DELAYED RECTIFICATION IN THE CARDIAC PURKINJE FIBER IS NOT ACTIVATED BY INTRACELLULAR CALCIUM

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ABSTRACT This study was designed to test the hypothesis that an outward current (I_x) responsible for action potential repolarization in the cardiac Purkinje fiber is activated by intracellular calcium (Ca_i). Pharmacological probes were combined with the measurement of membrane current and contractile activity under voltage clamp conditions. Experiments were designed to examine properties of I_x that have previously linked activation of this current to changes in Ca_i. The independence of I_x from Ca_i was demonstrated for each case tested. Thus, the results of these experiments support the view that I_x is not a calcium-activated current.

INTRODUCTION

Cation-selective ion channels are now known to exist in several different forms. Some are regulated by membrane potential, some by intracellular calcium, and some by chemical transmitters (for review see Latorre and Miller, 1983; Meech, 1978). Some cells contain only one class of cation channel, but, more often than not, cells contain a combination of these channels.

In the cardiac Purkinje fiber, repolarization of the action potential is initiated in part by the activation of an outward current carried largely by potassium ions and is referred to as the delayed rectifier, or I_x (Noble and Tsien, 1969). Some of the properties of I_x have led to its interpretation as a calcium-activated current (Salata and Jalife, 1982; Brown and DiFrancesco, 1980; Gelles and Aronson, 1977). The activation of I_x is preceeded by calcium influx through the calcium channel during depolarizing voltage steps (see Kass, 1983). Reduction of calcium current by several calcium channel blockers is accompanied by reduction of I_x (Kass and Tsien, 1975). Finally, catecholamine-induced increases in calcium channel current and contractile activity are parallelled by marked increases in I_x (Kass and Wiegers, 1982; Noma et al., 1980).

Evidence has also accumulated from previous studies to suggest that I_x activation does not rely on calcium entry (Kass, 1982*a*; DiFrancesco and McNaughton, 1979; Kass and Tsien, 1975), but this possibility has not been systematically addressed. This study combines pharmacological probes with measurement of contractile activity and membrane current to determine the role of Ca_i^{2+} in the activation of I_x. The results of these experiments provide strong evidence that I_x is not a calcium-activated current. Some of these results have been previously presented to the Biophysical Society (Kass, 1982*b*).

METHODS

Purkinje fiber strands were dissected from either ventricle of calf or dog hearts as previously described (Scheuer and Kass, 1983). Membrane current was recorded from shortened strands (0.5–1.5 mm) using a conventional two microelectrode voltage clamp arrangement with protocols that emphasize measurement of calcium current and I_x (Kass, 1982*a*). Contractile activity was monitored with an optical arrangement described in a previous study (Kass, 1981). Nisoldipine was dissolved in polyethyleneglycol 400 in a concentrated stock solution (10 m*M*), and aliquots of this stock were added to Tyrode's solution to obtain final concentrations. Experiments involving nisoldipine were carried out under subdued illumination (Kass, 1982*a*). All solutions were oxygenated with 100% O_2 and kept near 37°C. The modified Tyrode's solution consisted of (in millimoles per liter) 150 NaCl, 4 KCl, 5.4 $CaCl_2$, 0.5 $MgCl_2$, 5 glucose, and 10 Tris buffer (pH 7.4).

RESULTS

D600 Reduces I_x When Ca Channels Are Previously Blocked

When calcium channels are blocked by the organic compound D600, I_x tail currents are also reduced (Kass and Tsien, 1975). From this observation it is not clear whether this reduction of I_x is a direct action of the drug or an indirect consequence of the inhibition of calcium current by this compound.

In experiments desigend to distinguish between these possibilities another calcium channel blocker, nisoldipine, was first used to block calcium channels before applying

D600. Control experiments established that exposure to 10 μM nisoldipine alone completely blocked calcium channel current measured during test pulses applied from a −43 mV holding potential. Exposure to D600 after blocking calcium channels with nisoldipine produced no additional effects on calcium current because of the presence of nisoldipine, but did reduce the outward current tails due to I_x (Kass, 1982*a*; Figs. 5 and 9). Since calcium current was first blocked by nisoldipine, the reduction of the outward tails by this exposure to D600 must not depend on inhibition of Ca^{2+} influx by this drug.

I_x Persists When Contractile Activity is Abolished

The previous result showed that calcium influx is not necessary to activate I_x, but did not rule out the possibility that release of calcium from an intracellular store may be important for I_x activation. Fig. 1 shows the results of one experimental test for this possibility. In this experiment, contractile activity was monitored in addition to membrane current and was used as an assay for changes in intracellular calcium. As in the previous experiments, this preparation was first exposed to nisoldipine (10 μM) while depolarizing voltage steps were applied from a −40 mV holding potential. Although the drug blocked calcium channel current (compare Fig. 1 *A* inset current traces during applied voltage pulses), amplitudes of the outward current tails that follow the depolarizing pulses were not changed. The insensitivity of the I_x tails to calcium current blockade by nisoldipine in this experiment was confirmed over a broader range of voltage steps by measuring the 300-ms isochronal activation curve for I_x before and after application of this drug (curve, Fig. 1 *A*).

In the same experiment, contractile activation responded in a graded manner to depolarizing voltage pulses before exposing the fiber to nisoldipine (inset, Fig. 1 *B*, ○). The magnitude of the peak contractile response to each depolarization was measured and plotted against the test pulse voltage, and the curve that resulted (Fig. 1 *B*, ○) indicated that before applying the drug, the voltage dependence of contractile activation in this fiber was very similar to the voltage dependence of I_x activation. This similarity suggested a common underlying mechanism: namely, changes in Ca^{2+}_i. However, after application of nisoldipine, contractile activity, unlike I_x activation, was completely inhibited over all voltages examined. The results of this experiment make it unlikely that I_x activation depends on phasic changes in intracellular calcium.

It could be argued that nisoldipine blocked contractile activity, but not the intracellular release of calcium in this experiment. But this is not likely, since Morgan et al. (1983) have shown that calcium channel blockers inhibit cytoplasmic calcium transients as well as twitch tension in the dog Purkinje fiber.

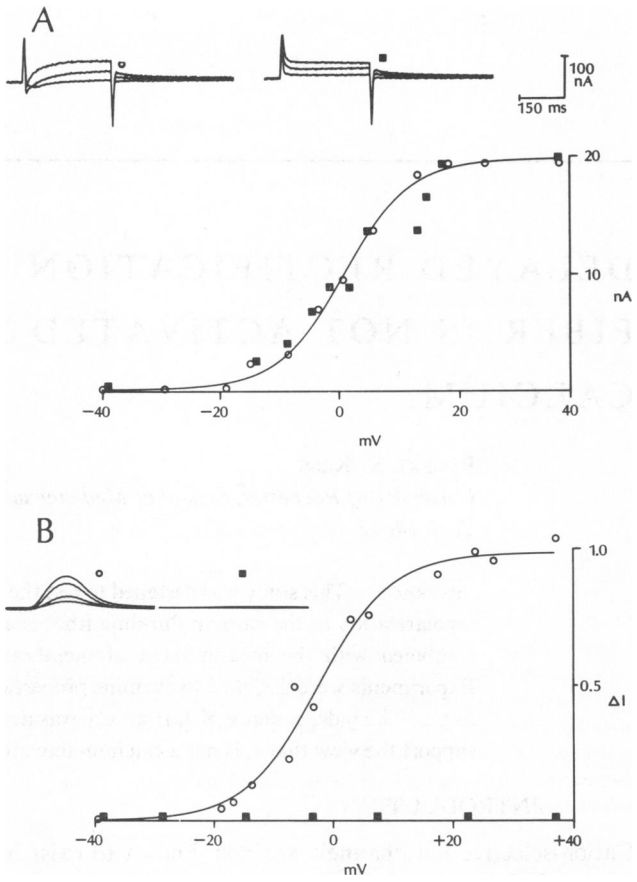

FIGURE 1 Nisoldipine blocks contractile activity without reducing I_x. (*A*) The insets show currents in response to voltage steps to −16, 0, and +10 mV in the absence (○) and presence (■) of nisoldipine (10 μM). Outward tail magnitudes measured after each voltage pulse are plotted against pulse magnitude in each solution. (*B*) Contractile activity was monitored by changes in detected light that had been focused onto the fiber (Kass, 1981). The inset shows the contractile response to voltage steps to −18, −7, 0, and +12 mV before (○) and after adding nisoldipine (10 μM) (■). The holding potential was −40 mV. Data obtained from preparation 206-1 in both panels.

Simultaneous measurements of changes in membrane current and contractile activity were also used to investigate the Ca_i-dependence of the response of I_x to norepinephrine. In this experiment nisoldipine was again used to block calcium current and the twitch, and, as in the previous experiments, I_x was not suppressed under these conditions (Fig. 2). The fiber was then exposed to a high concentration of norepinephrine (1 μM). Calcium current and contractile activity were not affected, but I_x was increased (Fig. 2). Thus, I_x can be modulated by this neurohormone under conditions when neither calcium influx nor contractile activity is affected.

In summary, each of these experiments has been designed to examine a different property of I_x in the Purkinje fiber that has linked this current to changes in Ca_i and suggested that it is a Ca_i^{2+}-activated current. In each case pharmacological interventions were used to dissociate

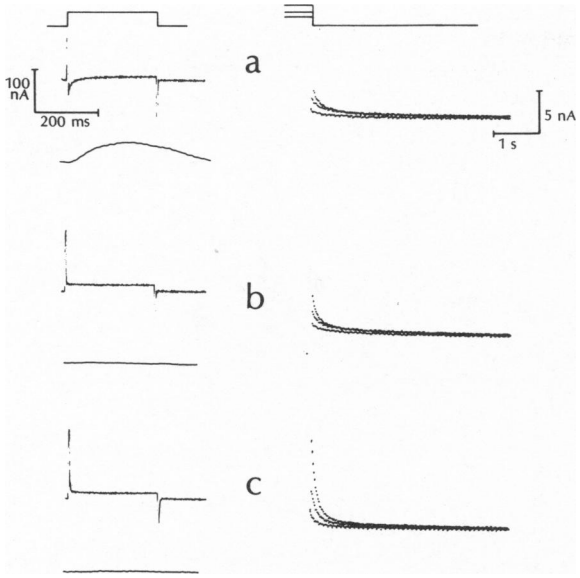

FIGURE 2 Norepinephrine increases I_x when contractile activity and calcium current (I_{si}) are inhibited by nisoldipine (10 μM). Contractile responses and membrane current recorded during depolarizing voltage steps to 0 mV are shown on the left. The right shows outward current tails after 1-s voltage steps to −15, −2, and +22 mV. Records are shown in control condition (*a*), in the presence of nisoldipine (10 μM) (*b*), and in the presence of nisoldipine (10 μM) plus norepinephrine (1 μM) (*c*). Holding potential was −50 mV. Preparation 208-1.

changes in the delayed rectifier from changes in calcium influx via calcium channel current or from changes in contractile activity. Although the possibility exists that very low background levels of Ca_i^{2+} or some membrane bound component of Ca_i^{2+} may affect I_x, this dissociation provides strong evidence that I_x is not a calcium-activated current.

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