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Electrophysiological effects of Ca antagonists, tetrodotoxin, $[Ca]_o$ and $[Na]_o$ on myocardium of hibernating chipmunks: possible involvement of Na-Ca exchange mechanism

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1 The electrophysiological performance of myocardium of hibernating chipmunks was investigated in the presence of Ca antagonists and tetrodotoxin, and the effects of high $[Ca]_o$ and low $[Na]_o$ were examined.

2 The action potential of the preparations was characterized by the low amplitude of the plateau phase (APp). Ca antagonists, nifedipine (10^{-6} M) and nitrendipine (2×10^{-6} M), did not significantly inhibit this APp or the contraction.

3 These nifedipine-insensitive electromechanical responses were completely abolished by an internal Ca release inhibitor, ryanodine.

4 Both increasing $[Ca]_o$ and lowering $[Na]_o$, by replacing Na by lithium or choline, also inhibited APp.

5 Tetrodotoxin (10^{-5} M) which markedly inhibited the initial rapid phase of the action potential slightly affected APp.

6 These results suggest that the plateau potential of the present preparations is controlled by a process linked to Ca release from internal stores, most likely the Na-Ca exchange mechanism.

Introduction

Recently, it has been demonstrated that the myocardium of hibernating chipmunks is an interesting model in which intracellularly derived calcium plays a major role in the activation of contraction (Kondo & Shibata, 1984; Kondo, 1986a). In this preparation, the membrane action potential plateau (APp) showed a reduced amplitude. Further studies on this electrical response (Kondo, 1986a,b) indicated that APp is regulated by a mechanism different from the slow Ca inward current and is highly sensitive to ryanodine, an inhibitor of internal calcium release. From these results, it has been suggested that the internal Ca release might be involved in the genesis of APp of this preparation (Kondo, 1986b). Ca released from internal stores has also been found to gate membrane currents, such as the transient inward and outward currents and Na-Ca exchange current, resulting in myocardial membrane action potential changes (Bassingthwaighte *et al.*, 1976; Kass *et al.*, 1978; Mullins, 1979; Siegelbaum & Tsien, 1980; Colquhoun *et al.*, 1981; Sutko & Kenyon, 1983). Thus, APp of hibernating animal myocardium is assumed to be generated by the membrane current linked to internal Ca release. Therefore, the present experiments were carried out to characterize further the electrophysiological properties of myocardium of hibernating chipmunks.

Methods

Asian chipmunks (*Tamias sibiricus*) of either sex were trapped in September and transferred to individual wiremesh cages. They were introduced to a darkened cold room (4 ± 1°C) with food, a standard diet of pelleted laboratory rat chow, and water available. Most of them exhibited preliminary bouts of hibernation within 3 weeks, and subsequently, they exhibited several consecutive bouts of hibernation greater than 1 week in duration until the following March. Animals in deep hibernation were used for the experiments.

Animals were killed following cervical dislocation. The heart was quickly excised, a papillary muscle, 2–3 mm in length and less than 1 mm in diameter, was isolated from the right ventricle. The preparation was mounted and equilibrated for 2 h in a tissue bath containing Krebs-Ringer solution aerated with

95% O_2 and 5% CO_2, with the ends impaled on two hooks, one of which was attached to a force displacement transducer (Kondo & Shibata, 1984). The composition of the Krebs-Ringer solution in mmol l^{-1} was: NaCl 120, KCl 4.8, $CaCl_2$ 1.2, $MgSO_4$, $7H_2O$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 24.2 and glucose 5.5 (pH 7.4). In high calcium medium, extracellular Ca concentration was increased to 6 or 8.2 mM. In low sodium medium, the Na concentration was reduced to 24 mM by replacing NaCl by lithium chloride or choline chloride. In the latter medium, atropine at concentration of 10^{-6} M was added. The temperature of the superfusate was maintained at 30°C. The preparations were stimulated at 0.2 Hz with pulses 1 ms in duration and twice the diastolic threshold. Membrane action potential was recorded through glass microelectrodes filled with 3 M KCl. The action potential was displayed on a storage oscilloscope (Tektronix 7613) and recorded on FM tapes (Sony recorder FE 3500). The recorded signal was digitized and displayed on a paper recorder. The mechanical tension was recorded on a polygraph (Nihon Kohden TB612T).

The agents used in the experiments were as follows: ryanodine (2×10^{-6} M; S.B. Penick Company, N.Y.), nifedipine (10^{-6} M), nitrendipine (2×10^{-6} M) and tetrodotoxin (TTX, 2×10^{-6} M and 10^{-5} M; Sankyo Co., Ltd).

Results

Effect of nifedipine and ryanodine

The effects of the Ca antagonist, nifedipine, and an internal Ca release inhibitor, ryanodine, were examined. Figure 1 shows the electromechanical effects of nifedipine (10^{-6} M) and ryanodine (2×10^{-6} M). Although nifedipine caused inhibitory effects on the electrical and the mechanical responses, these effects were weak. The maximum effects of nifedipine were observed within 14 min after application of the drug. The contraction was reduced to 80.0 ± 7.7% of control ($n = 4$). Another Ca antagonist, nitrendipine (2×10^{-6} M), also caused a similar effect (not shown). Under such conditions, the additional application of ryanodine abolished the action potential plateau (APp) and contraction.

Effect of a high calcium and a low sodium medium

The effects of increasing extracellular Ca and reducing extracellular Na concentrations on APp were examined (Figure 2). Increasing extracellular Ca concentration ($[Ca]_o$) to 6 mM caused an inhibitory effect on APp. Further increasing $[Ca]_o$ to 8.2 mM markedly inhibited APp (Figure 2A). This is in good agreement

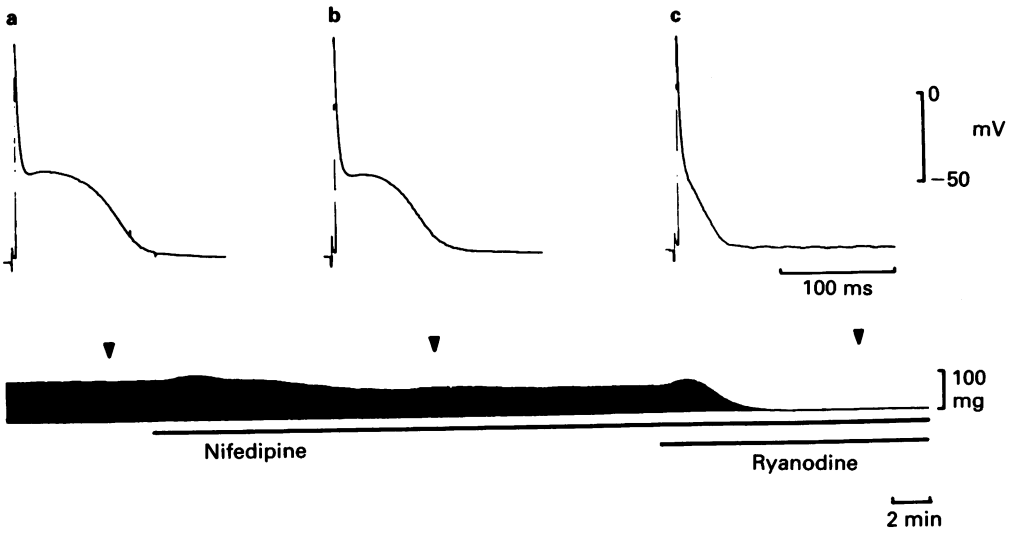

Figure 1 Effects of nifedipine and ryanodine on the membrane action potential (upper trace) and contraction (lower trace). Arrowheads indicate recording points of the membrane action potential. (a) Control, (b) 14 min after application of nifedipine (10^{-6} M), (c) 10 min after additional application of ryanodine (2×10^{-6} M).

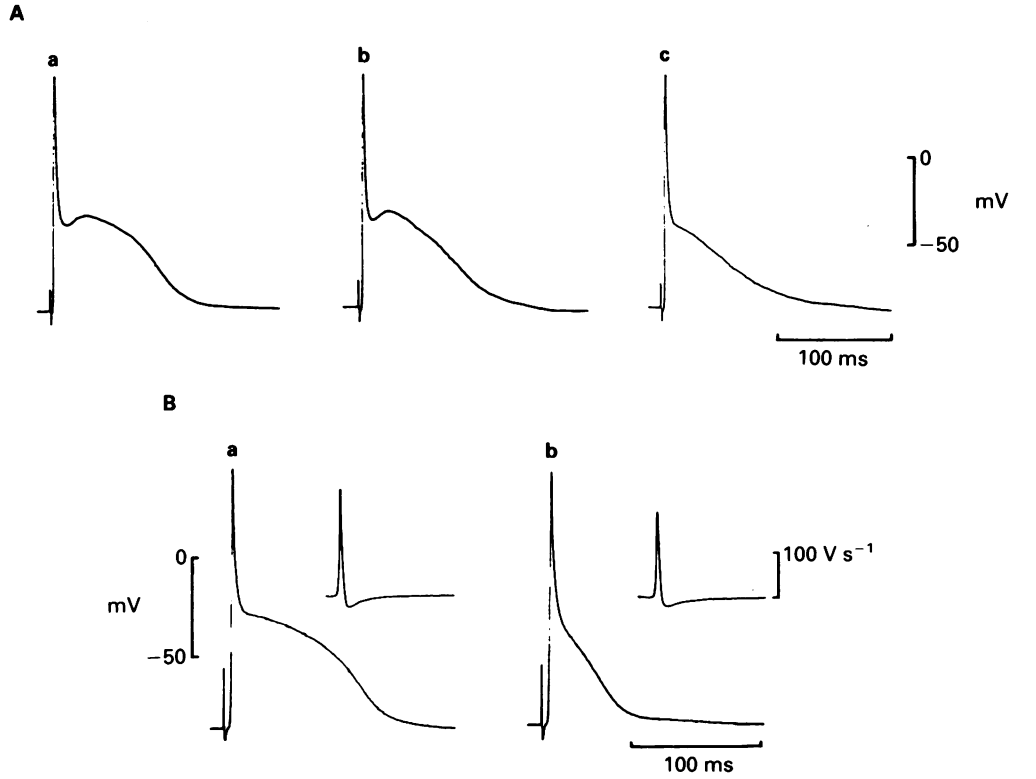

Figure 2 Effects of a high Ca and a low Na medium on the membrane action potential plateau. (A), (a) Control, (b) 6 min after exposure to 6 mM Ca^{2+}-containing medium, (c) 6 min after exposure to 8.2 mM Ca^{2+}-containing medium. (B), (a) Control, (b) 13 min after exposure to a low sodium medium (24 mM). The inset traces in (B) show the maximum upstroke velocity of the action potential.

with previous results that in a high calcium medium, the rate of repolarization of APp tended to be accelerated (Kondo, 1986a). In addition, under these conditions, the contraction was not substantially affected (not shown).

Lowering the extracellular Na concentration to 24 mM, by replacing NaCl by lithium chloride, markedly inhibited APp (Figure 2B). However, the maximum upstroke velocity of the action potential was slightly reduced by this procedure. This may be due to the replacement by lithium which can carry the inward current through the fast Na channels. Under this condition, the contraction was hardly affected (not shown). Na replacement by choline caused similar results, but the maximum upstroke velocity of the action potential was markedly decreased (not shown).

Effect of tetrodotoxin

The involvement of the fast Na channels in the electrical response was examined in the presence of high concentrations of TTX (Figure 3). When TTX 10^{-5} M was applied to the preparations, the initial rapid phase of the action potential was markedly reduced and the maximum upstroke velocity of the action potential, used as a measure of the fast Na

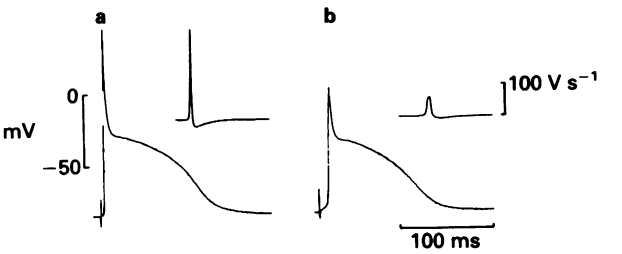

Figure 3 Effects of tetrodotoxin (TTX) on the membrane action potential. Each inset trace shows the maximum upstroke velocity of the action potential. (a) Control, (b) 13 min after application of TTX (10^{-5} M).

current, was decreased to about 16% of control. However, TTX also had little effect on the APp and the contraction was only slightly affected (not shown).

Discussion

In mammalian ventricular muscles a plateau depolarization of the action potential is generated by the following inward currents: the slow Ca inward current, the residual fast Na inward current during its inactivation process (Isenberg & Ravens, 1984), and TTX-sensitive Na window current (Attwell *et al.*, 1979). The present study indicated that Ca antagonists, nifedipine and nitrendipine, slightly affected the action potential plateau of the preparations from hibernating animals, suggesting less contribution of the slow Ca inward current to this action potential plateau. This is in good agreement with my previous suggestion that a flow of the slow inward current through Ca channels does not play a major role in regulating the plateau potential of hibernating animal preparations (Kondo, 1986a,b) Thus, the slow Ca inward current is unlikely to contribute to the present action potential plateau. The involvement of either the delayed inactivation of the fast Na current or the Na window current in this electrical response may also be eliminated by the following results. (1) The action potential plateau of this preparation was unaffected in the presence of TTX, an agent known to block the fast Na channel in an excitable cell membrane (Narahashi, 1974; Carmeliet & Vereecke, 1979). (2) Na replacement by lithium which can substitute for sodium as a charge carrier through the fast Na channels markedly suppressed the plateau potential in spite of only slightly affecting the maximum upstroke velocity of the action potential (used as a measure of the fast Na current). These results suggest that the action potential plateau of the present preparations is mediated by a process different from the fast Na and the slow Ca inward currents.

Recently, it has been shown that a Na-Ca exchange current, which is secondarily activated by intracellular Ca, plays a role in membrane depolarization (Mullins, 1979; Reeves & Hale, 1984; Kimura *et al.*, 1986; Mechmann & Pott, 1986). Na-Ca exchange inward current is stimulated by a transient rise in internal Ca caused by Ca release from internal stores (Mullins, 1979; Mechmann & Pott, 1986), resulting from Ca moving out of the cell in exchange for external Na (Reeves & Hale, 1984). Since this exchange current is driven by the concentration gradients of both Na and Ca, either increasing extracellular Ca or decreasing extracellular Na concentrations may reduce the inward current through Na-Ca exchange stimulated by the increased intracellular Ca. This results in an attenuation of the membrane depolarization. In the present experiments, ryanodine, an internal Ca release inhibitor (Sutko & Willerson, 1980; Sutko & Kenyon, 1983; Wier *et al.*, 1983; Marban & Wier, 1985) completely abolished the action potential plateau and the contraction of hibernating animal preparations. Either increasing extracellular Ca or decreasing extracellular Na concentrations also markedly inhibited this action potential plateau. Thus, the characteristics of the present action potential plateau are most probably due to a Na-Ca exchange current stimulated by internal Ca release.

To explain the transient membrane depolarization due to Ca release from internal stores, an alternative inward current carried through non-specific surface membrane ion channels has been suggested (Lederer & Tsien, 1976; Kass *et al.*, 1978; Colquhoun *et al.*, 1981). From the results of the present study, this inward current may not be the major mechanism in the genesis of the action potential plateau of the present preparations. The activation of this inward current could not account for the marked inhibition of the electrical response by increasing extracellular Ca concentration. However, further studies are necessary to elucidate precisely the involvement of this mechanism.

Based on the above discussion, it seems reasonable to conclude that the electrical response of hibernating animal myocardium linked to internal Ca release may be mediated by the stimulation of a Na-Ca exchange mechanism. This is not peculiar to the present preparations, since similar electrical activity has been demonstrated in rat cardiac muscles (Mitchell *et al.*, 1984a,b). In these studies by Mitchell *et al.* (1984a,b), it has also been suggested that the inward current, activated by internal calcium, may play a role in the plateau of action potentials in rat, guinea-pig and, perhaps, other mammalian ventricular muscles. The clear distinction of this inward current from other inward currents, such as the slow Ca and the fast Na inward currents, may be important when studying the role of this electrical change in cardiac function and the effects of various drugs on internal Ca release.

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