CONTRACTILE RESPONSE TO SYMPATHOMIMETIC AMINES IN ISOLATED RAT MUSCLES AFTER CHRONIC DENERVATION

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It was demonstrated by Bowman et al (1) that an intravenous injection of epinephrine (EPI) caused an increase or a decrease in the tone of the chronically denervated cat muscles and the effects were mediated by the β-receptor. In our previous study in vivo (2), denervated rat muscles responded to EPI by a contracture mainly through the α-receptor, while rabbit muscles responded by a fall in the resting tension through both α- and β-receptors. The reason for the discrepancy is not clear, though it may in part be due to species difference. In the present paper, effects of EPI and other adrenergic amines were further studied in vitro using chronically denervated rat muscles.

The sciatic nerve of male rats (Sprague-Dawly, 4-5 weeks of age) was sectioned under pentobarbital-Na anesthesia. The extensor digitorum longus muscle and the soleus muscle were isolated 10-21 days after the operation and were suspended in a 10 ml bath of mammalian Ringer's solution according to Isaacson et al (3), which was aerated with a mixture of 95% O₂ and 5% CO₂ and kept at 37±1°C (pH 7.5). In order to examine twitch response, field stimulation was applied to the muscle by platinum electrodes with the rectangular pulses of supramaximal voltage and 5 msec duration at 0.1 Hz frequency. The initial tension of the muscles was maintained within the range of 0.1-0.3 g. Semi-isometric responses of twitch and resting tension were recorded on an ink-writing oscillograph through a strain gauge. Drugs were added to the bath in 0.1 ml volume to make the final required concentrations. EPI, norepinephrine (NOR) and trimetoquinol (TMQ) were used as sympathomimetics. TMQ is known to possess more potent β-stimulating activity than isoproterenol in the tracheal muscle of guinea-pigs (4, 5), and the drugs also showed only a β-action in the skeletal muscles of rats and rabbits (2, 6), while isoproterenol had a weak α-action besides β-action (6).

EPI in a concentration of 10⁻⁷ g/ml caused an increase in the resting tension (contracture) of the chronically denervated extensor digitorum longus muscle, as shown in Figs. 1 and 2. Higher concentration (10⁻⁶- 10⁻⁵ g/ml) of the drug produced more remarkable contracture with a steeper slope of rising phase and a larger peak of the tension, while lower concentration (10⁻⁸ g/ml) sometimes failed to elicit the effect. This contractural response was differentiated into two components, a fast phase and a slow phase, which were sometimes observed very clearly as shown in Fig. 1-C. NOR produced similar contracture to that by EPI. TMQ caused only a slow phase of the contracture, the onset of
which was slower and the maximal tension developed was less than observed with the other two drugs. The denervated soleus muscle responded to the drugs in a qualitatively similar way as to the extensor muscle, though the fast phase of the contracture by EPI or NOR was not so clear (Fig. 1-B). Tachyphylaxis appeared to develop to the contractural effect of these amines more quickly than the muscle in vivo (2).

Fig. 1. Twitch and contractural response to epinephrine (EPI), norepinephrine (NOR) and trimetoquinol (TMQ) on the denervated rat muscles. In C, only the contractural responses were shown. A and C: The extensor digitorum longus muscles. B: The soleus muscle. Washing-out procedure was carried out after each incubation with the test drugs.

Fig. 2. Effects of propranolol (PROP) and phentolamine (PHENT) on the contractile response of the denervated muscles to EPI and TMQ. A, B, D and E: The extensor digitorum longus muscles. C: The soleus muscle.
Twitch responses to EPI in 10⁻⁷ g/ml varied in both muscles. In a lower concentration (10⁻⁸ g/ml), the drug produced a progressive increase in twitch heights, while, in 10⁻⁶-10⁻⁵ g/ml of the drug, a depressive response in twitch was observed. TMQ (10⁻⁶–10⁻⁵ g/ml) caused an increase in twitch heights only.

A β-blocking agent, propranolol, in 10⁻⁷ g/ml slightly suppressed the slow phase of the contracture by EPI and also completely depressed the contracture by TMQ (Fig. 2-A, B, C). This blocking agent always revealed a decrease in twitch heights by EPI and the effect was more marked when the control response was depressive (Fig. 2-A). The increase in twitch by TMQ vanished by pre-incubation of propranolol. On the other hand, phentolamine (10⁻⁵ g/ml) diminished the fast component of the contracture by EPI and the slow component still in part remained (Fig. 2-D, E). In addition, the α-blocking agent caused an increase in twitch heights by EPI.

According to these results, contractural response to EPI on the chronically denervated rat muscles appeared to consist of two components; a fast phase mediated through the α-receptor and a slow phase through both α- and β-receptors. EPI caused a dual action also in its twitch response; an increase by the β-action and a decrease by the α-action. NOR showed similar responses in resting tension and twitch, although the β-effect appeared weaker than that by EPI. TMQ showed only the β-effect on both responses.

In in vivo experiment with denervated rat muscles (2), contractual effect by administration of EPI corresponded to an increase in the frequency of the fibrillation potentials; both effects were mediated through the α-receptor, and the β-action introduced slight changes in the frequency of the potentials. NOR is also known to cause depolarization (7) or an increase in the frequency of the fibrillations (8) on the denervated gracilis muscle of the rat. Consequently, the fast component of the contracture by EPI and NOR appears to be strongly related to the electrical changes of the muscle membrane, probably the increase in the desynchronized spikes and/or depolarization of the muscle fibers. The slow component by the two drugs may be introduced partly by the persisting depolarization through the α-receptor and partly by other changes through the β-receptor including those in the contractile system.

The present results regarding the effect of EPI are in fairly good parallel with the brief communication by Paterson (9), who reported that, in isolated rat diaphragm, the contracture was susceptible to α-blocking agents and that β-blocking agents were less effective. However, the discrepant results between our experiments with rats and those by Bowman et al with cats (1), who showed the changes in muscle tone by EPI corresponded to electrical changes and were brought about through the β-receptor, still remains to be explained. Moreover, the results with rats in vitro did not always parallel those in vivo (2), in which β-action caused a slight decrease in the resting tension. Further studies are in progress the results of which may account for these discrepancies.

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REFERENCES

EFFECTS OF PHENTOLAMINE MESYLATE AND PROPRANOLOL HYDROCHLORIDE ON ASPIRIN-INDUCED GASTRIC LESIONS AND GASTRIC SECRETION IN THE RAT

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There is an increasing number of publications which deal with the effect of various adrenergic blocking agents on gastric secretion or lesions caused by acute stress or ulcerogenic drugs in man (1) and experimental animals (2-7). The present paper describes the effect of phentolamine mesylate, an alpha-blocker, and propranolol hydrochloride, a beta-blocker, on the aspirin-induced gastric lesion model which was recently established by the present authors and on the gastric secretion in pylorus-ligated rats.

Male Donryu strain rats, weighing 170-190 g, were deprived of food but allowed free access to water for 24 hr prior to experiments. Gastric lesions were produced by the method described previously (8). Briefly, the pylorus of the rat stomach was ligated under ether anesthesia according to the standard method by Shay et al. (9). Ten min later, aspirin (100 mg/kg), suspended in 1% carboxymethylcellulose solution, was given per os to the pylorus-ligated rats. Seven hr after being dosed with aspirin, during which time no food or water was allowed, the animals were sacrificed by an overdose of ether. Ten min before death, the animals under ether anesthesia were given 1 ml of a 5% solution of pontamine sky blue 6 BX dissolved in saline and injected into the tail vein (10). After sacrificing the animals, the stomach was removed and treated with 1% formalin solution as a routine procedure. Subsequently, the stomach was incised along the greater curvature, and the length of lesions in the glandular portion was measured under a dissecting microscope (×10). The sum of the length (mm) of the lesions per rat was used as an ulcer index. Phentolamine mesylate (Regitine, Ciba-Geigy) and propranolol hydrochloride (Inderal, Sumitomo) dissolved in saline solution were injected s.c., 10 min before pylorus ligation. The control animals were given the same amount of saline solution s.c. In order to deter-