LARGE CONDUCTANCE CA\textsuperscript{++}-ACTIVATED K\textsuperscript{+} CHANNELS IN SMOOTH MUSCLE CELL MEMBRANE

Reduction in Unitary Currents Due to Internal Na\textsuperscript{+} Ions

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To circumvent the many problems associated with recording and analyzing transmembrane ionic currents in syncytial tissue preparations, we have used a preparation of freshly dissociated, single-vertebrate smooth muscle cells for electrophysiological studies. Early in the course of these experiments on these cells the presence of a Ca\textsuperscript{++}-activated K\textsuperscript{+} conductance became apparent (11,13). In subsequent studies of macroscopic currents with conventional two-microelectrode voltage-clamp techniques, we found that this conductance gave rise to a large peak outward current whose magnitude depended on the amplitude of the preceding inward calcium current (14). To characterize this conductance further, we have recently examined single-channel currents in these smooth muscle cells using the patch-clamp technique (12,15). In these studies a channel with a large unitary conductance (~250 pS in symmetric 130 mM KCl) has been identified which resembles in its major features the Ca\textsuperscript{++}-activated K\textsuperscript{+} channel originally reported by Marty in cultured chromaffin cells (8) and subsequently found in a number of other preparations (2,7,10,16). We report here a characteristic of this channel in the smooth muscle cells, the reduction in the unitary current at positive potentials in the presence of internal Na\textsuperscript{+} ions, a property it shares with the large conductance Ca\textsuperscript{++}-activated K\textsuperscript{+} channels of cultured chromaffin cells (9) and apparently with the delayed rectifier channels in axons (3–5).

METHODS

Smooth muscle cells were enzymatically dissociated from the stomach muscularis of the toad Bufo marinus by minor modifications of the technique originally developed by Bagby and his collaborators (1). The cells were used on the same day as isolated to avoid long-term changes that might occur in culture. We used a List EPC-5 patch clamp and standard single-channel recording techniques (6), except that patch pipettes were usually not coated with insulator. Recordings were filtered at a high-frequency cutoff of 1 kHz. The studies were in the main carried out using inside-out excised patches whose inner surfaces were perfused with various solutions by a modification of the method originally described by Yellen (17). In this modification the perfusion pipettes have a larger tip diameter (150–200 µm) so that the recording pipette
RESULTS

Identification of the channel was made as follows (12, 15). First, it was shown with excised patches that the probability of the channel being open increased when \([Ca^{++}]\), was raised or when, at a constant \([Ca^{++}]\), the patch membrane potential was made more positive (that is, the curve relating the probability of the channel being open to the membrane potential is shifted along the voltage axis to negative potentials as \([Ca^{++}]\) is raised). Then, the zero current or reversal level for the unitary (i.e., single open-channel) current was shown to follow the Nernst relationship for a pure \(K^+\) electrode. One such demonstration of the \(K^+\) selectivity of the channel is shown in Fig. 1.

At potentials more positive than those shown in Fig. 1, it was noted that in the presence of lower \([K^+]\), and higher \([Na^+]\), the unitary current did not increase with potential, but declined. That is, a negative slope was observed in the \(I-V\) relationship at very positive potentials. This behavior, because it was observed in the presence of higher \([Na^+]\), was reminiscent of the interference by internal \(Na^+\) ions with the flow of \(K^+\) ions through the delayed rectifier in axons (3–5). Because it has been demonstrated that such interference is relieved by elevated \([K^+]_o\) (4), we undertook another set of experiments to examine this phenomenon, but with lower \([K^+]_o\). Results are shown in Figs. 2 and 3. Over the range of potentials investigated the negative slope conductance is evident in the presence of \([Na^+]\), but not in its absence. This observation may partially explain the inflection or decline in the whole cell macroscopic peak outward current seen in the \(I-V\) relationship at positive potentials under voltage clamp (14).

Marty (9) has recently reported similar behavior for \(Ca^{++}\)-activated \(K^+\) channels in cultured chromaffin cells. In that report, where the high frequency cutoff for recordings was 5 kHz, it was possible to discern a marked increase in open channel noise or flickering in the presence of \([Na^+]\); this was attributed to blocking by \(Na^+\) ions. We did not observe such flickering, but in light of the general similarity of the two channels this is likely due to the greater filtering (1 kHz high frequency cutoff) of our recordings.

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REFERENCES


TWO TYPES OF VOLTAGE DEPENDENT NA CHANNELS SUGGESTED BY DIFFERENTIAL SENSITIVITY OF SINGLE CHANNELS TO TETRODOTOXIN

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Tetrodotoxin (TTX) can reduce the upstroke velocity and decrease the duration of the plateau phase of myocardial action potentials. In fact, the plateau duration of canine Purkinje fibers is more sensitive to TTX than is the upstroke (Coraboeuf et al., 1979), suggesting that cardiac Na current has either a slowly inactivating component or a steady-state noninactivating voltage-dependent Na-leak component, and that the sensitivity to TTX of the channels involved in upstroke production may be different from that of the channels conducting current during the plateau phase (termed “window current”). We examined these hypotheses with single-channel Na currents elicited during voltage clamp by isolated cultured chick ventricular myocytes. We asked: (a) During sustained depolarization to potentials in the range of the action potential plateau voltage, do Na channels open and conduct TTX-sensitive current? If so, then (b) do the channels that carry current during sustained depolarization eventually fail to open (i.e., slowly inactivate), or does their probability of opening remain >0 despite sustained depolarization to voltages at which “window current” is elicited? (c) Are there any electrophysiologically definable differences between the channels that open early on during voltage clamp to the “window” level and those that open 20 or more ms later? And finally, (d) is the sensitivity to TTX of the early opening channels different from that of the later-opening channels?

METHODS

Voltage-dependent single Na-channels having short opening latencies (<20 ms) after the onset of depolarizing voltage-clamp steps were compared with those opening with long latencies (20–160 ms). We used outside-out membrane patches isolated from cultured cardiac ventricular myocytes obtained from 11-d old chick embryos. Myocytes were dispersed from the ventricles of 6–10 embryos by gentle agitation in a 0.25% trypsin solution, as described by Lehmkuhl and Sperelakis (1963). Harvested myocytes suspended in culture medium were transferred to 2-cm glass coverslips, placed in covered culture dishes; for 2–5 d at 37°C; then placed in a shallow chamber mounted on the moveable stage of an inverted microscope and perfused with a conventional HEPES-buffered physiological extracellular solution maintained at constant temperature (± 0.3°C) between 13° and 14°C. The composition of the extracellular solution was NaCl 135 mM, KCl 4 mM, CaCl2 1.8 mM, MgCl2 1.2 mM, HEPES 20 mM, Glucose 11 mM; the pH was adjusted to 7.30 ± 0.05.

Elementary single-channel TTX-sensitive Na currents were recorded during voltage clamp using the gigahm-seal technique of Hamill et al. (1981). Heat-polished machine-pulled glass pipettes having inside diameters of ~0.3 μm and DC resistances of 5–20 MΩ when filled with “internal solution” were used. The composition of the internal solution was K+ 100 mM, Na+ 1.0 mM, HEPES 20, EGTA 20, glutamate 54, sucrose ~100 (used to adjust osmolarity to 340 mosmoles) and pH was adjusted with KOH to 7.2 ± 0.05.

Isolated outside-out membrane patches were obtained by a technique similar to that used by Horn and Patlak, 1980. After good seal (2–20 GΩ)