Myotonia is characterized by prolonged contraction (delay in onset of relaxation) of skeletal muscle fibers with characteristic electromyographic findings. Calcium channel blocking drugs may be expected to reduce myotonia, should they promote the onset of relaxation in a contracted skeletal muscle. This study was aimed at evaluating the effect of diltiazem, a calcium channel blocking agent, on myotonia induced by 2,4-dichlorophenoxyacetic acid (2,4-D).

In rat diaphragm, exposed to 2.5 m*M* 2,4-D in a tissue bath, myotonia was quantified by documenting the contraction time in response to direct stimulation with supramaximal electric stimuli. At the peak of myotonia, different concentrations of diltiazem were added to the tissue bath and the effect on evoked contraction studied over a period of 6 minutes. A concentration of $5 \times 10^{-5}M$ was found to be the most effective, causing a decrease in contraction time of more than 90% in 3 minutes in 100% of specimens ($n = 7$).

The above findings raise the possibility of using diltiazem as an antimyotonic agent.

Keywords: myotonia • calcium channel blockers • diltiazem • 2,4-dicholorophenoxyacetic acid • skeletal muscle • sarcoplasmic reticulum • mitochondria

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EFFECT OF DILTIAZEM ON 2,4-D-INDUCED MYOTONIA IN RATS

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Myotonia, which is characterized by delayed relaxation of skeletal muscle with typical repetitive electric discharges, occurs in a number of disorders, notably in myotonia congenita and myotonic dystrophy. It may also be induced experimentally by a number of chemical agents. Monocarboxylic aromatic acids like 9 anthracine hydrochloric acid and 2,4-dichlorophenoxyacetic acid (2,4-D) are powerful myotonia-inducing agents both in vivo and in vitro.[3,7] Although the exact mechanism of myotonia may vary in different myotonic disorders, a reduced chloride conductance of the sarcolemma has been found to be a common underlying factor in the human and goat congenital myotonia as well as myotonia induced by aromatic carboxylic acids.[4]

Calcium channel blocking agents, in general, block the slow calcium channels of the cell membrane, leading to decreased calcium influx resulting in electromechanical uncoupling in the case of cardiac and perhaps skeletal muscle.[5] It is possible that these drugs may have a specific effect on intracellular sites as well.[2] It is conceivable that calcium channel blocking drugs, by decreasing the inward calcium flux and by promoting relaxation in a contracted skeletal muscle, may be capable of inhibiting myotonia. The present study was aimed at evaluating this hypothesis, using diltiazem a calcium channel blocker on the 2,4-D in vitro model of myotonia.

To further elucidate a possible mechanism of action, the effect of diltiazem on the 2,4-D in vivo model of myotonia was also investigated.

MATERIALS AND METHODS

In Vitro Experiments. Sprague-Dawley rats weighing 250–300 g were used in the experiments. Under pentobarbital anesthesia, triangular pieces of diaphragm with rib attached at the basal margin were removed from the rats. The rib margin was attached to a glass rod containing two platinum wires for direct stimulation of the muscle. The tendinous end of the specimen was connected to a force-displacement transducer (Grass FT 10 C) by

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a silk thread. The force-displacement transducer was in turn connected to a polygraph (Grass Model 5C). The specimen was kept immersed in an isolated tissue organ bath (Phipps & Bird 7053–400) containing 10 ml oxygenated (37°C, pH 7.4) Tyrode solution of the following millimolar composition: NaCl 119, KCl 5, $NaHCO_3$ 24, NaH_2PO_4 1, $MgCl_2$ 1, $CaCl_2$ 2, and glucose 11. The muscle was directly stimulated with 0.1-msec square wave electrical pulses and the baseline contractions recorded. The amplitude and the duration of contraction (contraction time C_t) measured from the peak of the contraction curve to the point of return to the baseline were noted. Myotonia was induced by replacing the normal Tyrode solution with modified Tyrode (pH 7.4) containing 2.5 m*M* 2,4-D and of the following millimolar composition: NaCl 119, KCl 2.5, $NaHCO_3$ 24, NaH_2PO_4 1, $MgCl_2$ 1, and glucose 11. Earlier experiments in our laboratory have shown that such modification of Tyrode was most conductive to development of maximum myotonia.[8] Muscle contractions in response to electrical stimuli were recorded at 3–5-minute intervals to detect the time of peak myotonia. It was found that maximum myotonia was reached in 10 minutes after exposure to 2,4-D and was maintained for the subsequent 9–12 minutes. In separate experiments, 5×10^{-5}, 5×10^{-6}, or 5×10^{-7} *M* diltiazem were added to the tissue bath at the onset of peak myotonia. The response to electrical stimulation was recorded at 3 and 6 minutes. For each concentration of diltiazem, from six to eight experiments were performed as indicated in Table 1.

Control experiments included measuring the effect of identical concentrations of diltiazem on contractions of nonmyotonic muscle.

To facilitate comparison between different muscle specimens, the degree of myotonia for a given specimen at a given time was expressed as the ratio of the observed C_t to the C_t at peak myotonia for that specimen. The significance of changes in C_t and amplitude was calculated using the *t*-test, analysis of variance, and Tukey Honest test.

In Vivo Experiments. Myotonia was induced by intraperitoneal injection of 200 mg/kg of 2,4-D (dissolved in normal saline, 15 mg/ml, pH 7.4) in 4 male Sprague-Dawley rats weighing 200–300 g. Myotonia was detected with a concentric needle electrode placed in the gastrocnemius muscles, which was connected to a TECA 42 electromyograph (TECA Corp., Pleasantville, NY). The occurrence of waxing and waning trains of potentials with the characteristic "dive bomber" sound was considered the evidence for myotonia. This was quantified on the basis of the ease of eliciting myotonic discharges by needle insertion on a 1–4 scale during 4 consecutive needle insertions: namely, grade 1, single discharge; grade 2, two discharges; grade 3, three discharges; grade 4, four or more discharges per needle reinsertion. At the peak of myotonia 2 ml of 5×10^{-6} solution of Diltiazem were injected in each rat, and myotonic discharges were assessed every 5 minutes for 30 minutes. Three myotonic rats injected similarly with normal saline served as controls.

RESULTS

In the in vitro experiments diltiazem did not produce any change in amplitude or contraction time in nonmyotonic muscle during the 6 minutes. Decrease in the duration of contraction time was significant in myotonic specimens for all three concentrations of diltiazem with maximum effect for $5 \times 10^{-5}M$ concentration (Table 1, Fig. 1). Comparison of the three concentrations using analysis of variance showed a highly significant variance ratio ($F = 87.13$, $P < 0.0001$). The Tukey Honest test indicated that each of the concentrations was significantly different from the other. There was

Table 1. Changes in contraction time after exposure to diltiazem.

Concentration (*M*)	Number of tests	Mean contraction time*		
		Control	At 3 min	At 6 min
5×10^{-5}	7	2	9	7
5×10^{-6}	6	2	83	62
5×10^{-7}	8	2	92	85

**Expressed as percentage of the contraction time at peak myotonia.*

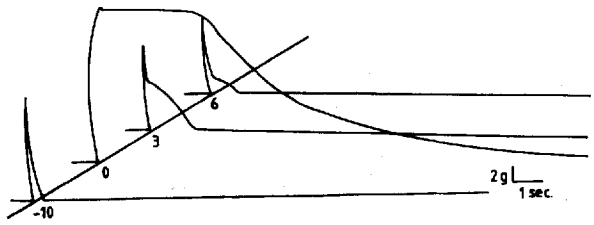

FIGURE 1. Control and experimental evoked contractions after exposure of a myotonic hemidiaphragm to $5 \times 10^{-5}M$ diltiazem. Baseline indicates time in minutes after addition of 2,4-D to the medium. Trace at 0 shows peak myotonia at which time diltiazem was added. Traces at 3 and 6 minutes show the marked decrease in contraction time.

also a significant decrease in the amplitude of contraction at 6 minutes for each of the concentrations compared with that at peak myotonia ($P < 0.0001$ for 5×10^{-5}, $P < 0.005$ for 5×10^{-6}, and $P < 0.005$ for 5×10^{-7}); the Tukey test showed a significant difference between the three groups. However, comparison of the premyotonic amplitude to the postdiltiazem amplitude at 6 minutes did not show a significant decrease.

In the in vivo experiments no significant reduction in electrical myotonia was observed following injection of diltiazem.

DISCUSSION

Calcium channel blocking drugs selectively inhibit calcium ion influx through the cell membrane.[5] There are at least four classes of such drugs: phenylalkylamines (verapamil), dihydropyridines (nifedipine, nimodipine), benzothiazepines (diltiazem), and diphenylalkylamines (flunarizine).

It is generally accepted that the mode of action of the calcium channel blockers in smooth and cardiac muscle may be at the level of voltage-dependent calcium channels in the cell membrane and perhaps at other intracellular sites, leading to relaxation of the muscle, probably by electromechanical uncoupling.[2,5] Although this may be true for skeletal muscle also, there is controversy as to their exact mode of action and their effect on skeletal muscle. Walsh, Bryant, and Schwartz reported that diltiazem in concentration of 0.1–1 μM enhanced the mechanical activity in skeletal muscle and lowered the mechanical threshold.[9,10] Higher concentrations (100 μM of *d-cis*-diltiazem) were found to cause inhibition of inward calcium currents. The concentrations of diltiazem, verapamil, and D-600 required to inhibit inward calcium currents in skeletal muscle were found to be higher than those required to block calcium currents in cardiac cells. Walsh et al. studied calcium currents of rabbit sternomastoid under the influence of different calcium channel blockers and found that diltiazem reversibly blocked inward calcium influx in a concentration-dependent manner. However, they did not find such an effect with nitrendipine, indicating possible differences in modes of action among the various calcium channel blocking agents.[11] The present authors noted in earlier studies that nifedipine, unlike diltiazem, did not exhibit any significant effect on myotonic rat diaphragm;[1] however, there was difficulty in evaluating the finding since the solvent of nifedipine, ethanol, by itself showed some degree of antimyotonic effect.

The present study has demonstrated that diltiazem in a concentration $5 \times 10^{-5} M$ markedly reduces the duration and amplitude of contraction of myotonic muscle, indicating an inhibitory effect on continuous contraction. This effect may be the result of blockage of inward calcium flux, leading to a decrease in intracellular calcium, thus prompting the onset of relaxation. However, this may not be the mechanism, since the modified Tyrode was very low in calcium and due to the sarcoplasmic reticulum being quite rich in calcium. It is possible that diltiazem causes inhibition of the release of adequate Ca^{2+} from the sarcoplasmic reticulum vesicles or mitochondria also,[2,6] leading to a decrease of tension. In the in vivo experiments, diltiazem did not cause any significant reduction in electrical myotonia. This would seem to suggest an action similar to that of dantrolene. Whatever the exact mode of action, our findings raise the possibility of using diltiazem as an antimyotonic agent in human congenital myotonia.

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