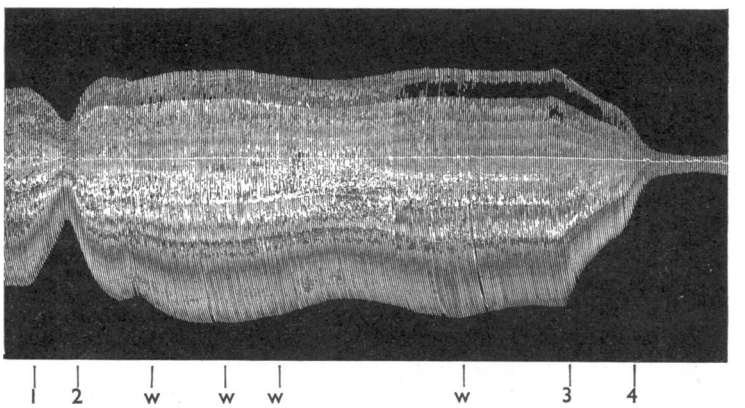

FIG. 6.—Isolated rat phrenic nerve-diaphragm preparation. Bath volume 50 ml.; frequency of stimulation 12/min. Contractions downward. 1, addition of 0·2 mg. tubocurarine to the bath. 2, 0·175 mg. neostigmine. 3, 5·0 mg. 48/80. 4, 0·175 mg. neostigmine. W, fresh Tyrode solution.

histamine failed to cause a similar paralysis in rabbits and isolated rat diaphragms; 2, if 48/80 was given repeatedly to the same rabbit or isolated rat diaphragm the depression became more profound with each subsequent administration, whereas the amount of histamine that is liberated diminishes with repeated exposure (Feldberg and Paton, 1951; Mongar and Schild, 1952); 3, high doses of mepyramine did not counteract the effect of 48/80 in rabbits. It is interesting in this respect that Wastl (1928) found fatigue of the artificially stimulated nerve-muscle preparation of a cat after the injection of histamine, an effect which she thought to be directly due to the drug. Eppinger, László and Schürmeyer (1928), who performed similar experiments on dogs, supposed that the decrease of the muscular contractions in histamine and peptone shock was due to decreased blood flow through the muscle.

Concerning the mechanism that is involved in the muscular paralysis caused by 48/80, no satisfactory explanation has yet been found. When tested in rabbits, some of its properties suggested a relation with tubocurarine; moreover, addition of the effects of 48/80 and tubocurarine was observed upon the isolated rat diaphragm. However, the inability of the cholinesterase inhibitors, physostigmine and neostigmine, to antagonize the paralysis by 48/80 is a strong argument against the view that the latter acts by competition with acetylcholine. On the other hand, the lack of stimulating effect upon denervated rat muscle makes it very improbable that 48/80 could have a depolarizing action.

A third known mechanism by which neuromuscular conduction can be blocked is inhibition of acetylcholine release at the motor nerve terminals. Gertner (1955) in his study of the effect of 48/80 on autonomic ganglia showed the release of acetylcholine to be unimpaired. It was therefore considered unlikely that the blocking effect of 48/80 on skeletal muscle would be due to inhibition of the release of acetylcholine at the motor endplate, although of course this drug might affect different tissues in different ways. Although our experiments did not exclude the possibility of axonal block by 48/80, we consider this rather improbable.

Most substances which block the conduction of impulses at the neuromuscular junction contain one or more quaternary or tertiary amino groups. An exception is mephenesin, which contains no nitrogen atom at all and yet exhibits some neuromuscular-blocking activity besides its action on the internuncial neurones (Berger, 1947). Compound 48/80 is a condensation product of *p*-methoxyphenylmethylamine with formaldehyde (Baltzly *et al.*, 1949). Being a secondary amine, it differs chemically from most other neuromuscular-blocking agents, and the question arises whether its action too is fundamentally different from the three main classes of peripheral paralysing drugs.

Summary

1. 48/80 causes a marked inhibition of the contractions of indirectly stimulated skeletal muscle in the rabbit. The direct excitability of the muscle is not affected.

2. Isolated rat diaphragms are depressed by 48/80 if stimulation is indirect, but not if it is direct.

3. Evidence is presented that the paralysis caused by 48/80 is due to the action of the drug itself and not to the liberation of histamine.

4. The results indicate that 48/80 does not act either by competition with acetylcholine or by depolarization at the motor endplate.

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