Effects of Cholinergic Drugs and 4-Aminopyridine on Cat Ciliary Muscle Contractility

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The effects of some cholinergic agents and 4-aminopyridine (4-AP) on neurally mediated contractions of in vitro cat ciliary muscle preparations were studied. The contractile response to trains of stimuli was enhanced by eserine and completely blocked by tetrodotoxin or atropine. Low concentrations of carbachol did not modify muscle resting tension but clearly attenuated contractile response to electrical stimuli, while higher concentrations increased the resting tonus leading to contracture which did not respond to further stimulation. 4-AP is known to be a potassium-channel blocking drug that increases neurotransmitter release at nerve terminals during the action potential. This substance exhibited a dose-related potentiation of the evoked ciliary muscle contractions without changing resting tension. The eventual reducing effect of 4-AP on the accommodative convergence/accommodation ratio (AC/A) is discussed in relation to its potential clinical application in certain strabismus patients. Invest Ophthalmol Vis Sci 26:1309–1313, 1985

The fundamental role played by the ciliary muscle in the visual accommodative process is well known. Most experimental studies concerning mechanical activity of isolated preparations of this muscle have measured the changes induced by different substances on its basal tension in the absence of electrical stimulation. We have considered that a useful tool to study the physiology and pharmacology of accommodation would be in vitro neuromuscular preparations of ciliary muscle in which contraction is elicited by electrical stimulation. The function of this smooth muscle is mainly due to the activity of cholinergic mediators, and the contribution of adrenergic transmitters has not been fully established although it has been demonstrated that catecholamines inhibit contractile response.1

Aminopyridine compounds, particularly 4-aminopyridine (4-AP) have received special attention in recent years because of their facilitatory actions upon neurotransmitter release. This substance has been clinically employed as an anticholinergic agent and in some diseases where neuromuscular transmission is impaired. For instance, it has proven to be effective in the treatment of myasthenia gravis, botulism and other pathologic conditions of the motor endplate. These effects of aminopyridines have been ascribed to blockade of potassium channels at the nerve membrane.2

The purpose of the present experiments was to test the actions of some cholinergic drugs and 4-AP on the electrically induced contractions of the cat ciliary muscle. Our results showed that carbachol diminished the evoked contraction of this muscle while raising its resting tension, and that 4-AP enhanced the evoked response of the preparation without modifying the resting tonus.

Materials and Methods. Healthy adult cats were anesthetized with 35 mg/kg pentobarbital sodium ip and then killed by an air injection into the femoral vein. Procedures were carried out conforming to the ARVO Resolution on the Use of Animals in Research. Both eyes were immediately enucleated and placed in oxygenated Krebs solution. Meridional strips 5 mm in width were cut from the optic nerve to the center of the cornea and then the iris and retina were

Fig. 1. Schematic representation of the experimental set up employed for stimulation of in vitro preparations of cat ciliary muscle.

Ciliary muscle preparations were obtained by carefully dissecting choroid and ciliary body from sclera preserving the normal insertion of the muscle to the scleral spur. The adjacent cornea was fixed to a plastic clamp and the posterior end of the muscle was caught by means of a silk thread tied to the choroid just behind the ora serrata (Fig. 1).

Two similar muscle strips were suspended in individual 20 ml organ baths containing Krebs solution thermostatically controlled at 32°C (5) and bubbled with a 95% O₂-5% CO₂ gas mixture. Silver wire electrodes 5 mm apart were placed close to the tissue in a fixed position for field stimulation. Contractile force was expressed in milliNewtons (mN). One mN is equivalent to 101.9 mg.

Ciliary muscle contractions were registered under isometric conditions by means of Grass FT.03 force displacement transducers and recorded on a D-7 Grass polygraph (Quincy, MA). A resting tension of 5 mN was applied and experiments were started after a 30-min stabilization period.

Stimuli were applied using two coupled S-48 Grass stimulators (Quincy, MA) through stimulus isolation units. Preparations were subjected either to single pulses (0.5 msec, 0.05 Hz) or to train pulse stimulation. This last form of stimulation was preferred. Each train consisted of 0.5-msec pulses at 10 Hz during 3 sec and frequency of trains was one per minute. Stimulus strength was twice the threshold voltage and ranged between 10 and 15 V.

Krebs solution had the following composition (mM): Na⁺, 142; K⁺, 4.6; Mg²⁺, 1.18; Ca²⁺, 2.5; HCO₃⁻, 24; Cl⁻, 125; H₂PO₄⁻ 1.18 and glucose 11.1; pH = 7.4. Drugs employed were: atropine sulphate (Sigma; St. Louis, MO); eserine, sulphate (Nutritional Biochemicals; Cleveland, OH); carbachol chloride (Sigma), tetrodotoxin (Sigma), and 4-aminopyridine (Sigma). Drugs tested were expressed in molar concentration of the bases.

**Results.** Characteristics of the electrically evoked ciliary muscle contraction: In accordance with reports of other authors, no spontaneous activity of isolated preparations was observed. When ciliary muscle strips were stimulated with single pulses, rapid and transient twitches reaching about 0.7 mN tension were recorded. The average time elapsed between the stimulus and beginning of contractile activity was 160 msec; time to peak tension was 400 msec; maximum rate of rise was 2.5 mN·sec⁻¹ and twitch at 50% amplitude lasted 620 msec (n = 8) (Fig. 2A).

Trains of 3 sec duration with 0.5-msec pulses at 10 Hz produced summed and sustained contractions. After ending each train, relaxation occurred, and the muscle recovered its previous resting tension. Train pulse stimulation was repeated every 1 min providing stable and uniform recordings throughout several hours under control conditions (Fig. 2B). Maximal contractile amplitude of responses to this form of stimulation was 3.1 ± 0.34 mN (n = 27). Muscle response to trains of 5 sec or more showed a slow decline of contractile force (Fig. 2C).

Since electrical stimulation was applied in the proximities of the muscle strip, the sodium channel blocking agent tetrodotoxin (0.1 μM) was used to test whether contractions were neurally mediated or caused by a direct response of the muscle fiber. This drug completely abolished evoked activity of preparations. However, in the tetrodotoxin-blocked preparations, direct cholinergic stimulation with carbachol produced a tonic increase in tension as illustrated in Figure 2F. Furthermore, stimuli longer than 5 msec at supra-maximal voltage also did elicit recordable contractions in tetrodotoxin-blocked preparations. These last results were interpreted as consequence of direct activation of the muscle fibers.

**Effects of cholinergic drugs:** Figures 2D and 2E depict representative chart recordings of the effects of eserine and atropine upon ciliary muscle contractions induced by train stimulation. The respective effects of carbachol are shown in Figure 3.

Eserine 2.5 μM induced about 60% increase of isometric contractile force. In fact, amplitude changed from 3.1 ± 0.34 mN to 4.9 ± 0.51 mN (P < 0.005; n = 27). No significant change in the resting tension
was observed with this concentration (Fig. 2D). However, higher doses (10 \mu M) increased muscle basic tension.

Atropine (0.5 \mu M) completely abolished the muscle response to electrical stimulation. At this concentration, the time required for the full blocking effect of atropine was about 90 min (Fig. 2E). Blockade by atropine of in vitro ciliary muscle preparations subjected or not to electric stimuli is well documented and confirms the cholinergic nature of this muscle (Fig. 2E).\(^4\)

Carbachol in concentrations ranging from 0.1 to 5.0 \mu M showed a dual effect: a gradual decrease of amplitude of evoked contractions and also an increase of the basal tension. As shown in Fig. 3, doses of 0.1 to 0.5 \mu M induced a dose-related decrease of amplitude of contractions with only a slight change of the resting tension. Higher concentrations (1.0 \mu M) further decreased amplitude of evoked contractions and produced a sustained increase of contractile force. Concentrations of 5.0 \mu M or higher caused a steady contracture and the muscle failed to respond to electrical stimulation probably due to the fact that the contractile machinery had reached near its maximal capability by the drug action. These effects of carbachol were quickly reverted after removal of the drug from the bathing solution.

**Effects of 4-aminopyridine (4-AP):** In order to potentiate the cholinergic activity of the ciliary muscle, eserine 2.5 \mu M was applied 30 min before exposure to 4-AP.

As shown in Figure 4, 4-AP enhanced the electrically elicited activity of ciliary muscle, this effect being observed either in the contractions evoked by single pulses or by train stimulation.

When single pulses were applied, 4-AP 100 \mu M induced a 122 \pm 12\% increase of the maximal amplitude of contractions. Time to peak tension was prolonged by 23 \pm 3\% and the maximum rate of rise increased 80 \pm 8\% while stimulus–contraction time decreased 14 \pm 2\%, \(n = 8\). (Fig. 4A).
Representative chart recordings of the response to different concentrations of 4-AP in preparations subjected to train stimulation are illustrated in Figure 4B.

4-AP in concentrations from 6.25 to 100 μM induced a dose-related increase of amplitude of contractions evoked by train pulse stimulation. Maximal response was observed at concentrations of 250 μM 4-AP, reaching about 100% increase in contractile force. Figure 4C shows the dose–response curve obtained with 4-AP.

No significant change in the resting tension was observed with any of the tested concentrations of 4-AP. The effects of this drug were longlasting and complete recovery did not occur even after 45–60 min washing.

Discussion. These results are concerned with the effects of some drugs on electrically stimulated cat ciliary muscle neuromuscular preparations. The neurally mediated nature of the contractile response of the ciliary muscle was evidenced by the blocking effect of tetrodotoxin and by the carbachol-induced contraction observed in the tetrodotoxin-treated preparation.

The anticholinesterase agent eserine enhanced amplitude of evoked contractions and higher doses also increased resting tension. On the contrary, the cholinergic agonist carbachol induced a decrease of amplitude of evoked contractions and a rise in the resting tonus. The inhibitory action upon amplitude of contractions was observed with concentrations lower than those which increased the muscle tonus. These apparently opposed effects on contractile response could be interpreted as a consequence of partial agonism between carbachol and endogenous acetylcholine released by electrical pulses. As previously reviewed, carbachol when compared with acetylcholine appears 1.2 times less active in contracting the rabbit ileum and 6.6 times less active in lowering rabbit blood pressure, indicating that the activity of the former drug is lower than that of acetylcholine. Interaction of carbachol with the postsynaptic muscarinic receptor would prevent the action of the more active agonist acetylcholine, thus causing a partial antagonism. The possibility of a mechanism involving presynaptic cholinergic receptors could also be considered. The existence of presynaptic cholinergic receptors has been documented at various cholinergic and noncholinergic nerve endings, having a modulatory action by inhibiting neurotransmitter release. Also, decrease of contraction amplitude observed in the presence of carbachol can be ascribed to an acute desensitizing effect.

4-Aminopyridine (4-AP) is known to increase neuronal excitability by blocking potassium channels and thus increasing the duration of the action potential. In the context of this study, 4-AP was shown to enhance ciliary muscle contractility in a dose-dependent manner, with maximal effects at higher concentrations. The drug's effects were long-lasting and recovery did not occur even after prolonged washing.
neurotransmitter release by nerve impulses at synapses. The mechanism of action of 4-AP is related to its potassium channel blocking property, thus prolonging the action potential. This allows a greater calcium ion entrance through the cell membrane promoting the release of vesicles containing the neurotransmitter. This substance seems to be devoid of anticholinesterase activity at low concentrations. 4-AP increased the neurally mediated ciliary muscle contractions without modifying its resting tonus. Therefore, the enhancement of contractile force can be ascribed to the known stimulatory effect of this drug on neurotransmitter release. The absence of change in the resting tension suggests that the amount of neurotransmitter released under nonstimulated conditions in the presence of 4-AP is insufficient to induce a sustained depolarization of the muscle cell membrane.

A drug that enhances the neurally mediated contraction of the ciliary muscle without modifying its resting tonus appears potentially ideal to reduce the stimulus AC/A ratio when indicated in certain strabismus patients. At present, this is achieved by the administration of longlasting anticholinesterase drugs. However, as it is well known, these agents are not devoid of untoward effects.

Our experimental findings suggest that 4-AP and analog substances could allow a different pharmacologic approach to certain strabismus patients with high AC/A ratio. However, further work on ocular efficacy and toxicity of these drugs is obviously required before testing their clinical effects on human eyes.

**Key words:** 4-aminopyridine, cholinergic drugs, ciliary muscle, accommodation

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