PHARMACOLOGICAL EXPERIMENTS WITH THE ISOLATED EXTRAOCULAR MUSCLE FROM RABBIT

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There have been some articles in regard to the pharmacological study on the extraocular muscles. Katz and Eakins (1) reported the in situ effect of drugs on electrically stimulated extraocular muscle of cats, and Kern (2, 3) studied the effect of sympathomimetic amines on the isolated extraocular muscles.

However, there has been no publication, to our knowledge, in regard to the electrical stimulation of the isolated extraocular muscle-nerve preparations. The investigations on the neuromuscular junction in the extraocular muscle were generally carried out in the in situ experiments. In the in situ experiments a part of responses of the extraocular muscles to drugs may be due to secondary effects accompanied with responses of the intraocular muscles to the drugs. In the present study, the effects of drugs on the electrically stimulated extraocular muscle-nerve preparations isolated from rabbits were investigated and were compared with actions of the drugs in the in situ experiments. Furthermore, actions of drugs on the isolated extraocular muscles were examined.

METHODS

Transmural stimulation of the isolated inferior oblique muscle-oculomotorius nerve preparation

Male rabbits weighing 2 to 3 kg were killed by blow and exsanguination. The inferior oblique muscle or one of extraocular muscles was excised with care to avoid injury. The isolated muscle was mounted between two platinum wire electrodes in a 30 ml organ bath filled with Tyrode solution kept at a constant temperature of 32.0 ±0.5°C and gassed with a mixture of 95% O2 and 5% CO2. The oculomotorius nerve was stimulated indirectly with the rectangular pulses of 0.05 msec duration at a frequency of 0.3 cps. Changes of twitch responses and base-line tension were recorded through a mechano-electric transducer or RCA-5734.

The experiments with the isolated extraocular muscle

In some experiments, isolated inferior oblique muscle and inferior rectus muscle

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were used as test preparations. Their responses were recorded through an isotonic lever. The results in this paper were collected from at least 10 experiments.

**Drugs used:** acetylcholine chloride, methacholine chloride, pilocarpine hydrochloride, succinylcholine chloride, decamethonium bromide, atropine sulfate, eserine hydrochloride, norepinephrine hydrochloride, epinephrine hydrochloride, isoproterenol hydrochloride, dibenamine hydrochloride, phenoxybenzamine hydrochloride, phenoxybenzamine hydrochloride, tolazoline hydrochloride, and propranolol hydrochloride. They were obtained as powder forms.

**RESULTS**

*Effects of the curare-like drugs on the electrically stimulated inferior oblique muscle-oculomotorius nerve preparation.*

d-Tubocurarine in concentrations of $10^{-4}$ to $10^{-3}$ g/ml depressed twitch responses of the electrically stimulated inferior oblique muscle-oculomotorius nerve preparation. The inhibitory effects of d-tubocurarine on the twitch responses was antagonized by eserine ($10^{-5}$ to $10^{-4}$ g/ml) but the twitch responses could not be restored even with eserine, after completely depressed by d-tubocurarine. d-Tubocurarine was without any effects on the base-line tension in most of the preparations but slightly lowered the base-

**Fig. 1.** Effects of d-tubocurarine, eserine (A), succinylcholine (B and C) and decamethonium (D) on tension and twitch responses of the electrically stimulated inferior oblique muscle-oculomotorius nerve preparation from the rabbit.

d-Tc : d-tubocurarine, SCh : succinylcholine, C10 : decamethonium
line tension in only one of 17 preparations. Eserine increased the base-line tension in 11 of 16 preparations. This increased tension was lowered to the initial level after several washings of the preparation. The typical registrogram was shown in Fig. 1A.

Twitch responses of the preparation stimulated with the rectangular pulses of 1.0 to 3.0 msec duration was not affected by d-tubocurarine \((10^{-5} \text{ g/ml})\). This indicates that the twitch responses of the preparations stimulated with the pulses of 0.05 msec duration are due to stimulation of the oculomotorius nerve but not to the muscle stimulation.

The actions of succinylcholine on the base-line tension and twitch responses of the stimulated inferior oblique muscle-oculomotorius nerve preparation were found to be divided into three different types depending on the concentrations of succinylcholine applied in the bath fluid. Low concentrations \((10^{-7} \text{ to } 2 \times 10^{-7} \text{ g/ml})\) of succinylcholine increased both base-line tension and twitch responses, as shown in Fig. 1B. Succinylcholine in concentrations of \(2 \times 10^{-7} \text{ to } 10^{-6} \text{ g/ml}\) increased the base-line tension but slightly increased twitch responses. High concentrations \((10^{-6} \text{ g/ml or more})\) of succinylcholine depressed the twitch responses but greatly increased the base-line tension (Fig. 1C). The increase of the twitch responses and of the base-line tension induced by succinylcholine in comparatively lower concentrations could be readily decreased to the initial level by washings, but the twitch responses after depressed by succinylcholine in higher concentrations were found to be difficult to restore the initial level. As in the case of d-tubocurarine, the twitch responses induced by muscle stimulation with the rectangular pulses of longer duration \((1.0 \text{ to } 3.0 \text{ msec})\) were not modified by succinylcholine.

The effect of decamethonium \((10^{-5} \text{ g/ml})\), as shown Fig. 1D, were found to be quite similar to those of succinylcholine in that the former increased the base-line tension and depressed the twitch responses of the preparation. The difference between decamethonium and succinylcholine, however, was that the former in any concentrations did not cause any increase in the twitch responses which was observed to occur after application of succinylcholine in lower concentrations \((10^{-7} \text{ to } 2 \times 10^{-7} \text{ g/ml})\). Decamethonium was without any effects on the twitch responses produced by the direct stimulation of muscle.

**Effect of sympathomimetic amines**

The effects of the sympathomimetic amines were investigated. In this study, the effects of these amines on the base-line tension of isolated inferior oblique muscles in the absence of an electrical stimulation were first examined. Epinephrine, norepinephrine and isoproterenol in concentrations of \(10^{-6} \text{ to } 10^{-4} \text{ g/ml}\) were without any actions on the base-line tension.

The effects of these amines on the tension induced by acetylcholine were tested. The base-line tension was raised by acetylcholine \((10^{-6} \text{ to } 10^{-4} \text{ g/ml})\) in the presence or absence of eserine \((10^{-5} \text{ g/ml})\) or by methacholine \((10^{-5} \text{ g/ml})\). As the results, no consistent effects of the amines were observed. It was shown in only 9 of 43 preparations that tension induced by acetylcholine or methacholine was modified by these amines,
FIG. 2. Effect of epinephrine.

A: on the twitch responses of the electrically stimulated inferior oblique muscle-oculomotorius nerve preparation from the rabbit.

B: on the continuous contracture of the isolated inferior oblique muscle produced by ACh in the presence of eserine (2 x 10\(^{-6}\) g/ml).

C: on the continuous contracture of the muscle produced by ACh in the presence of eserine (2 x 10\(^{-6}\) g/ml), after 30 minutes treatment with propranolol (3 x 10\(^{-6}\) g/ml).

Epi: epinephrine

and the modification was mild and inconsistent one, i.e. either further increase or decrease (Fig. 2B).

The same experiments were repeated on the muscle pretreated with α- or β-adrenergic blocking agent, dibenamine (5 x 10\(^{-8}\) to 10\(^{-5}\) g/ml), tolazoline (3 x 10\(^{-7}\) to 10\(^{-6}\) g/ml) or propranolol (10\(^{-8}\) to 3 x 10\(^{-4}\) g/ml), for 10 to 30 minutes before application of acetylcholine or methacholine. The effects of the sympathomimetic amines in the presence of the adrenergic blocking agent were quite similar to those observed in the absence of the adrenergic blocking agent (Fig. 2C).

The effects of sympathomimetic amines on twitch responses of the electrically stimulated inferior oblique muscle-oculomotorius nerve preparation were also studied. In these experiments, the oculomotorius nerve was stimulated indirectly with the rectangular pulses of 0.05 msec duration at a frequency of 0.3 cps.

Epinephrine (10\(^{-7}\) to 10\(^{-1}\) g/ml) slightly facilitated the twitch responses, as shown in Fig. 2A. Norepinephrine (10\(^{-8}\) to 10\(^{-4}\) g/ml) was found to have little effect on the twitch responses. Isoprenaline (10\(^{-6}\) to 10\(^{-4}\) g/ml) gradually decreased the twitch res-
DRUG ACTIONS ON EXTRAOCULAR MUSCLE 501

responses and in some preparations completely abolished them, after washings of the preparation. The similar finding was obtained with norepinephrine in some preparations. The facilitation of the twitch responses by epinephrine was found not to be modified by pretreatment of the preparations with phenoxybenzamine (10⁻⁶ to 10⁻⁵ g/ml), dibenamine (2 × 10⁻³ g/ml) or propranolol (10⁻⁴ to 5 × 10⁻⁴ g/ml) for 10 to 30 minutes.

Effects of some drugs on the isolated inferior oblique and inferior rectus muscles

In isolated rabbit inferior oblique and inferior rectus muscles, acetylcholine in concentrations of 10⁻⁷ to 10⁻³ g/ml induced the dose-dependent contraction. The acetylcholine-induced contraction was not antagonized by a 3 minutes pretreatment with atropine in concentrations of higher than 10⁻⁶ g/ml, but to be considerably inhibited by a 3 minutes pretreatment with d-tubocurarine in a concentration of 2 × 10⁻⁶ g/ml and the dose-response curve for acetylcholine was shifted in parallel towards higher concentrations. d-Tubocurarine of the same concentration (2 × 10⁻⁶ g/ml) did not inhibit the acetylcholine-induced contraction of the muscle which had been pretreated with 10⁻⁹ g/ml of eserine for 10 minutes. Pilocarpine (10⁻⁷ to 10⁻⁹ g/ml), on the contrary, did not induce the contraction of the extraocular muscle preparation. The inferior oblique muscle and the inferior rectus muscle were found to respond to these agents in a quite similar manner. The results shown in Fig. 3 are similar to those obtained on the rectus abdominis muscle from the frog (4).

DISCUSSION

The modes of actions of d-tubocurarine, succinylcholine, decamethonium, acetylcholine and eserine obtained in our experiment suggest the presence of two different
types of muscle fibers or fast muscle fibers and slow muscle fibers in the inferior oblique muscle of the rabbit. The presence of these two different neuromuscular systems in the extraocular muscles of various species of animals has been known as the results of morphological and in situ pharmacological studies. Dietert (5) speculated that the fast fibers were characterized by Fibrillenstruktur fibers and "en plaque" nerve endings, and the slow fibers were characterized by Ferderstruktur and "en grappe" nerve endings, basing on the results of his studies on human extraocular muscles by means of electronmicroscopy and histochemical demonstration of cholinesterase.

The results obtained in the present study are similar to those obtained by Katz and Eakins (1) in their in situ experiments, except one discrepancy. They reported that d-tubocurarine induced an increase in the base-line tension of the superior rectus muscle of the cat in some 50% of animals studied, whereas d-tubocurarine unaffected the base-line tension in this paper.

Kern (2, 3) showed the presence of the adrenergic receptors in extraocular muscles of the monkey, rabbit and cat in their isolated muscle preparations. In addition, he concluded the predominance of α-adrenergic receptors in extraocular muscles of the rabbit, the predominance of β-adrenergic receptors in those of the cat and exclusive presence of β-adrenergic receptors in those of the monkey. Eakins and Katz (6), and Sanghui (7) reported that the intravenously injected epinephrine produced the contracture of the superior rectus muscle of pentobarbital-anesthetized cat and this contracture was blocked by β-adrenergic blocking agent. Eakins and Katz (6) speculated, as the mechanism of this contracture, the presence of smooth muscle-like elements in the extraocular muscles, the direct action of epinephrine on multiple nerve endings of the slow fiber, ACh release from the prejunctional storage site of the superior rectus muscle by epinephrine, and the secondary contracture accompanied with contracture of the intraocular muscles. The present result obtained with the isolated extraocular muscle that adrenergic blocking agent did not modify the action of sympathomimetic amines suggests the absence of adrenergic receptors in the extraocular muscle of the rabbit. As described before, Eakins and Katz (6) demonstrated in their in situ experiment the predominance of β-adrenergic receptors in superior rectus muscle of the cat, and Kern (2, 3), on the contrary, demonstrated in his in vitro experiment the predominance of α-adrenergic receptors in the same muscle. Differences in the results still remain to be investigated.

**SUMMARY**

The isolated inferior oblique muscle-oculomotorius nerve preparation of the rabbit was indirectly stimulated with the rectangular pulses of 0.05 msec duration at a frequency of 0.3 cps. The twitch responses of the preparation were depressed by d-tubocurarine ($10^{-4}$ to $10^{-5}$ g/ml), decamethonium ($10^{-5}$ g/ml) and succinylcholine ($10^{-5}$ g/ml or more). The inhibition of the twitch responses by d-tubocurarine was antagonized by eserine ($10^{-4}$ g/ml). Decamethonium ($10^{-5}$ g/ml) and succinylcholine ($10^{-7}$ to $10^{-8}$ g/ml) in-
increased the base-line tension. These results indicate that the extraocular muscle consists of the fast and slow fibers. The existence of the adrenergic receptors could not be confirmed in the extraocular muscles used in this work.

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