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RAPID PUBLICATION

Two Types of BK Channels in Immature Rat Neocortical Pyramidal Neurons

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SUMMARY AND CONCLUSIONS

1. The properties of large conductance Ca^{2+}-activated K^+ channels (BK channels) were investigated in neocortical infragranular pyramidal neurons by the use of inside-out patch recordings. Neurons were acutely isolated from slices of newborn to 28-day-old rats (*P0–P28*) by using minimal protease exposure followed by trituration with a vibrating glass probe. Two types of BK channels, slow-gating and fast-gating, were observed in immature neurons (*P0–P5*), whereas only slow-gating BK were found in more mature neurons. Fast-gating BK channels differed in conductance, voltage dependence, and kinetics from the slow-gating ones.

2. The properties of fast-gating channels included a conductance of 145 ± 12.9 (SE) pS; frequent openings with short mean open times that were relatively voltage-independent, mean closed times that showed a voltage-dependent increase, a voltage-dependent decrease in open probability (P_o). The properties of slow-gating channels contrasted with those of the fast-gating ones, in that the former had a conductance of 181 ± 3.9 pS, longer mean open times that showed a voltage-dependent increase, mean closed times that showed a marked voltage-dependent decrease, a voltage-dependent increase in P_o, and slight inward rectification. The significance of these developmental variations in channel properties is discussed.

INTRODUCTION

Ca^{2+}-activated K^+ channels (K_{Ca}) are widely distributed in neurons, muscle, and gland cells (Blatz and Magleby 1987; Latorre et al. 1989; Marty 1989). One such channel, the large conductance K_{Ca} channel (BK), is ubiquitous in a variety of cells including neurons. Evidence suggests that there are subtypes of BK channels. For example, studies in which rat brain plasma membrane vesicles were incorporated into planar lipid bilayers demonstrated that two types of BK channels with different gating rates and sensitivities to charybdotoxin were present (Reinhart et al. 1989). Further, alternatively spliced channel variants expressed in oocytes were also functionally heterogeneous with different conductances, kinetics, and Ca^{2+}-sensitivities (Lagrutta et al. 1994). The differential expression of subtypes of BK channels could be one factor that contributes to the different electrophysiological properties of neurons in mature and immature animals (Kriegstein et al. 1987; McCormick and Prince 1987). In the present study, we investigated BK channels in deep layer rat neocortical pyramidal neurons that were isolated from brain slices by the use of a vibrating glass microprobe (Vorobjev et al. 1991) after minimal exposure to proteolytic enzymes. We found that there were two types of BK channels in immature neurons from animals <6 days of age (<*P6*), fast gating and slow gating, whereas only slow-gating BK channels were present in more mature neurons. Both fast-gating and slow-gating BK channels were selectively permeable to K^+ and activated by intracellular Ca^{2+}. Fast-gating channels showed kinetic and voltage-dependent properties that were opposite to those of slow-gating channels.

METHODS

Neurons were isolated by the use of previously described techniques (Kang et al. 1996). Brain slices (300 μm thick) were cut on a vibratome, and those containing sensorimotor cortex were incubated at 37°C for 20 min in a piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) buffer solution to which 0.10–0.15 mg/mL protease XXIII (Sigma) and 0.10–0.15 mg/mL collagenase had been added (pH adjusted to 7.2 with NaOH). Neurons were isolated with a vibrodissociation device similar to that described previously by Vorobjev (1991). Single-channel current recordings were obtained at room temperature (21–22°C) with the use of the inside-out configuration of the patch-clamp technique (Hamill et al. 1981). Recording electrodes had resistances of 4–7 MΩ. The channel activity in inside-out patches was recorded during perfusion of the intracellular face of the patch with the intracellular solution containing different Ca^{2+} concentrations via a multitube system that allowed rapid changes in the bath solution. The extracellular solution (pipette solution for inside-out patch recordings) was the same intracellular solution, but with no added Ca^{2+}. Signals were filtered through an 8-pole Bessel low-pass filter with 1–2 kHz cutoff frequency. Membrane potentials are conventionally expressed and refer to the difference between the intracellular and extracellular side of the membrane. The intracellular solution contained (in mM) 130 KCl, 5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 2 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 4 dextrose (pH adjusted to 7.2 with KOH). Estimated final [K^+] was 140 mM after adding KOH to adjust pH. Calcium-free solution containing 5 mM EGTA was assumed to have 3–5 nM free [Ca^{2+}]. Free calcium levels in EGTA buffer were calculated on the basis of pH, [Mg], [Ca], and [EGTA]. Free [Ca^{2+}] of 0.05, 0.1, 0.5, 1, and 10 μM were obtained by adding a total of 0.86, 2.47, 2.50, 2.96, and 2.99 mM of $CaCl_2$, respectively, to a 5-mM EGTA and 2-mM Mg^{2+}-containing solution (Stockbridge 1987). Unitary current traces were sampled every 50 μs with PCLAMP6.0-Fetchan (Axon Instruments), and a 50% threshold criterion was used to determine the durations of open and closed events. Amplitudes of fast-gating channel openings were manually measured by cursors through relative long-lasting events.

RESULTS

Inside-out patch configurations were excised from neocortical pyramidal neurons, and when possible, neurons were

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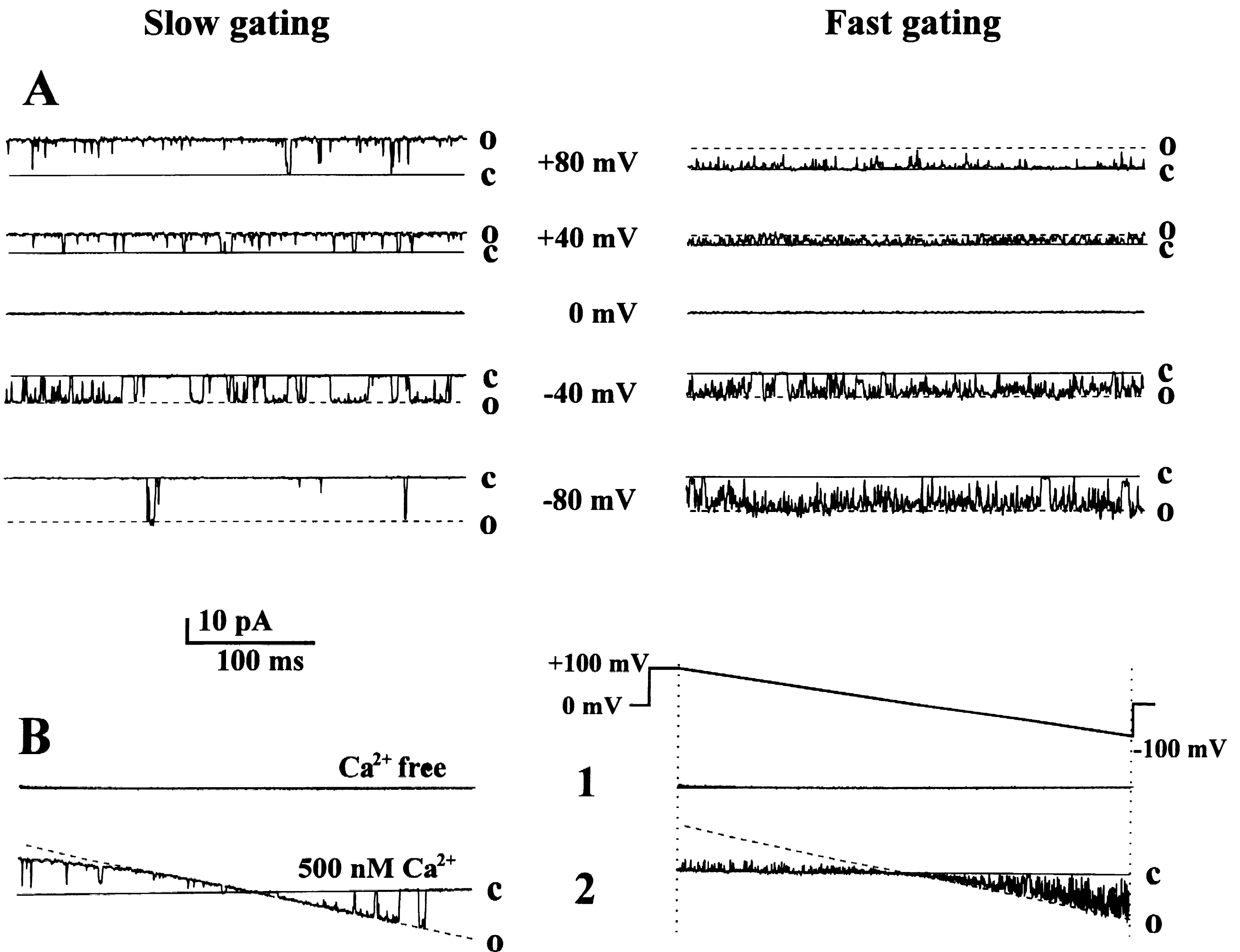

FIG. 1. Inside-out patch recordings of slow and fast channels from 1-day-old (*P1*) neurons. Voltage values refer to membrane potentials. *Left* and *right panels* in *A* and *B* show recordings from slow and fast channels, respectively. Solid lines indicate the channel closed state (c), and dashed lines show the open state (o). *A*: steady holding potentials with 0.5 μM Ca^{2+} in the bath solution. *B*: recordings with ramp voltage commands. Current segments were taken from the beginning to just before the end of voltage ramps as indicated by the vertical dotted lines in *B, right.* Current recordings and ramp commands are on the same time scale. The data were leak subtracted so that any deviation from the closed state (horizontal solid line) denotes channel openings. *B1*: Ca^{2+}-free solution. *B2*: 0.5 μM Ca^{2+}. V_h: 0 mV.

repatched and several patches were obtained from the same neuron. Three hundred ninety-six slow-gating (''slow'') and 9 fast-gating (''fast'') BK channels were observed in 218 inside-out patches excised from *P1–P28* neurons. The slow channel, which was the most prominent type in both mature and immature neurons, has properties similar to those expected for BK channels (Farley and Rudy 1988; Reinhart et al. 1989). However, fast channels were only observed in immature neurons. Eight channels in 43 patches from *P0–P1* neurons were fast gating, whereas only 1 was found in patches from a *P5* animal and none at older ages. Twenty-four channels in 43 patches were slow gating. Most patches from immature neurons showed either single-channel activity or no channel activity. Patches that contained both types of events (3 patches) were not used for analysis. The slow channel had a conductance of 181.6 ± 3.9 pS (mean ± SE, $n = 21$, measured at membrane potentials between −20 and −80 mV), compared with the fast channel, which had a conductance of 145 ± 12.9 pS ($P < 0.01$, $n = 6$, measured at membrane potentials between −20 and −80 mV). Recordings during both steady and ramp voltage commands showed that fewer openings of the slow channel occurred when the membrane was hyperpolarized than when depolarized (Fig. 1, *A* and *B, left panel*). By contrast, the fast channel opened more frequently as the membrane was hyperpolarized (Fig. 1, *A* and *B, right panel*). The fast channel also showed inward rectification; unitary currents were smaller at positive than at negative membrane potentials (Fig. 1, *A* and *B, right panel*). The activity of both the slow and fast channels was Ca^{2+} dependent. When the intracellular surfaces of patches were exposed to Ca^{2+}-free solution, no channel activity was observed (Fig. 1*B1*), but when the solution contained 0.5 μM Ca^{2+}, both the slow and fast channels showed openings when ramp pulses were delivered

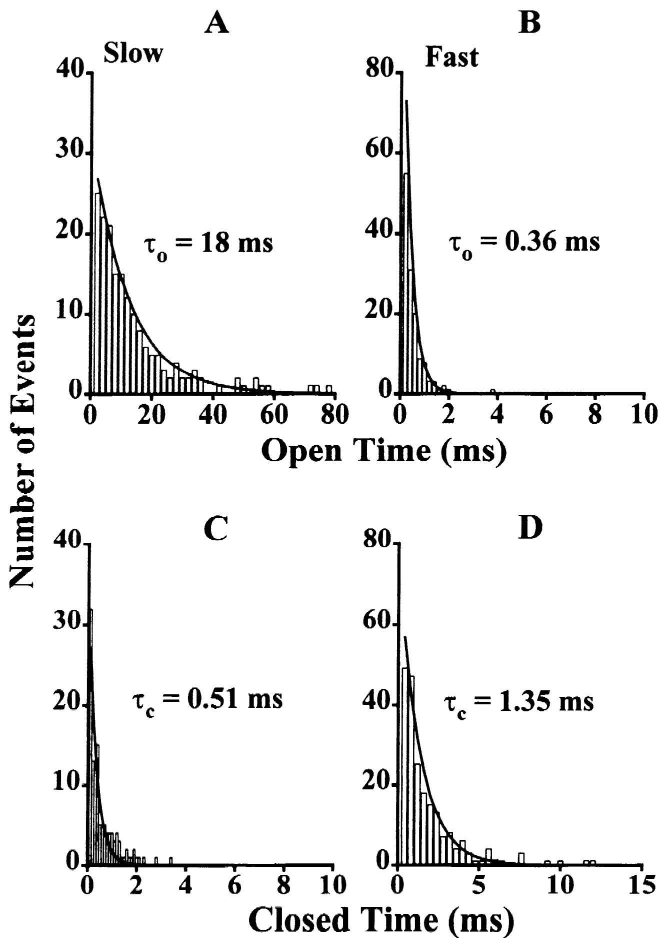

FIG. 2. Kinetics of slow and fast large conductance Ca^{2+}-activated K^+ channels (BK) channels from *P1* neurons. *A* and *B*: open time distributions. *C* and *D*: closed time distributions. *A* and *C* are from a slow channel. *B* and *D* are from a fast channel. V_h = −40 mV; $[Ca^{2+}]$ = 0.5 μM. Solid lines are exponential fittings with a single exponential.

to the patches (Fig. 1*B2*). Kinetic analysis showed that the slow channel had a longer mean open time (τ_o) than the fast channel (Fig. 2, *A* and *B*). At a membrane potential of −80 mV, τ_o for the slow channel (0.51 ± 0.8 ms) was similar to that for the fast channel (0.88 ± 0.3 ms, note different scales for $\tau_{o,slow}$ and $\tau_{o,fast}$ in Fig. 3*A*), whereas, at +80 mV the slow channel τ_o was much greater than that for the fast channel (Fig. 3*A*, $P < 0.01$). This was due to a voltage-dependent increase in the mean open time for the slow channel, together with a slight decrease for the fast channel (Fig. 3*A*). The mean closed time (τ_c) for the slow channel showed a marked voltage-dependent decrease at holding potentials positive to −80 mV, whereas the mean closed time for the fast channel increased at membrane potentials more positive than 40 mV (Fig. 3*B*). Open probability-voltage relationships (P_o-V) showed a voltage-dependent increase in P_o for the slow channel compared with a voltage-dependent decrease in P_o for the fast-gating channel (Fig. 3*C*). Ca^{2+}-dependent activation curves revealed that the slow channel had a higher maximum P_o than the fast channel, whereas the Ca^{2+} concentration to obtain half-maximal P_o (EC_{50}) was similar for these two types of BK channel (Fig. 3*D*). Both the slow and fast channels were highly selective to K^+. When the solution bathing the intracellular side of inside-out patches was changed from high K^+-low Na^+ solution (140 mM K^+ and 0 mM Na^+) to low K^+-high Na^+ solution (5 mM K^+ and 140 mM Na^+), the reversal potential shifted from 0 mV to +61.5 ± 1.1 mV (n = 27) and to +59 ± 2.5 mV (n = 6) for the slow and fast channel, respectively. Thus, according to Goldman-Hodgkin-Katz relationship, the ratio of K^+ to Na^+ permeable for both slow gating and fast gating channels is greater than 15:1.

DISCUSSION

We observed two types of BK channels in immature neurons, slow gating and fast gating. The kinetic, conductance, rectification, and voltage-dependent properties of the fast-gating BK channel are different from those of the fast-gating BK channel reported by Reinhart et al. (1989). The different properties of the fast-gating BK channels in our experiments could not be due to effects of the enzymes used to dissociate neurons, because we also recorded fast-gating BK channels with the same properties in inside-out patches directly ex-

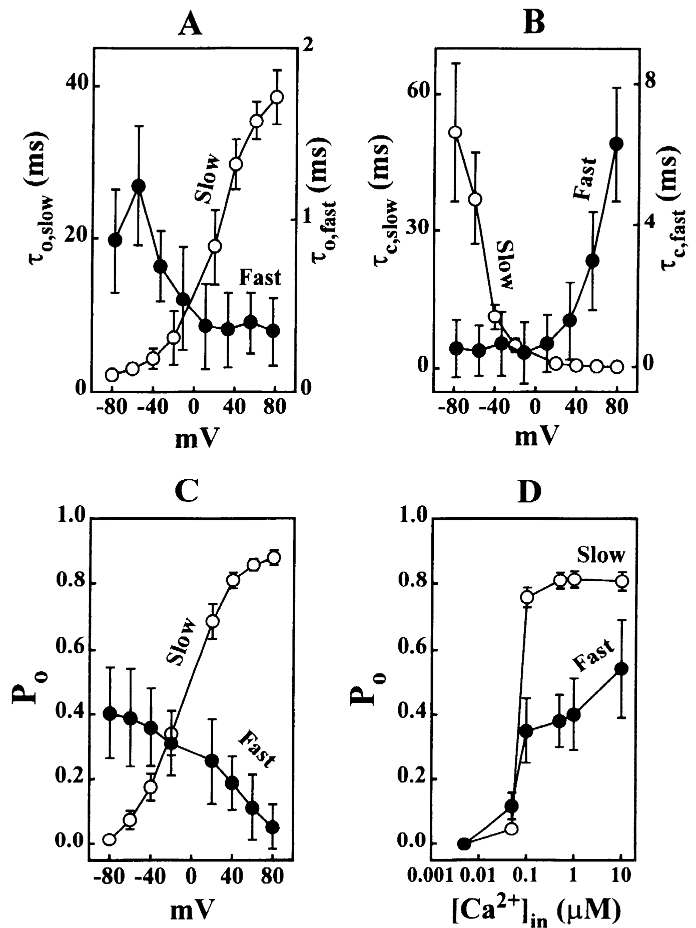

FIG. 3. Voltage and Ca^{2+} dependence of slow and fast BK channels. Open circles, slow channels; filled circles, fast channels. *A*: mean open time (τ_o)−voltage relationships. *B*: mean closed time (τ_c)−voltage relationships. *C*: open probability (P_o)-voltage relationships. $[Ca^{2+}]$ = 0.5 μM. *D*: Ca^{2+}-dependent activation. Membrane potentials were +40 mV for slow gating and −40 mV for fast gating, respectively. Sample numbers for slow and fast channels were 5 patches and 3 patches, respectively in *A*–*D*. Error bars omitted when smaller than symbol size.

cised from neurons in slices that were not exposed to enzymes (unpublished data). The fast-gating BK channel in immature neurons may be due to expression of a different gene, or alternate splicing. Studies of alternately spliced variants of *Slowpoke,* a *Drosophila* large-conductance K^+ channel expressed in oocytes, indicate that alternate splicing of a common RNA precursor can produce a diversity of functional properties of the expressed channel, including conductance, Ca^{2+} sensitivity and kinetics.

It should be pointed out that the low-pass filtering used for reducing noise in this study would compromise the detection of brief events and therefore affect our analysis of fast-gating channel activity. The major effect of filtering was to reduce amplitudes of fast events, but it had minor effects on their duration. Therefore open and closed time analyses were still used on fast-gating channels in this study. In terms of kinetic analysis, the consequences of such filtering (Heinemann 1995) would be missed short closures (Fig. 2, *C* and *D*) and an overestimate of open times, especially for the fast-gating channels (Fig. 2*B*). Thus the values for $\tau_{o,fast}$ presented in Fig. 3*A,* which are up to 80 times smaller than those for $\tau_{o,slow}$, are likely to be too large. Further, because filtering reduced amplitudes of fast events more than that of slow events, we could not reliably determine subconductance states, although there were occasionally differences in observed event amplitudes (Fig. 1, *top right panel*). Finally, filtering could have contributed to the apparent rectification of the fast gating channels (Fig. 1) because their depolarization-dependent decrease in open time would have resulted in a decreased event amplitude. These consequences of limited bandwidth in the recorded signal do not affect any basic conclusions of this study. That is, the findings that both channel types are K^+ and Ca^{2+} dependent (Figs. 1*B* and 3*D*), but have opposite voltage dependence (Fig. 1*A* and 3*C*), are not compromised by this technical limitation.

A BK channel with properties similar to those of the fast-gating channel described above has not been previously reported. This channel is only transiently expressed in very young ages (*P0–P5*) where it is found in ~20% (8/40) of patches. Without more information about the developmental state of other voltage-dependent channels at times when the fast-gating BK channel is expressed, it is only possible to speculate with respect to its functional significance. The voltage-dependent increase in mean closed times and decrease in open probability would tend to decrease the efficacy of fast-gating channels in repolarizing neurons during periods of intense depolarization, such as might occur in the course of epileptiform discharges. These properties would make K^+ currents through BK channels less effective in offsetting calcium currents evoked by depolarized membrane potentials, even though slow- and fast-gating channels have similar sensitivities to calcium concentrations within the range of those expected in these neurons (Fig. 3*D*). Slow- and fast-gating channels also must make significantly different contributions to the conductance of neurons at resting membrane potentials (Fig. 1*A*). The fast-gating channel is mostly open at rest and thus contributes to the resting membrane potential and would be largely open at threshold for evoking action potentials (approximately −45 mV). It might also serve to shunt excitatory postsynaptic potentials evoked at the resting membrane potential or in the subthreshold range, whereas the slow-gating channels would not have such effects. Finally, the fast-gating channel will act as an inward rectifier, becoming increasingly activated with larger hyperpolarizations. The functional result of this inverse BK channel activation function will be a Ca^{2+}-dependent increase in conductance that would be "regenerative"; once activated it would tend to clamp membrane potential near the potassium equilibrium potential for as long as $[Ca^{2+}]$ remains elevated. This unique activation of BK channels may somehow subserve action-potential repolarization in immature neurons.

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