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Activation and deactivation properties of rat brain K^+ channels of the *Shaker*-related subfamily

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Abstract. We studied the activation properties of members of the *Shaker*-related subfamily of voltage-gated K^+ channels cloned from rat brain and expressed in *Xenopus* oocytes. We find that Kv1.1, Kv1.4, Kv1.5, and Kv1.6 have similar activation and deactivation kinetics. The K^+ currents produced by step depolarisations increase with a sigmoidal time course that can be described by a delay and by the derivative of the current at the inflection point. The delay tends to zero and the logarithmic derivative seems to approach a finite value at large positive voltages, but these asymptotic values are not yet reached at +80 mV. Deactivation of the currents upon stepping to negative membrane potentials below −60 mV is fairly well described by a single exponential. The decrease of the deactivation time constant at increasingly negative voltages tends to become less steep, indicating that this parameter also has a finite limiting value, which is not yet reached, however, at −160 mV. The various clones studied have very similar voltage dependencies of activation with half-activation voltages ranging between −50 and −11 mV and maximum steepness yielding an *e*-fold change for voltage increments between 3.8 and 7.0 mV. The shallower activation curve of Kv1.4 is likely to be due to coupling with the fast inactivation process present in this clone.

Key words: Potassium channels – Rat brain – *Shaker* – Oocyte expression

Introduction

Several members of the RCK *Shaker*-related subfamily found in the rat genome and encoding K^+-channel proteins (Kv1.1 to Kv1.6)[1] have been extensively characterised (Stühmer et al. 1988, 1989a; Betshold et al. 1990; Christie et al. 1989). Between each other and compared to members of the other K^+-channel families from *Drosophila* (*Shaker, Shab, Shal,* and *Shaw*), from rat brain, mouse, human, etc. all *Shaker*-related channels have a very high percentage of sequence identity in the putative membrane-spanning core region of the polypeptide, whereas very extensive differences exist in the N- and C-terminal domains (for reviews see Pongs 1992; Jan and Jan 1992). These differences are mainly responsible for the physiological diversities that are principally related with fast (N-type) and slow (C-type) inactivation.

Within the *Shaker*-related subfamily, Kv1.4 is characterised by a much longer N-terminus, containing about 155 initial residues that are not present in other family members. Correspondingly, Kv1.4 is the only channel of the family that allows I_A-like currents that inactivate within fractions of a second (Stühmer et al. 1989a) and addition of the N-terminal peptide of Kv1.4 to a tandem mutant (RCK4-1) which has been deprived of inactivation (Ruppersberg et al. 1991a) restores the latter process (Ruppersberg et al. 1991b). Apart from inactivation, Kv1.4 was also reported to have different activation properties and single channel conductance (Stühmer et al. 1989a). The single channel conductance is changed by the amino acid substitution K533Y that renders Kv1.4 identical to Kv1.6 in the pore (P) segment (Ludewig et al. 1993). We investigate here the other apparently distinct feature of Kv1.4, of having a much shallower voltage dependence. For this study we find that it is mandatory to use protocols allowing more than 30 s between successive stimulations. The experiments by Ruppersberg et al. (1991b) show that the rate at which Kv1.4 channels close when the membrane is repolarised is particularly slow, to the extent that during the recovery from inactivation a significant fraction of channels dwell in the open state long enough to show an appreciable inward current. This slow closing could allow the accumulation of a significant degree of long lasting inactivation after repolarisation, as suggested by Ruppersberg et al. (1990), biasing the results of the repetitive stimulations that are normally used to study the current-voltage

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[1] We shall use throughout this paper the nomenclature suggested by Gutman and Chandy (1993): Kv1.1 for RCK1; Kv1.4 for RCK4; Kv1.5 for RCK7; Kv1.6 for RCK2

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characteristics of voltage-activated channels. With our protocol we find that Kv1.4 channels have activation and deactivation properties much more similar to those of other members of the same subfamily.

Methods

Aqueous solutions (0.1–0.3 µg/µl) of cRNA encoding rat brain potassium channels were microinjected (46 nl) in *Xenopus laevis* oocytes (Stühmer et al. 1989a). Ionic currents through expressed channels were recorded by the patch clamp technique from macro-patches in the cell-attached configuration using standard patch-clamp amplifiers (EPC-7, List; Axopatch-200, Axon Instruments). Patch pipettes were pulled from aluminium-silicate glass capillaries (Hilgemberg), coated with silicone rubber (General Electric), and fire polished to a resistance of 0.6 to 1.2 MΩ. Double-electrode voltage-clamp recordings from the whole oocyte were occasionally obtained using a commercial voltage-clamp amplifier (TEC-1, NPI) and 0.5–0.7 MΩ intracellular micropipettes filled with 3 M KCl. Stimulation and data acquisition were performed with a 16 bit AD/DA converter (ITC-16, Instrutech) controlled by a microcomputer (Macintosh II). Before acquisition, the output of the recording amplifier was filtered through a low-pass four-poles Bessel filter (4302, Ithaco or 902 Frequency Devices) with a cut-off frequency of 5 kHz or 10 kHz (patch-recording) or 3 kHz (whole-oocyte recording). The data were sampled at 20 to 50 kHz. For patch-clamp recordings the oocytes were maintained in a bathing solution with the following composition (in mM): KCl 120, TRIS-Cl 20, EGTA 5; pH 7.4. Owing to the high K^+-concentration of the bath, the resting cell membrane potential was close to zero (±2 mV), as checked in a few cases by penetrating the oocyte with a voltage measuring micropipette. Therefore, the patch membrane potential, V, was assumed to be just equal and opposite to the pipette potential. In a few experiments the patch-pipette solution was normal frog Ringer (NFR) containing (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 10, pH 7.4. More often, for better resolution of tail currents at negative potentials, the potassium equilibrium potential was made less negative by using 6 K- or 20 K-NFR solutions, where the KCl concentration was raised to 6 or 20 mM (and NaCl was reduced consequently). Whole-oocyte recordings were usually obtained with the cell immersed in 6 K-NFR. The holding potential, V_H, was usually kept between –100 and –120 mV. Linear capacity and leakage currents remaining after analog compensation were digitally subtracted using the P/4 responses from a control potential of –120 mV. All measurements were performed at 19–21 °C.

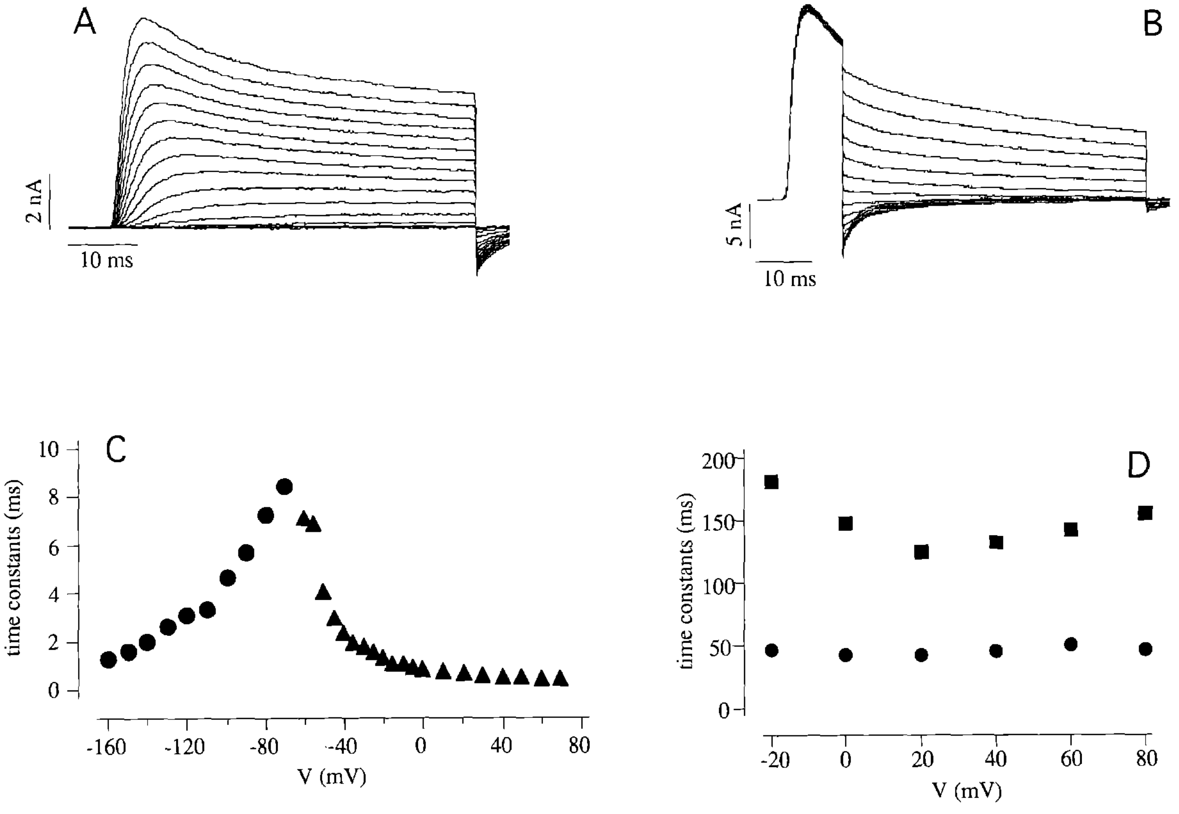

Fig. 1A–D. Potassium currents recorded from a *Xenopus* oocyte injected with cRNA encoding Kv1.4 channels. **A** Outward currents evoked by depolarising pulses from a holding potential of –100 mV to a test potential of –30 mV to +60 mV for 20 ms. **B** Tail currents recorded from repolarisations to different potentials (from –80 to –140 mV), after a test pulse of 10 ms to +40 mV. **C** Activation (*triangles*) and deactivation (*circles*) time constants (see text for definition) are plotted against potential. **D** Time constants of the fast (*circles*) and slow (*squares*) components of inactivation measured at different test potentials

Results

In oocytes injected with Kv1.4–cRNA active currents elicited by depolarising voltage steps to various test potentials, V_P, were readily appreciable for $V_P >= -65$ mV. In the experiment illustrated in Fig. 1 A the current activated by a step to 0 mV from a holding potential of –100 mV reached its maximum of 7.4 nA in about 5 ms. For the purpose of quantitative analysis this initial time course was fitted by a Hodgkin-Huxley type of kinetics plus a delay, δ:

$$I(t)=I_0(1-\exp[-(t+\delta)/\tau_a])^4. \qquad (1)$$

The time constant τ_a was in this case 0.8 ms and δ was 580 µs. Upon depolarisation the Kv1.4 channels returned to the closed state with a time course that could be best seen if the final potential, V_{tail}, was far from the potassium equilibrium potential, as shown by the tail current measurements presented in Fig. 1 B. For $V_{tail} < -70$ mV such a time course was well characterised by a single deactivation time constant, τ_d, according to the expression:

$$I(t)=I_\infty+(I_0-I_\infty)\exp(-t/\tau_d). \qquad (2)$$

The deactivation process was slightly slower at higher extracellular K^+ concentrations; the value of τ_d measured at $V_{tail}=-120$ mV changed from about 2.5 ms with 2.5 mM–K^+ to about 4 ms with 20 mM–K^+. This is consistent with previous reports about the effects of extracellular K^+ concentration on the deactivation kinetics of potassium channels (Swenson and Armstrong 1981; Cahalan et al. 1985). However, the changes in activation and deactivation kinetics that we observed were relatively minor because of the small range of K^+ concentrations used.

The voltage dependence of the activation and deactivation time constants measured in 20 K-NFR is presented in Fig. 1 C. Notice that both τ_d and τ_a tend to approach finite asymptotic values both at low and at high membrane potentials, consistent with the suggested presence of a voltage-independent closed-open transition (Zagotta et al. 1994).

The inactivation of the Kv1.4 current was much slower than activation. In some cases the time course of inactivation could be described by a single exponential decay of the currents, but clearly a double exponential fit was more adequate in general to describe the time course of the inactivation of outward currents evoked by 1 s test pulses. This was quite variable, even when measured in the same batch of oocytes injected with the same cRNA. This phenomenon has been already observed in rat brain potassium channel clones (Ruppersberg et al. 1991 a). The main variability appears to occur in the relative amplitudes of the fast and slow components, whose ratio changed between 0.7 and 5.6. The time constant of each component was less variable, with a fast time constant between 40 and 60 ms, and a slow time constant between 140 and 280 ms, measured at $V_P=+20$ mV. There was no obvious correlation between the changes in time course of inactivation and the extracellular K^+ concentration, in the range 2.5 to 20 mM. An extreme example of the variability of the extent and the kinetics of the inactivation process can be observed by comparing Fig. 1 A, showing a record from a patch with a large slow component, with Fig. 2, showing a case of

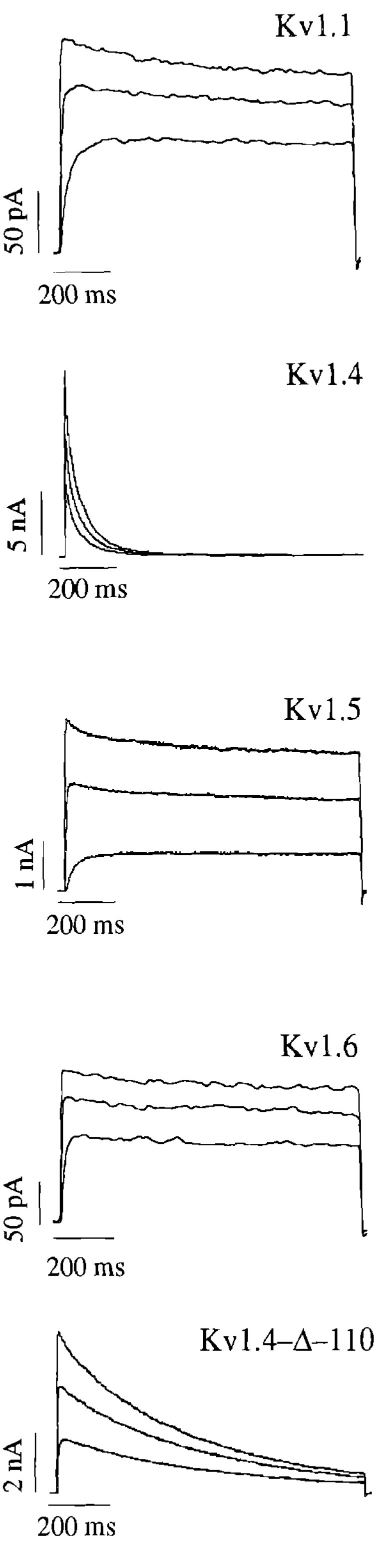

Fig. 2. Currents elicited by long depolarising pulses for the potassium channel clones Kv1.1, Kv1.4, Kv1.5, Kv1.6 and Kv1.4–Δ-110. The membrane potential was stepped from $V_H=-120$ mV to test potentials of –20 mV, 0 mV and +20 mV for 1 s. Observe the very slow and small inactivation in Kv1.1, Kv1.5, Kv1.6, the slow inactivation in the mutant Kv1.4–Δ-110 and the fast inactivation in Kv1.4

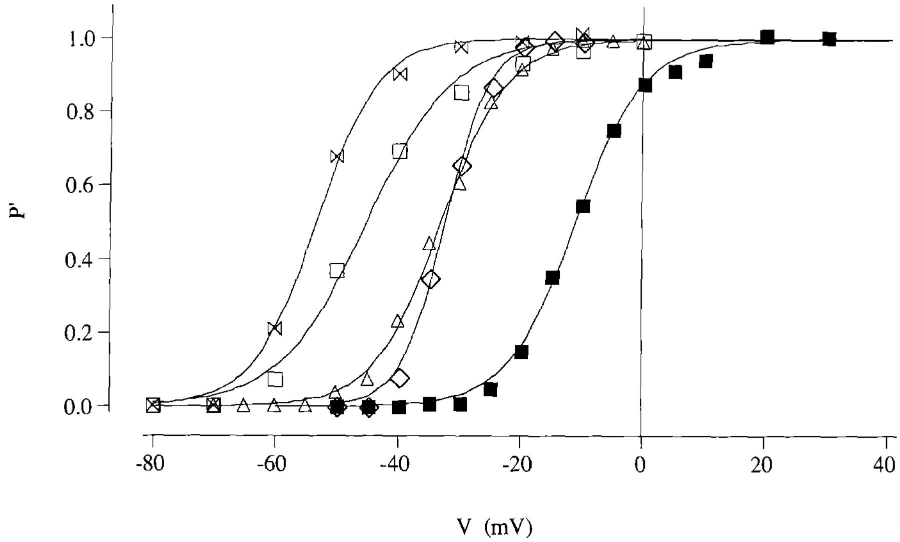

Fig. 3. Activation curves for different rat brain *Shaker*-related K$^+$ channel clones. Peak activation probabilities were calculated as described in results. The half-activation voltages and the *e*-fold slopes of the fitting curves are –33 mV and 4.5 mV (Kv1.1, *triangles*), –45 mV and 7 mV (Kv1.4, *empty squares*), –11 mV and 5.5 mV (Kv1.5, *filled squares*), –32 mV and 3.5 mV (Kv1.6, *diamonds*), –53 mV and 5.1 mV (Kv1.4–Δ-110, *butterflies*)

Kv1.4 currents where the fast component is predominant and inactivation is almost complete in 250 ms. Figure 1 D shows inactivation time constants measured at different V_P from –20 mV to +80 mV in one experiment. Both inactivation time constants were fairly voltage independent in the range of potential studied.

The repetitive stimulation of the Kv1.4 channels also produced a reduction of the peak outward current, due to cumulative long lasting inactivation (Bertoli et al. in preparation). Consequently, we used stimulation intervals ≥30 s when studying the expression of the Kv1.4 channel.

Injection of oocytes with Kv1.1, Kv1.5 and Kv1.6–cRNA expressed currents that showed very little decay during a 1 s test pulse (see Fig. 2). On the other hand, expression of Kv1.4–Δ-110, a deletion Kv1.4 mutant missing 110 residues at the N-terminal (Rettig et al. 1994), resulted in a current that lacked only the fast component of inactivation, while maintaining a slow decay with a time constant similar to that of the Kv1.4 wild type (see Fig. 2).

Activation curves were constructed by dividing the peak current evoked at each test potential, V_P, by the instantaneous tail current obtained when stepping to $V_{Tail} = V_P$ after a conditioning depolarising pulse that opened a large fraction of the channels. These data were normalised to their asymptotic value for large positive voltages and plotted as P′ (apparent peak open channel probability) as a function of V_P. This activation curve was fitted empirically by a simple Boltzmann distribution, using the equation:

$$P' = 1/(1 + \exp[(V_{1/2} - V_P)/V_a]) \tag{3}$$

where $V_{1/2}$ is the potential at which $P' = 0.5$ and V_a is the *e*-fold slope of the voltage dependence. Activation curves obtained for 4 different wild type clones, Kv1.1, Kv1.4, Kv1.5 and Kv1.6, and the mutant Kv1.4–Δ-110 are shown in Fig. 3 and the relative activation parameters are listed in Table 1. Observe that, in contrast to the previously publised data (Stühmer et al. 1989 a), the Kv1.4 clone has a voltage dependence with a slope that is similar to other members of the rat brain *Shaker*-related potassium channel subfamily.

Table 1. Activation parameters of the rat brain *Shaker*-related potassium channel clones and the mutant Kv1.4–Δ-110. $V_{1/2}$ is the potential to achieve half of the maximum activation, V_a is the *e*-fold slope of the voltage dependence of activation, τ_a is the activation time constant measured at 0 mV, and τ_d is the deactivation time constant measured at –160 mV. Values indicate mean±s.e.m. (number of experiments)

	$V_{1/2}$ (mV)	V_a (mV)	τ_a (ms)	τ_d (ms)
Kv1.1	–34±8 (3)	6.1±1 (3)	4 ±1 (3)	0.5±0.05 (2)
Kv1.4	–44±4 (3)	7.0±2 (3)	1.3±0.7 (4)	1.6±0.2 (4)
Kv1.4–Δ-110	–50±5 (3)	4.5±0.5 (3)	2.3±0.4 (3)	1.8±0.4 (3)
Kv1.5	–11±5 (3)	5.5±0.4 (3)	8.4±1 (4)	0.7±0.1 (4)
Kv1.6	–30±6 (3)	3.8±0.5 (3)	2.1±0.6 (3)	1.1±0.4 (2)

Discussion

The main purpose of this work was to correct a previous analysis of the voltage dependence of the activation of Kv1.4 channels (Stühmer et al. 1989 a), which concluded that these channels are about three times less sensitive to voltage than the other members of the same family, Kv1.1, Kv1.5 and Kv1.6. We find for Kv1.1 activation parameters ($V_{1/2} = -34 \pm 8$ mV, $V_a = -6.1 \pm 1$ mV) in perfect agreement with those reported by Stühmer et al. (1989 a) ($V_{1/2} = -30 \pm 7$ mV, $V_a = -6.5 \pm 1.8$ mV). However, from our measurements on Kv1.4 we obtain a $V_{1/2}$ more negative by 22 mV and a V_a about 2.4 times smaller than the respective estimates given in the previous work. Our data make the Kv1.4 channel much more similar to the other clones of the same family and are more consistent with the present view of the structural correlate of the voltage sensitivity of outward rectifying K$^+$ channels. As first proposed for sodium channels (Noda et al. 1986; Stühmer et al. 1989 b) it is presently widely accepted that the voltage sensing structure of voltage-gated channels is the highly conserved segment S4, present with its stereotypic recurrence of basic residues with a periodicity of 3 in all four repeats of the sodium and calcium channels and in all known clones of outward rectifying K$^+$ channels (for review see Pongs 1992). Accordingly, a difference in the voltage sensitivity of Kv1.4 could be expected on the basis of differences in its S4 segment relative to the other channels. However, segment S4 is completely conserved between Kv1.1, Kv1.4, Kv1.5 and Kv1.6, except for a single amino acid towards its amino terminal end (valine at position 293 in Kv1.1) which is changed to isoleucine in Kv1.4. Simple models ascribing the voltage sensitivity to transmembrane movements of S4 relative to the rest of the channel protein, either as a sliding helix (Guy and Seetharamulu 1986; Catterall 1988) or a propagating helix (Guy and Conti 1990), predict that the slope of activation curves should be modified only as result of modifications of the positively charged residues of S4, as is indeed observed in this type of mutagenesis experiment (Stühmer et al. 1989b; Papazian et al. 1991). On the other hand, changes in the hydrophobic amino acids of the S4 segments produce shifts of the activation curves without drastic changes in the slope of the voltage dependence (McCormack et al. 1991; Lopez et al. 1991).

Although much less pronounced than previously reported, a shallower voltage sensitivity of Kv1.4 is also found in the present work. Rather than casting serious doubts on the major role of segment S4 in voltage-dependent transitions, we suggest that these functional characteristics of Kv1.4 may be strictly correlated to its stronger inactivation properties. The existence in Kv1.4 of inactivated states coupled sequentially to the activated states through relatively fast and voltage-independent transitions are expected to alter the apparent activation of the channels as measured from peak currents. At small depolarisations the rate at which the channels reach the open state is comparable with that at which they inactivate, and the peak open probability is only a fraction of the steady-state probability that would be reached in the absence of inactivated states. The latter situation is approached more closely with increasing depolarisation, when the transitions from closed to activated states become much faster and the channels can be fully activated before they have enough chance to inactivate. By this mechanism the peak open probability may keep increasing beyond depolarisations that already saturate the equilibrium between closed and activated states in favour of the latter. This may be the main cause of the significant decrease of slope that we observe in the activation curve of Kv1.4 channels in comparison to the other clones, which have very much slower inactivation kinetics and whose activation slopes should reflect more closely the size of charge movement associated with the conformational transitions of the voltage-sensing structures. In agreement with this we find that the mutant Kv1.4–Δ-110, lacking the fast N-type inactivation, has much steeper activation, which is practically identical to that of Kv1.1, Kv1.5 and Kv1.6.

The above discussion suggests one possible way to explain the discrepancy between our results and those of Stühmer and collaborators (1989a) concerning Kv1.4. One suggestion is that their data could be inadvertedly biased by the phenomenon of cumulative inactivation, that is particularly pronounced in Kv1.4 channels as we describe elsewhere (Bertoli et al. in preparation). It is possible that using protocols of successive stimulations at increasing voltages without waiting long enough between stimuli may produce not only a progressive reduction of the activatable channels, but also change the kinetics of activation (because the starting state is not an equilibrium one), and thus somehow make the above effects of interaction between activation and inactivation more serious.

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