POTASSIUM CHANNEL "INACTIVATION" INDUCED BY SOFT-GLASS PATCH PIPETTES

GABRIEL COTA AND CLAY M. ARMSTRONG
Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6085

ABSTRACT We have studied the potassium currents of rat pituitary pars intermedia cells kept in primary culture using whole-cell recording with patch pipettes. The potassium current recorded with hard-glass pipettes is mainly carried by voltage-dependent channels that show slow inactivation in the presence of 0.5 mM internal EGTA. Fast "inactivation" of the potassium current is seen with patch pipettes fabricated from soft glass (soda glass or potash lead glass), and is probably caused by block of the potassium channels by di- or multivalent cations released from the glass.

INTRODUCTION

Choosing the glass to make patch pipettes for single-channel or whole-cell recording with the patch clamp technique (Hamill et al., 1981) is not usually a simple matter. Consideration of the thermal, electrical, and sealing properties of glasses commonly dictates the selection (Corey and Stevens, 1983; Sakmann and Neher, 1983; Rae and Levis, 1984). We present here results that call attention to an additional property of glasses that is important for patch clamping: chemical inertness. Specifically, we have studied the whole-cell potassium currents of cultured pituitary cells, and found that currents rapidly "inactivate" when certain types of soft glass (soda glass or Corning No. 8161 [Gardner Glass, Claremont, CA]) are used to fabricate the patch pipette and the EGTA concentration in the internal solution is low (0.5 mM). Rapid "inactivation" is not observed with high (10 or 20 mM) internal EGTA or with borosilicate glass pipettes. We propose that the soft glasses used release water-soluble components, probably di- or multivalent cations, which affect the functional properties of the potassium channels under study.

METHODS

Whole-cell potassium currents were recorded at 20°–21°C from pituitary pars intermedia cells (PI) using patch pipettes. PI cells were dissociated from adult rat neurointermediate lobes and maintained for 2–10 d in primary culture before the experiments. The techniques used to obtain and culture PI cells and to perform electrical recordings were similar to those described previously (Cota, 1986).

Patch pipettes were fabricated from one of the following glasses: (*a*) Hard, borosilicate glass (KIMAX-51; Kimble Div., Owens-Illinois, Inc., Toledo, OH); (*b*) soft, soda glass (VWR hematocrit tubing, blue band; VWR Scientific Div., Univar, San Francisco, CA), and (*c*) soft, potash lead glass (Corning No. 8161; Gardner Glass). The electrodes had a resistance of 0.8–1.2 MΩ.

The external recording solution contained 135 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 1–5 μM tetrodotoxin to block sodium channel activity, and 10 mM Hepes-NaOH (pH 7.30). The internal solution contained, in mM: 0.5, 10, or 20 EGTA-KOH, 20 KF, 20 KCl, 70–110 K-glutamate, 2 $MgCl_2$, and 10 or 20 Hepes-KOH. The pH of the internal solutions was adjusted to either 7.30 (solutions with 0.5 or 10 mM EGTA and 10 mM Hepes) or to 8.20 (solution with 20 mM EGTA and 20 mM Hepes). It should be recalled that the Ca-buffering capacity of EGTA-containing solutions can be increased by raising either the EGTA concentration or the pH (see Marty and Neher, 1985). The calculated reversal potential for potassium ions was close to −90 mV.

Outwards currents were absent when all internal K was replaced by Cs and *N*-methylglucamine. The remaining current was an inward calcium current that had a maximum amplitude of 60 pA, on average, at +20 mV (see Cota, 1986). This corresponds to 5% or less of the magnitude of the steady outward potassium current measured at the same voltage in different cells.

RESULTS AND DISCUSSION

Examples of potassium outward currents recorded from PI cells using hard-glass pipettes are shown in Fig. 1. Channel opening was induced by voltages steps to +60 mV applied from two different holding potentials. Potassium current turns on with a sigmoidal rise (this is better resolved using a faster sampling rate) reaches a maximum value and then tends to decay slowly as a function of time. Records in Fig. 1 *A* were obtained in the presence of 0.5 mM EGTA in the internal solution. The change in holding potential from −80 to −40 mV reduces the magnitude of the outward current by ~20% without modifying its time course. Thus,

Dr. Cota's present address is Dept. of Physiology, CINVESTAV-IPN, AP 14-740, Mexico, D.F. 07000, Mexico.

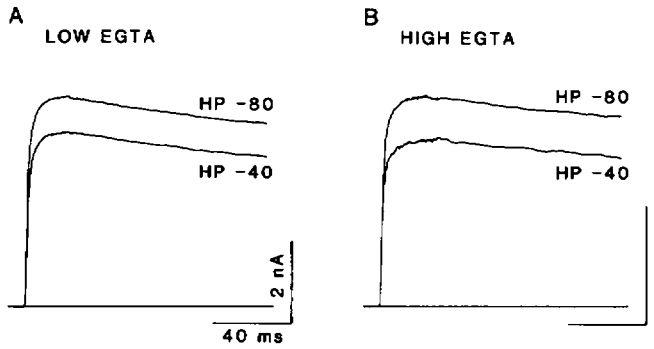

FIGURE 1 Potassium outward currents recorded with hard-glass pipettes. The traces are the current during a pulse from the holding potential (HP, indicated by numbers in millivolts) to +60 mV, obtained in the presence of 0.5 mM EGTA (*A*), or 20 mM EGTA (*B*) in the internal solution. In this and in subsequent figure legends, *T* corresponds to the age of the culture, and *t* is the time elapsed since breaking into a cell with the patch electrode. (*A*) Cell 14J12186; $T = 2$ d; $t = 2.0$–3.2 min. (*B*) Cell 13J12186; $T = 2$ d; $t = 2.2$–3.5 min.

unlike GH3 cells (May and Oxford, 1986; Cota, G., unpublished results) and other pituitary pars distalis cells (Lingle et al., 1986; Marchetti et al., 1987), a transient, I_A-like current is not a major component of potassium current in PI cells. In addition, the time course of the outward current as well as its voltage dependence are practically independent on the internal EGTA concentration (0.5, 10, or 20 mM), which suggests a very minor calcium-dependent component. For instance, records in Fig. 1 *B* were obtained from a different PI cell with high internal EGTA (20 mM). The change in holding potential modifies the magnitude of the long-lasting outward current with no alteration of time course, in a way similar to that observed in Fig. 1 *A*.

Fig. 2 presents potassium currents recorded with soft-glass pipettes. The pipettes were fabricated either from soda glass (*upper panels*) or from Corning No. 8161 glass (*lower panels*). With 0.5 mM EGTA in the internal solution (Fig. 2 *A*), the current induced by depolarization to +60 mV from a holding potential of −90 mV is somewhat less maintained than with hard glass. The decay of the current is much faster when the holding potential is −40 mV, even though the onset of the current seems to be normal. This is particularly pronounced with Corning No. 8161 glass. Raising the internal EGTA concentration to 10

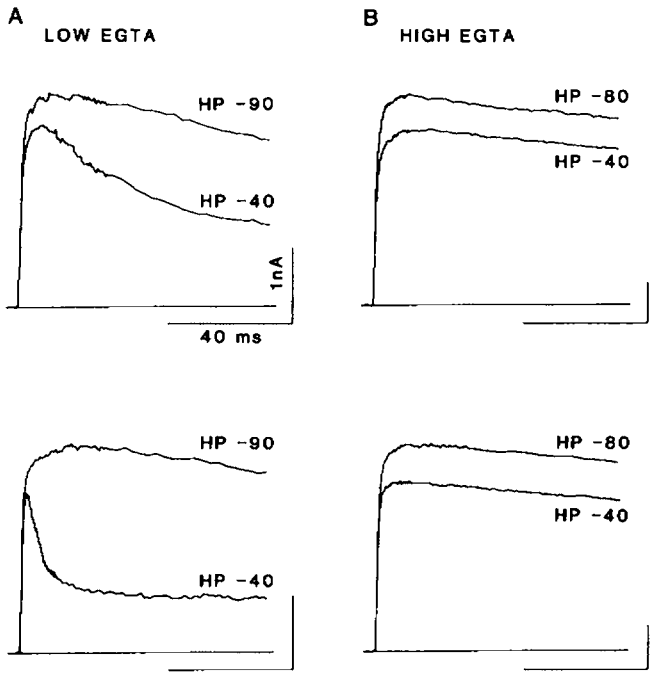

FIGURE 2 Experiments similar to those in Fig. 1, this time with soft-glass pipettes. The traces shown in the upper panels were obtained with pipettes fabricated from soda glass, and those in the lower panels with pipettes fabricated from Corning No. 8161 glass. (*A*) *Top*, cell 5Dc1685; $T = 6$ d; $t = 5.7$–8.5 min. *Bottom*, cell 1Ag0585; $T = 9$ d; $t = 2.7$–13.6 min. (*B*) *Top*, cell 4J12186; $T = 2$ d; $t = 7.0$–8.7 min. *Bottom*, cell 2J12186; $T = 2$ days; $t = 6.7$–11.4 min.

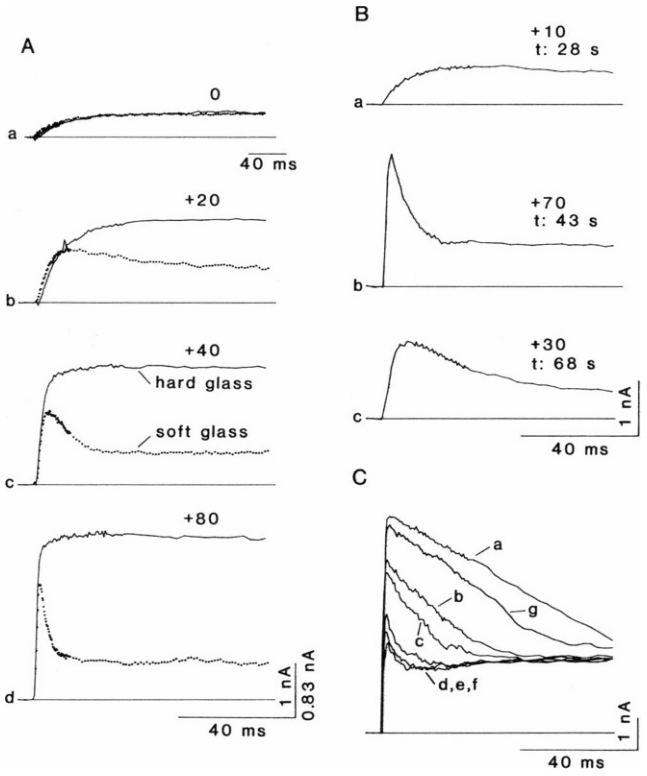

FIGURE 3 Potassium currents using Corning No. 8161 glass-pipettes, with 0.5 mM EGTA in the internal solution. (*A*) The current observed at various voltages (indicated by numbers, in millivolts) using a Corning No. 8161 glass-pipette (*dotted traces*) is compared with that recorded from a different cell using a pipette fabricated from hard glass (*continuous traces*). HP −40 mV in both cases. The traces obtained with the hard-glass pipette were scaled up by a factor of 1.2. The vertical bar represents 1 nA for the dotted traces, and 0.83 nA for the continuous ones. Hard-glass experiment: cell 2Sp0186, $T = 9$ d; $t = 9.6$–12.3 min. Soft-glass experiment: cell 1Ag0585; $T = 9$ d; $t = 6.8$–13.1 min. (*B*) Currents recorded in the sequence shown using a HP of −30 mV. Cell 11Dc1685; $T = 6$ d. (*C*) Currents during pulses to +100 mV from a HP of −80 mV (see text). There was a 390-s interval without pulses before trace *a*. Cell 5Sp1086; $T = 8$ d; $t = 28$–31 min.

or 20 mM prevents the fast decay of the outward current associated with soft-glass pipettes, as can be seen in Fig. 2 *B*.

Fig. 3 illustrates three more characteristics of the "inactivation" of the potassium current observed with Corning No. 8161-glass pipettes. (*a*) Above +20 mV the rate of decay of the current increases steeply with voltage, as shown in Fig. 3 *A*; (*b*) the soft-glass effect is rapid: it can be observed within 1 min after breaking into the cell with the patch pipette (Fig. 3 *B*); and (*c*) clear cumulative effects are observed by rapid pulsing to voltages of +40 mV or more. Fig. 3 *C* shows such cumulative effects during a series of pulses to +100 mV applied from a holding potential of −80 mV. Pulsing every 20 s progressively reduces the peak amplitude of the current and increases its rate of decay (traces *a–c*). Following trace *c*, a more severe "inactivation" is observed on reducing the interval between steps to 5–6 s (traces *d–f*). Recovery is still incomplete after a 60-s interval (trace *g*). In contrast, a wait of 5 s between pulses was generally enough to induce reproducible currents when recording with hard-glass pipettes.

The results suggest very strongly that soft-glass pipettes can release water-soluble components that have the ability to block open potassium channels, causing rapid "inactivation" of the potassium current. Such glass components are probably di- or multivalent cations. This is supported by the following: (*a*) Increasing the current flowing through the pipette into the cell by using relatively positive holding potentials makes "inactivation" more pronounced, which suggests the iontophoretic application of a blocker cation inside the cell (Fig. 2 *A*). (*b*) High internal EGTA eliminates fast "inactivation" (Fig. 2 *B*). (*c*) The "inactivation" process is strongly voltage-dependent and full recovery from "inactivation" appears to be slow (Fig. 3, *A* and *C*). These characteristics resemble the blocking of potassium channels by internally applied barium ions in squid giant axons (Armstrong and Taylor, 1980; Armstrong et al., 1982). (*d*) Several divalent cations, including calcium, magnesium, and lead, are commonly present in ordinary soda glasses (Corey and Stevens, 1983), and Corning No. 8161 glass contains a high amount of lead (51% PbO; Corning Glass Works, Corning, NY).

A study of the effect of lead ions and other di- and multivalent cations, added to the internal medium, on the potassium currents recorded with hard-glass pipettes may help to decide whether this view is correct or not. It is nevertheless clear that diffusible components of the glass used to make the patch pipette should be considered as a possible source of artifact during interpretation of patch-clamp data.

Received for publication 14 September 1987.

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