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Effect of Stannous Chloride on the Twitch of Frog Cardiac Muscle

TOSHIMI HATTORI and HIROSHI MAEHASHI

Department of Dental Pharmacology, Matsumoto Dental College
(Chief : Prof. H. Maehashi)

Summary

Stannous chloride ($SnCl_2$) increases the calcium (Ca) entry into motor nerve terminals. Three kinds of Ca channels have been found in nerves (i. e., L-, N-, and T-types). To determine whether or not L-type Ca channels participate in the $SnCl_2$-induced increase in Ca entry, the effect of $SnCl_2$ on the twitch of the bullfrog auricular muscle was investigated and compared with that of the modulator selective to each type of the Ca channels. Both nifedipine (20μM) and nicardipine (20μM), L-type Ca channel blockers, inhibited the twitch. Although Bay K 8644 (20μM), a Ca agonist of the L-type Ca channels, augmented the twitch, $SnCl_2$ (0.1mM) did not. $NiCl_2$ (0.1mM), a T-type Ca channel blocker, had no effect on the twitch. The results obtained suggest that $SnCl_2$ may not activate the L-type Ca channels and that $SnCl_2$ might be likely to enhance the Ca entry into the motor nerve terminals by activating the N-type channels.

Introduction

Fluorides generally augment the skeletal muscle twitch by facilitating the neuromuscular transmission. Among the fluorides investigated, stannous fluoride exerts the most powerful action. Moreover, from the investigations using stannous chloride ($SnCl_2$) as the agent to be examined, stannous ions have been found to be involved in this action by enhancing the transmitter release from the motor nerve terminals[1]. We have recently shown that $SnCl_2$ accelerates the transmitter release by increasing the calcium (Ca) entry into the nerve terminals through the voltage-dependent Ca channels[2].

Nowycky *et al.*[3] have demonstrated that neurons have 3 different types of Ca channels, each with their own unique properties and pharmacology. The first type of channels, designated L, is modulated by dihydropyridine agonists and antagonists but the second (N) and the third (T) types are not. L- and N-type channels are potently blocked by cadmium, whereas T-type ones are much less sensitive to cadmium. ω-Conotoxin, a venom of the marine snail *Conus geographus*[4,5], blocks both N- and L-type channels, but not T-type ones[3,6]. Nickel ions inhibit the T-type channels more strongly than L- or N-type ones[7].

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Activated with weak depolarizations, T-type channels have been shown to continue to the generation of pace maker depolarizations in heart cells[8] and in neurons in the mammalian central nervous system[9]. In many cases, their contribution to the total Ca influx is relatively small. Ca entry through L-type channels has been shown to link excitation to a variety of different responses, for example, release of substance P from dorsal root ganglion neurons[10] and release of noradrenaline from undifferentiated PC-12 cells[11]. N-type channels play a dominant, if not exclusive, role in controlling transmitter release in certain cases, e. g., noradrenaline release from sympathetic neurons, which is dihydropyridine-resistant, but sensitive to both cadmium and ω-conotoxin[12]. N-type channels could also explain the dihydropyridine-resistance of transmitter release from other preparations such as brain slices, synaptosomes, and neuromuscular junction[13].

Thus, the N-type channels seem likely to participate in the $SnCl_2$-induced increase in the Ca entry. In the present study, to determine whether or not $SnCl_2$ does act on the L-type channels, the effect of $SnCl_2$ was investigated on the twitch of cardiac muscles which have predominantly the L-type channels[14] but not the N-type ones[7], and was compared with that of the modulator selective to each type of the Ca channels.

Materials and Methods

Isolated auricular muscle strips (3×10 mm) from bullfrogs (*Rana catesbeiana*) weighing between 150 g and 200 g were used as the material. The material was horizontally mounted in a chamber containing 3 ml perfusate. The muscle twitch was evoked by electrical stimulation (voltage, 100 V ; duration, 50 msec ; frequency, 0.5 Hz) using a pair of platinum wire electrodes (0.5 mm in diameter and 15 mm in length) placed in a longitudinal direction on the both sides of the muscle. The twitch tension was measured with a force-displacement transducer. Drugs were added to the perfusate to examine their effects. Composition of the perfusate (Ringer's solution) was as follows: (in mM) NaCl, 110 ; KCl, 1.9 ; $CaCl_2$, 1.1 ; NaH_2PO_4, 0.5 ; $NaHCO_3$, 2.4 and glucose, 5.6. pH was adjusted at 7.3. All experiments were carried out at room temperature (20-25℃). Chemicals used in this experiment were $SnCl_2$ and $NiCl_2$ (Nacalai Tesque, Japan), Bay K 8644 (Wako Pure Chemicals, Japan), and nifedipine and nicardipine hydrochloride (Sigma, U. S. A.). Immediately before the experiment, $SnCl_2$ was dissolved in 10 mM tartaric acid to make a 4 mM $SnCl_2$ solution. Nifedipine was dissolved in 50 % acetone to make a 4 mM nifedipine stock solution.

Statistical analyses of the data were performed by the Student's 2-sided pired t-test. Differences between mean values were considered significant if the probability of error was less than 0.05.

Results

The effects of nifedipine and nicardipine on the twitch were examined to ensure that inhibitors of the L-type Ca channels depress the twitch. Fig. 1 illustrates these results. As was expected, both 20 μM nifedipine (Fig 1A) and 20 μM nicardipine (Fig. 1B) significantly decreased the twitch tension to about 0.7 and 0.6 times as compared with the control value (taken as 1.0) obtained before each drug application, respectively.

Bay K 8644 (20 μM) significantly increased the twitch tension to about 1.6 times the control value (Fig. 2A). On the other hand, $SnCl_2$ even at a concentration as high as 0.1 mM never strengthened the twitch (Fig. 2B). This result suggests that $SnCl_2$ did not act on the L-type channels.

$NiCl_2$ (0.1 mM) had no significant effect on the twitch (Fig. 3).

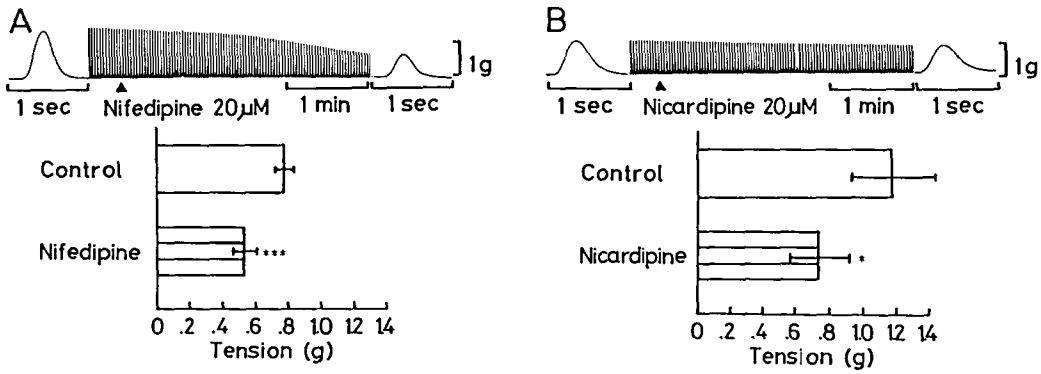

Fig. 1: Inhibition of the twitch of the bullfrog auricular muscle by nifedipine (A) and nicardipine (B). Upper figures: Time course and wave form of the twitch tension. Lower figures: Comparison between the twitch tensions before and 3 min after the drug application. Columns and horizontal bars represent the mean value and the standard error. Number of the data obtained from each experiment is 5. * and ***: Significantly different from the value obtained before the drug application at $p<0.05$ and $p<0.005$, respectively.

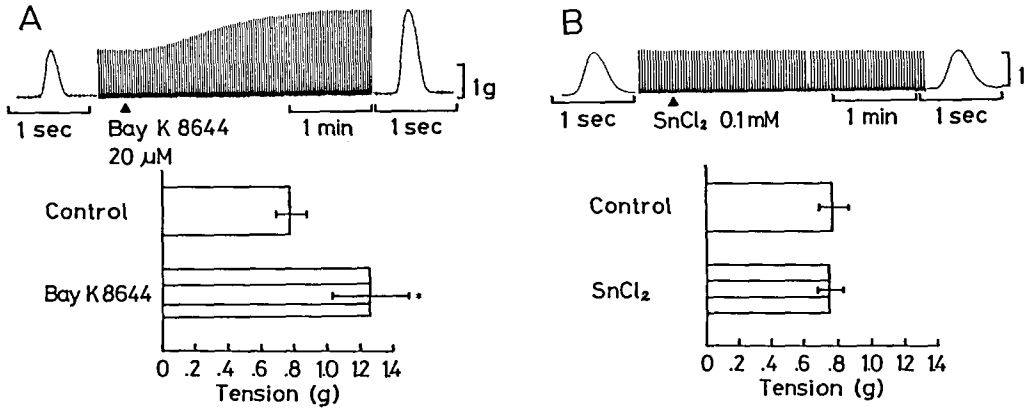

Fig. 2: Effects of Bay K 8644 (A) and $SnCl_2$ (B) on the twitch. Both upper and lower figures show the same as in Fig. 1. Numbers of the data obtained from the experiments concerning Bay K 8644 and $SnCl_2$ are 5 and 10, respectively. *: $p<0.05$.

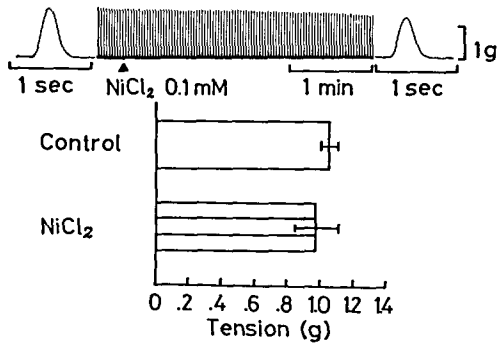

Fig. 3: Effect of $NiCl_2$ on the twitch. Both upper and lower figures show the same as in Fig. 1. Number of the data is 10.

Discussion

Hess *et al.*[14] have found that Bay K 8644 (1 μM) , a Ca agonist compound, strongly enhances the Ca current in frog ventricular cells. Fox *et al.*[15] have explained that the Ca current enhanced by Bay K 8644 is due to the Ca influx through the L-type channels and that nifedipine (10 μM), a dihydropyridine Ca antagonist, inhibits the L-type Ca channel activity.

In our present experiment also, Bay K 8644 significantly augmented the auricular muscle twitch. Nifedipine and nicardipine inhibited it. These results show clearly that Ca entry through the L-type channels is closely related to the heart motility and that the modulators of L-type channels have a significant effect on the twitch. Thus, it is shown that the method used in this experiment is appropriate to determine whether or not $SnCl_2$ acts on the L-type Ca channels.

If $SnCl_2$ can activate the L-type channels, $SnCl_2$ as well as Bay K 8644 must augment the twitch. However, the result that $SnCl_2$ at the concentration high enough to increase the Ca entry into the nerve terminals[2] had no augmentative effect on the twitch implies that $SnCl_2$ does not activate the L-type channels.

In addition, the effect of $NiCl_2$ on the twitch was investigated to see the relation between $SnCl_2$ and T-type channels. Fox *et al.*[16] have observed that inorganic nickel ions (100 μM) largely abolish the current evoked by the Ca entry through the T-type channels selectively in neurons of chick dorsal root ganglion. However, $NiCl_2$ at the same concentration as in this report had no inhibitory influence on the twitch. This result suggests the possibility that there are no T-type channels in the frog heart or at least T-type channels may not strongly participate in the heart motility.

From these findings, it is concluded that $SnCl_2$ does not activate the L-type Ca channels. Moreover, it is very likely that $SnCl_2$ may increase the Ca entry into the motor nerve terminals by acting on the N-type channels. However, the possibility that $SnCl_2$ may stimulate the T-type channels cannot be completely ruled out. To decide whether $SnCl_2$ acts on the N- or T-type channels, it will be necessary to examine the detailed effects of $SnCl_2$ on these channels by the use of different techniques.

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抄録：塩化第一スズのカエル心筋攣縮に対する作用

服部敏己，前橋　浩（松本歯大・歯科薬理）

フッ化物は一般に骨格筋攣縮を増強するが，その中でもとりわけフッ化第一スズはその作用が著しく強力である．これまでの検討により，それには第一スズイオンが関与しており神経末端内へのカルシウム（Ca）流入を促進することにより伝達物質遊離量を増大することがわかっている．神経にはCaの流入経路として3種類のCaチャネル（L型，N型およびT型）が存在し，これらの中のどの型のチャネルがこの作用に関係しているのか興味が持たれる．今回の実験では主にL型チャネルとの関係の有無を明らかにすることを目的として，ウシガエルの心房筋攣縮に対する塩化第一スズ（$SnCl_2$）とCaチャネルの各々の型に特異的な作用薬の作用とを比較した．

Nifedipine（20μM）およびnicardipine（20μM）（いずれもL型Caチャネルブロッカー）は攣縮を抑制した．Bay K 8644（20μM，L型Caチャネルアゴニスト）は攣縮を増強したが，$SnCl_2$（0.1mM）は増強させなかった．$NiCl_2$（0.1mM，T型チャネルブロッカー）は攣縮に影響を与えなかった．

得られた結果は$SnCl_2$がL型チャネルには作用しないことを示唆している．また伝達物質遊離にはN型チャネルを介したCa流入が関係しているとの報告があることから，$SnCl_2$によるCa流入の増大にはN型チャネルが関与している可能性が高まった．