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Original Paper

The Voltage Sensitive Ca^{2+}-Channel Blocker Affected the Ca^{2+}-Release from Ca^{2+}-Channel of the Sarcoplasmic Reticulum Membrane in the Skeletal Muscle

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Abstract

The effects of nicardipine as the Ca-antagonist in the dihydropyridine receptor (DHPR) of the transverse (T) tubular voltage sensor (Ca^{2+}channel) were studied in isolated frog skeletal muscle fibers. Nicardipine suppressed the twitch tension about 40% with repeated tetanic stimulation, and suppressed more than 80% with repeated applications of high potassium. When caffeine contracture on the nicardipine treated muscle fibers was induced by concentrations of lower than 5 mM caffeine, an inhibitory effect was seen with repeated tetanic stimulation, . On the contrary, an activation effect was seen on the nicardipine treated muscle fibers with repeated potassium deporalization. However, caffeine contracture with concentrations of more than 5 mM had not the different effects on the both nicardipine treated muscles. These results show that the voltage sensor in the T-tubular membranes has influence on the Ca-release from sarcoplasmic reticulum (SR) with caffeine, and was suggested that the SR membranes has the Ca induced Ca release channels which was inhibited by the voltage sensor on the T-tubles.

Introduction

In skeletal muscle cells, the signal for Ca-release in excitation contraction coupling is transferred from the T-tubular membrane across the triadic junction to the terminal cisterunae of the sarcoplasmic reticulum (SR)[1,2]. It is believed that the physiological Ca release from the SR, primarily regulated by depolarization of T-tubular membranes is the richest source of voltage sensitive Ca-channels[3]. It is not yet clear, however, how a change in potential across the T-tubular membranes leads to the Ca-release from the

SR. The current notion about this process is that the voltage sensitive receptor (Ca-channel) in the T-tubular membrane senses a change in membrane voltage and undergoes a molecular rearrangement that is postulated to directly gate the Ca release channel in the SR membrane.

The purpose of the present study was to

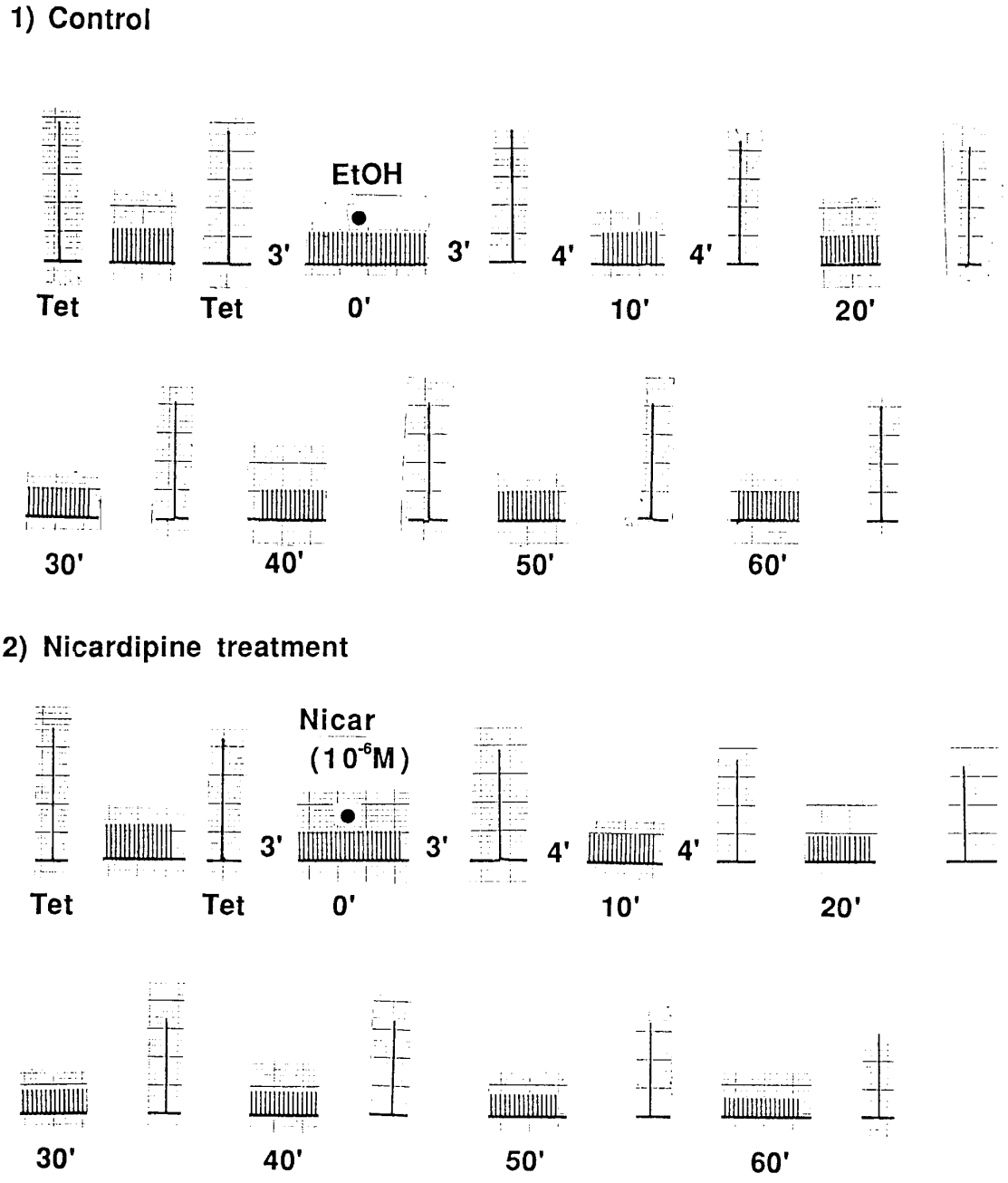

Fig. 1 Effects of nicardipine on the twitch and tetanus tension of frog skeletal muscle.
After the isolated fast skeletal muscle fibers of the frog were incubated in normal Ringer solution, tetanus tension were produced by tetanic stimulation using a subthreshold voltage with a duration of 10 msec. After 3 min., twitch response of this muscle was produced for 2 min. using a drive frequency of 0.1 Hz, at a duration of 1 msec. ● EtOH is the 100% ethanol solution used as a solvent for Nicardipine. ● Nicar (10^{-6}M) was made by adding 30 μl of 10^{-3}M nicardipine in ethanol, into 30 ml of normal Ringer's solution in a glass vessel.

investigate the functional interaction in both Ca-channels. It was known that the dihydropyridine (DHP) bound in the voltage sensitive Ca-channels and inhibited the function of this channel. Therefore, it was explored with frog's fast skeletal muscle whether the Ca-release channel undergoes by DHP.

Material and Methods

Small bundles containing thirty to forty fibers 15 mm in length and 0.4-0.5 mm in diameter were dissected from the Extensor dig.long IV muscle (EDL) of Rana japonica. The muscle bundles were mounted vertically in 30 ml glass vessel containing normal Ringer's solution. One of the tendons was connected to the sensitive arm of a force transducer (Nihon-Kohdenn), and the other end was fixed to a manipulator to adjust its length. Isometric tension responses were recorded on a direct writing oscillograph. Supermaximal current pulses of 1 msec duration delivered from an electric stimulator (Nihon kohden) were applied through a pair of ring-shaped platinum electrodes placed at each end of the muscle. Tetani were produced by a train of 500 msec pulses at a frequency of 50/sec. The normal Ringer's solution had the following composition (mM): NaCl, 111.2: KCl, 3.0; $CaCl_2$, 2.0; Tris, 5.0 mM and pH7.2. All solution contained 2×10^{-5} g/100ml d-tubocurarin. Submaximal potassium contractures were elicited by a 96 mM K_2SO_4 solution. Nicardipine blocked L-type Ca-channel in T-tubular membrane, was diluted to 10^{-9} to 10^{-4} M shortly before the application according to the experimental purpose.

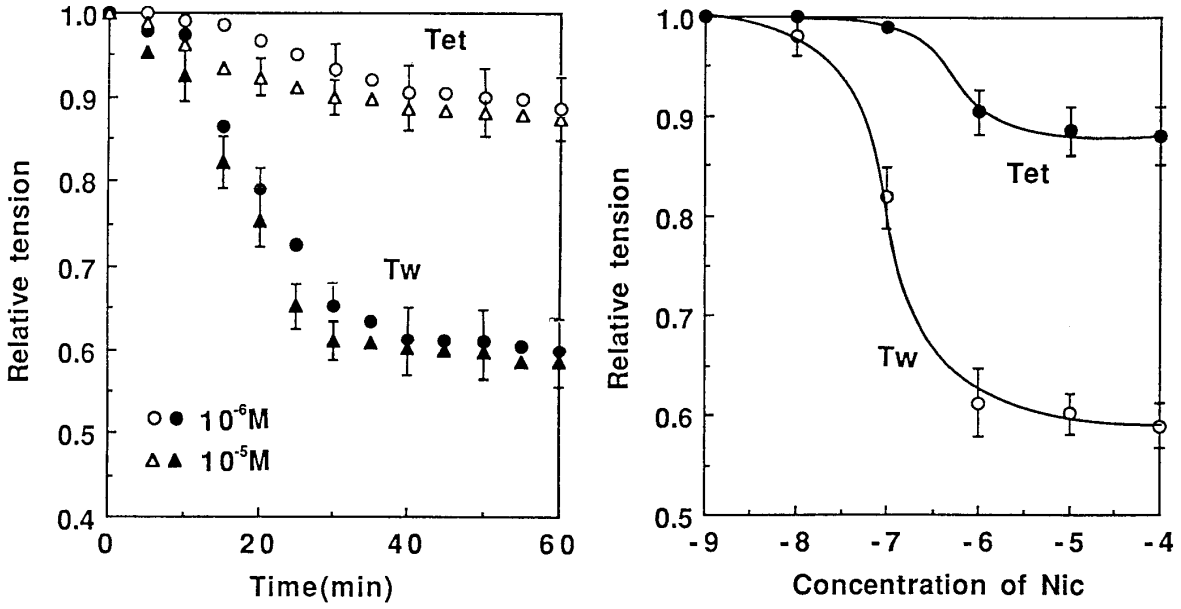

Fig. 2 Changes of the twitch and tetanus tension on the nicardipine treated skeletal muscle fibers.
A) The time course of the effects of the nicardipine on the peak amplitude of twitch and tetanus tension. In each plot the control is expressed as 100%. Circles and triangles indicate the changes of twitch and tetanus tension with the 10^{-6} M and 10^{-5} M nicardipine, respectively. Significant differences in inhibitory effect was not observed with 10^{-5} and 10^{-6} M of nicardipine. The closed circle and triangle are the changes of twitch tension, and the open circle and triangle are the changes of tetanus tension. Data were obtained from 4-5 different tests, with means ±S.E. indicated.
B) Dose response curves of twitch and tetanus tension with application of nicardipine. The open and closed circles indicates the change of twitch and tetanus tension, respectively. Data was obtained from 3-6 different tests, with means ±S.E. indicated.

Results and Discussion

The effect of nicardipine on twitch and tetanus tension are shown in Fig. 1. In normal Ringer solution, skeletal muscle fibers were stimulated tetanically by a train pulse (500 msec) at a frequency of 50/sec. After 3 min., fibers were stimulated once every 10s for 2 min. Using this procedure, the tetanus and twitch stimulations were repeated six times. The twitch and tetanic tension in normal Ringer solution declined slightly with the

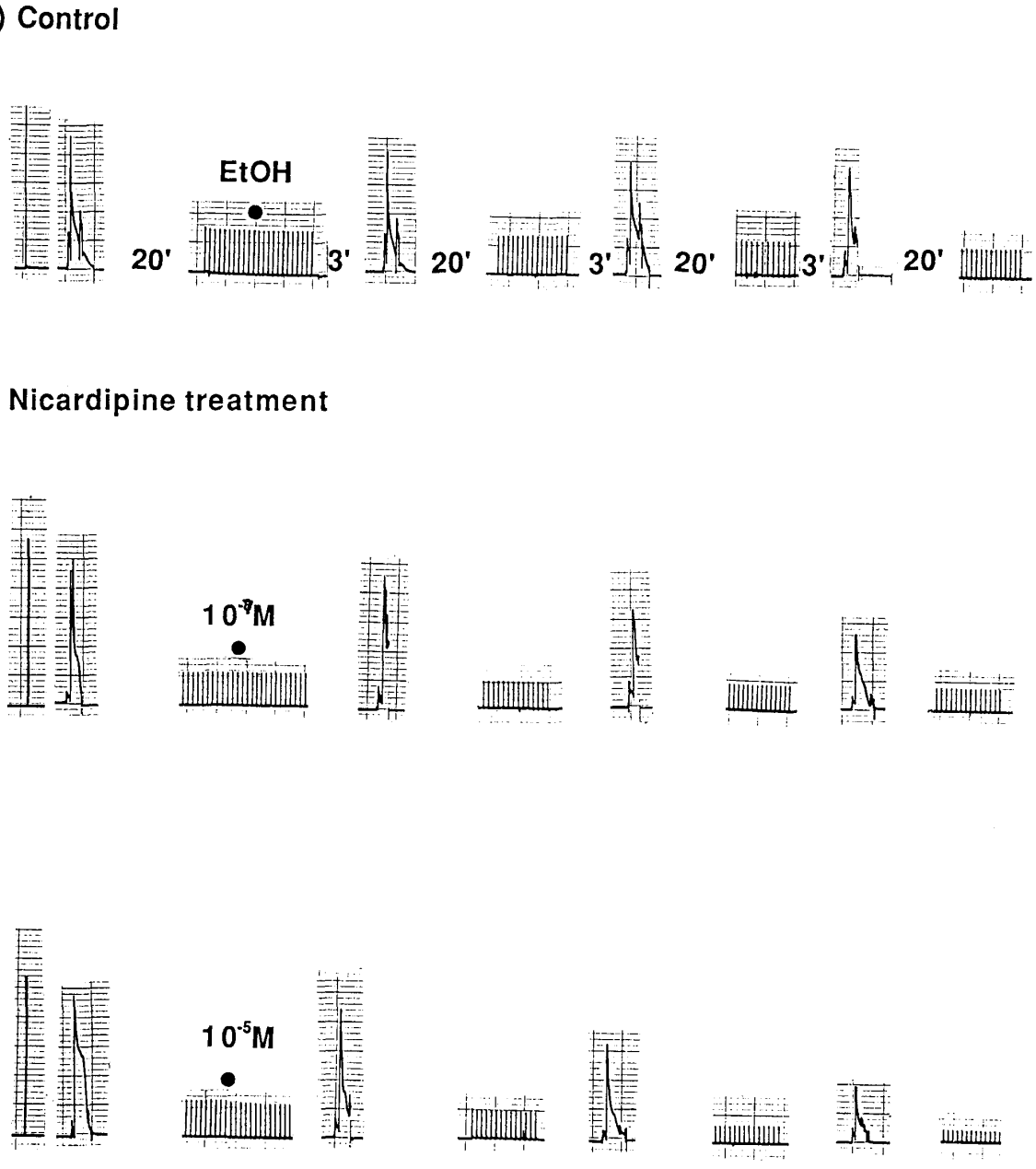

Fig. 3 Effects of nicardipine on the twitch and potassium contractures of skeletal muscle fibers. After the tetanus response of muscle fibers certified in normal ringer solution, the potassium contracture was induced in 96 mM K_2SO_4 solution. The muscle fibers were incubated in normal Ringer solution for 20 min. Twitch response of this muscle was produced by a drive frequency of 0.1 Hz, at a duration of 1 msec. ● EtOH is the 100% ethanol solution used as a solvent for Nicardipine. Effects of nicardipine on the twitch and potassium contractures were examined with 10^{-7} M and 10^{-5} M nicardipine.

repeated stimulation. On the other hand, on the nicardipine treated muscle fibers, the tension with twitch and tetanus stimulation decreased exponentially and reached a steady level in 60 min., and this suppression was dependent on the concentration of nicardipine. In this experiment, the suppression of twitch tension was much greater than that with tetanus stimulation. The suppression of twitch tension was 40%, and that of tetanus tension was 25%. It has been estimated that the Ca-release from SR with twitch[4] and tetanus[5] stimulation were 25-30% and about 60% of Ca, respectively, including SR in the resting level. It is known that nicardipine exerts its Ca^{2+} antagonistic effect via a blockade of the transmembrane of the slow inward Ca-current, and that this suppressant effect can be counteracted by increased $[Ca^{2+}]_o$[6]. Therefore, there is quite a possibility that the slightly suppressant effect on the nicardipine treated muscle fibers was dependent on the released Ca^{2+} from the SR. These results indicated that the nicardipine affects one or several steps in E-C coupling subsequent to membrane depolarization.

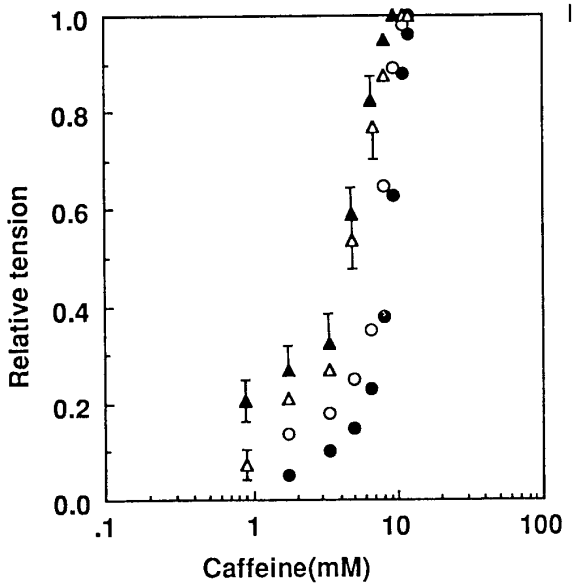

Fig. 4 Effects of nicardipine on the caffeine contracture.
The tension height of contracture induced by 10 mM caffeine on the muscle fibers, without nicardipine in normal Ringer solution was taken as unity. Open and filled circles show the relative values of caffeine contracture on the muscle fibers with repeated twitch and tetanus stimulation. Filled circles (average of 3 experiments) and triangles (average of 6 experiments) show that in the presence of nicardipine (10^{-5} M). Filled triangles show that with twitch tension and potassium contraction were completely inhibited by the nicardipine (10^{-5} M).

If nicardipine exerts its Ca-antagonistic effect, depolarization on the transmembrane by high potassium would be inhibited by nicardipine. Fig. 3 shows the effects of nicardipine on the potassium contractures and twitch responses of skeletal muscle fibers. A repeat of potassium depolarization on the skeletal muscle fibers induced only a slight suppression of the contracture and the tension of twitch stimulation in normal Ringer solution.

However, in the nicardipine treated muscle fibers, the peak tension of potassium contracture markedly decreased with a repeat of potassium depolarization, and the tension with twitch stimulation in normal Ringer solution was also suppressed markedly. As shown in Fig. 3, on the 10^{-7} and 10^{-5} M nicardipine treated muscle fibers, the peak tension of potassium contracture was suppressed more than 80% and 90%, respectively. The decrease in twitch tension was more than 80%. These suppressions were more than those induced by repeated tetanic stimulation. It is well known that DHP derivative, nicardipine, is strongly bound in the transmembrane by depolarization. In this experiment, the strong suppression of contracture with potassium depolarization in the nicardipine treated muscle fibers is further support for this idea. It is known that the Ca-release

channel can be activated by an increase in myoplasmic Ca (Ca-induced Ca-release-CICR), and that this channel was potentiated by caffeine[2]. It was a interest subject whether caffeine controlled the Ca-release from the channel by a voltage sensor in the transmembrane.

The relative changes of caffeine contracture on the nicardipine treated muscle fibers with repeated tetanic stimulation and potassium depolarization are shown in Fig. 4. At concentrations lower than 5 mM caffeine, an inhibitory effect was seen in the nicardipine treated muscle fibers with repeated tetanus stimulation. On the contrary, an activation effect was seen on the nicardipine treated muscle fibers with repeated the potassium depolarization. At higher caffeine concentrations the action of the nicardipine was slight. These results show that the nicardipine certainly functions as a voltage sensing molecule. However, as shown in Fig. 4, the different effects on contracture by the low concentration of caffeine on the nicardipine treated muscle fibers suggested that the Ca-release channels in the SR membrane may have two different functions depending on the voltage sensor in the T-tubular membrane.

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