

Properties of single M-type KCNQ2/KCNQ3 potassium channels expressed in mammalian cells

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1. The single channel properties of KCNQ2/KCNQ3 channels underlying neuronal voltage-dependent M-type potassium currents were studied in cell-attached patches from transfected Chinese hamster ovary (CHO) cells. Macroscopic currents produced by homo- and heteromeric KCNQ2/KCNQ3 channels were measured using the perforated-patch whole-cell technique.
2. Compared with heteromeric KCNQ2 + KCNQ3 channels, homomeric KCNQ2 channels had lower slope conductance (9.0 ± 0.3 and 5.8 ± 0.3 pS, respectively) and open probability at 0 mV (0.30 ± 0.07 and 0.15 ± 0.03 , respectively), consistent with their 3.8-fold smaller macroscopic currents. By contrast, homomeric KCNQ3 channels had the same slope conductance (9.0 ± 1.1 pS) as KCNQ2 + KCNQ3 channels, and higher open probability (0.59 ± 0.11), inconsistent with their 12.7-fold smaller macroscopic currents. Thus, KCNQ2 and KCNQ3 subunits may play different roles in the expression of M-type currents, with KCNQ2 ensuring surface expression of underlying channels and KCNQ3 modifying their function.
3. Both in homo- and heteromeric KCNQ2/KCNQ3 channels the shut time distributions were fitted with three, and the open time distributions with two, exponential components. By measuring these and other parameters (e.g. conductance and open probability) KCNQ2/KCNQ3 channels can be shown to resemble previously characterised neuronal M-type channels.

KCNQ2 and KCNQ3 subunits form heteromeric potassium channels that underlie slow, subthreshold M-type potassium currents in autonomic (and possibly central) neurones (Wang *et al.* 1998). They are related to KCNQ1 (Yang *et al.* 1997) and KCNQ4 (Kubisch *et al.* 1999) subunits, mutations of which produce one form of the cardiac long QT syndrome and deafness, respectively. KCNQ2 and KCNQ3 subunits are expressed exclusively in the nervous system and their mutations produce a benign form of epilepsy in newborns (Biervert *et al.* 1998; Charlier *et al.* 1998; Singh *et al.* 1998; Lerche *et al.* 1999). This is consistent with the role that the M-type channels play in limiting neuronal excitability (Brown, 1988; Marrion, 1997).

When expressed in *Xenopus* oocytes (Wang *et al.* 1998) or mammalian cells (Hadley *et al.* 2000; Selyanko *et al.* 2000; Shapiro *et al.* 2000), heteromeric KCNQ2 + KCNQ3 channels generate currents with biophysical and pharmacological properties characteristic of M-type currents. Currents produced by individually expressed KCNQ2 and KCNQ3 subunits also have appropriate M-like biophysical properties, but they are much smaller and show different sensitivities to tetraethylammonium (TEA) (Wang *et al.* 1998; Yang *et al.* 1998; Hadley *et al.* 2000; Selyanko *et al.* 2000; Shapiro *et al.* 2000).

In the present experiments, we have examined the properties of single hetero- and homomeric KCNQ2/KCNQ3 channels in transfected CHO cells, to see how far the differences in macroscopic current amplitude might result from differences in single channel conductance and open probability. The results suggest that, in these mammalian cells, KCNQ2 subunits might drive the surface expression of the heteromeric channels whereas KCNQ3 subunits might have the predominant influence on their function. During the course of these experiments, Schwake *et al.* (2000) published the results of some similar experiments on expressed KCNQ2/KCNQ3 channels in frog oocytes; the similarities to and differences from the present results are considered in the Discussion.

METHODS

The methods of cell culture and transfection, as well as whole-cell and cell-attached patch recording, were as described previously (Selyanko & Brown, 1999; Hadley *et al.* 2000; Selyanko *et al.* 2000). Briefly, Chinese hamster ovary (CHO) cells were grown in minimum essential medium (α -MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 1% penicillin–streptomycin with 5% CO₂ at 37°C. Cells were plated in 35 mm plastic dishes and transfected 1 day later using LipofectAmine Plus (Gibco BRL). KCNQ and CD8 cDNA plasmids, in pCR3.1 vector (Invitrogen, The Netherlands), were co-

transfected in a 10:1 ratio. For expression of heteromultimers, equal amounts of KCNQ2 and KCNQ3 cDNAs were used. Transfected cells were identified by adding CD8-binding Dynabeads (Dyna, UK) before recording.

Macroscopic KCNQ2/KCNQ3 currents were recorded at room temperature (20–22 °C) 1 day after transfection. (At longer times the currents were too large for an adequate voltage clamping.) Cells were bath perfused with a 'low-K⁺' solution of the following composition (mM): NaCl 144, KCl 2.5, CaCl₂ 2, MgCl₂ 0.5, Hepes 5, glucose 10; pH 7.4 with Tris base. Pipettes (2–3 MΩ) were filled with an 'internal' solution containing (mM): potassium acetate 80, KCl 30, Hepes 40, MgCl₂ 3, EGTA 3, CaCl₂ 1; pH 7.4 with NaOH. Amphotericin B was added to permeabilise the patch (Rae *et al.* 1991). Series resistance was compensated (90%). Currents were filtered at 1 kHz and sampled at 4–8 kHz.

Single channel activity was recorded 2–4 days after transfection. (This extended period increased the chances of obtaining functional channels in the patches.) Cell-attached pipettes were filled with the above 'low-K⁺' bath solution whereas cells were bathed in a solution with an elevated (25 mM) [K⁺], in order to clamp the membrane potential near to –30 mV. The methods of recording and analysis were similar to those previously employed for studying M-channels (Selyanko & Brown, 1999). Records were filtered at 1 kHz and sampled at 4.46 kHz. Pipette resistance was 2–3 MΩ.

Data were acquired and analysed using pCLAMP software (versions 6.0 and 8.0; Axon Instruments, CA, USA). Currents were recorded using an Axopatch 200A patch-clamp amplifier (Axon Instruments). Whole-cell KCNQ2/KCNQ3 currents were measured from the amplitudes of the deactivation tails recorded during a step from –20 to –50 mV. An estimate of cell capacitance was obtained by reading the setting for whole-cell input capacitance neutralisation directly from the amplifier. Expression of KCNQ2/KCNQ3 channels was estimated as the macroscopic current–cell capacitance ratio.

The presence of a single channel in a patch was deduced from the lack of current superpositions over several minutes of recording, particularly at positive membrane potentials where the activity would be strongly facilitated. When current superpositions were observed, the lower limit for the number of active channels in the patch was estimated from the maximal number of superimposed openings.

Mean single channel current (i), open probability (P_o) and kinetic components were determined for the activity from single channels.

Open and closed states were nominated when current amplitudes were above or below, respectively, the 50% level. Records were filtered at 500 Hz. Channel openings and closings shorter than 1 ms were ignored and only events ≥ 1.5 ms were included for analysis. i was obtained by fitting open-points amplitude histograms with single Gaussian curves. Distributions of open and closed times were logarithmically binned and were fitted with exponential densities by the method of maximum likelihood (see Colquhoun & Sigworth, 1995). The number of exponential densities used for fitting the histograms was determined in pSTAT (pCLAMP) on the basis of examination of the 'F statistic' ($P > 0.99$).

Where activity was produced by more than one channel, only i and an apparent P_o were measured. Current levels were estimated by fitting all-points amplitude histograms with multiple Gaussian curves, whereas P_o was calculated according to the following equation:

$$NP_o = \left(\sum_{j=1}^N t_j \right) / T,$$

where t_j is the time spent at each current level corresponding to $j = 0, 1, 2 \dots N$; T is the duration of the recording; N is the number of current levels (minimum number of active channels).

The program Origin (version 5.0, Microcal Software Inc., MA, USA) was used for constructing linear and Boltzmann fits and creating the figures.

cDNAs for human KCNQ2 and rat KCNQ3 were obtained from Dr D. McKinnon (as in Wang *et al.* 1998) and for human KCNQ1 from Dr M. T. Keating. All drugs and chemicals were obtained from Sigma or BDH (Poole, UK).

RESULTS

Expression of whole-cell KCNQ2/KCNQ3 currents in CHO cells

When transfected into CHO cells, both hetero- and homomeric KCNQ2/KCNQ3 channels produced M-type potassium currents with characteristic sustained activation on membrane depolarisation to –20 mV and slow deactivation/reactivation following long (1 s) hyperpolarising voltage steps (Fig. 1). However,

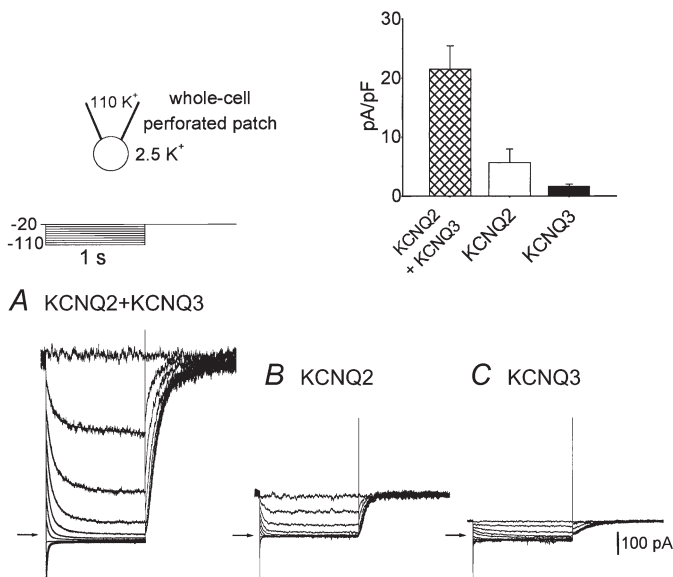


Figure 1. KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 currents in CHO cells

KCNQ2 + KCNQ3 (A), KCNQ2 (B) and KCNQ3 (C) currents recorded 1 day after transfection at different membrane potentials using the whole-cell perforated-patch technique and standard deactivation M-current protocol: holding level, –20 mV; step duration, 1 s; interval, 15 s; increment, –10 mV (voltage protocol (mV) is shown in A). Arrows show the zero-current levels. Inset, mean densities of KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 currents (mean \pm S.E.M.) recorded 1 day after transfection and expressed as the ratio between the current amplitude at –50 mV and cell capacitance. Current densities were 21.6 ± 3.9 pA pF⁻¹ ($n = 13$), 5.7 ± 2.3 pA pF⁻¹ ($n = 10$) and 1.7 ± 0.4 pA pF⁻¹ ($n = 7$), respectively (mean \pm S.E.M.).

Table 1. KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 currents and properties of underlying channels

		KCNQ2 + KCNQ3	<i>n</i>	KCNQ2	<i>n</i>	KCNQ3	<i>n</i>
<i>I</i>	pA pF ⁻¹	21.6 ± 3.9*,**	13	5.7 ± 2.3*,***	10	1.7 ± 0.4**,***	7
	Relative	1		0.3		0.08	
<i>P_o</i>		0.30 ± 0.07 †,††	9	0.15 ± 0.03 †,†††	12	0.59 ± 0.11 ††,†††	12
<i>i</i>	pA	0.71 ± 0.04 †,††	9	0.57 ± 0.02 †,†††	12	0.53 ± 0.13 ††,†††	12
<i>iP_o</i>	pA	0.21 ± 0.03 §,§§		0.09 ± 0.02 §,§§§		0.36 ± 0.08 §§,§§§	
	Relative	1		0.43		1.71	

Values are means ± S.E.M. (*n* = number of observations). Relative values are the ratio relative to KCNQ2 + KCNQ3. Comparison of results with same symbols: * *P* < 0.01; ** *P* < 0.001; *** *P* > 0.05; † *P* > 0.05; †† *P* < 0.05; ††† *P* < 0.001; ‡ *P* < 0.01; ‡‡ *P* < 0.05; ‡‡‡ *P* > 0.05; § *P* < 0.05; §§ *P* > 0.05; §§§ *P* < 0.01.

KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channel currents (measured from the deactivation tails at -50 mV; see Methods) were clearly different in amplitude. When recorded 1 day after transfection, the current density ratios were (KCNQ2 + KCNQ3 : KCNQ2 : KCNQ3) 1 : 0.3 : 0.08 (see inset to Fig. 1 and Table 1).

Single channel properties of KCNQ2/KCNQ3 currents

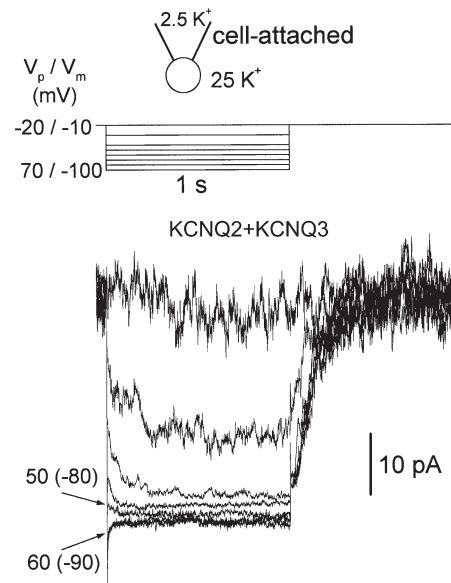
Multi-channel KCNQ2 + KCNQ3 currents recorded with cell-attached pipettes (Fig. 2) were similar to whole-cell currents (Fig. 1) in their time and voltage dependencies (as well as in their muscarinic sensitivity; Selyanko *et al.* 2000) which suggests that the channel properties were well-preserved in the cell-attached patch configuration. Furthermore, the deactivation tails of these currents reversed at the same membrane potential (between -90 and -80 mV; *n* = 4; see Fig. 2) as the whole-cell current tails (see Fig. 1), which confirmed our supposition that with 25 mM K⁺ in the extra-patch (bath) solution the cell membrane potential was set within the range of KCNQ2/KCNQ3 channel activation, at ~ -30 mV.

As expected from previous observations on native neuronal M-type channels, which in low external K⁺ had medium-range conductances of 7–11 pS (Selyanko *et al.* 1992), single channel current levels were clearly resolved even when recorded from patches containing multiple KCNQ2/KCNQ3 channels, especially when channels were deactivated by hyperpolarising voltage steps (upper traces in Fig. 3A–C). Thus, averaging of many responses was required to produce ‘smooth’ KCNQ2/KCNQ3 deactivation currents (lower traces in Fig. 3A–C). By contrast, deactivation of currents produced by the related ‘cardiac’ KCNQ1 channels (which in low external K⁺ have a very small conductance of < 1 pS; Pusch, 1998; Sesti & Goldstein, 1998; Yang & Sigworth, 1998), showed a ‘smooth’ deactivation relaxation even in response to a single voltage step, with no resolvable single channel events (*n* = 4; Fig. 3D).

Activity was produced by single channels in some of the patches studied (4/15, KCNQ2 + KCNQ3; 3/15, KCNQ2; 6/13, KCNQ3); the rest had more than one channel in the patch. Examples of steady-state single channel activities recorded at 0 mV (Fig. 4Aa–Ca), as well as their open-

Figure 2. KCNQ2 + KCNQ3 currents produced by multi-channel activity and recorded from a cell-attached membrane patch

Currents were recorded using a cell-attached pipette and a deactivation protocol (top panel) similar to that shown in Fig. 1. Note that these currents matched the corresponding whole-cell currents in their time dependence, negative threshold of activation (~ -60 mV) and reversal potential (between -90 and -80 mV). With 25 mM K⁺ in the bath, the cell membrane potential (*V_m*) was assumed to be close to -30 mV. *V_p*, pipette potential.



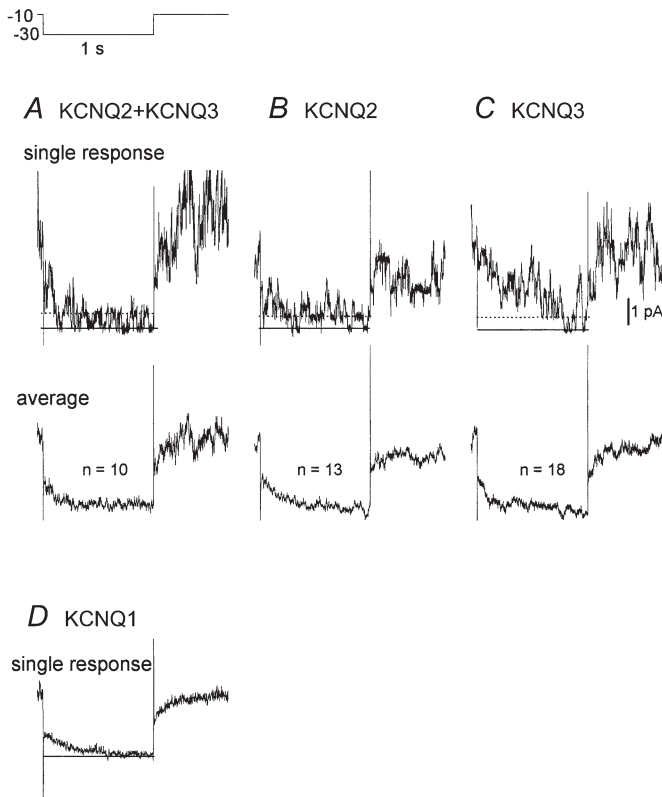


Figure 3. Deactivation of currents produced by KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels, but not by KCNQ1 channels, shows resolvable single channel current levels

KCNQ2 + KCNQ3 (A), KCNQ2 (B) and KCNQ3 (C) channels were pre-activated by holding the membrane potential in the patch at -10 mV and then deactivated by 1 s steps to -30 mV. Upper traces in A–C, single responses; lower traces in A–C, averaged responses (number of averaged records is indicated near each response). D, KCNQ1 current; note that when recorded under the same conditions, the deactivating KCNQ1 current showed no resolvable single channel events. Data in A–D were obtained from four different cell-attached membrane patches.

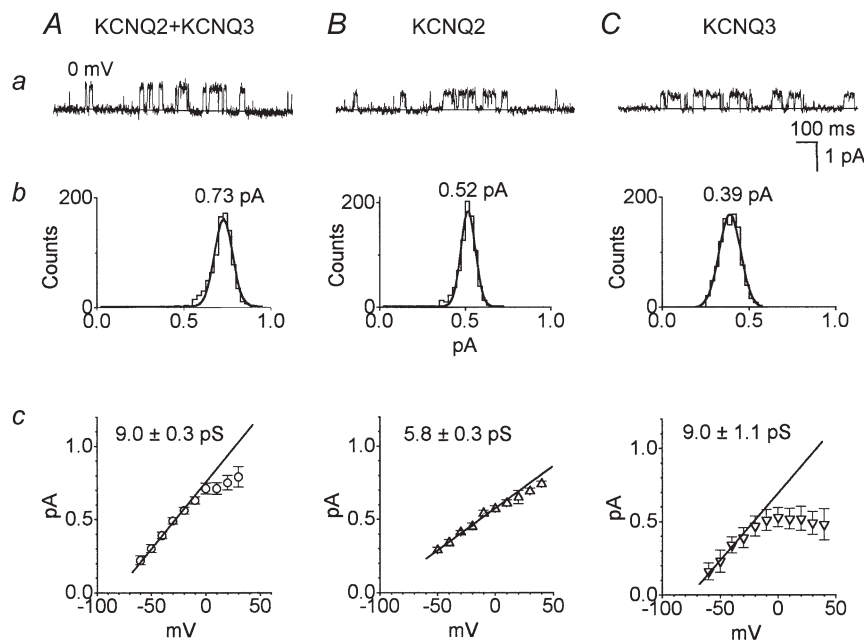


Figure 4. Conductance of single KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels

Upper row (a) shows examples of openings of single KCNQ2 + KCNQ3 (A), KCNQ2 (B) and KCNQ3 (C) channels recorded from three different cell-attached membrane patches at 0 mV. Middle row (b), open-point amplitude histograms for the activities shown in a fitted with Gaussian curves (smooth lines). The current components were 0.73 ± 0.10 pA (Ab), 0.52 ± 0.08 (Bb) and 0.39 ± 0.12 (Cb) (mean \pm S.E.M.). Lower row (c), mean single channel currents (means \pm S.E.M.) for currents shown in a plotted against patch membrane potential. In view of the inward rectification of the current–voltage relationships, slope conductances were measured between around -60 and -20 mV; they were 9.0 ± 0.3 pS (Ac), 5.8 ± 0.3 pS (Bc) and 9.0 ± 1.1 pS (Cc) (mean \pm S.E.M.).

points amplitude histograms (fitted by single Gaussian distributions; Fig. 4*Ab-Cb*), show that single KCNQ2/KCNQ3 channels have only one predominant conductance level in the open state. (In some records descents to lower conductances could also be detected but they were very infrequent (< 1%) and their contribution to the overall activity was negligible.)

$i-V_m$ relationships for all three types of channel showed an inward rectification at positive potentials which was most pronounced in KCNQ3 channels (Fig. 4*Ac-Cc*). This rectification correlated with previously noted rectification of whole-cell KCNQ currents which showed 'saturation' or a reduction in the response to strong membrane depolarisation, and either remained unchanged or increased in response to moderate repolarisation (see Fig. 1 in Selyanko *et al.* 2000). Thus, slope single channel conductances were measured at negative membrane potentials (between about -60 and -20 mV), where the relationships were linear and where expression of whole-cell currents was measured. Slope conductances obtained from pooled data were found to be 9.0 ± 0.3 pS (KCNQ2 + KCNQ3; mean \pm S.E.M.; $n = 13$ patches),

5.8 ± 0.3 pS (KCNQ2; $n = 15$ patches) and 9.0 ± 1.1 pS (KCNQ3; $n = 13$ patches). When measured in individual patches, slope conductances ranged between 6.8 and 11.9 pS (KCNQ2 + KCNQ3), 4.1 and 7.8 pS (KCNQ2), and 5.5 and 13.1 pS (KCNQ3). This suggests that KCNQ2 + KCNQ3 and KCNQ3 channels may correspond to both '7 pS' and '11 pS' M-type channels (Selyanko & Brown, 1993) whereas KCNQ2 channels may correspond to only the '7 pS' channels.

Thus, conductances of KCNQ2 + KCNQ3 and KCNQ3 channels were similar, whereas the conductance of KCNQ2 channels was about 50% lower (*t* test, $P < 0.01$).

KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels showed sustained voltage-dependent activation at ≥ -60 mV, although at each potential tested the degree of activation of these channels was different: mean P_o was highest for KCNQ3 channels and lowest for KCNQ2 channels, with KCNQ2 + KCNQ3 channels having an intermediate P_o . Figure 5*A* shows examples of steady-state activities of single KCNQ2 + KCNQ3, KCNQ2, and KCNQ3 channels recorded at 0 mV, where they reached

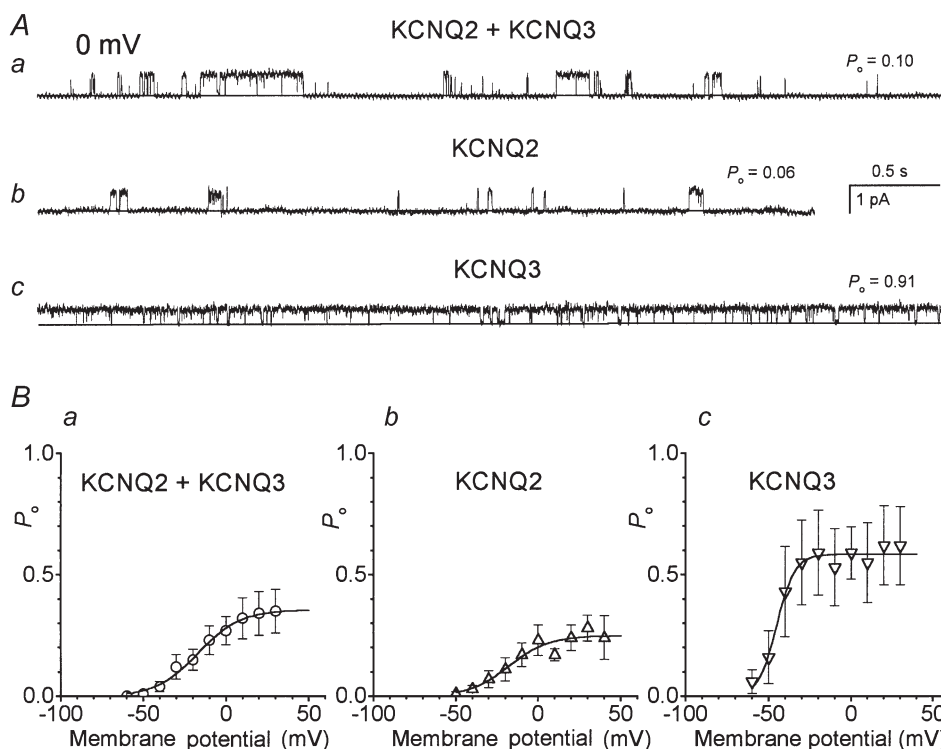


Figure 5. Open probability for single KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels

A, examples of steady-state activities of single KCNQ2 + KCNQ3 (*a*), KCNQ2 (*b*) and KCNQ3 (*c*) channels recorded from three different cell-attached membrane patches at 0 mV. Open probabilities (P_o) for these activities are indicated near each record. *B*, mean P_o (means \pm S.E.M.) for KCNQ2 + KCNQ3 (*a*), KCNQ2 (*b*) and KCNQ3 (*c*) channels plotted against patch membrane potential. P_o-V_m relationships were fitted by Boltzmann equations with maximal P_o , half-activation potential and slope factor of, respectively, 0.36 ± 0.01 , -17.0 ± 1.7 mV and 12.7 ± 1.3 mV for KCNQ2 + KCNQ3 (*a*; 7 patches), 0.25 ± 0.02 , -17.9 ± 4.1 mV and 12.1 ± 3.4 mV for KCNQ2 (*b*; 5 patches), and 0.58 ± 0.01 , -45.3 ± 1.1 mV and 5.5 ± 0.9 mV for KCNQ3 (*c*; 6 patches) (mean \pm S.E.M.). P_o was estimated as described in the Methods.

their near-maximal activation with P_o values of 0.1, 0.06 and 0.91, respectively. Figure 5B illustrates the mean P_o - V_m relationships. When fitted by Boltzmann equations, these relationships for KCNQ2 + KCNQ3 and KCNQ2 channels had similar half-activation potentials (~ -17 mV) and slope factors (~ 12 mV), but different maximal P_o values (0.36 and 0.25, respectively). By comparison, the P_o - V_m relationship for KCNQ3 channels had a more negative half-activation potential (-45 mV), a steeper slope factor (6 mV) and a higher maximal P_o (0.58) (Fig. 5Bc). Parameters of activation of single KCNQ2/KCNQ3 channels were similar to those for corresponding whole-cell currents previously reported from CHO (Selyanko *et al.* 2000) and tsA-201 (Shapiro *et al.* 2000) cells. Thus, in CHO cells activation curves for KCNQ2/KCNQ3 channels were fitted by the Boltzmann equation with the half-activation potential and the slope factor equal to -17.7 ± 0.6 mV and 11.9 ± 0.5 mV (KCNQ2 + KCNQ3); -13.8 ± 0.4 mV and 12.1 ± 0.4 mV (KCNQ2); -36.8 ± 1.3 mV and 5.5 ± 1.1 mV (KCNQ3) (Selyanko *et al.* 2000).

As noted above, individual patches containing KCNQ2 + KCNQ3 channels showed a nearly 2-fold difference in slope conductances (range between 6.8 and 11.9 pS). This raises the question of whether low- and high-conductance channels also have different P_o values. In order to test this possibility, the mean current amplitudes and open probabilities for channels with low (< 9 pS) and high (≥ 9 pS) conductances were plotted against membrane potential. Figure 6 shows that the channels with low conductance have the higher maximal P_o .

The amplitude of the whole-cell current is equal to NiP_o , where N is the number of expressed channels and i and P_o are their mean single channel current amplitude and open probability. In order to establish whether the difference in expression of whole-cell KCNQ2/KCNQ3 currents was related to the difference in the number of expressed

channels or the difference in their abilities to generate currents, we examined the second possibility by measuring an integral current (iP_o) through individual KCNQ2/KCNQ3 channels. At 0 mV iP_o for KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 was 0.21 ± 0.03 pA ($n = 9$), 0.09 ± 0.02 pA ($n = 12$) and 0.36 ± 0.08 pA ($n = 12$), respectively. Thus, the ratio of the ability of these channels to generate currents was 1 : 0.43 : 1.71. By comparing this ratio with the whole-cell current density ratio (1 : 0.3 : 0.08) one can suggest that only in the case of homomeric KCNQ2 could their smaller whole-cell currents be largely due to reduced single channel conductance and P_o . In the case of homomeric KCNQ3 channels their smaller whole-cell currents must have been due to a 21-fold lower density of expressed channels. For comparison with expressed whole-cell currents, parameters of the underlying channels are summarised in Table 1.

M-type potassium channels have multiple kinetic components to their activity, suggesting the presence of at least three shut and two open states (Selyanko & Brown, 1993, 1999). Because M-type channels are heteromers, such a complex kinetic behaviour might be related to participation of two different subunits in forming the channel. On the other hand, the differences in P_o for KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels might be due to variation in the number of kinetic states assumed by these channels, resulting in shifts in the equilibrium between the open and the shut states. Thus, the steady-state kinetics of KCNQ2/KCNQ3 channels were examined. The results showed that homomeric KCNQ2 and KCNQ3 and heteromeric KCNQ2 + KCNQ3 channels have the same number of kinetic components to their activity, and that these were similar to those in native M-type channels. Figure 7 exemplifies the distributions of shut and open times obtained from the activity of single KCNQ2 + KCNQ3 channels recorded at 0 mV. These distributions were

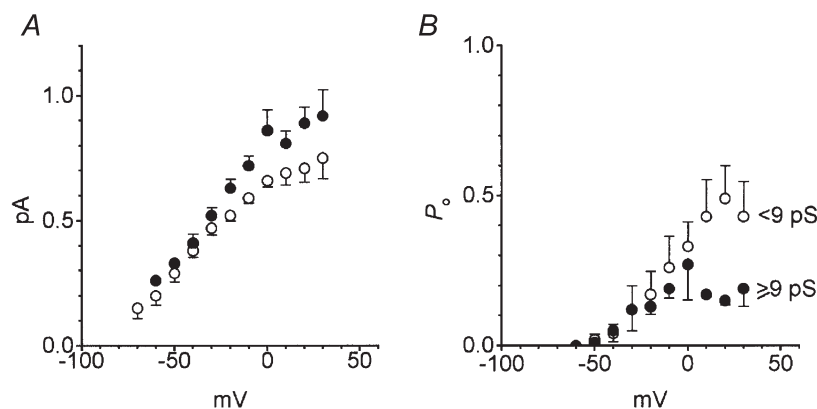


Figure 6. Heteromeric low- and high-conductance KCNQ2 + KCNQ3 channels have different open probabilities

Single channel current amplitudes (A) and open probabilities (B) plotted against membrane potential for channels with low (< 9 pS, \circ ; $n = 9$) and high (≥ 9 pS, \bullet ; $n = 4$) conductances. Conductances in individual patches were measured as slope conductances (see text). Data are means \pm S.E.M.

Table 2. Kinetic components of the activity of single KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 channels

Channel	n	Shut times			Open times	
		τ_{s1} (ms) (%)	τ_{s2} (ms) (%)	τ_{s3} (ms) (%)	τ_{o1} (ms) (%)	τ_{o2} (ms) (%)
KCNQ2 + KCNQ3	4	1.4 ± 0.2 (34.2 \pm 8.7)	13.8 ± 8.4 (32.0 \pm 3.4)	128.6 ± 39.0 (33.8 \pm 11.4)	3.2 ± 0.9 (26.8 \pm 10.3)	21.9 ± 5.0 (73.2 \pm 10.3)
KCNQ2	3	1.3 ± 0.3 (23.5 \pm 2.8)	14.6 ± 5.6 (26.7 \pm 1.8)	297 ± 137 (49.8 \pm 4.5)	4.9 ± 0.8 (30.7 \pm 6.1)	42.2 ± 6.5 (69.3 \pm 6.1)
KCNQ3	6	2.0 ± 0.6 (39.1 \pm 9.5)	15.5 ± 6.4 (32.0 \pm 7.0)	85.3 ± 39.5 (28.9 \pm 9.6)	4.5 ± 2.8 (35.6 \pm 8.0)	28.8 ± 9.4 (64.4 \pm 8.0)

Activities were recorded at 0 mV. τ_{s1} , τ_{s2} and τ_{s3} are short, medium and long kinetic components in the distribution of channel shut times, whereas τ_{o1} and τ_{o2} are short and long kinetic components in the distribution of open times. Values are means \pm S.E.M. (n = number of patches). Values in parentheses are percentages of the total shut or open times.

fitted by three and two exponentials, respectively. Mean kinetic components for all three forms of KCNQ2/KCNQ3 channel measured at 0 mV are shown in Table 2. Hence, both hetero- and homomeric KCNQ2/KCNQ3 channels are capable of producing the complex kinetic behaviour characteristic of neuronal M-type potassium channels.

DISCUSSION

In the present work we have tried to establish the link between the expression of M-type potassium currents and functional properties of the participating subunits. Expression of whole-cell KCNQ2/KCNQ3 currents had the following ratio: 1 : 0.3 : 0.08 (KCNQ2 + KCNQ3 : KCNQ2 : KCNQ3). Previously reported expression of KCNQ2 + KCNQ3, KCNQ2 and KCNQ3 currents in *Xenopus* oocytes yielded the same sequence but with much larger differences between hetero- and homomeric channels (expression ratio: 1 : 0.08 : <0.01; Wang *et al.* 1998; Yang *et al.* 1998). This raises the possibility that homomeric forms of KCNQ2 and KCNQ3 channels might contribute to the 'heteromeric' KCNQ2 + KCNQ3

currents studied in the present experiments. However, this seems unlikely, at least with the expression system employed in the present experiments, since in previous experiments using CHO cells we found that expressed KCNQ2 + KCNQ3 currents had a TEA sensitivity intermediate between those for homomeric KCNQ2 and KCNQ3 channels (IC_{50} , 3.8, 0.3 and > 30 mM respectively) while the slope of the concentration–inhibition curve remained close to that (1.0) for inhibition of the homomeric KCNQ2 current (Hadley *et al.* 2000). This implies a homogeneous (or near-homogeneous) population of KCNQ2 + KCNQ3 channels with a unique (possibly 2 + 2) stoichiometry. (By contrast, when expressed in tsA-201 cells KCNQ2 + KCNQ3 currents were less sensitive to TEA and had a shallow dependence on [TEA], consistent with expression of a significant population of homomeric channels; Shapiro *et al.* 2000.) Though we cannot totally exclude the possibility of some contribution by homomeric KCNQ2 channels to our KCNQ2/KCNQ3 currents, a contribution by homomeric KCNQ3 channels is most unlikely in view of the observed low expression of whole-cell KCNQ3 currents, and an even lower estimated expression of KCNQ3 channels.

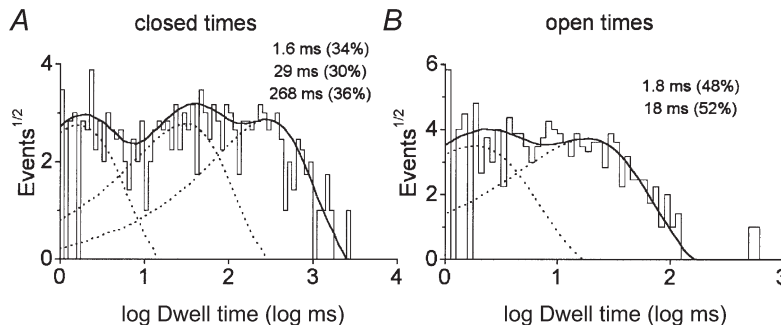


Figure 7. Kinetics of KCNQ2 + KCNQ3 channel activity

Shut (A) and open (B) time distributions for the activity recorded at 0 mV. Distributions were fitted by exponential densities. Individual components are shown by dotted lines and their sums by smooth lines. Kinetic components are indicated above each distribution.

Possible contributions by different stoichiometric assemblies of co-expressed KCNQ2 and KCNQ3 subunits might partly be resolved in future experiments by comparison with currents generated using tandem KCNQ2/KCNQ3 constructs; see Wickenden *et al.* (2000).

The properties of single heteromeric KCNQ2 + KCNQ3 channels appeared to be consistent with those previously reported, under similar conditions, from native M-type potassium channels (Selyanko & Brown, 1993, 1999). The two types of channel showed the same range of conductances (7–11 pS, with openings to only one conductance level), a comparable maximal P_o (~ 0.2 – 0.3) and five kinetic components to their steady-state activities. To this extent, therefore, our single channel data accord with the view that native M-channels in rat sympathetic neurones might be composed of a heteromeric assembly of KCNQ2 and KCNQ3 channels (Wang *et al.* 1998). This result was not entirely predictable since the properties of M-channels in neurones are probably affected by expression of different splice variants of KCNQ2 (Pan *et al.* 2001; Smith *et al.* 2001), KCNQ5 (Lerche *et al.* 2000; Schroeder *et al.* 2000a; Wickenden *et al.* 2001), or expression of an accessory subunit KCNE2 (Tinel *et al.* 2000).

Homomeric KCNQ2 channels had a somewhat lower mean slope conductance (5.8 pS) than the heteromeric channels (9.0 pS). This, together with their lower maximum P_o (0.15), would go a considerable way towards explaining the smaller KCNQ2 macroscopic currents without the necessity of postulating any very large difference in channel expression (see Table 1). However, this was not true for homomeric KCNQ3 channels, since individual KCNQ3 channels showed a high, although variable, P_o (0.59) and a comparable slope conductance to the heteromeric channels (although the current through these channels was more strongly affected by inward rectification at positive potentials). Hence, it would appear from this that the much smaller macroscopic KCNQ3 currents observed in CHO cells result primarily from a reduced level of membrane expression of the homomeric KCNQ3 channels. One might then speculate that, whereas co-assembly of KCNQ2 is necessary for trafficking and expression of the heteromeric (KCNQ2 + KCNQ3) channels, the KCNQ3 subunit exerts the stronger influence on the biophysical properties (conductance and open probability) of the heteromeric channels.

Somewhat different conclusions have been drawn from recent experiments on HEK cells (Cooper *et al.* 2000) and *Xenopus* oocytes (Schwake *et al.* 2000), which showed an enhanced expression of both KCNQ2 and KCNQ3 subunits when they were co-expressed. This would accord with the fact that, in the latter cells, homomeric KCNQ2 currents were very much smaller than the heteromeric

KCNQ2 + KCNQ3 currents (Wang *et al.* 1998; Yang *et al.* 1998). In contrast, the larger KCNQ2 currents in our experiments clearly imply a more substantial expression of homomeric KCNQ2 channels in CHO cells. Another difference in the oocyte experiments is that the apparent conductance of homomeric KCNQ3 channels was half that of homomeric KCNQ2 or heteromeric KCNQ2 + KCNQ3 channels. However, these conductance measurements were deduced from inward currents measured in high potassium solutions: since the degree of rectification (or otherwise) was not determined, it is difficult to compare these inward currents both with the macroscopic currents recorded from oocytes in normal Ringer solution and with the single channel currents recorded in the present experiments. Further, differences in the apparent conductances of the same molecular species of ion channel when expressed in frog oocytes and mammalian cells have been noted previously (e.g. Lewis *et al.* 1997).

The ability to form heteromers, with different functional properties, is characteristic of KCNQ channels. Thus, co-assembly of KCNQ1 and KCNE1 (which does not form functional channels on its own) results in larger whole-cell currents (owing to an increase in single channels conductance; Pusch, 1998; Sesti & Goldstein, 1998; Yang & Sigworth, 1998), a negative shift in the activation curve and slowed kinetics of activation/deactivation (Barhanin *et al.* 1996; Sanguinetti *et al.* 1996; Yang *et al.* 1997). Some inherited mutations of KCNQ1 channels both reduce the expression of KCNQ1 channels and change their deactivation kinetics (Chouabe *et al.* 1997; Schmitt *et al.* 2000). Co-assembly of KCNQ1 and KCNE3 subunits results in constitutively active channels which do not show voltage- or time-dependence of activation (Schroeder *et al.* 2000b). KCNQ2 + KCNQ3 heteromers produce larger currents with essentially the same kinetics and parameters of activation as KCNQ2 or KCNQ3 homomers (Chouabe *et al.* 1997; Wang *et al.* 1998; Kubisch *et al.* 1999; Selyanko *et al.* 2000; Shapiro *et al.* 2000) and mutations which cause epilepsy produce a very moderate (20–30%) reduction in the expression of KCNQ2/KCNQ3 channels without obvious changes in the parameters of whole-cell currents (Schroeder *et al.* 1998). Co-assembly of KCNQ4 and KCNQ3 subunits results in larger whole-cell currents with faster activation/deactivation and a more negative threshold of activation compared with homomeric channels (Kubisch *et al.* 1999). Heteromers of KCNQ5 subunits with KCNQ3 produce larger currents with only slight changes in activation parameters (Lerche *et al.* 2000; Schroeder *et al.* 2000b). Thus, the relationship between changes in expression of whole-cell currents and their biophysical parameters varies with different KCNQ heteromers. Whether changes in the parameters of single channels are also variable remains to be established.

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