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Membrane and contractile properties of the dog ciliary muscle

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1 Membrane properties and excitation-contraction coupling mechanisms of the dog ciliary muscle were investigated by use of microelectrode and isometric tension recording methods.

2 The mean resting membrane potential of the smooth muscle cell was -59.6 ± 1.6 mV and the membrane was electrically quiescent. With applications of inward and outward current pulses, by the partition stimulating method (pulse duration, 1.0 s), electrotonic potentials, but not action potentials (spike) were evoked even in the presence of tetraethylammonium (TEA, 10 mM).

3 The space and time constants calculated from the current-voltage relationship observed at various distances from the stimulating electrode were 0.43 ± 0.07 mm ($\pm$ s.d. $n = 5$) and 82.5 ± 10.6 ms ($\pm$ s.d. $n = 4$), respectively.

4 Electrical field stimulation of short duration (50–300 μs) evoked excitatory junction potentials (e.j.ps) followed by twitch contraction, both of which were potentiated by neostigmine (10^{-7} M) and abolished by tetrodotoxin (TTX, 10^{-7} M) or atropine (10^{-6} M). However, e.j.ps of amplitude over 3–5 mV did not evoke action potentials.

5 Excess $[K]_o$ solution dose-dependently depolarized the membrane (the maximum slope of the depolarizations produced by a tenfold increase in $[K]_o$ plotted on a log scale was 52.5 mV) and evoked contractions. Atropine (10^{-6} M) had no effect on the $[K]_o$-induced membrane depolarization, however, it greatly reduced the amplitude of $[K]_o$-induced contraction.

6 Nisoldipine (5×10^{-7} M) had no effect on the contraction evoked by field stimulation, however, this agent suppressed the $[K]_o$-induced contraction to $78.3 \pm 6.8\%$ of the control value. Combined application of nisoldipine and atropine completely inhibited the $[K]_o$-induced contraction.

7 Although the membrane depolarization evoked by carbachol ($> 10^{-7}$ M) was small (10 mV depolarization at 10^{-5} M), the contraction evoked by 10^{-5} M carbachol was larger than that evoked by 118 mM $[K]_o$ ($185 \pm 50\%$, $n = 9$).

8 Ca-free 3 mM EGTA-containing solution did not alter the resting tension, but greatly reduced the carbachol-induced contraction (to $8.6 \pm 2.3\%$ of the control value).

9 The present results indicate that the membrane of dog ciliary muscle behaves as an electrical syncytium, and that the contractile responses occur mainly through activation of the receptor-operated Ca channels and partly through activation of voltage-dependent Ca channels.

Introduction

The near point accommodation of the eye is the result of contraction of the ciliary muscle (Moses, 1981), and initiation of the contraction largely depends on parasympathetic nerve activity (Ruskell & Griffiths, 1979). Although electrical or mechanical properties of various smooth muscle cells have been extensively investigated (see for example, Jones, 1981), little attention has been given to the ciliary muscle. Recently, it was reported that contractile activity of the bovine ciliary muscle is solely dependent on cholinergic nerve activity and not on depolarization of the muscle membrane, *per se* (Suzuki, 1983).

However, little is known of the electrical membrane properties and excitation-contraction coupling of the ciliary smooth muscle cells. In an attempt at elucidation, we isolated dog ciliary muscle tissue and microelectrode and isometric tension recording methods were used in both the presence and absence of various chemicals or excess $[K]_o$ solutions. We report evidence that the ciliary muscle is an electrical syncytium, and that the membrane depolarization due to excess $[K]_o$ solutions in the presence of atropine

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(10^{-6}M) initiates a small but discrete contraction, thereby indicating that the muscle contraction is evoked mainly by activation of the receptor-operated Ca channels, and partly by voltage-dependent ones.

Methods

Mongrel dogs of either sex (1–2 years of age, 10–15 kg) were anaesthetized by giving pentobarbitone Na (30 mg kg^{-1} i.v.) and were then exsanguinated from the femoral artery. The eyes were immediately enucleated and kept in oxygenated Krebs solution. Under the microscope, the ciliary body was carefully dissected from the scleral spur, lens and vitreous body, and muscle specimens (0.5–1.0 mm wide and 3–4 mm long) were prepared. To measure the development of isometric tension, ciliary muscle specimens were mounted in a 0.9 ml organ bath through which the test solution, at a temperature of 34°C, flowed continuously (0.3 ml s^{-1}). The preparations were placed vertically, both ends were tied with silk thread and one end of the strip was tied to a mechano-transducer (Nihon-Koden Ltd. RCA5734) and the other to a hook at the bottom of the bath. The resting tension was 50–100 mg. To investigate neural effects on the motility of the muscle tissues, electrical field stimulations were applied through a pair of electrodes consisting of silver plates, separated by 5 mm and placed so that a current pulse would pass transversely across the tissue. Single and repetitive stimulations at 10 Hz were applied, with a current pulse of 0.1–0.8 ms in duration and 50–100 V in strength.

To record the membrane potential, muscle preparations (0.4–0.8 mm wide and 5–8 mm long) were mounted in an organ bath with a volume of 2 ml through which the solution flowed continuously at a rate of 3 ml min^{-1} at 34°C. The conventional glass microelectrode with a resistance of 40–70 MΩ and filled with 3 M KCl was inserted from the scleral side. Application of electrical stimulation to the muscle tissue was made by the partition stimulating electrode, as described by Abe & Tomita (1968).

Modified Krebs solution of the following ionic concentration was used (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, HCO_3^- 15.5, $H_2PO_4^-$ 1.2 and glucose 11.5. The solution was aerated with 97% O_2 and 3% CO_2, and the pH was adjusted to 7.2–7.3. Excess $[K]_o$ solution was prepared by replacing equimolar NaCl with KCl, isotonically. The Ca-free 3 mM EGTA-containing solution was prepared by replacing $CaCl_2$ with equimolar $MgCl_2$ and adding 3 mM EGTA.

The following drugs were used: tetrodotoxin (TTX, Sankyo), atropine sulphate (Daiichi), neostigmine bromide (Sigma), tetraethylammonium-Cl (TEA, Tokyo Kasei), nisoldipine (Bayer), carbachol (Tokyo Kasei) and ethyleneglycol*bis* (β-aminoethylether)-N, N'-tetraacetic acid (EGTA, Dozin).

Results

Electrical membrane properties of the ciliary muscle

The resting membrane potential of the dog ciliary muscle ranged between −55 to −64 mV, with a mean value of −59.6 ± 1.6 mV (mean ± s.d., $n = 105$). With application of inward and outward current pulses (1.0 s in pulse duration) to the tissues by the partition stimulating method (Abe & Tomita, 1968), electrotonic potentials could be recorded at distances of up to 2 mm beyond the stimulating compartment, suggesting that this muscle was an electrical synctium. However, generation of action potentials was not observed in response to the outward current pulses, due to the rectifying property of the membrane. Figure 1a shows the current-voltage relationships observed at three different distances (0.15, 0.22, 0.50 mm) from the stimulating electrode. There was a fairly linear relationship between the applied inward current intensity and the amplitude of electrotonic potential up to about 20 mV of membrane hyperpolarization. If the cable equations are applicable to the tissue, the relationship between the amplitude of electrotonic potentials plotted on a log scale and the distance from the stimulating electrode should be linear (Tomita, 1970). A linear relationship was observed in the dog ciliary muscle at any given current intensity (Figure 1b). From the slope of the decay with distance, the length constant (λ) was calculated to be 0.43 ± 0.07 mm ($n, = 5$).

To measure the time constant of the tissue, the time required to reach half of the final steady amplitude of the electrotonic potential was plotted against the distance from the stimulating electrode. As shown in Figure 1c, a linear relationship was observed. On the assumption that the cable equation is applicable to this tissue, the slope can be expressed as $\tau/2\lambda$ (Hodgkin & Rushton, 1946) where τ and λ are the time and length constants of the membrane, respectively. The mean time constant of the dog ciliary muscle was 82.5 ± 10.6 ms ($n = 4$).

In the presence of TEA (10 mM), the membrane was depolarized from −59 mV to −55 mV; however, the generation of an action potential was not observed in response to outward current pulses.

Effects of field stimulation on the electrical membrane properties of ciliary smooth muscle

With application of field stimulation (100 μs in duration), a brief membrane depolarization (excitatory

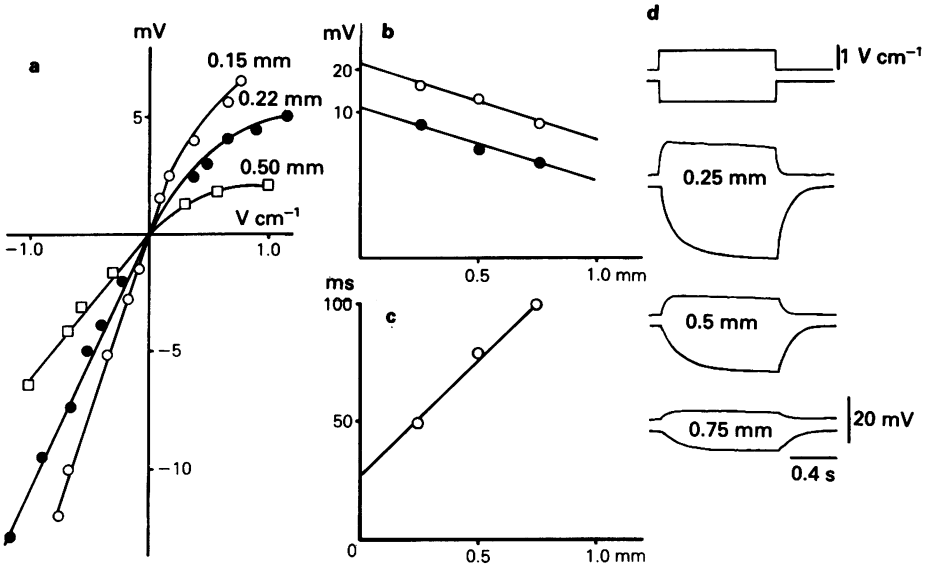

Figure 1 Measurements of passive membrane properties of dog ciliary muscle. (a) Current-voltage relationship measured at three different distances from the stimulating electrode (0.15–0.05 mm); (b) relationship between the amplitude of the electrotonic potential plotted on a log scale against the distance from the stimulating electrode, two different current intensities were used; (c) relationship between the time to reach half of the final steady-state amplitude of electrotonic potential and the distance from the stimulating electrode; (d) actual records of electrotonic potentials evoked by inward and outward current pulses, and recorded at three different distances from the stimulating electrode (0.75, 0.5 and 0.25 mm). (b) and (c) were plotted from the same experiments.

junction potential, e.j.p.) was observed. The amplitude of e.j.p. recorded by the microelectrode was in a wide range between 0.5 and 5 mV, and depended mainly on the distance from the stimulating electrode, the stimulus intensity or duration. E.j.ps could be recorded when the microelectrode was inserted into the cells within 2 mm from the stimulating electrode. As shown in Figure 2, the amplitude of the e.j.p. increased in proportion to the increase in the stimulus intensity, at a constant duration of stimulation (100 μs). Increase in the stimulus number at a constant stimulus intensity and frequency also enhanced the amplitude of e.j.p. (data not shown). When a single stimulation with supermaximal intensity was applied, the latency between the stimulus artifact and the onset of the e.j.p. was in the range between 64 to 80 ms, giving the mean value of 70.4 ± 5.2 ms ($n = 8$).

When the amplitude of e.j.ps exceeded 3 or 4 mV, twitch contractions of the muscle tissue were microscopically evident and mechanical artifacts followed e.j.ps (Figure 2 a-d). However, generation of the action potential was never observed following e.j.ps. This means that in the ciliary muscle, the generation of action potential is not a pre-requisite in the initiation of contraction. Application of atropine (10^{-6}M) abolished both the e.j.ps and the following contractions.

Properties of the mechanical response evoked by field stimulation

When specimens of ciliary muscle (under a resting tension of 50 to 100 mg) were mounted in the organ bath, the tissue gradually relaxed to a steady tension after 0.5 to 1 h superfusion with Krebs solution. Spontaneous contraction did not occur at any time during the experiment. Application of a single field stimulation (100 μs in duration and 100 V in strength) evoked a phasic contraction. When repetitive stimulation (5 or 10 stimuli at 10 Hz) was applied, amplitudes of the phasic contraction were enhanced in proportion to the number of stimuli (Figure 3a). These responses were completely blocked by application of TTX (10^{-7}M), thereby indicating that the mechanical response was neurogenic in origin.

Figure 3 also shows the effect of neostigmine and atropine on mechanical response of the ciliary muscle evoked by electrical field stimulation. Application of neostigmine (10^{-7}M) increased the resting tension of the muscle and enhanced the amplitude (to 1.4–2.0 times that of the control) and duration (to 6–10 times that of the control) of the phasic contraction evoked by field stimulation (Figure 3b). Additional application of atropine (10^{-6}M) gradually reduced the resting

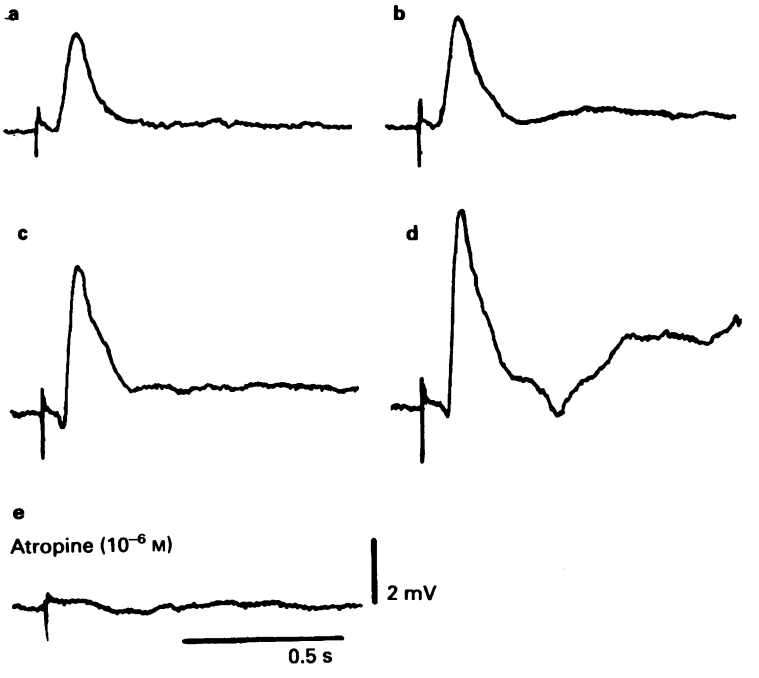

Figure 2 Excitatory junction potentials evoked by brief stimulations (pulse duration; 50 μs; stimulus intensities were increased in a step wise manner from (a) to (d), (e) effects of atropine on the e.j.p..

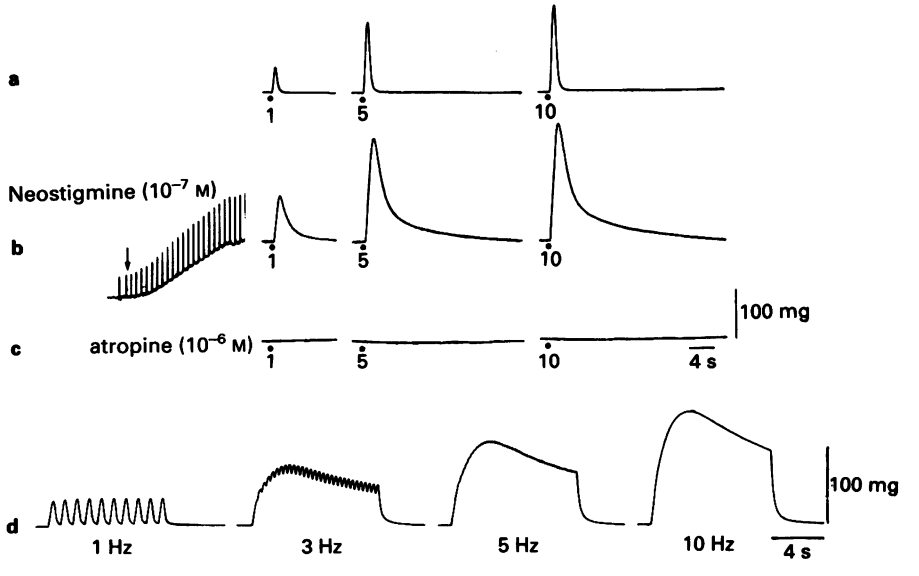

Figure 3 Effects of field stimulations (50 μs duration and 50 V strength) on the mechanical properties of dog ciliary muscle. (a-c) Numbers of field stimulations used were 1, 5 and 10 at 20 Hz, and dots indicate application of field stimulation. In (b) neostigmine (10^{-7} M) elevated the muscle tone, enhanced the amplitude and prolonged the duration of twitch contractions. In (c) atropine abolished the twitch contractions. In (d) repetitive field stimulation at 1, 3, 5 or 10 Hz was applied for 10 s.

tension, to the control level and suppressed the twitch contraction evoked by electrical field stimulation (Figure 3c).

Repetitive field stimulations at high frequency produced summation of the twitch contraction (Figure 3d), showing incomplete (<3 Hz) or complete tetanus (>5 Hz).

Effects of excess concentrations of $[K]_o$ on the ciliary muscle

Figure 4 shows the effects of excess concentrations of $[K]_o$ solutions on mechanical properties of the ciliary muscle. The minimum concentration of $[K]_o$ required to evoke the contraction of the muscle tissue was in the range between 20.2 to 39.2 mM (Figure 4a), and the amplitude of excess $[K]_o$-induced contraction increased, dose-dependently. With increased concentrations of $[K]_o$ (77.1 mM or 118 mM), the phasic contraction was followed by tonic contraction.

Figure 4 also shows the effects of atropine (10^{-6}M) on the excess $[K]_o$-induced contraction, or on the twitch contractions evoked by the electrical field stimulation (Figure 4b). Atropine (10^{-6}M) completely suppressed the generation of twitch contractions evoked by field stimulation, and reduced the amplitude of $[K]_o$-induced contraction to about 35–70% of the control value, depending on the concentration of excess $[K]_o$, but did not abolish the response. In the presence of atropine (10^{-6}M), excess $[K]_o$ solutions still evoked both phasic and tonic contractions (Figure 4c).

Figure 5a shows the relationships between the relative amplitude of contraction and concentration of excess $[K]_o$, observed before and after the application of atropine (10^{-6}M), where the amplitude of the $[K]_o$-induced contraction (118 mM) was taken as a relative amplitude of 1.0. These observations indicate that the $[K]_o$-induced contractions are mainly due to acetylcholine (ACh) released from cholinergic nerve terminals, and that the direct effects of excess $[K]_o$ solutions is to evoke small phasic and tonic contractions of the ciliary muscle.

To investigate the effect of excess $[K]_o$ solutions on the membrane potential in the presence or absence of atropine (10^{-6}M), the microelectrode method was

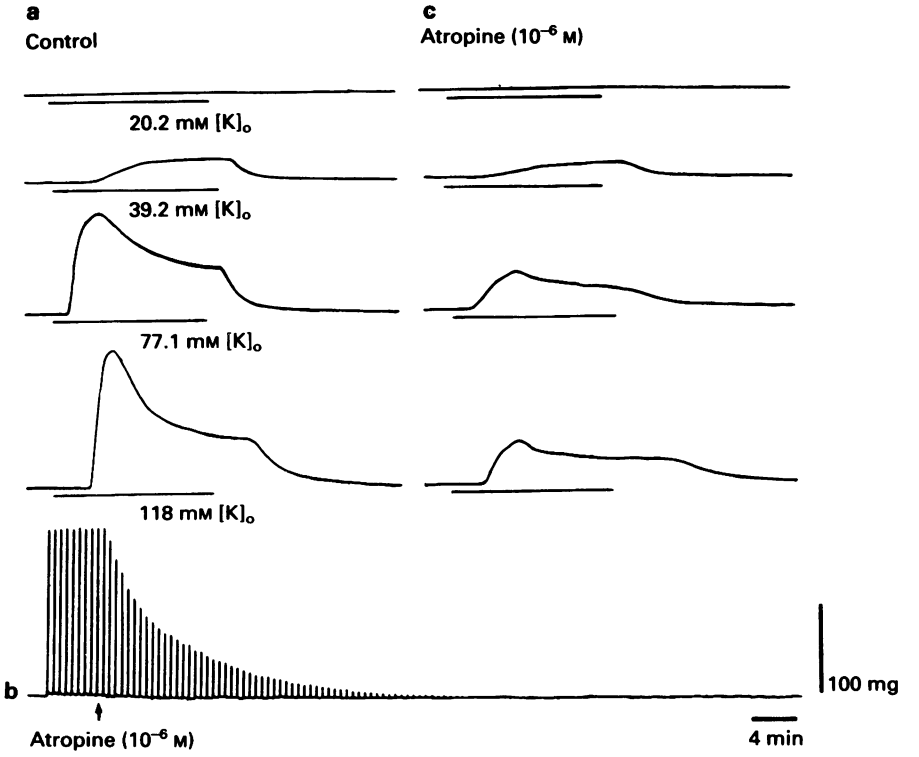

Figure 4 Effects of excess $[K]_o$ solutions on mechanical properties of the dog ciliary muscle in the absence (a) or presence (c) of atropine (10^{-6}M) In (b) atropine (10^{-6}) was applied to observe the effects on mechanical responses evoked by field stimulation (10 stimuli at 20 Hz applied every 1 min).

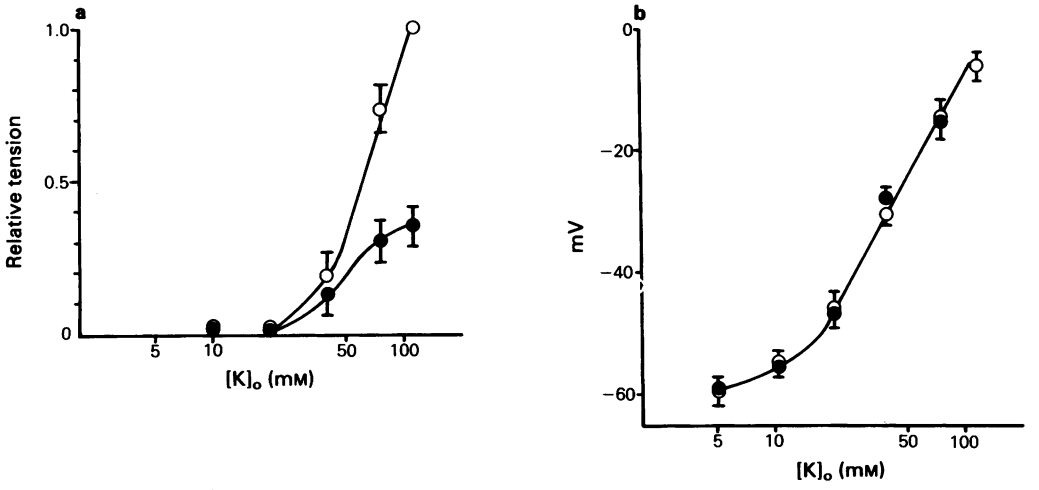

Figure 5 (a) Relationship between the relative tension development and concentrations of excess $[K]_o$ in the absence (○) or presence (●) of atropine (10^{-6}M), where the maximum tension evoked by 118 mM $[K]_o$ was taken as a relative tension of 1.0. Each point indicates mean value of six experiments, and vertical bars are 2 × s.d. (b) Relationship between the membrane potential and concentrations of excess $[K]_o$ in the absence (○) or presence (●) of atropine (10^{-6}M). Each point indicates mean value of resting membrane potentials recorded from 10–47 cells, and vertical bars are 2 × s.d.

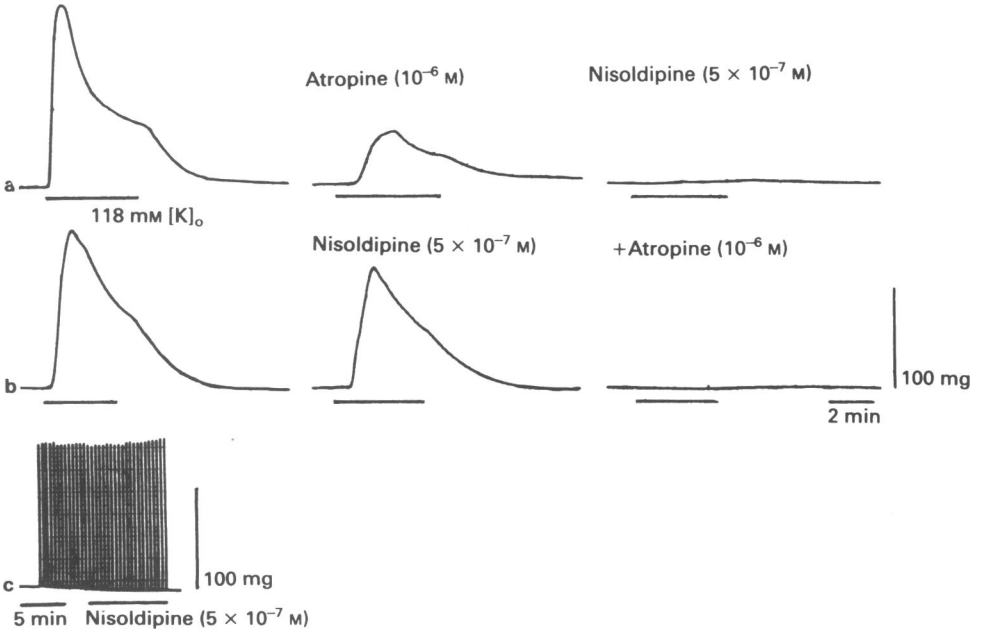

Figure 6 Effects of nisoldipine (5×10^{-7} M) on the excess $[K]_o$-induced contraction in the presence or absence of atropine (10^{-6}M). (a) Atropine (10^{-6}M) reduced the amplitude of excess $[K]_o$-induced contraction to 30% of the control value, and additional application of nisoldipine (5×10^{-7}M) abolished the response. (b) Nisolidipine (5×10^{-7}M) reduced the amplitude of excess $[K]_o$-induced contraction to 70% of the initial value, and additional application of atropine (10^{-6}M) abolished the response. (c) Effects of nisoldipine (5×10^{-7}M) on the twitch contraction evoked by field stimulation.

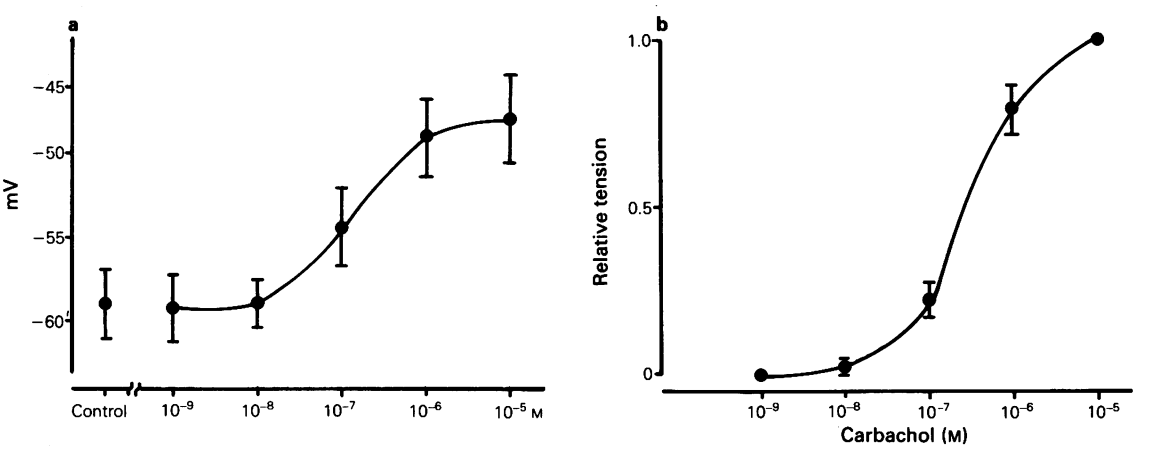

Figure 7 Effects of carbachol (10^{-9}–10^{-5}M) on the membrane and mechanical properties of the dog ciliary muscle. (a) Relationship between the membrane potential and concentrations of carbachol. Each point indicates mean value of resting membrane potentials recorded from 10–53 cells, and vertical bars are 2 × s.d. (b) Relationship between the relative tension development and concentrations of carbachol, where the maximum tension evoked by 10^{-5}M carbachol was taken as a relative tension of 1.0. Each point indicates mean value of six experiments, and vertical bars are 2 × s.d.

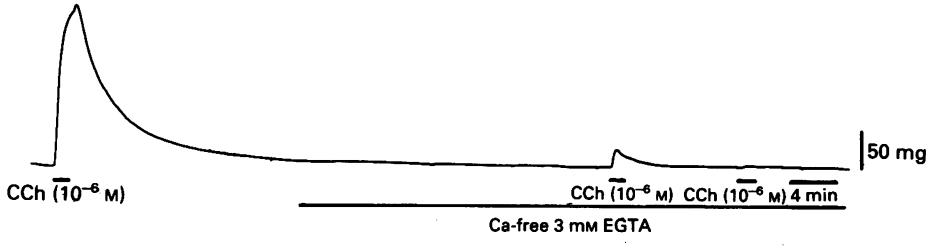

Figure 8 Effects of Ca-free 3 mM EGTA-containing solution on the carbachol CCh-induced contraction. Horizontal bars indicate the application of carbachol (10^{-6}M) or Ca-free 3 mM EGTA-containing solution.

used. As shown in Figure 5b, excess $[K]_o$ solutions dose-dependently depolarized the membrane, and the potential change induced by a tenfold increase in $[K]_o$ concentration plotted on a log scale was 52.5 mV. Atropine (10^{-6}M) did not modify the membrane potential recorded, at any given concentration of $[K]_o$. In the presence of atropine (10^{-6}M), a mechanical response was evoked with 39.2 mM excess $[K]_o$ solution, in which solution the membrane depolarized from −59 to −29 mV. Therefore, the membrane depolarization required for the generation of contraction (mechanical threshold) was 30 mV.

Effects of nisoldipine on the excess $[K]_o$-induced contraction

To investigate mechanisms involved in the genesis of the excess $[K]_o$-induced contraction, we observed the effects of nisoldipine (5×10^{-7}M), a calcium antagonist, on the excess $[K]_o$-induced contraction, in the presence or absence of atropine. As described above, atropine (10^{-6}M) suppressed the amplitude of the $[K]_o$-induced contraction (118 mM) to 35.0 ± 6.9% ($n = 6$) of the control value, and additional application of nisoldipine (5×10^{-7}M) completely blocked the generation of 118 mM $[K]_o$-induced contraction (Figure 6a). On the other hand, nisoldipine (5×10^{-7}M) reduced the amplitude of the $[K]_o$-induced contraction (118 mM) to 78.3 ± 6.8% ($n = 6$) of the control value, and combined application of nisoldipine and atropine abolished the 118 mM $[K]_o$-induced contraction (Figure 6b). However, nisoldipine had no effect on the twitch contraction evoked by electrical field stimulation. These results indicate that the $[K]_o$-induced contraction is mainly due to ACh released from the nerve terminals and partly due to the voltage-depen-

dent Ca channels distributed on the smooth muscle cells. Nisoldipine completely suppressed the latter but had no effect on the former.

Effects of carbachol on the ciliary muscle

To investigate the effects of cholinomimetic agents on the membrane and contractile properties of the ciliary muscle, we used carbachol. Carbachol ($>10^{-7}$M) depolarized the membrane dose-dependently, however, the amplitude of membrane depolarization evoked by this agent was relatively small and was only 10 mV at a concentration of 10^{-5}M (Figure 7a). The minimum concentration of carbachol that evoked the contractile response was also in the range between 10^{-8} to 10^{-7}M, and the amplitude of carbachol-induced contraction increased, dose-dependently. The amplitude of carbachol-induced contraction was relatively large in comparison to that of the excess $[K]_o$-induced contraction, and the amplitude was 1.85 ± 0.5 ($n = 9$) relative to that of the 118 mM $[K]_o$-induced contraction at 10^{-5}M. These results indicate that carbachol is more effective than excess $[K]_o$ solutions for initiating the contractile response, although the latter depolarizes the membrane of the ciliary muscle to a greater extent

To investigate the source of Ca^{2+} contributing to activation of contractile proteins in the ciliary muscle, we examined the effects of Ca-free solution containing 3 mM EGTA on the carbachol-induced contraction of the ciliary muscle. After pre-treatment of the tissue with Ca-free solution containing 3 mM EGTA, the amplitude of the carbachol-induced contraction (10^{-6}M) was reduced to about $8.4 \pm 2.3\%$ ($n = 4$) of the control value, and the contraction was not observed in response to the second application of carbachol, in the presence of Ca-free solution (Figure 8).

Discussion

The smooth muscle cells of dog ciliary muscle had an average membrane potential of -59.3 mV, and the membrane was electrically quiescent. In other words, neither spontaneous fluctuations of the membrane potential nor spontaneous action potential were observed. However, the present studies revealed that membranes of the smooth muscle cells of the dog ciliary muscle behave as a typical synctium (Bozler, 1948) as in the case of other visceral smooth muscle cells, and the cable equation is applicable to the tissue (Abe & Tomita, 1968; Kuriyama & Ito, 1975). The length constant and the time constant of the dog ciliary muscle were 0.43 mm and 82.5 ms, respectively. As compared with other visceral smooth muscles, the smooth muscle membrane of the dog ciliary muscle has a small value of space and time constants (see for example, Jones, 1981). In the portal vein of the guinea-pig, for example, the space constant is also short (0.6 mm; Kuriyama, *et al.*, 1971) and it was considered that the portal vein possesses a less well developed cable than the other visceral smooth muscle such as taenia coli (Abe & Tomita, 1968; Jones, 1981). The dog ciliary muscle may also be classified into this category of smooth muscle cells.

In the dog ciliary muscle, neither depolarizing current pulses applied in the presence of TEA to the smooth muscle cells nor field stimulation to cholinergic nerves evoked an action potential, as has also been noted for tracheal smooth muscle cells in various animal species, including the ox (Kirkpatrick 1981), dog (Suzuki *et al.*, 1976; Ito & Tajima, 1981) and cat (Ito & Itoh, 1984b). However, in smooth muscle cells, an action potential is not an essential requirement for the initiation of contraction. For example, in pulmonary or mesenteric arteries, a low concentration of noradrenaline induces contraction without depolarization of the membrane and this phenomenon was termed pharmaco-mechanical coupling, while high concentrations induce contraction with depolarization of the membrane (Su *et al.*, 1964; Somlyo & Somlyo, 1968; Kitamura *et al.*, 1976; Casteels *et al.*, 1976). On the other hand, it was reported that ACh hyperpolarizes the membrane and yet consistently produces contractions in the rabbit superior mesenteric artery (Kuriyama & Suzuki, 1978). Responses of smooth muscle cells of the coronary artery to ACh differ markedly with the species. In the guinea-pig, ACh significantly hyperpolarizes the membrane and yet produces contraction (Kitamura & Kuriyama, 1979), in the porcine coronary artery, ACh has no effect on membrane potential and resistance, but does evoke contraction (Ito *et al.*, 1979). In the dog iris sphincter or dilator muscles, exogenously applied carbachol or noradrenaline in concentrations below 3×10^{-5}M did not modify the membrane potential, either muscle tissue, yet these agents evoked muscle contraction or relaxation, respectively, in the sphincter muscle and the opposite sequences of mechanical responses were observed in the dilator. Moreover, electrical field stimulations evoke contraction or relaxation with no change in the membrane potential of the smooth muscle cells in the iris sphincter or dilator muscles (Yoshitomi & Ito, 1986).

In the present experiments, 3–5 mV membrane depolarization evoked by the e.j.p., but not electrical membrane depolarization evoked by outward current pulses produced a phasic contraction. When the membrane was depolarized by excess $[K]_o$ solutions in the presence of atropine, contractions were evoked only when the amplitude of membrane depolarization exceeded 30 mV. On the other hand, relatively low concentrations of carbachol (10^{-8}M–10^{-7}M) evoked contraction of the dog ciliary muscle by 3–5 mV

membrane depolarization (Figure 7). Carbachol (10^{-5}M)-induced contraction relative to that of the 118 mM-$[K]_o$ induced contraction was 1.85 ± 0.5 ($n = 9$), and the membrane depolarization due to this agent (10^{-5}M) was only 10 mV. All these data taken together indicate that the contraction of the dog ciliary muscle evoked by field stimulation is not due to membrane depolarization and that pharmaco-mechanical coupling (for review see Kuriyama, *et al.*, 1982) plays an important role in the initiation of the mechanical responses. In the dog ciliary muscle, agonist-induced contractions were greatly suppressed in the presence of Ca-free solutions containing 3 mM EGTA. This means that the contraction may be evoked by activation of receptor-operated calcium channels. These observations differ from events with airway smooth muscle, where the ACh- or caffeine-induced contractions were largely unaffected in Ca-free (2 mM EGTA-containing) solution (Ito & Itoh, 1984a, b).

In the case of the bovine ciliary muscle, it was reported that the contractions evoked by either electrical stimulation or excess $[K]_o$ solutions were completely inhibited by atropine, thereby indicating that depolarization induced by electrical currents or by excess $[K]_o$ is ineffective in evoking contraction (Suzuki, 1983). However, in the present experiments, atropine (10^{-6}M) reduced the amplitude but did not abolish excess $[K]_o$-induced contractions of the dog ciliary muscle. Therefore, $[K]_o$-induced contractions of the dog ciliary muscle are mainly due to ACh released from nerve terminals by excess $[K]_o$, as has been proposed in the case of bovine ciliary or iris dilator muscles (Suzuki, 1983; Suzuki *et al.*, 1983). In the dog ciliary muscle, excess $[K]_o$-solution, *per se*, evoked phasic and tonic contractions which were suppressed by nisoldipine. The contractions evoked by excess $[K]_o$ solutions in the presence of atropine are therefore due to membrane depolarization, hence probably voltage-dependent Ca channels located in the ciliary muscle (Itoh *et al.*, 1984). Similarly, in the dog iris sphincter muscle, excess $[K]_o$ solution evoked minute but discrete contractions in the presence of atropine, phentolamine and timolol, indicating the presence of voltage-dependent Ca channels (Yoshitomi & Ito, 1986). On the other hand, in human or dog iris dilator muscles, excess $[K]_o$ solutions evoked combined mechanical responses of contraction and relaxation, which were mainly due to noradrenaline or ACh released from the nerve terminals, and these mechanical responses were completely blocked by combined application of adrenergic and cholingeric blocking agents (Yoshitomi *et al.*, 1985; Yoshitomi & Ito, 1986). Therefore, it is considered that the voltage-dependent Ca channel is poorly distributed on the iris dilator muscles of various animal species.

The present study provides evidence that the muscle membrane of the dog ciliary muscle behaves as an electrical syncytium, as in the case of other visceral smooth muscle cells, and that the contractile responses occur mainly through activation of the receptor-operated Ca channels and partly by activation of the voltage-dependent Ca channels.

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References

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.*, **196**, 87–100.

BOZLER, E. (1948). Conduction, automaticity and tonus of visceral muscles. *Experientia.*, **4**, 213–218.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977). Excitation-contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.*, **271**, 63–79.

HODGKIN, A.L. & RUSHTON, W.A.H. (1946). The electrical constants of a crustacean nerve fibre. *Proc. R. Soc.*, **B 133**, 444–479.

ITO, Y., KITAMURA, K. & KURIYAMA, H. (1979). Effects of acetylcholine and catecholamines in the smooth muscle cell of the porcine coronary artery. *J. Physiol.*, **294**, 595–611.

ITO, Y. & ITOH, T. (1984a). The roles of stored calcium in contractions of cat tracheal smooth muscle produced by electrical stimulation, acetylcholine and high K^+. *Br. J. Pharmac.*, **83**, 667–676.

ITO, Y. & ITOH, T. (1984b). Effect of isoprenaline on the contraction-relaxation cycle in the cat trachea. *Br. J. Pharmac.*, **83**, 677–686.

ITO, Y. & TAJIMA, K. (1981). Actions of indomethacin and prostaglandin on neuroeffector transmission in the dog trachea. *J. Physiol.*, **000**, 379–392.

ITOH, T., KANMURA, H., KURIYAMA, H. & SUZUKI, H. (1984). Nisoldipine-induced relaxation in intact and skinned smooth muscle of rabbit coronary arteries. *Br. J. Pharmac.*, **83**, 243–258.

JONES, A. W. (1981). Vascular smooth muscle and alterations during hypertension. In *Smooth Muscle*, ed. Bülbring, E., Brading A.F., Jones, A.W. & Tomita, T. pp. 397–430. London: Edward Arnold.

KIRKPATRICK, W. (1981). Tracheobronchal smooth muscle. In *Smooth Muscle*, ed. Bülbring, E., Brading, A.F. Jones, A.W. & Tomita, T. pp. 385–396. London: Edward Arnold.

KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1976). Effect

of noradrenaline on the membrane properties and on the contraction of the rabbit main pulmonary artery. *J. Physiol.*, **263**, 164–165.

KITAMURA, K. & KURIYAMA, H. (1979). Effects of acetylcholine on the smooth muscle cell of isolated main coronary artery of the guinea-pig. *J. Physiol.*, **293**, 119–133.

KURIYAMA, H., ITO, Y., SUZUKI, H., KITAMURA, K. & ITOH, T. (1982). Factors modifying contraction-relaxation cycle in vascular smooth muscles. *Am. J. Physiol.*, **243**, H641–H662.

KURIYAMA, H. & ITO, Y. (1975). Recording of intracellular electrical activity with microelectrodes. In *Methods in Pharmacology*, Vol. 3. Smooth Muscle. ed. Daniel, E. E. & Palon, D. M. pp. 201–203. New York & London: Plenum Press.

KURIYAMA, H., OSHIMA, K. & SAKAMOTO, Y. (1971). The membrane properties of the smooth muscle of the guinea-pig portal vein in isotonic and hypertonic solutions. *J. Physiol.*, **217**, 179–199.

KURIYAMA, H. & SUZUKI, H. (1978). The effect of acetylcholine on the membrane and contractile properties of smooth muscle cells of the rabbit superior mesenteric artery. *Br. J. Pharmac.*, **64**, 493–501.

MOSES, R. A. (1981). Accommodation. In *Adler's Physiology of the Eye*, ed. Moses, R.A. pp. 304–325. St. Louis: The CV Mosby Company.

RUSKELL, G.L. & GRIFFITHS, T. (1979) Peripheral nerve pathway to the ciliary muscle. *Exp. Eye Res.*, **28**, 277–284.

SOMLYO, A.P. & SOMLYO, A.V. (1968). Electrochemical and pharmacochemical coupling in vascular smooth muscle. *J. Pharmac. exp. Ther.*, **159**, 129–145.

SU, C., BEVAN, J.A. & URISILLO, R.C. (1964). Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. *Circulation Res.*, **15**, 20–27.

SUZUKI, H., MORITA, K. & KURIYAMA, H. (1976). Innervation and properties of the smooth muscle of the dog trachea. *Jap. J. Physiol.*, **26**, 303–320.

SUZUKI, R., OSA, T. & KOBAYASHI, S. (1983). Cholinergic inhibitory response in the bovine iris dilator muscle. *Invest. Ophthal. Vis. Sci.*, **24**, 760–765.

SUZUKI, R. (1983). Neuronal influence on the mechanical activity of the ciliary muscle. *Br. J. Pharmac.*, **78**, 591–597.

TOMITA, T. (1970). Electrical properties of mammalian smooth muscle. In *Smooth Muscle*. ed. Bülbring, E., Brading A.F., Jones, A.W. & Tomita, T. pp. 198–243. London: Edward Arnold.

YOSHITOMI, T. ITO, Y. & INOMATA, H. (1985). Adrenergic excitatory and cholinergic inhibitory innervation in the human iris dilator. *Exp. Eye Res.*, **40**, 453–459.

YOSHITOMI, T & ITO, Y. (1986). Double reciprocal innervations in dog iris sphincter and dilator muscles. *Invest. Ophthalmol. Vis. Sci.*, **27**, 83–91

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