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SPECIAL REPORT

Ca^{2+} release from isolated sarcoplasmic reticulum of guinea-pig psoas muscle induced by K^+-channel blockers

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A Ca^{2+}-sensitive electrode was used to measure the Ca^{2+} concentration of the medium containing the heavy fraction of the fragmented sarcoplasmic reticulum (SR) prepared from guinea-pig psoas muscle. Among K^+-channel blockers tested, 4-aminopyridine (4-AP), tetraethylammonium (TEA) and charybdotoxin elicited Ca^{2+} release from the SR, but apamin and glibenclamide did not. These results suggest that a reduction of SR K^+ conductance leads to Ca^{2+} release from the SR.

Keywords: K^+-channel blocker; 4-aminopyridine; tetraethylammonium; charybdotoxin; apamin; glibenclamide; Ca^{2+}-release; sarcoplasmic reticulum; guinea-pig skeletal muscle

Introduction Striated muscle contraction is initiated by a rapid release of Ca^{2+} through Ca^{2+} channels on the sarcoplasmic reticulum (SR) membrane (Endo, 1985). The SR also possesses K^+ channels which it has been suggested form a counter-ion pathway during Ca^{2+} movement upon contraction, following extensive studies using planar phospholipid bilayer membranes (Miller *et al.*, 1984). In the present experiments, we investigated the effects of various potassium channel blockers on Ca^{2+}-filled fragmented SR prepared from guinea-pig psoas muscle to determine if a relationship exists between K^+ and Ca^{2+} channels on the SR membrane.

Methods The heavy fraction of the fragmented SR was prepared by the modified method of Kim *et al.* (1983). Male guinea-pigs (about 400 g) were stunned and exsanguinated. The psoas muscle (a white muscle) was isolated and homogenized. The homogenate in 5 volumes of 5 mM Tris-maleate (pH 7.0) was fractionated by differential centrifugation. The SR heavy fraction remaining in the supernatant after centrifugation at 5000 *g* for 5 min were pelleted at 12000 *g* for 30 min. The pellet was washed with a buffer containing 0.1 M KCl and 5 mM Tris-maleate by centrifugation at 70000 *g* for 30 min. The heavy fraction of the fragmented SR was stored at 0°C and used within 3 days.

A Ca^{2+}-sensitive electrode was used to measure the Ca^{2+} concentration of the medium containing the fragmented SR as described elsewhere (Kobayashi *et al.*, 1988). The electrode showed Nernstian responsiveness (slope, 27–29 mV/pCa unit) in the calibration solutions between pCa 3 to 6.5 with Ca^{2+} and ethylene glycol-*bis*(β-aminoethylether)-N,N,N,N-tetraacetic acid (EGTA). The time for 90% response was approximately 0.6 s when pCa was lowered from 6 to 4. For the assay, $CaCl_2$ 50 μM, fragmented SR 0.5–1 mg protein ml^{-1}, phosphocreatine 5 mM and ATP 1 mM plus creatine kinase 0.1 mg ml^{-1} were successively added to the basic solution of the following composition (mM): KCl 100, $MgCl_2$ 0.5 and 4-morpholinepropane sulphonic acid (MOPS) 50 (pH 7.0, 30°C). After Ca^{2+} in the medium was taken up by the SR, various drugs were administered (number of experiments, at least 3). The Ca^{2+} concentration in the nominally Ca^{2+}-free solution was estimated to be 1.28 ± 0.21 μM (mean ± s.e. mean, $n = 6$).

To make the Ca^{2+}-sensitive electrode, a small amount of the Ca^{2+} sensor, a mixture of Ca^{2+}-cocktail (Fluka 21048, Switzerland) and polyvinyl chloride in tetrahydrofuran, was attached at the tip of a polyethylene pipette (C20, Gilson, France) ~0.3 mm in diameter. Then, the pipette was filled with an internal solution of the following composition (mM): $CaCl_2$ 5, EGTA 5, $MgCl_2$ 5, KCl 100 and MOPS 50 (pH 7.0). The reference electrode (glass pipette) was filled with 1.5% agar in the internal solution without $CaCl_2$ and EGTA. The electrodes were fed into a pH meter (model GT-01, Mitsubishi Kasei, Tokyo).

Drugs used were: 4-aminopyridine and tetraethylammonium chloride (Tokyo Kasei, Tokyo), caffeine (Wako Pure Chemicals, Tokyo), apamin and glibenclamide (Sigma, St Louis, MO). Charybdotoxin was synthesized as reported elsewhere (Lambert *et al.*, 1990). Cromakalim and nicorandil were generous gifts from Beecham Pharmaceuticals, Surrey, U.K. and Chugai Pharmaceutical Co., Tokyo, Japan, respectively.

Results The effects of various K^+-channel blockers on the SR prepared from the guinea-pig psoas muscle were investigated by directly monitoring the Ca^{2+} concentration in the medium with a Ca^{2+}-sensitive electrode (Figure 1). The prompt reduction and the subsequent gradual decrease in Ca^{2+} concentration after addition of ATP are due to the formation of the Ca-ATP complex and active Ca^{2+} uptake by SR, respectively. The early part of the Ca^{2+} uptake was almost linear in an antilogarithmic plot (data not shown). When the Ca^{2+} concentration was reduced to submicromolar levels, the rate of Ca^{2+} uptake decreased. Addition of caffeine (0.5 mM), which is known to release Ca^{2+} from SR (Endo, 1985), elicited a transient increase in the Ca^{2+} concentration of the medium (Figure 1), indicating Ca^{2+} release from SR followed by reuptake, as reported previously (Kobayashi *et al.*, 1988). Addition of 4-AP (1 mM), TEA (10 mM) or charybdotoxin (1 μM) elicited a similar increase in the Ca^{2+} concentration of the medium. High concentrations of 4-AP (10 mM) and TEA (30 mM) prolonged the duration of the increase in the Ca^{2+}-concentration of the medium. 4-AP (10 mM) caused oscillation of the Ca^{2+}-level to occur. Lower concentrations of 4-AP (less than 0.3 mM) and TEA (3 mM) did not release Ca^{2+} from the fragmented SR. Apamin (1 μM) and glibenclamide (10 μM) did not elicit Ca^{2+} release from the fragmented SR. The presence of cromakalim or nicorandil (both 10 μM), so-called K^+-channel activators (Weston & Abbot, 1987), did not elicit Ca^{2+} release or affect the Ca^{2+} release induced by 4-AP, TEA, charybdotoxin or caffeine (data not shown).

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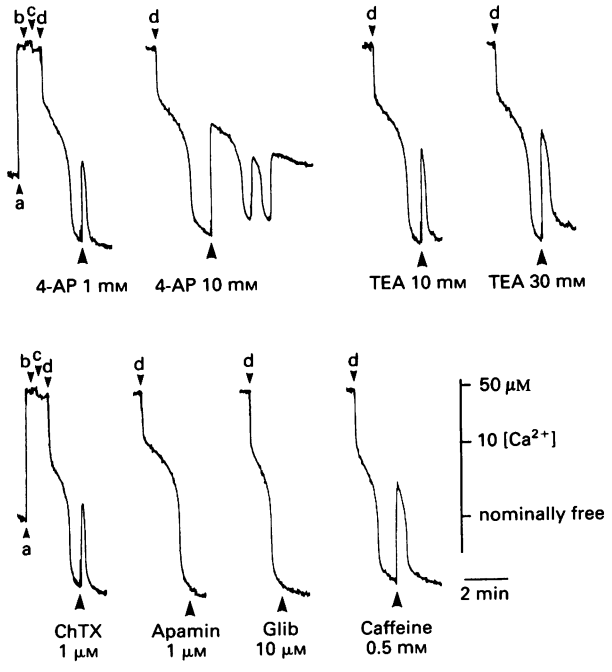

Figure 1 Effects of various K^+-channel blockers and caffeine on the Ca^{2+}-filled sarcoplasmic reticulum (SR) prepared from guinea-pig psoas muscle. Following accumulation of Ca^{2+} from the medium by the SR, 4-aminopyridine (4-AP), tetraethylammonium (TEA), charybdotoxin (ChTX), apamin, glibenclamide (Glib) and caffeine were administered. The following additions were made: (a) $CaCl_2$; (b) fragmented SR; (c) phosphocreatine and (d) ATP and creatine kinase. Calibration of the Ca^{2+} concentration of the medium is shown in the logarithmic scale on the right of the lower panel. The level before addition of Ca^{2+} represents the nominally Ca^{2+}-free level (1.25 μM).

Discussion TEA has been reported to be able to impede the K^+ conductance of channels when applied to the inside of the lipid bilayer, presumably representing the inside membrane of the SR (Miller *et al.*, 1984). Thus, one might speculate that TEA as well as 4-AP, although administered to the cytosolic side in the present experiments, could gain entry to the SR and by an action on the inner membrane elicit Ca^{2+} release. The large molecular size of charybdotoxin is likely to preclude its entry into the SR, but it too elicited Ca^{2+} release. It seems likely, therefore, that all three K^+ channel blockers can trigger the Ca^{2+} release from the SR by an action on the outer, cytosolic membrane of the SR. It is possible that K^+ channel blockade produces a depolarization of the SR membrane which leads to Ca^{2+} release (Endo, 1985), although the role of depolarization in SR is not yet fully understood.

Miller *et al.* (1985) reported that charybdotoxin, an inhibitor of the large-conductance Ca^{2+}-activated K^+ channel, had no effect on K^+ channels from mammalian SR. Presumably, the concentration of charybdotoxin used by these workers was inappropriately low (the highest concentration described was 1.3 $\mu g\ ml^{-1}$ of the partially purified toxin). The concentration of 1 μM used here was higher than that required to block K^+ channels on the plasma membrane, however, the same concentration of another peptide blocker, apamin, did not elicit Ca^{2+} release from SR, suggesting that the inhibitory effect of charybdotoxin is rather specific. Recently, Uehara *et al.* (1991) found a class of Ca^{2+}-activated K^+ channels in cardiac SR with a large conductance of 184 pS. This SR K^+ channel had properties distinct from other reported Ca^{2+}-activated K^+ channels on the plasma membrane with respect to the channel open time and voltage sensitivity. Thus, the need for a high concentration of charybdotoxin in our experiments may be because the K^+ channel in the SR is atypical.

In skeletal muscle, cromakalim was reported to open the ATP-sensitive K^+ channels of the plasma membrane (Spuler *et al.*, 1989). Glibenclamide is known to inhibit ATP-sensitive K^+ channels (Sturgess *et al.*, 1988). Neither cromakalim nor glibenclamide elicited Ca^{2+} release from the SR or affected the Ca^{2+} release induced by caffeine and the K^+ channel blockers described above. These results suggest that K^+ channels distinct from the ATP-sensitive K^+ channel play a substantial role in the regulation of Ca^{2+} movements in the SR.

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