Potassium channel activity from bovine chromaffin granules.

Renata Hordejuk[a], Adam Szewczyk[b] and Krzysztof Dolowy[a]

a. Department of Biophysics, Agriculture University SGGW, Warszawa.
b. Laboratory of Intracellular Ion Channels.
Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland.

Correspondence: Dr. Renata Hordejuk, Department of Biophysics, Agriculture University SGGW, 159 Nowoursynowska St., 02-776 Warszawa, Poland
Phone: 48-504242684; **Fax:** 48-226-491676; **Email:** hordejuk@delta.sggw.waw.pl

Cell Biology of the Chromaffin Cell
R. Borges & L. Gandía Eds.
Instituto Teófilo Hernando, Spain, 2004

We characterized the large conductance potassium channel present in chromaffin granule membranes and the effect of pH on its kinetic. Granule membrane "ghosts" were incorporated into black lipid membrane (BLM) made of asolectin. In 450 / 150 mM KCl gradient the current / voltage relationship appeared to be linear for membrane potentials between -70 mV and +70 mV with conductance of ~360 pS. The effect of pH on the potassium channel was examined. After lowering pH value to pH = 6.4 from the intravesicular face potassium channel activity was blocked. Results show that K^+ channel with large conductance can play an important role in acidification of chromaffin granules interior by V-ATPase.

RESULTS AND DISCUSSION

The chromaffin granule membranes were added to the *trans*-side chamber. Upon reconstitution the potassium current flowing through membrane channels was observed. The conductance of the channel was determined on 360 ± 7 pS for 450 mM / 150 mM KCl and 432 ± 9 pS in symmetric 450 mM KCl, both in pH 7.0. Close and open- time distributions were voltage dependent. The channel activity was blocked by TEA^+ applied to the *cis* side (data not shown).

Figure 1A shows channel recordings in asymmetric 450 mM / 150 mM KCl at holding potential of 30 mV in control and after lowering pH to 6.4 from the *cis* side. KCl solution pH value in the *trans*-side chamber was always equal pH = 7.0. Traces 1 to 5 in the Figure 1B were measured at various pH of experimental solution of both sides of the planar lipid membrane as marked on the Figure 1. On the right side of each current trace the distribution of single channel current amplitude is presented. Lowering the pH from the *cis* side results in diminishing of both the open probability and the current amplitude. Only small changes of single channel current amplitude were observed when pH = 6.4 was applied to the *trans*-side chamber.

Our results indicate that measured channel activity was strongly inhibited by low pH applied to the *cis* side. Inhibition of K^+ channel by pH lowering was observed from the same side as TEA^+ inhibition. It was earlier shown that chromaffin granule K^+ channel is blocked by TEA^+ from intragranular side[1]. Thus, it seems that lowering pH in the intragranular side blocks the channel activity. Findings of the present study may be important for understanding physiological role of potassium conductance in chromaffin granules.

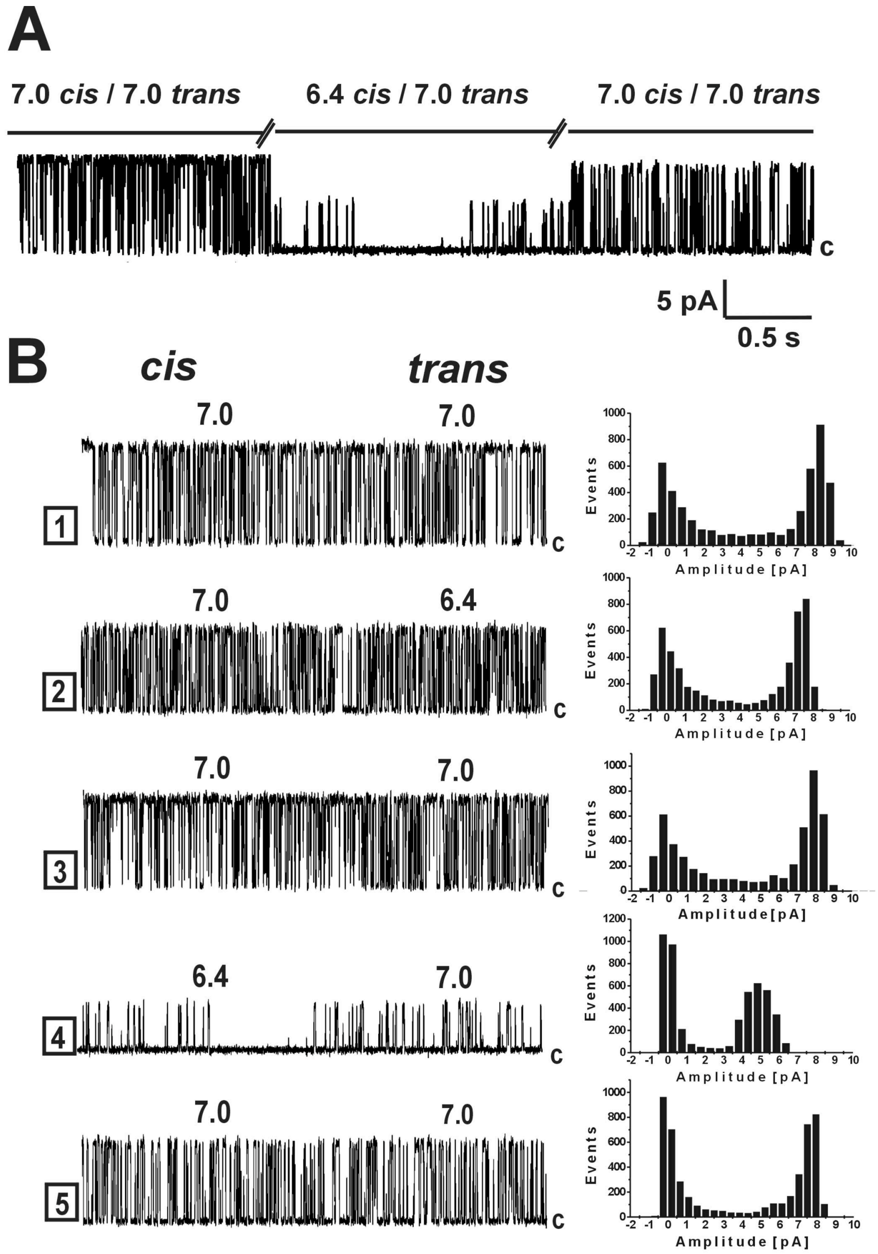

Figure 1. Regulation of large conductance chromaffin granules potassium channel by pH. Single channel recordings at a holding potential of 30 mV from "ghost" membranes incorporated into BLM in asymmetric 450 / 150 mM KCl. The closed levels, corresponding to currents though the lipid bilayer are indicated with **c**. **A** Changes in channel kinetic after changing pH from the *cis* side only. **B** Traces 1 to 5 measured at various pH of experimental solution of both sides of lipid bilayer as marked on the figure. All recordings filtered at 200 Hz. On the right side of each trace the distribution of single channel current amplitude is shown.

The chromaffin granule membranes contain a vacuolar-type (V-type) V-ATPase, which generates an electrochemical proton gradient, acidifying the granule interior. Potassium channel can play physiological role by compensating electric charge produced by the V-ATPase[1]. This enables the formation of a membrane potential (□□) and □pH, sufficient to drive catecholamine uptake into the chromaffin granules. This hypothesis is also supported by the experiments on the effects of intra-granular cation composition on ATP-dependent acidification of chromaffin granules[1]. In fact, a much higher □pH was observed with K^+ inside than with TEA^+ [1]. Our present observation on the pH-dependence of K^+ transport points to the fact that low pH should block this "charge compensation" mechanism. Blockage of K^+ channels by low pH would block further acidification of chromaffin granules. This channel would play a role of protective fuse to diminish over-acidification of granular lumen.

REFERENCES

1. Ashley, R.H., et al., *Evidence for a K^+ channel in bovine chromaffin granule membranes: single-channel properties and possible bioenergetics significance.* Eur Biophys J, 1994. **23**:263-275.