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SPECIAL REPORT

Differential tetraethylammonium sensitivity of KCNQ1–4 potassium channels

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In *Shaker*-group potassium channels the presence of a tyrosine residue, just downstream of the pore signature sequence GYG, determines sensitivity to tetraethylammonium (TEA). The KCNQ family of channels has a variety of amino acid residues in the equivalent position. We studied the effect of TEA on currents generated by KCNQ homomers and heteromers expressed in CHO cells. We used wild-type KCNQ1–4 channels and heteromeric KCNQ2/3 channels incorporating either wild-type KCNQ3 subunits or a mutated KCNQ3 in which tyrosine replaced threonine at position 323 (mutant T323Y). IC_{50} values were (mM): KCNQ1, 5.0; KCNQ2, 0.3; KCNQ3, >30; KCNQ4, 3.0; KCNQ2+KCNQ3, 3.8; and KCNQ2+KCNQ3(T323Y), 0.5. While the high TEA sensitivity of KCNQ2 may be conferred by a tyrosine residue lacking in the other channels, the intermediate TEA sensitivity of KCNQ1 and KCNQ4 implies that other residues are also important in determining TEA block of the KCNQ channels.
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Abbreviations: CHO (hm1), Chinese hamster ovary cell (stably expressing human M_1 muscarinic receptors); EGTA, ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; IC_{50}, drug concentration giving 50% block of current; M-channel/M-current, muscarinic-inhibited potassium channel/current; NG108-15, neuroblastoma-glioma hybrid cell line; n_H, Hill slope; pIC_{50}, negative log of IC_{50}; TEA, tetraethylammonium

Introduction The KCNQ family of channels includes the cardiac slow delayed rectifier KCNQ1 (KvLQT1) (Barhanin *et al*., 1996; Sanguinetti *et al*., 1996), the neural-specific channels KCNQ2 and KCNQ3 (Biervert *et al*., 1998; Charlier *et al*., 1998; Yang *et al*., 1998), which have been proposed as components of the M-channel (Wang *et al*., 1998), and KCNQ4, whose mRNA is expressed in the cochlea (Kubisch *et al*., 1999). The TEA sensitivity of KCNQ2 is higher than that of the M-channel, that of KCNQ3 is lower, and that of the KCNQ2/3 heteromultimer closely matches that of the M-current (Yang *et al*., 1998; Wang *et al*., 1998). Hence, differential TEA sensitivity can provide helpful information regarding the contribution of different members of this gene family to native K^+ channels.

The high TEA sensitivity of KCNQ2 might result from the presence of a tyrosine residue in the pore loop of the channel (see Kavanaugh *et al*., 1991). Thus, the *Shaker* potassium channel, which is weakly blocked by TEA (IC_{50} 27 mM), has a threonine at position 499 (just downstream of the GYG pore sequence at positions 444–446), but can be made much more sensitive to TEA (0.59 mM) by replacing T449 with a tyrosine (MacKinnon & Yellen, 1990). KCNQ2 also has a tyrosine in the corresponding position, whereas KCNQ3 has a threonine (Wang *et al*., 1998) and KCNQ1 and KCNQ4 have a valine and a threonine, respectively (Kubisch *et al*., 1999).

Using a CHO cell expression system and the perforated-patch configuration of the patch-clamp, we quantified the effect of TEA on currents generated by homomeric wild-type KCNQ1–4 channels, on heteromeric wild-type KCNQ2/3 channels and on heteromeric KCNQ2/3 channels incorporating a mutated KCNQ3 subunit in which tyrosine replaced threonine at position 323.

Methods Methods for culture and transfection of CHO hm1 cells have been described previously in Selyanko *et al*. (1999). CHO cells (a derivative of the CHO-K1 line; Mullaney *et al*., 1993) were grown at 37°C and 5% CO_2 in α-MEM supplemented with 10% foetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin. For recording, cells were plated in 35 mm plastic dishes, and transfected 1–2 days after plating using Lipofect Amine Plus (Life Technologies) as suggested by the manufacturer. Plasmids (driven by CMV promoter) containing cDNAs for KCNQ channels, and cDNA for CD8 as a marker, were co-transfected at a channel : marker ratio of 10 : 1. Cells for patch-clamping were identified using CD8-binding Dynabeads (Dynal (U.K.) Ltd) 1 day after transfection.

The perfusing solution contained (mM): NaCl 144, KCl 2.5, $CaCl_2$ 2, $MgCl_2$ 0.5, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) 5, and glucose 10 plus Tris base to pH 7.4. The pipette solution contained (mM): K acetate 80, KCl 30, HEPES 40, $MgCl_2$ 3, ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA) 3, $CaCl_2$ 1 and NaOH to pH 7.4. Amphotericin B was used to perforate the patch (Rae *et al*., 1991). TEA was applied cumulatively, using 0.2 M and 2 M aqueous TEA solutions added to the reservoir of perfusing fluid.

Data were acquired and analysed using pClamp software (version 6.0.3). Currents were recorded using an Axopatch 200A (or 200) patch-clamp amplifier, filtered at 1 kHz, and digitized at 1–4 kHz. One second steps to −50 mV were applied from a holding potential of −20 mV and the total deactivation relaxation amplitude was measured at −50 mV in control and for each dose of TEA. Data were normalized and plotted in Origin (version 5.0, Microcal Software), and curves

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were fitted with the equation: $y = 1/(1 + (x/x_0)^p)$ where y is the relative current amplitude; x is the concentration of the blocker; x_0, the IC_{50}, is the concentration at which y is 0.5; and p is the power (equivalent to the Hill slope, n_H). Additionally, data were plotted against log concentrations and fitted with the Boltzman equation: $y = 1/(1 + e^{(x-x0)/dx})$, where x0 is the pIC_{50} (negative log of the IC_{50}), as a check on the true (geometric) spread of the standard error.

TEA was from Lancaster Synthesis. All other chemicals were obtained from Sigma or BDH Chemicals. Human KCNQ1 cDNA was kindly provided by Dr M.T. Keating; human KCNQ2 and rat KCNQ3 (as studied in Wang *et al*., 1998) by Dr D. McKinnon; and human KCNQ4 (as in Kubisch *et al*., 1999) by Dr T.J. Jentsch. We thank Dr T.J. Jentsch and Tatjana Kharkovets for the mutated KCNQ3(T323Y), which was based on the human sequence.

Results We find that the TEA sensitivity of KCNQ2 is high, that of KCNQ3 is low, and that of KCNQ1 and KCNQ4 is intermediate. Figure 1Aa–d shows representative data records for TEA inhibition of homomeric KCNQ channel currents. Figure 2A and Table 1 show the potencies for these channels. Figure 1Ba,b shows data records for the heteromeric channels produced by co-transfecting KCNQ2 with wild-type KCNQ3 or with the mutated KCNQ3 subunit KCNQ3(T323Y). As is clear from Figure 2B and Table 1, the potency for the heteromeric wild-type KCNQ2 and KCNQ3 was intermediate between those of homomeric KCNQ2 and KCNQ3. The potency for heteromers containing the mutated KCNQ3(T323Y) was increased, approaching that for the wild-type KCNQ2.

Discussion Our results for TEA inhibition of KCNQ2 and KCNQ2/3 currents in CHO cells give IC_{50} values of 0.3 ± 0.02 mM and 3.8 ± 0.2 mM (pIC_{50} values 3.53 ± 0.03 and 2.42 ± 0.02), respectively. These values accord well with previously reported K_d values for these channels expressed in *Xenopus* oocytes, which were 0.16 ± 0.02 mM and 3.5 ± 0.7 mM, respectively (Wang *et al*., 1998). The sensitivity of KCNQ3 to TEA blockade has not been quantified, but 5 mM TEA had a negligible effect on KCNQ3 currents in oocytes (Yang *et al*., 1998). Even the highest concentration of TEA tested in the present experiments, 30 mM, which could completely block KCNQ1, 2 and 4, had a minimal effect on KCNQ3 (Figure 2A).

TEA block does not appear to have been tested before on KCNQ1 and KCNQ4 channels, so it was of interest to compare TEA sensitivity of these channels with that of the

Table 1 Half-maximal blocking concentrations of TEA (IC_{50}) in mM, pIC_{50} (negative log of the IC_{50} in M, fitted separately), and Hill slopes (n_H) for block of KCNQ channels expressed in CHO cells; *n* = number of cells tested

	KCNQ1	*KCNQ2*	*KCNQ3*	*KCNQ4*	*KCNQ2,3*	*KCNQ2,3 (T323Y)*
$IC_{50} \pm$ s.e.mean (mM)	5.0 ± 0.2	0.3 ± 0.02	>30	3.0 ± 0.3	3.8 ± 0.2	0.5 ± 0.05
$pIC_{50} \pm$ s.e.mean	2.30 ± 0.02	3.53 ± 0.03	–	2.53 ± 0.04	2.42 ± 0.02	3.29 ± 0.05
n_H	1.8 ± 0.1	0.9 ± 0.06	–	2.0 ± 0.4	0.8 ± 0.03	1.0 ± 0.09
n	4	4	4	3	4	3

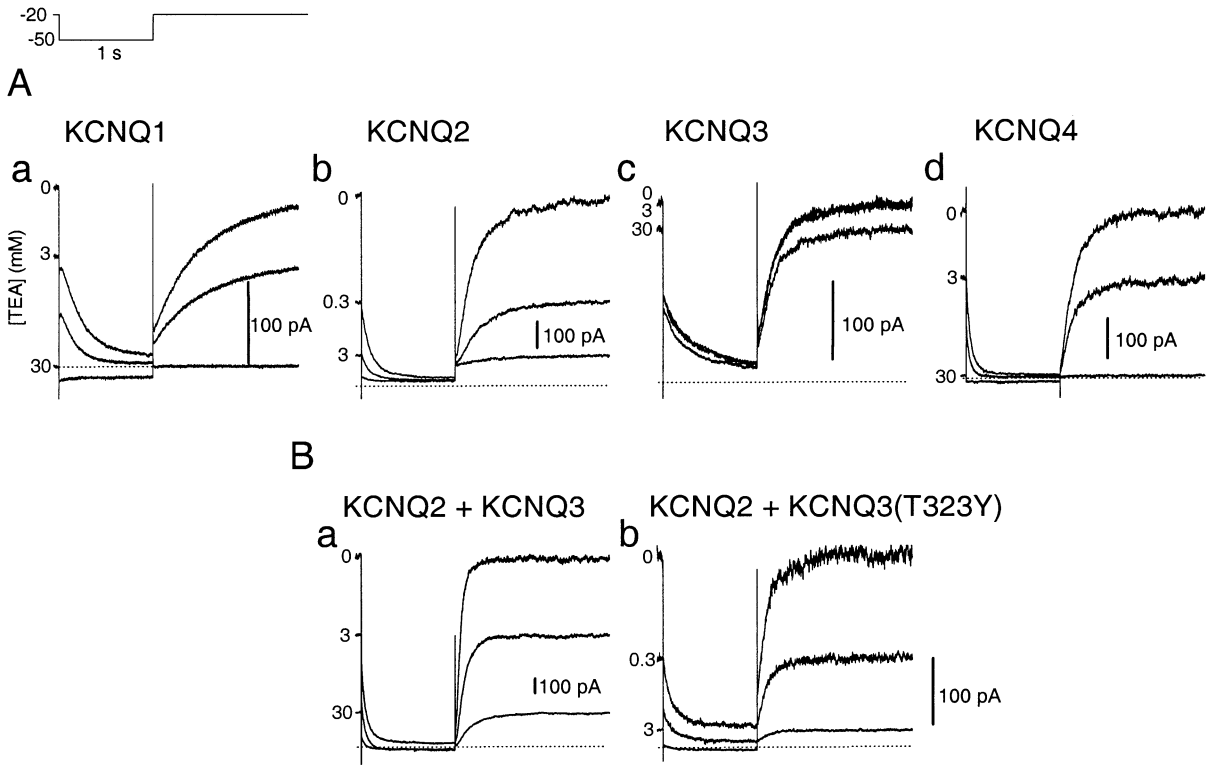

Figure 1 Differential TEA sensitivities of KCNQ1–4 (Aa–d) and heteromeric KCNQ2/KCNQ3 and KCNQ2/KCNQ3(T323Y) currents (Ba,b), expressed in CHO cells. Deactivations were recorded in response to 1 s steps from the holding level of −20 to −50 mV, in the absence and presence of different concentrations of TEA as indicated (in mM) on the left side of each trace.

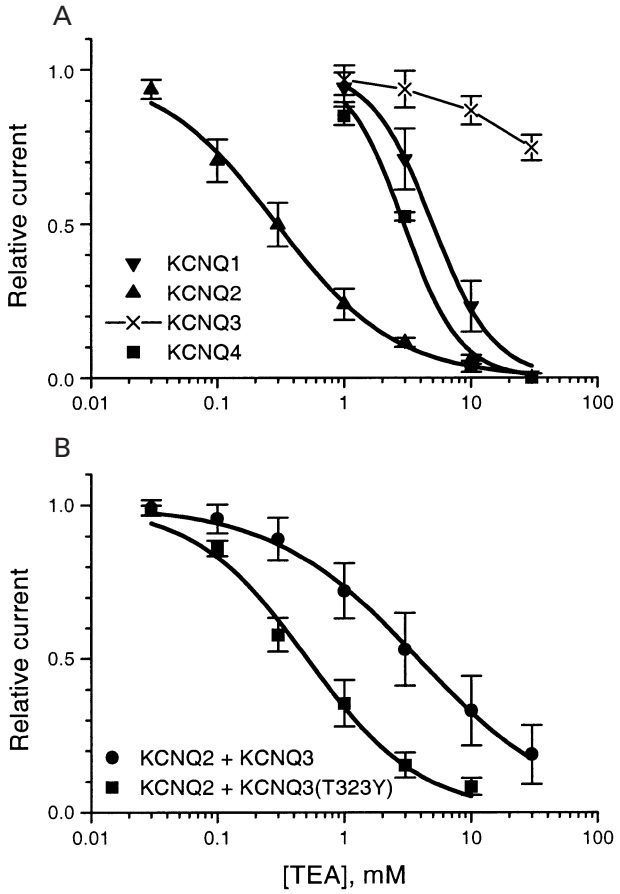

Figure 2 Concentration dependences of inhibition of KCNQ1–4 currents (A), and coexpressed KCNQ2/KCNQ3 and KCNQ2/KCNQ3(T323Y) channels (B) in CHO cells. All values are mean ± s.e.mean. Smooth lines are least squares fits to the equation $y = 1/(1 + (x/x_0)^p)$, superimposed for all data sets except KCNQ3, where the points have been joined by straight lines for clarity.

M-channel components KCNQ2 and KCNQ3. Figure 2 and Table 1 indicate that the slope of the inhibition curve is considerably steeper for KCNQ1 and KCNQ4, with Hill coefficients of around 2, compared with values of about 1 for KCNQ2, KCNQ3 and their heteromers. Thus, the nature of the molecular interaction between TEA and the channel protein may differ between different KCNQ channels. Our Hill coefficient of 0.8 for TEA inhibition of KCNQ2/3 heteromers matches the previously reported value of 0.8 for these channels (Yang *et al*., 1998), and is comparable with values of 0.6 for both the mouse superior cervical ganglion M-current and the fast component of the neuroblastoma-glioma hybrid (NG108-15) M-like current (Selyanko *et al*., 1999).

We find that substituting tyrosine for threonine at position 323 in KCNQ3 apparently increases the TEA sensitivity of the heteromeric KCNQ2/3 channel, as predicted from previous work on *Shaker* channels. This implies that homomeric KCNQ3(T323Y) currents should also be considerably more sensitive to TEA than wild-type KCNQ3 channels, but unfortunately we were unable to obtain homomeric KCNQ3(T323Y) currents of sufficient amplitude to test their TEA sensitivity directly. However, the fact that KCNQ1 and KCNQ4, lacking a tyrosine in this position, also have a higher TEA sensitivity than KCNQ3, suggests that other residues are important in determining TEA block of KCNQ channels.

Finally, it is worth noting that both KCNQ1 and KCNQ4 yield currents with a similar appearance to M-currents (Figure 1). Since their sensitivity to TEA (and to the M-channel blocker, linopirdine: Kubisch *et al*., 1999; A.A. Selyanko, unpublished observations) is similar to that of native M-currents, these may also be regarded as representing species of 'M-channels'. The characteristics of TEA blockade described here may be useful in establishing whether KCNQ1 and KCNQ4 participate in the formation of any native M-like channels.

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