Slow inactivation conserved in heteromultimeric voltage-dependent K^+ channels between *Shaker* (Kv1) and *Shaw* (Kv3) subfamilies

Mohammad Shahidullah, Naoto Hoshi, Shigeru Yokoyama, Tetsuro Kawamura, Haruhiro Higashida*

Department of Biophysics, Neuroinformation Research Institute, Kanazawa University School of Medicine, 13-1 Takara-machi, Kanazawa 920, Japan

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Abstract **Single K^+ channels were recorded under the cell-attached mode in *Xenopus* oocytes injected with an equal amount of mRNAs coding for NGK1 (Kv1.2) and NGK2 (Kv3.1a) voltage-dependent K^+ channels. A new class of channels of 20 pS in conductance with three degrees of inactivation was observed. The results suggest that voltage-dependent NGK1 *Shaker* and NGK2 *Shaw* K^+ channels, from different subfamilies, assemble to form heteromultimeric K^+ channels in *Xenopus* oocytes and show characteristics inherited from two parental channels.**

Key words: Single K^+ channel; Delayed rectifier; α Subunit assembly; mRNA; Heterologous expression; Oocyte

1. Introduction

Association of four α subunits of the *Shaker* voltage-dependent K^+ channel protein which seems to form the ion pore has been shown as a negative-staining image by electron microscopy [11]. K^+ channels in the four subfamilies of *Shaker* (Kv1), *Shab* (Kv2), *Shaw* (Kv3), and *Shal* (Kv4) result in the formation of homomultimeric assemble [2,9]. Heteromultimeric K^+ channels can be formed by K^+ channel proteins belonging to the same subfamilies [1,6,16,19], but not between ones belonging to different subfamilies [20,21]. However, in oocytes injected with a mixture of mRNAs encoding rat *Shaker* NGK1 (Kv1.2) and mouse *Shaw* NGK2 (Kv3.1a) K^+ channels [22], a whole-cell outward current which cannot be explained by a simple sum of NGK1 and NGK2 current components has been recorded [7]. This suggests that proteins of NGK1 and NGK2 voltage-dependent K^+ channels, belonging to different subfamilies, form heteromultimers [7]. Here, we attempted to record single K^+ channels in the cell-attached configuration of the patch voltage-clamp method [3] in oocytes injected with the both mRNAs, in order to obtain more direct evidence for our hypothesis. In this paper, we describe single K^+ channels with a unit conductance of about 20 pS which showed electrophysiological characteristics derived from NGK1 and/or NGK2 channels.

2. Materials and methods

Oocytes were harvested from adult *Xenopus laevis*, and the vitelline envelopes were peeled off under a dissecting microscope 2–3 h before starting electrophysiological recording. NGK1 (Kv1.2)- and NGK2 (Kv3.1a)-specific mRNAs were synthesized in vitro, using *Xho*I-cleaved pSPNGK1 and pSPNGK2, respectively [22]. Transcription was primed with the cap dinucleotide (G(5′)ppp(5′)G). Each mRNA or the mixture of NGK1- and NGK2-specific mRNA at a 1:1 ratio was injected into denuded oocytes (concentration, 1 or 2 ng/μl; volume injected per oocyte, about 50 nl), using a microinjector from calibrated glass micropipettes (tip width = 30 μm). The oocytes were then incubated at 20°C, and were usually examined 2 to 5 days after injection.

Single K^+ channel currents were measured using the cell-attached patch recording technique [3]. For single channel recording, the cells were perfused with high K^+ solution containing (in mM): KCl 100, $MgCl_2$ 1, EGTA 10, HEPES 10, pH 7.4. The patch electrodes were filled with a solution containing (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, HEPES 10, pH 7.4. All single channel recordings were performed in the cell-attached configuration using an Axopatch-1D amplifier (Axon Instruments Inc., Foster City, CA, USA). The membrane potentials of these oocytes were 0 mV during the experiments ($n = 5$). Thus, the membrane potential could be controlled accurately. Data were usually low-pass filtered at 1 kHz, digitized at 5 or 10 kHz with a 12 bit A/D converter. To process single channel records, pClamp (Axon Instruments Inc.) was used to determine unit conductance, the open probability and ensemble averages of the single channel records. All records were corrected for capacitive and leak currents by subtracting an average of 10 null traces evoked by hyperpolarization.

3. Results

Single K^+ channels were recorded under the cell-attached condition in oocytes injected with a mixture of mRNAs of NGK1 (Kv1.2) and NGK2 (Kv3.1a). Their unitary conductances ranged from 13 to 27 pS, showing a trimodal distribution. 59% (24 of 41) of channels ranged from 18 to 22 pS, while 2 channels (5%) ranged from 13 to 17 pS and 15 channels (37%) from 23 to 27 pS. The most abundant channels were those of a unit conductance of 20 pS, with a mean value of 19.6 ± 0.22 pS (mean ± S.E.M, $n = 24$). This value of 20 pS is approximately the mean of the average conductance of NGK1 channels (14.3 ± 0.47 pS, $n = 9$) and of NGK2 channels (25.0 ± 0.93 pS, $n = 26$) recorded in oocytes injected with NGK1 or NGK2 mRNA alone. Channels of >23 pS conductance in co-injected oocytes were identified as NGK2 homomultimeric channels, whereas channels with < 17 pS as NGK1 homomultimeric ones. Thus, 20-pS K^+ channels expressed in oocytes injected with a mixture of NGK1 and NGK2 mRNAs are believed to be a new class of channels which are distinct from homomultimeric NGK1 and NGK2 channels. We therefore carried out a more detailed investigation of these 20-pS K^+ channels.

Fig. 1 shows current traces of typical NGK1 and NGK2 K^+ channels and two examples of 20-pS channels. One type of 20-pS channels opened at potentials around −40 mV were observed in 21 oocytes (55%) (panel C), while another type opened at about −20 mV ($n = 17$ cells, 45%) (panel D). To confirm the difference in gating properties, the open probability of several channels during the initial 400 ms of step pulses to various potentials from a holding potential of −100 mV was calculated

*Corresponding author. Fax: (81) (762) 34-4236/4202.

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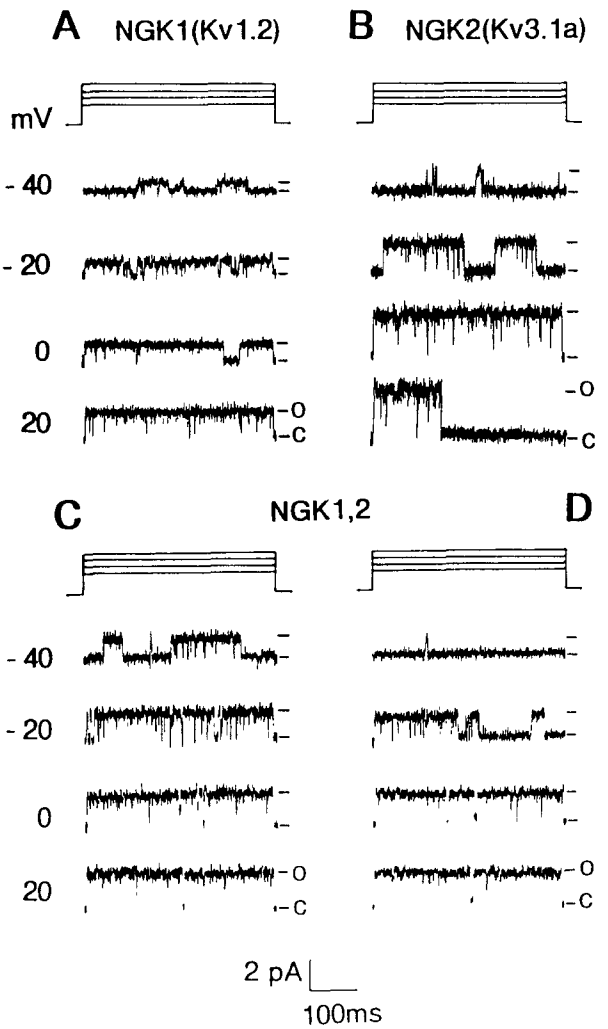

Fig. 1. Current traces of single K^+ channels recorded in oocytes injected with mRNAs. Currents obtained at various depolarized potentials from the holding potential of −100 mV are indicated on the left in mV. (A,B) Typical traces of single channel currents recorded from oocytes injected with either NGK1 (Kv1.2) mRNA (A) or NGK2 (Kv3.1a) mRNA (B). Note that the currents in A show subconductance states and those in B show a large degree of inactivation. (C,D) Two examples of 20-pS K^+ channels recorded from oocytes injected with a 1:1 mixture of NGK1 and NGK2 mRNAs. Typical examples of channels opening at a threshold of about −40 mV (C) and about −20 mV (D). Note that the currents in C and D show little or no subconductance states with less inactivation during depolarized steps. Outward voltage steps for 400 ms are shown at the top. Outward current is upward.

(data not shown). With a step potential to −20 mV, a group of channels like the one in panel C showed an open probability of 0.70 ± 0.15 (n = 11), while those in panel D were as low as 0.21 ± 0.10 (n = 8), being significantly different at $P < 0.001$ (t test). These distinct low and high threshold potentials of 20-pS channels seem to reflect the original opening thresholds of about −40 mV for NGK1 channels (Fig. 1A) and −20 mV for NGK2 channels (Fig. 1B) in oocytes, as described previously [4,13,18,22].

Subconductance states are frequently observed in NGK1 homomultimeric channels but are very rare in NGK2 homomultimeric channels (Fig. 2, left and right), as reported previously [22]. Of nineteen 20-pS channels with a threshold voltage of −40 mV, 3 channels (16%) showed subconductance states and 16 channels (84%) showed few or no subconductance levels in their open states (Fig. 2, middle). Similarly, three 20-pS channels out of 15 records (20%) with a threshold voltage of −20 mV showed subconductance states while the remainder (n = 12, 80%) did not (Fig. 2, middle). Therefore, although NGK1-like properties were also observed, more than 80% of 20-pS channels with either high or low threshold voltages possessed NGK2-like open properties.

20-pS channels showed three degrees of inactivation. Inactivation was observed as either short, intermediate or long closures when channels were depolarized to potentials of > +20 mV from a holding potential of −100 mV (see Fig. 1B). Inactivation was further analyzed by ensemble averaging channel currents evoked by depolarizing pulses of 10 s to 40 mV. In 20-pS channels with the low threshold potential, inactivation at the end of depolarizing test pulses was 18 ± 1.1% in 11 cells (58%), 31 ± 1.2% in 4 cells (21%) and 60 ± 0.4% in 4 cells (21%) (Fig. 3, middle). 20-pS channels with the high threshold potential also showed three degrees of inactivation: 16 ± 2.3% (n = 7, 47%), 35 ± 2.4% (n = 3, 20%), and 60 ± 0.5% (n = 5, 33%), respectively (Fig. 3, middle). The inactivation of NGK1 channels ranged from 11 to 25%, with an average value of 19 ± 2.0% (n = 7) (Fig. 3, left), while NGK2 channels had two inactivation rates of 35 ± 3.2% (n = 7, 62%) and 61 ± 0.6% (n = 5, 38%) (Fig. 3, right). Therefore, the three degrees of inactivation of 20-pS channels seem to correspond to those of either NGK1 or NGK2 channels.

4. Discussion

The results of these experiments show strong evidence that coexpression of cloned rat NGK1 (rKv1.2) and mouse NGK2 (mKv3.1a) K^+ channels in *Xenopus* oocytes, which belong to two different subfamilies, *Shaker* and *Shaw*, results in assembly of heteromultimeric NGK1 and NGK2 (NGK1,2) channels detected in a functional assay. The modal unit conductance of heteromultimeric NGK1,2 channels was about 20 pS, which is roughly intermediate between the conductance of NGK1 (14 pS) and of NGK2 (25 pS) channels [22]. This might be expected if the ion pore structure of heteromultimeric NGK1,2 channels were determined by the interaction of the two types of α subunits. However, the gating property in the threshold potential (−40 and −20 mV) of the parental channels was conserved rather than mixed in discrete NGK1,2 channels.

NGK1,2 channels in isolated patches, with either high or low threshold activation potentials, both showed larger and smaller degrees of inactivation (Fig. 3). A high degree of inactivation observed in NGK1,2 channels seems to be determined by NGK2 subunits, since such inactivation was not observed in NGK1 channels. Inactivation of NGK2 and NGK1,2 channels corresponds well to that observed in macroscopic currents reported in oocytes by Ito et al. [7] and Lopez et al. [12], and resembles the inactivation observed in NG108-15 cells [17,23], differing from the typical A-type inactivation [5]. Although inactivation of > 60% was not observed in NGK2 channels in isolated patches (unpublished data), such larger inactivation was frequently observed in NGK2 channels in the cell-attached configuration, suggesting that channels recorded under the cell-attached configuration reflect more physiological properties.

It has been reported that the NGK2 current does not decay appreciably during the first 200 ms [4,12–15,18]. Unlike the

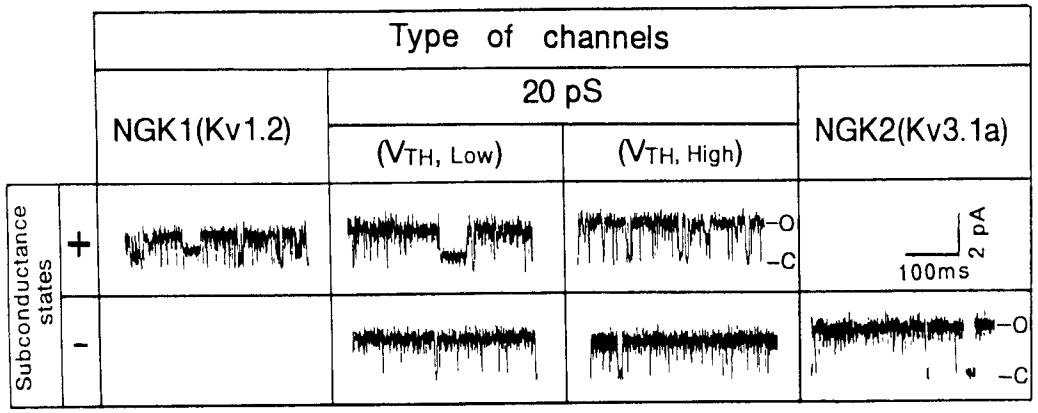

Fig 2. Subconductance states in single K^+ channels recorded from oocytes injected with mRNAs for NGK1 (Kv1.2) and NGK2 (Kv3.1a), and a 1:1 mixture of them. Traces of typical NGK1 or NGK2 channel currents and of currents of two types of 20-pS channels with low ($V_{TH, Low}$) and high ($V_{TH, High}$) threshold potentials were recorded at a test potential of +20 mV from a holding potential of −100 mV. Upper traces designated (+) show currents with subconductance states. Lower traces indicated (−) show currents with very few or no subconductance states. Note that both types of 20-pS channels possessed two (+ and −) states of subconductance levels, one is NGK1-like and the other is NGK2-like.

rapid inactivation of A-type K^+ currents [5], the inactivation mechanism for our currents is not clear at this moment, although inactivation was removed in mutant NGK2 channels with deletion of either the N- or C-terminus regions or of both ends [10]. At the end of 10 s-depolarizations, the outward current of NGK1 channels was decreased by 15–25% and the two types of NGK2 current were decreased by about 35% and 60%, respectively. From our inactivation experiments, we conclude that inactivation properties are essentially conserved in NGK1,2 heteromultimeric channels: one half of 20-pS channels had the slowest inactivation rate (16 or 18%), while the other half had faster inactivation (31, 35 or 60%). Thus, with respect to inactivation of 20-pS channels, both NGK1 and NGK2 parental channel subunits may contribute equally.

In conclusion, NGK1 (Kv1.2) and NGK2 (Kv3.1a) α subunit proteins seem to form heteromultimeric channels in heterologously expressed oocytes and they function as voltage-dependent K^+ channels, differing from parental channels. Further evidence is necessary for that these NGK1,2 heteromultimeric channels could serve as K^+ channel diversity in vivo.

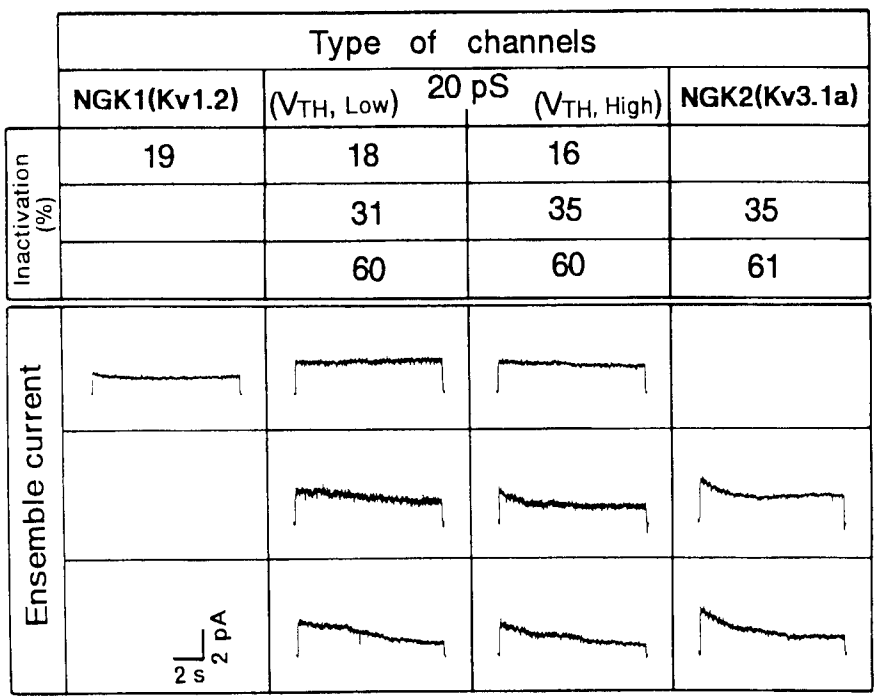

Fig. 3. Comparison of inactivation of NGK1 (Kv1.2) and NGK2 (Kv3.1a) channels and two types of 20-pS channels with low ($V_{TH\ Low}$) and high ($V_{TH, High}$) threshold potentials recorded from oocytes injected with NGK1 mRNA, NGK2 mRNA, or a 1:1 mixture. Ensemble currents are averages of 20 traces of single channel currents during 10 s depolarizing test potentials to +40 mV from a holding potential of −100 mV. The inactivation ratio (%) was calculated as (peak current−current remaining at the end of test pulse)/peak current × 100. Average values of inactivation in ensemble averages of 4 different channels are shown in percentages at upper half of the table. Typical examples of ensemble averages in each case are shown in the lower half of the table.

References

[1] Christie, M.J., North, R.A., Osborne, P.B., Douglass, J. and Adelman, J.P. (1990) Neuron 2, 405–411.
[2] Covarrubias, M., Wei, A. and Salkoff, L. (1991) Neuron 7, 763–773.
[3] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflugers Arch. 391, 65–100.
[4] Hartmann, H.A., Kirsch, G.E., Drewe, J A., Taglialatela, M., Joho, R.H. and Brown, A.M. (1991) Science 251, 942–944.
[5] Hoshi, T., Zagotta, W.N. and Aldrich, R.W. (1990) Science 250, 533–538.
[6] Isacoff, E.Y., Jan, Y.N. and Jan, L.Y. (1990) Nature 345, 530–534.
[7] Ito, Y., Yokoyama, S. and Higashida, H. (1992) Proc. R. Soc. Lond. B 248, 95–101.
[8] Jan, L.Y. and Jan, Y.N (1990) Trends Neurosci. 13, 415–419.
[9] Jan, L.Y. and Jan, Y.N. (1994) Nature 371, 537–555.
[10] Kawamura, T., Yokoyama, S., Yamashita, J. and Higashida, H. (1992) Soc. Neurosci. Abstr. 18, 77.
[11] Li, M., Unwin, N., Stauffer, K.A., Jan, Y -N. and Jan, L Y. (1994) Curr. Biol. 4, 110–115.
[12] Lopez, G.A., Jan, Y.N and Jan, L.Y. (1994) Nature 367, 179–182.
[13] Luneau, C.J., Williams, J.B., Marshall, J., Levitan, E.S., Oliva, C., Smith, J S., Antanavage, J., Folander, K , Stein, R B., Swanson, R., Kaczmarek, L.K. and Buhrow, S.A. (1991) Proc Natl. Acad. Sci. USA 88, 3932–3936.
[14] McCormack, T., Vega-Saenz de Miera, E.C. and Rudy, B. (1990) Proc. Natl. Acad. Sci. USA 87, 5227–5231.
[15] Rettig, J., Wunder, F., Stocker, M., Lichtinghagen, R., Mastiaux, F., Beckh, S , Kues, W., Pedarzani, P., Schröter, K.H., Ruppersberg, J.P., Veh, R. and Pongs, O. (1992) EMBO J. 11, 2473–2486.
[16] Ruppersberg, J.P., Schröter, K.H., Sakmann, B., Stocker, M., Sewing, S. and Pongs, O. (1990) Nature 345, 535–537.
[17] Robbins, J and Sim, J.A. (1990) Pflügers Arch. 416, 130–137.
[18] Rudy, B., Kentros, C., Weiser, M., Fruhling, D., Serodio, P., Vega-Saenz de Miera, E., Ellisman, M.H., Pollock, J.A. and Baker, H. (1992) Proc. Natl. Acad. Sci. USA 89, 4603–4607
[19] Ruppersberg, J P., Schröter, K.H., Sakmann, B., Stocker, M., Sewing, S. and Pongs, O. (1990) Nature 345, 535–537.
[20] Salkoff, L., Baker, K., Butler, A., Covarrubias, M., Pak, M.D. and Wei, A. (1992) Trends Neurosci. 15, 161–166.
[21] Shen, N.V. and Pfaffinger, P.J. (1995) Neuron 14, 625–633
[22] Yokoyama, S., Imoto, K., Kawamura, T., Higashida, H., Iwabe, N., Miyata, T and Numa, S. (1989) FEBS Lett. 259, 37–42.
[23] Yokoyama, S., Kawamura, T., Ito, Y., Hoshi, N., Enomoto, K. and Higashida, H. (1993) Ann. New York Acad Sci. 707, 60–73