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Sensitivity of high-conductance potassium channels in synaptosomal membranes from the rat brain to intracellular pH

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Abstract. High-conductance potassium channels have been studied in inside-out patches excised from proteoliposomes reconstituted from giant liposomes and rat brain synaptosomes. Acid pH in the medium reduced single channel current amplitude and increased the mean open probability and the frequency of channel opening. This was accompanied by a shortening of the open time constant at positive potential and by shortening of the longer closed time constant. The decrease of channel amplitude, the increase of the open probability and the decrease in the longer closed time constant can be explained by neutralization of negative charges of the membrane and by a decrease in the surface membrane potential which mimics membrane depolarization. The shortening of the mean open time is apparently due to a channel blockade by protons.

Key words: Synaptosomal membrane – K^+ channels – pH – Surface potential – Reconstituted proteoliposomes

Introduction

There are several types of potassium channels (K^+ channels) in excitable membranes which play an essential role in the maintenance of the resting membrane potential, in the repolarization of the cell during action potentials and in the modulation of neurotransmitter release (Kolb 1990). Some of these membrane properties are sensitive to a physiologically relevant increase in cytoplasmic H^+ concentration (Moody 1984). For example, it has been well documented that transmitter release from the motor nerve terminal is enhanced by low intraterminal pH (Anderson et al. 1983; Edwards et al. 1985) as well as by inhibition of nerve terminal K^+ channels (Kumamoto and Kuba 1985; Glavinovic 1987). It would be of interest to know whether the effect of low pH on nerve terminal K^+ channels may help to explain the H^+ enhancement of transmitter release.

There are only a few reports characterizing single K^+ channels in the nerve terminal because the small size of this membrane eliminates the possibility of direct recording. In rat brain synaptosomes incorporated into the planar bilayer, four different K^+ channels with unit conductance ranging from 8 to 40 pS (Nelson et al. 1983) and several types of Ca^{2+}-activated K^+ channels of large unitary conductance 200–250 pS (Farley and Rudy 1988; Reinhart et al. 1989; Nomura et al. 1990) have been found. In giant proteoliposomes from rat brain synaptosomes, three K^+ channels which differ in their conductance ranging from 15 to 91 pS were detected (Hirashima and Kirino 1988). Data concerning the effect of intracellular pH on single K^+ channel activity in the nerve terminal are lacking.

We report here the effect of pH on high-conductance potassium channels in synaptosomal membranes from the rat brain incorporated into giant liposomes. The standard patch clamp technique in an inside-out configuration was used to follow the amplitude and kinetics of single channel currents.

Materials and methods

Preparation of synaptosomes

Synaptosomes were prepared from cerebral cortices of adult male rat according to the method of Jones and Matus (1974). During homogenization of the nervous tissue in a solution of 37 mM succrose/10 mM TRIS HCl (pH 7.3), presynaptic nerve terminals were observed to form sealed structures, synaptosomes, which were then isolated by centrifugal density gradient separation. The synaptosomes thus obtained were suspended in a buffer of 150 mM KCl/10 mM HEPES-KOH (pH 7.3) and stored frozen at −80 °C.

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Preparation of liposomes

Liposomes were prepared according to the method of Correa and Andrew (1988) so that 7 mg pure phosphatidylethanolamine (Avanti Polar Lipids) and 3 mg phospatidylserine (Sigma) from bovine heart and brain were mixed, dissolved in 100 µl of ether and carefully dried in a stream of N_2. The dry lipids were hydrated by 200 µl of 0.1 mM EGTA/10 mM HEPES, pH 7.3 buffer. After intensive vortexing, the sample was sonicated to clarity for about 4 min. Ionic strength was raised by adding 400 µl of 450 mM KCl/0.1 M EGTA/10 mM HEPES, pH 7.3 to a final concentration of 350 mM KCl. The mixture was sonicated for 10 s and used as the stock solution of liposomes.

Reconstitution of proteoliposomes

Giant proteoliposomes were prepared by the freezing-thawing procedure (Hirashima and Kirino 1988; Correa and Agnew 1988). Synaptosomes and liposomes were mixed at room temperature in the ratio 9:1. The mixture was divided into 50 µl plastic tubes and frozen slowly (−2 °C/min) in a −20 °C freezer and after 20 min placed into a −55 °C freezer. Before each experiment one of these tubes was thawed by the reverse process: placed first in a −20 °C freezer for 20 min and then exposed to room temperature. To create expanded structures, a 5 µl suspension of proteoliposomes was smoothly mixed with 200 µl of 140 mM KCl/10 mM HEPES/3 mM EGTA, pH 7.3. After swelling (15 min), 5 µl of this suspension was placed in the centre of a 35 mm plastic tissue culture dish containing 2 ml of the bath solution (see later). Vesicles of approximately 10–50 µm in diameter were allowed to settle to the bottom of the dish before starting electrophysiological recordings. Vesicles were visualized with an inverted microscope (magnification 312.5 ×).

Single channel recording

Single channel currents from inside-out patches were recorded using a standard patch-clamp technique (Hamill et al. 1981). In all experiments, the pipettes with an outer tip diameter of about 2 µm were filled with a solution containing 120 mM KCl, 10 mM HEPES and 1 mM $CaCl_2$, pH 7.3 The proteoliposomes were initially placed in a NaCl-saline solution containing 140 mM NaCl, 10 mM HEPES and 0.1 mM $CaCl_2$ adjusted to pH 7.3. Relatively high amounts of calcium in the bath solution were used in order to facilitate seal formation (Correa and Agnew 1988). The effect of pH was studied after substituting 140 mM KCl for 140 mM NaCl in the bath. The required pH was obtained with KOH and HCl. Different pH solutions were applied into the bath using a slow perfusing system. Single channel currents were recorded 5–10 min after exchange of the solution.

Data analysis

Data were stored in digitized form on a videocasette using a modified digital-audio processor (Sony PCM-501 ES, 20 kHz) and analysed using the TL-1-125 program FETCHEX (Axon Instruments, Foster City, CA, USA). Computer digitization was carried out at a sampling rate of 10 kHz after filtering with an analogue filter (Bessel, 8 pole low pass 1.2 kHz −3 dB). Digital data were analyzed using program FETCHAN and pSTAT (version 5.5.1). The transition between the open and closed state was defined using the half-amplitude threshold-crossing method. Closings shorter than 140 µs (0.5 filter rise time, T_r) and openings shorter than 0.69 ms (2.5 T_r) were excluded from the analysis. Single channel current amplitude was obtained by approximation of the amplitude histogram with a sum of Gaussian curves.

Results

Excised inside-out patches from reconstituted proteoliposomes were obtained at 0 mV with 140 mM NaCl in the bath and 120 mM KCl in the pipette. Single K^+ currents were observed in 38% of patches (59 patches out of 151) and the current-voltage relationship was used to determine the reversal potential (E_{rev}) of the channel. The ratio of the channel permeability for K^+ and Na^+ ($p_K:p_{Na}$) was calculated from the value of E_{rev} according to the equation derived from the Goldmann-Hodgkin-Katz equation:

$$E_{rev} = \frac{RT}{zF} \ln \frac{p_K[K^+]}{p_{Na}[Na^+]}$$

where R = gas constant, T = absolute temperature, z = ion valency, F = Faraday's constant. The effect of a pH change from 8 or 7.3 to 5.5 was studied in channels having a conductance of 158 ± 25 pS which showed a selectivity ratio $p_K:p_{Na} = 7 \pm 3$ ($n = 6$). Experiments with pH were performed with 140 mM KCl solution in the bath.

The variation of unitary current amplitude with pH is shown in Fig. 1A. It has been found that the single channel current amplitude at +40 mV was reduced by 12% with a change of pH 7.3 to pH 6.5 and by 30% with a change to pH 5.5. An increase of 24% was observed at pH 8. Practically no changes in current amplitude with pH were observed at negative potentials. The voltage-current curves (Fig. 1B) were not well fitted by the Goldman-Hodgkin-Katz equation unless a variable potassium permeability (p) and an inner surface potential (Φ) were used (Adrian 1969):

$$I = \frac{p(E+\Phi)z^2F^2}{RT} \cdot \frac{C_I \exp(zFE/RT) - C_0}{\exp(zF(E+\Phi)/RT) - 1}$$

where E = membrane potential, C_I and C_0 = intracellular and extracellular potassium concentration. The calculated surface potential decreased by about 40 mV (Fig. 2A) and channel permeability increased by about 60% (Fig. 2B) at low pH 5.5. We assume that only the inner surface potential is changing as the pH of the extracel-

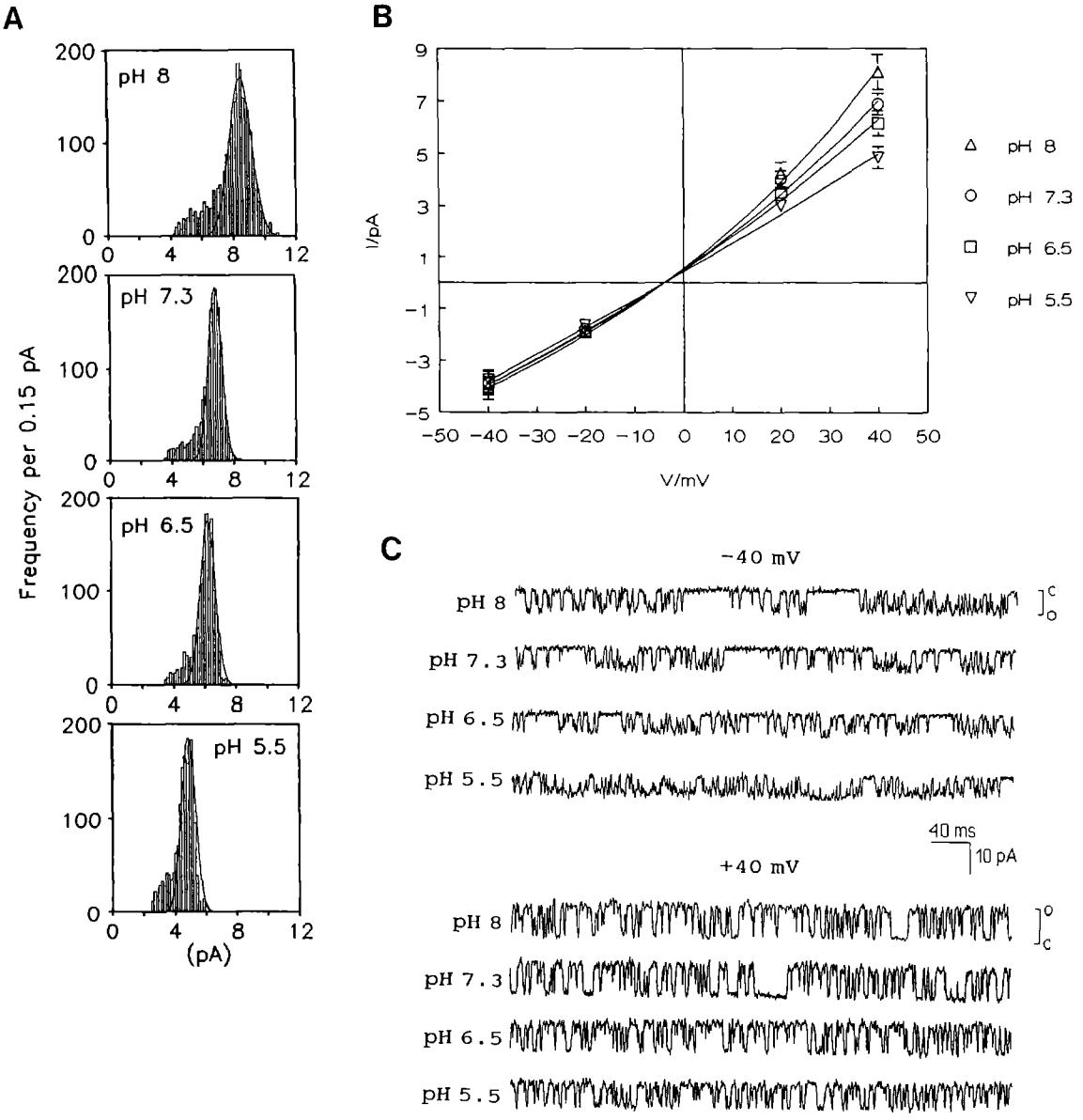

Fig. 1. **A** The amplitude histograms of the K^+ channel currents at +40 mV for different pH values in the bath. Each histogram was determined from a 10–20 s segment of the current record. **B** Current-voltage relationships for 3 channels at different pH. I–V curves were fitted to the Goldman-Hodgkin-Katz equation with variable K^+ permeability and inner surface potential (see results), mean value ± SD, the slope conductance at positive potentials is 160 ± 5 pS. **C** Single channel current traces for one inside-out patches from reconstituted proteoliposomes with 140 mM KCl in the bath and 120 mM KCl in the pipette. Membrane potentials are indicated with respect to the cell; *c = closed level, o = open level* of the channel

lular (pipette) solution is maintained at 7.3 by 10 mM HEPES. Changes in the surface potential were used to calculate the surface concentration of the cation (C_s) which is related to the bulk concentration (C_0) and surface potential by Boltzmann's factor $C_s = C_0 \exp -(z\Phi F/RT)$. It has been found that the concentration of cations near the inner surface of the membrane is reduced by about 72% at low pH (Fig. 2C).

Figure 3 shows the mean open probability (p_0) as a function of the membrane potential at different pH. At negative (hyperpolarizing) potentials, the mean open probability was lower and at positive (depolarizing) potentials the mean open probability was higher. This corresponds to an intact cell and indicates that in our system the synaptosomal membranes were incorporated with their "cytoplasmic" sides facing into liposomes. Acid pH 5.5 increased p_0 by about 30% at −40 mV and by about 20% at +40 mV.

The mean frequency of channel openings, which was almost identical at positive and at negative potentials at pH 7.3, increased at low pH 5.5 by 60% at −40 mV and by 32% at +40 mV (Fig. 4).

The increase of p_0 with H^+ concentration might be due to an increase of channel open time and/or to a decrease in channel closed time. The distribution histograms of the channel open times which fitted well with a single exponential (Fig. 5A) did not show any increase of the open time constant (τ_0) at negative potentials and

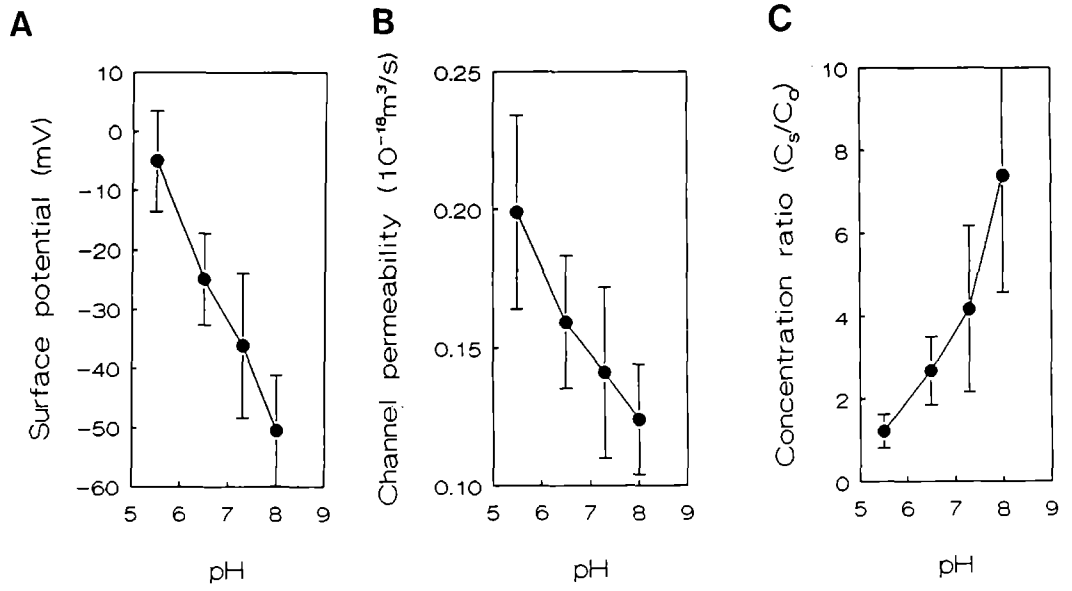

Fig. 2. The effect of pH on **A** surface potential, **B** channel permeability and **C** surface cation concentration ratio (ratio of surface concentration of cations C_s to the bulk concentration C_0). Values are given as the mean $\pm$SD

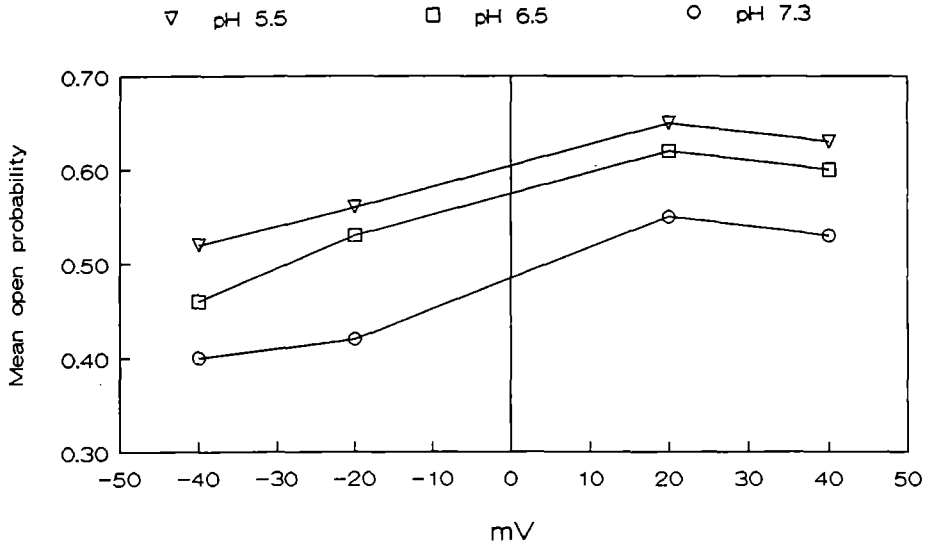

Fig. 3. The potential dependence of the mean open probability which was estimated by the ratio of total open time to total recording time (60 s) at different pH in 3 channels. Note the equivalence of the pH change from 7.3 to 5.5 to the effect of membrane depolarization from -40 to $+40$ mV

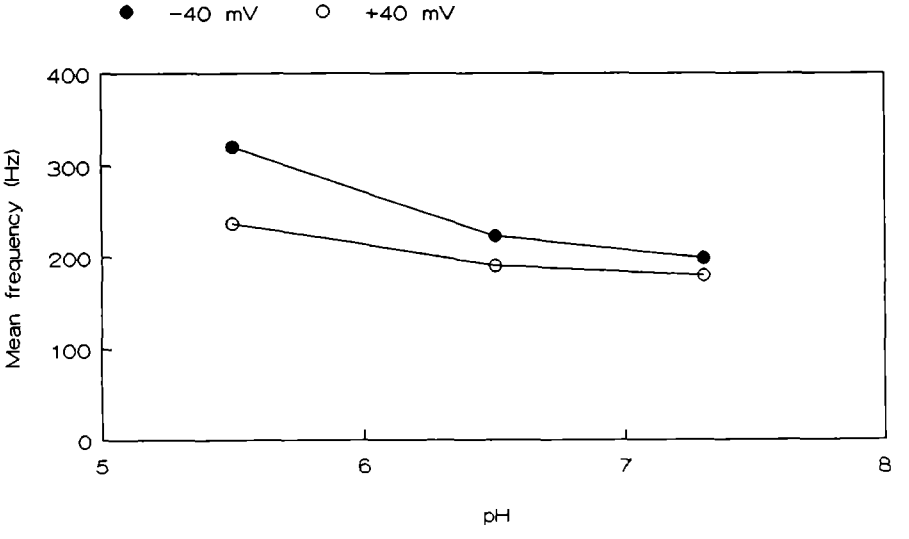

Fig. 4. The influence of pH on the frequency of channel opening at different pH. The membrane potential was held at -40 mV or at $+40$ mV

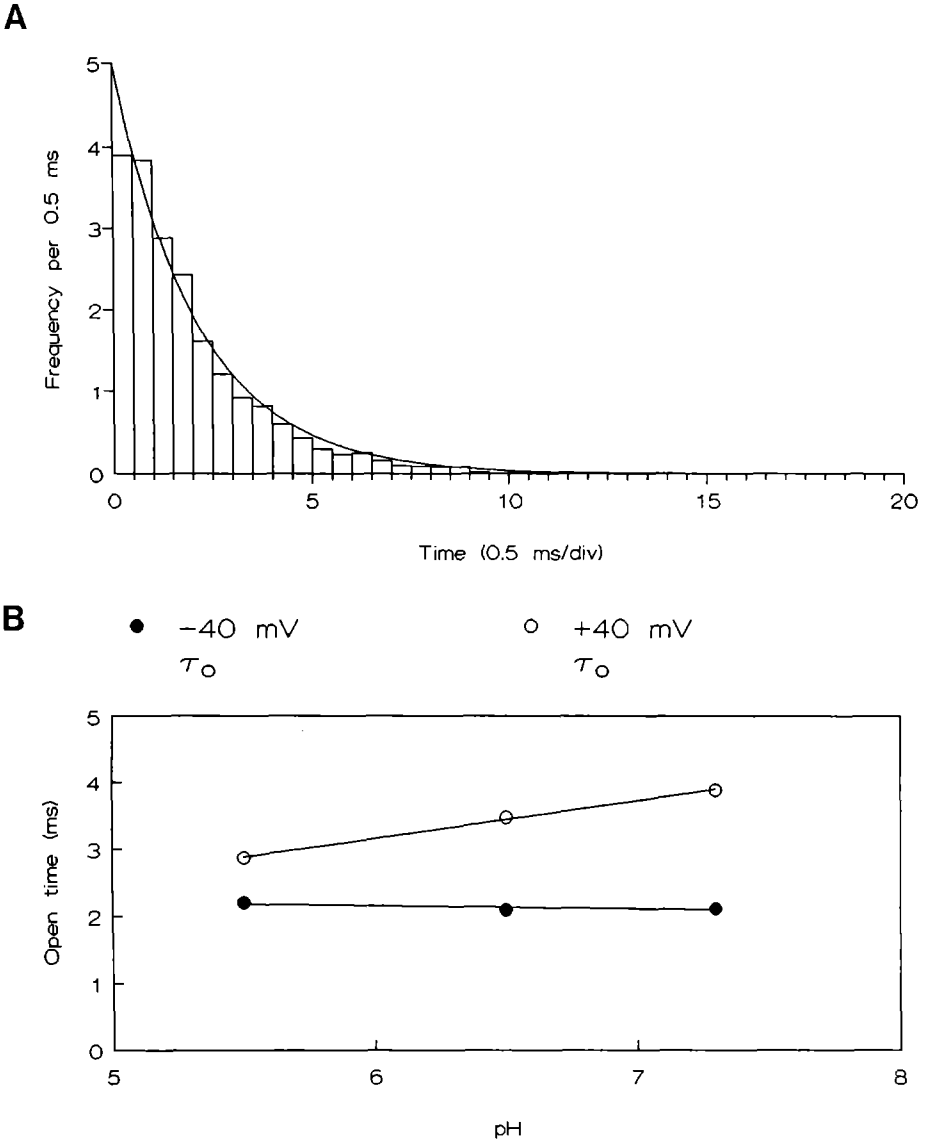

Fig. 5. **A** Example of a distribution histogram of the open times with a fitting curve at −40 mV at pH 7.3 Total number of openings in each sample ranged between 3081–5485. **B** The channel open time constant (τ_0) at different pH. The data are mean values for 3 channels measured at pH 7.3, 6.5 and 5.5. The membrane potential was held at +40 mV or at −40 mV

even showed a decrease (by 26%) at positive potentials (Fig. 5B).

The distribution histograms of closed times required two exponentials with time constants τ_{C1} and τ_{C2} for a good fit (Fig. 6A). The first (shorter) component did not change with pH (Fig. 6B) while the second (longer) component decreased at low pH both at positive potentials (by 42%) and at negative potentials (by 46%).

Discussion

In the present study we tested the effect of intracellular pH on the current amplitude and kinetic properties of large-conductance K^+ channels in rat brain synaptosomal membranes incorporated into giant liposomes.

Acid pH was found to decrease and alkaline pH to increase the amplitude of single currents. When we calculated parallel changes in the surface membrane potential and in the surface concentration of effective cations, in this case of K^+, we found that the decrease in the current amplitude might be explained by the neutralization of negative charges of membrane proteins and surrounding lipids and by the decreases of the transmembrane ionic gradient near the mouth of the channels as proposed by Kostyuk et al. (1982). The increase in the open probability of K^+ channels at acid pH also corresponds to a decrease of the surface potential because the mean open probability increases in control experiments when the membrane is depolarized and declines with hyperpolarization. We found two exponential functions in the histogram of the closed time distribution which indicate two closed states: the shorter one corresponding to flicker closures and the longer one corresponding to well-resolved closures (Colquhoun and Hawkes 1983; Christiensen and Zeuthen 1987). The flicker closed time was independent of pH, which has also been reported for the flicker component of Ca^{2+}-activated K^+ channels in the ventricular membrane of the choroid plexus epithelium (Christensen and Zeuthen 1987). On the other hand, the longer closed time which decreases with depolarization was found to be dependent on the pH with a decrease towards higher

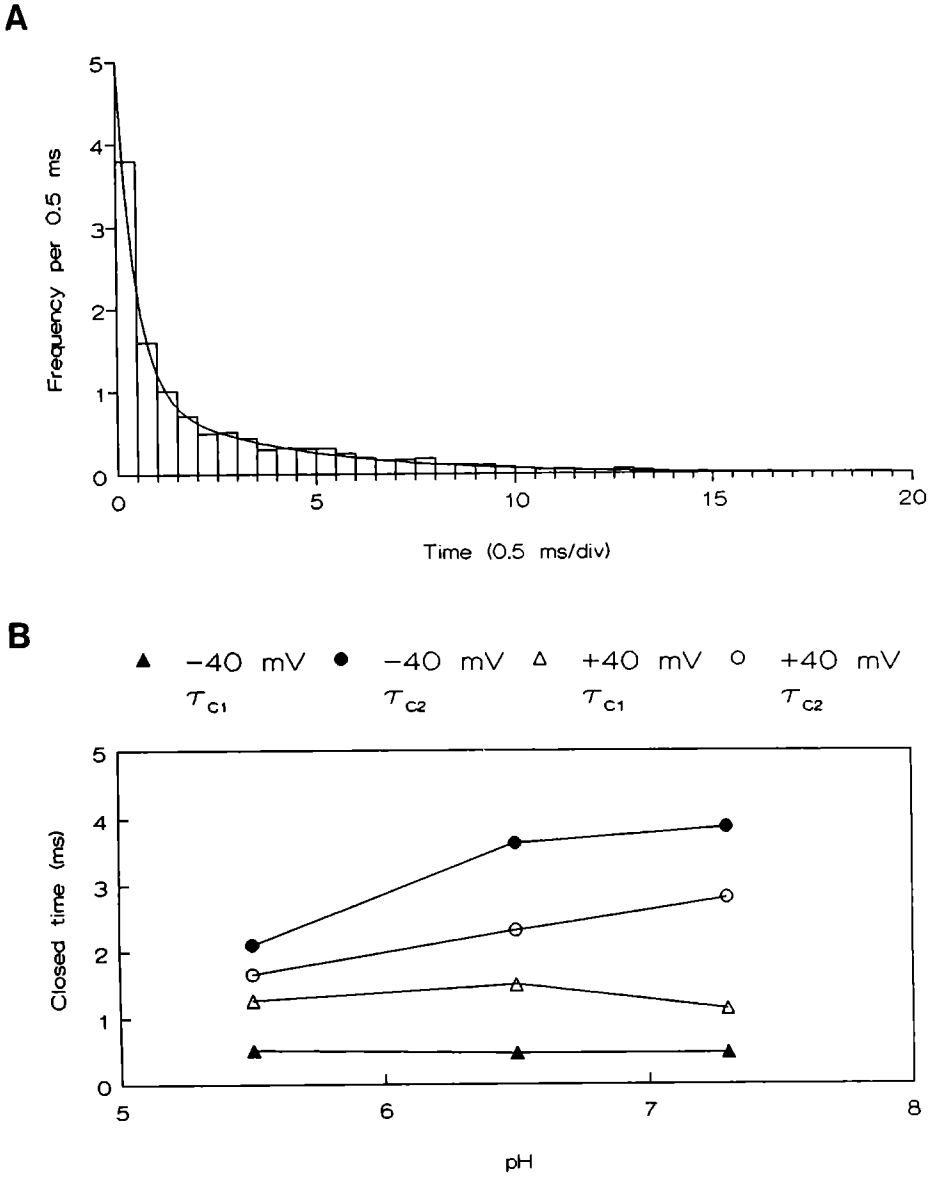

Fig. 6. **A** Example of a distribution histogram of the closed times fitted by two exponentials at −40 mV at pH 7.3. Total number of closing in each sample ranged between 3073–5484. **B** Two channel closed time constants (τ_{c1} and τ_{c2}) at different pH. The membrane potential was held at +40 mV and at −40 mV

acidity. In this case, the effect of acid pH also resembles that of depolarization and supports the surface charge hypothesis of the pH effect (Kostyuk et al. 1982).

The open time constant at low pH was only reduced at positive but not at negative potentials, indicating that this effect of pH cannot be simply explained by surface potential changes. At positive membrane voltages it is possible that the outward K^+ current is conducted through a channel which might be partially blocked by higher H^+ concentrations on the inner side of the membrane, while at negative potentials the inward current is not necessarily affected. Shortening of the mean open time due to the blocking action of H^+ has also been reported for Ca^{2+}-dependent K^+ channels (Kume et al. 1990; Laurido et al. 1991).

Under conditions when the open time constant is not changed and/or is reduced and the closed time is shortened at low pH, the increase of open probability might only be due to the increase in frequency of channel opening. We actually found that the frequency is increased at acid pH. This was not a depolarization-like effect and the only explanation is that H^+ binds to the channel to activate channel opening.

The mean open probability of our K^+ channels displays voltage-dependence characteristics of the Ca^{2+}-dependent K^+ channel (Barrett et al. 1982) or a delayed-rectifier channel (Reuter and Stevens 1980) which indicates that these channels could play a role in the maintenance of the membrane potential with a tendency to repolarize the membrane.

We found that intracellular H^+ ions produce two opposite effects on the voltage-dependent synaptosomal K^+ channels: they decrease the single current amplitude and the open time constant but increase the mean open probability of the channel. It is therefore difficult to predict, on the basis of contemporary knowledge, what is the effect of intracellular pH on K^+ permeability in intact nerve terminals unless the effect of intracellular pH on other types of K^+ channels in the nerve terminal is studied.

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