Acta Medica Okayama

Volume 51, *Issue* 3 1997 *Article* 1

JUNE 1997

The Role of Na^+ Channels in Twitch Generation During Exposure of the Frog Rectus Abdominis to Ca-Free Ringer Solution with Na_2EDTA

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Abstract

 The aim of the study was to investigate whether Na^+ channels play a role in the twitch component of the response of the isolated frog rectus abdominis to Ca^{2+}-free Ringer solution with 0.2 mM Na_2EDTA by using tetrodotoxin and some other well known drugs that exhibit a blocking action on Na^+ channels. In the presence of 5 x 10^{-7} M tetrodotoxin, the twitch component, measured isotonically, disappeared. Although 10^{-7} M d-tubocurarine was found to be ineffective, a complete blockage of twitch amplitude was observed at 5 x 10^{-6} M concentration of the drug. The inhibitory action of d-tubocurarine on twitch response was not antagonized by 10^{-6} and 10^{-5} M carbachol. Propranolol (10^{-6} - 10^{-5} M), lidocaine (2 x 10^{-6} - 10^{-5} M), quinine (10^{-6} - 2 x 10^{-5} M) and quinidine (10^{-6} - 2 x 10^{-5} M) inhibited maximal twitch amplitude in a concentration dependent manner. These findings strongly suggest that activation of tetrodotoxin sensitive Na^+ channel may play a primary role at twitch generation during exposure of the frog rectus abdominis to Ca^{2+}-free Ringer solution with Na_2 EDTA.

KEYWORDS: tetrodotoxin, Ca^{2+}-free medium with Na_2 EDTA, isolated frog rectus abdominis, Na^+ channel blocking activity

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*PMID: 9227789 [PubMed - indexed for MEDLINE]

ACTA MED OKAYAMA 1997; 51(3): 115-120

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The aim of the study was to investigate whether Na^+ channels play a role in the twitch component of the response of the isolated frog rectus abdominis to Ca^{2+}-free Ringer solution with 0.2 mM Na_2EDTA by using tetrodotoxin and some other well known drugs that exhibit a blocking action on Na^+ channels. In the presence of 5×10^{-7} M tetrodotoxin, the twitch component, measured isotonically, disappeared. Although 10^{-7} M d-tubocurarine was found to be ineffective, a complete blockage of twitch amplitude was observed at 5×10^{-6} M concentration of the drug. The inhibitory action of d-tubocurarine on twitch response was not antagonized by 10^{-6} and 10^{-5} M carbachol. Propranolol ($10^{-6} - 10^{-5}$ M), lidocaine ($2 \times 10^{-6} - 10^{-5}$ M), quinine ($10^{-6} - 2 \times 10^{-5}$ M) and quinidine ($10^{-6} - 2 \times 10^{-5}$ M) inhibited maximal twitch amplitude in a concentration dependent manner. These findings strongly suggest that activation of tetrodotoxin sensitive Na^+ channel may play a primary role at twitch generation during exposure of the frog rectus abdominis to Ca^{2+}-free Ringer solution with Na_2 EDTA.

Key words: tetrodotoxin, Ca^{2+}-free medium with Na_2 EDTA, isolated frog rectus abdominis, Na^+ channel blocking activity

It has been demonstrated that Ca^{2+}-free Ringer solution (Ca_FRS) with Na_2EDTA causes a response characterized by irregular phasic contractions superimposed on a slow sustained contracture in the isolated frog rectus abdominis (1, 2). Bianchi (2) used procaine successfully to suppress oscillations, supporting the finding that an increased amount of Na^+ is taken up by sartorial muscles incubated in Ca_FRS with 4 mM Na_2 EDTA for 30 min (3). On the other hand, responses become marked when the temperature of the bathing medium was kept at 30°C instead of 25°C (4).

However, it might be interesting to shed a new light on the mechanism of the basic phenomenon. The purpose of the present study was to investigate the role of Na^+ channels in the twitch component of the response of the isolated frog rectus abdominis to Ca_FRS with 0.2 mM Na_2 EDTA by examining the effects of tetrodotoxin (TTX) and some other well known substances that have Na^+-channel blocking activity.

Materials and Methods

Both sexes of fresh water frogs (*Rana pipiens*) weighing 15-25 g were used in this study. The rectus abdominis was removed after the animal was decapitated and pithed. The muscle was divided into two halves by dissecting along the linea alba and both preparations were separately mounted under 0.5 g tension in organ baths containing Ringer solution (as mM: NaCl 112, KCl 1.87, $CaCl_2$ 1.08, NaH_2PO_4 0.08, $NaHCO_3$ 2.38 and glucose 11.1). The bathing medium was continuously oxygenated. Temperature was maintained at 30°C. The tissue was equilibrated for 1 h. Changes in the preparation length were recorded on the smoked drum via an isotonic lever (× 8-10 magnification). After the basal length of the muscle was recorded for 5 min, the response of the tissue to Ca_FRS (as mM: NaCl 112, KCl 1.87, NaH_2PO_4 0.08, $NaHCO_3$ 2.38 glucose 11.1 and Na_2EDTA 0.2) was monitored for 15 min. Thereafter, the tissue was washed out with fresh normal Ringer solution. This procedure was repeated 4 times at 45 min intervals.

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In another series of the experiments, after the first response of the preparation was recorded as a control, the second, third and fourth responses were measured in the presence of TTX (5×10^{-7} M), d-tubocurarine (d-TC; 10^{-7} or 5×10^{-6}M), propranolol (10^{-6}, 2×10^{-6}, 5×10^{-6}, 7×10^{-6} or 10^{-5}M), lidocaine (2×10^{-6}, 5×10^{-6}, 7×10^{-6} or 10^{-5}M), quinine (10^{-6}, 7×10^{-6}, 10^{-5} or 2×10^{-5}M) or quinidine (10^{-6}, 7×10^{-6}, 10^{-5} or 2×10^{-5}M) by using both solutions (normal Ringer and Ca_FRS). Some trials were designed to clarify possible interaction between d-TC and carbachol. In such experiments, 10^{-6} or 10^{-5}M carbachol was present only in Ca_FRS, though both solutions contained d-TC at the same concentration. A separate experimental group was used for each concentration. In addition, control experiments were performed for vehicles (dimethyl sulfoxide and acetate buffer solution).

Twitch amplitude and frequency displayed variation from preparation to preparation. However, the maximal twitch amplitude induced by four successive exposure of a single tissue to Ca_FRS at 45 min intervals was not significantly different from each other, although twitch frequency tended to decrease; contracture length evoked by the second, third and fourth exposure of the tissue to Ca_FRS was always greater than that of the first application in all trials. Therefore, regardless of contracture amplitude, maximal twitch length in the response was the only parameter taken into consideration for the results and expressed as a percentage (mean ± S.E.) of the value obtained from the first response in the absence of drugs used. The unpaired Student's t test was used for statistical calculations; P values less than 0.05 were considered to be statistically significant. Propranolol hydrochloride, adrenaline hydrochloride, quinine hydrochloride and quinidine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, USA and dissolved in distillated water. Ten percent lidocaine hydrochloride ampoules (Aritmal ampoules, TEMS, Istanbul, Turkey) were used as stock solution and diluted with bathing fluid. D-tubocurarine chloride (Sigma) was dissolved in dimethyl sulfoxide. TTX (Sigma) was dissolved in acetate buffer (pH: 4–5), divided into portions and stored at −20°C.

Results

Ca_FRS caused reproducible responses characterized by irregular phasic contractions superimposed upon a slow, sustained contracture. Maximal twitch lengths in the second, third and fourth responses of the tissue exhibited no significant alteration, while contracture amplitude became markedly higher (Fig. 1). Values obtained as percentages of the first response for maximal twitch amplitudes were 112.2 ± 14.8, 93.4 ± 9.3 and 99.0 ± 11.6 %, respectively, (n = 42).

TTX (5×10^{-7} M), (n = 4) caused disappearance of the twitch component. d-TC (10^{-7}M, n = 8) was found to be ineffective; values obtained were 86.5 ± 13, 76.2 ± 9.2 and 65.4 ± 12.3 %, respectively, and 5×10^{-6}M (n = 5) concentration of the drug blocked twitches completely (Fig. 2). Carbachol did not antagonize the blocking action of d-TC at a dose of 10^{-6} (n = 4) or 10^{-5}M (n = 5) (data not presented).

Propranolol inhibited twitch lengths significantly at doses of 10^{-6} (n = 9), 2×10^{-6} (n = 16), 5×10^{-6} (n =

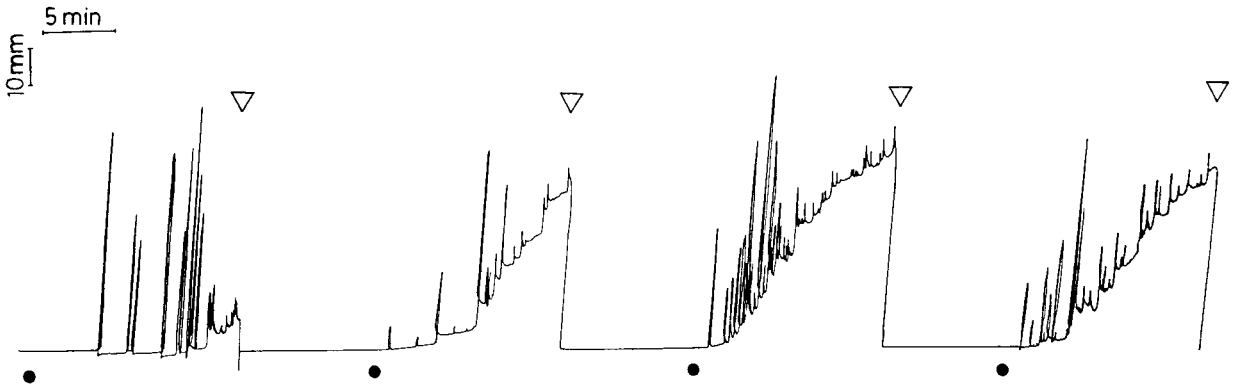

Fig. 1 A typical tracing showing the responses of the isolated frog rectus abdominis. (●) and (▽) indicate exposure of the tissue to Ca_FRS for 15 min at 45 min intervals and washing out with normal Ringer solution, respectively.

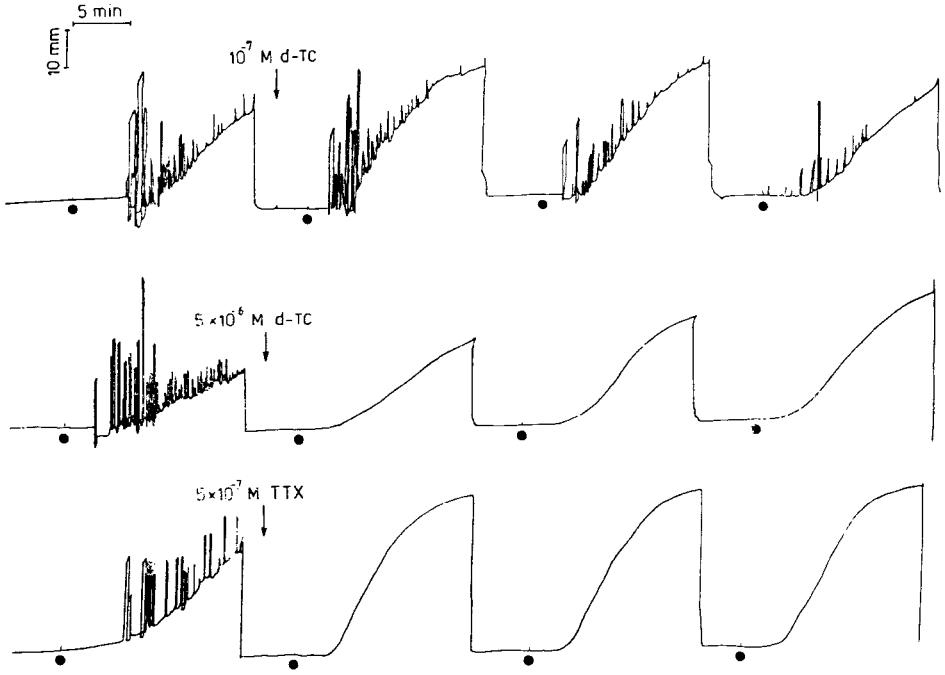

Fig. 2 Records showing effects of d-tubocurarine (d-TC) and tetrodotoxin (TTX) on the twitch component of the response. (●) indicates exposure of the tissue to Ca^{2+}-free Ringer solution (Ca_FRS) for 15 min.

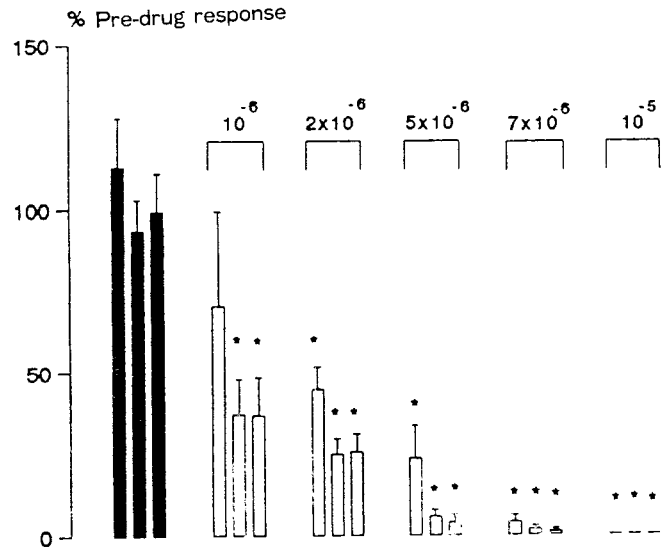

Fig. 3 The effect of propranolol on maximal twitch lengths, shown as a percentage of the response to the first exposure of the tissue to Ca_FRS. From left to right, each column in triads represents mean maximal twitch amplitude evoked by the second, third and fourth applications in the absence (dark columns) or presence (light columns) of propranolol. Vertical lines indicate standard errors. Numbers on the top of light triads show molar concentrations of the drug. *: $P < 0.05$. Ca_FRS: See legend to Fig. 2.

10) and 7×10^{-6} M (n = 11) in a dose-dependent manner. No measurable oscillation was observed at 10^{-5} M (n = 10) concentration of the drug (Fig. 3).

Lidocaine exhibited concentration-dependent inhibitory

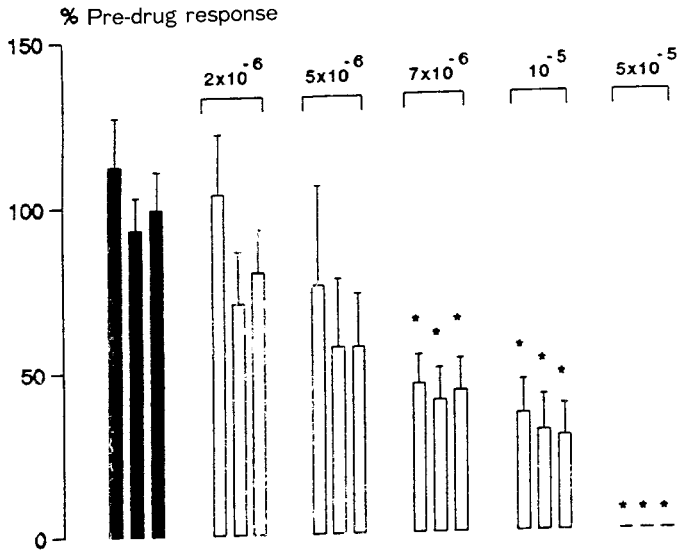

Fig. 4 The effect of lidocaine on maximal twitch lengths, shown as a percentage of the response to the first exposure of the tissue to Ca_FRS. From left to right, each column in triads represents mean maximal twitch amplitude evoked by the second, third and fourth applications in the absence (dark columns) or presence (light columns) of lidocaine. Vertical lines indicate standard errors. Numbers on the top of light triads show molar concentrations of the drug. *: $P < 0.05$. Ca_FRS: See legend to Fig. 2.

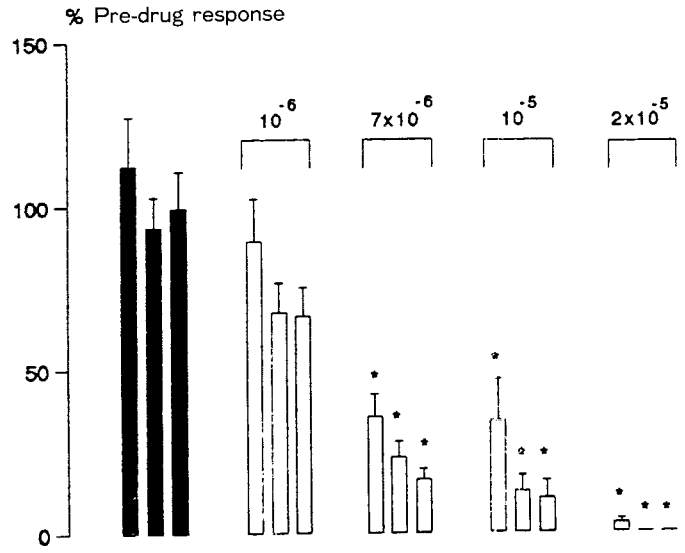

Fig. 5 The effect of quinine on maximal twitch lengths, shown as a percentage of the response to the first exposure of the tissue to Ca_FRS. From left to right, each column in triads represents mean maximal twitch amplitude evoked by the second, third and fourth applications in the absence (dark columns) or presence (light columns) of quinine. Vertical lines indicate standard errors. Numbers on the top of light triads show molar concentrations of the drug. *: $P < 0.05$. Ca_FRS: See legend to Fig. 2.

action on the maximal twitch length at doses of 2×10^{-6} (n = 11), 5×10^{-6} (n = 10), 7×10^{-6} (n = 10) and 10^{-5} M (n = 9). The drug at a concentration of 5×10^{-5} M (n = 10) blocked the twitch component of the responses completely (Fig. 4). Quinine reduced the maximal twitch length significantly, at doses of 10^{-6} (n = 10), 7×10^{-6}

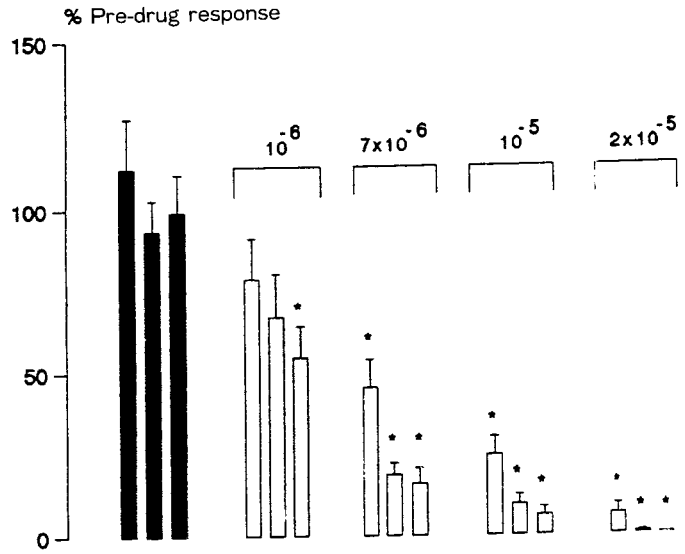

Fig. 6 The effect of quinidine on maximal twitch lengths, shown as a percentage of the response to the first exposure of the tissue to Ca_FRS. From left to right, each column in triads represents mean maximal twitch amplitude evoked by the second, third and fourth applications in the absence (dark columns) or presence (light columns) of quinidine. Vertical lines indicate standard errors. Numbers on the top of light triads show molar concentrations of the drug. *: $P < 0.05$. Ca_FRS: See legend to Fig. 2.

($n = 10$), 10^{-5} ($n = 9$) and 2×10^{-5}M ($n = 10$) in a dose-dependent manner (Fig. 5). Similar values were obtained with quinidine at doses of 10^{-6} ($n = 9$), 7×10^{-6} ($n = 10$), 10^{-5} ($n = 10$) and 2×10^{-5}M ($n = 10$) (Fig. 6).

Discussion

Results of the present study clearly show that a TTX-sensitive mechanism is responsible for twitch generation during exposure of the isolated frog rectus abdominis to Ca_FRS. On the other hand, a TTX-insensitive contracture which gradually increases in amplitude is also triggered by the same treatment.

TTX-sensitive Na^+-channels in innervated skeletal muscles are blocked by nM concentrations of TTX, whereas μM concentrations of the drug are needed for blockage of TTX-resistant Na^+-channels in denervated skeletal muscles (5). Hence, disappearance of twitches in the presence of TTX 5×10^{-7}M suggests that Na^+-channels in the tissue may be sensitive to TTX or they may become sensitive in Ca_FRS. In a previous study on the same preparation, the twitch component of the response to Ca_FRS was eliminated by withdrawal of sodium from the external medium (6). The former and the latter findings support the view that Na^+-channels play a primary role in twitch generation. Removal of external Ca^{2+} and addition of Na_2EDTA to the external medium may cause an impairment in the stability of Na^+ channels leading to spontaneous and irregular channel opening resulting in an increase in Na^+ influx and pulsatile Ca^{2+} release from store sites. This hypothetical chain of events appears to be responsible for twitch generation.

d-TC (5×10^{-6}M) eliminated twitches and this effect was unaffected by 10^{-6} or 10^{-5}M of carbachol, which has been found to be an effective opener of Na^+-channels in the frog endplate (7). This finding suggests a noncompetitive antagonism. This phenomenon is to be expected, since it is well known that high concentrations of d-TC can block the channel directly in a noncompetitive fashion (8). Nicotinic cholinergic mechanism does not contribute to twitch generation under these experimental conditions, as shown in a previous study on the same tissue (1), in which gallamine had no effect on Ca_FRS-induced twitches at a concentration of 5 μg/ml that caused marked inhibition in the responses due to acetylcholine. The results obtained from the present study suggest that Na^+ channels responsible for twitch generation can be

blocked by d-TC.

The inhibitory effect of propranolol on the twitch component appears to be due to its membrane stabilizing activity rather than β-adrenergic receptor blocking action, since it was found to be ineffective at concentrations lower than 10^{-6} which is higher than those necessary for β-adrenergic blockage. Some other agents having membrane stabilizing activity, lidocaine, quinine and quinidine, also produced similar effects, supporting the idea that this preparation may be useful in evaluation of Na^+ channel blocking activity of various substances. In frog skeletal muscles, quinidine and lidocaine at the same concentrations used in our study inhibited Na^+ channels in a dose-dependent manner (9, 10). Quinine attenuated neostigmine-induced twitch augmentation in the cat soleus muscle *in situ* (11). These observations appear to be consistent with our results. The gradual increase of inhibitory effects on twitches may suggest a use-dependent inhibitory effect, the characteristic of local anesthetic action on Na^+ channels (12).

In summary, the results of the present study, performed at the temperature of 30°C, clearly show that agents possessing Na^+-channel blocking action may selectively depress twitches superimposed on the contracture brought about by exposure of the frog rectus abdominis to Ca_FRS.

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Received April 19, 1996; accepted November 29, 1996.