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SPECIAL REPORT

The role of the I_{sK} protein in the specific pharmacological properties of the I_{Ks} channel complex

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I_{Ks} channels are composed of I_{sK} and KvLQT1 subunits and underly the slowly activating, voltage-dependent I_{Ks} conductance in heart. Although it appears clear that the I_{sK} protein affects both the biophysical properties and regulation of I_{Ks} channels, its role in channel pharmacology is unclear. In the present study we demonstrate that KvLQT1 homopolymeric K^+ channels are inhibited by the I_{Ks} blockers 293B, azimilide and 17-β-oestradiol. However, I_{Ks} channels induced by the coexpression of I_{sK} and KvLQT1 subunits have a 6–100 fold higher affinity for these blockers. Moreover, the I_{Ks} activators mefenamic acid and DIDS had little effect on KvLQT1 homopolymeric channels, although they dramatically enhanced steady-state currents through heteropolymeric I_{Ks} channels by arresting them in an open state. In summary, the I_{sK} protein modulates the effects of both blockers and activators of I_{Ks} channels. This finding is important for the action and specificity of these drugs as I_{sK} protein expression in heart and other tissues is regulated during development and by hormones.

Keywords: Heart; arrhythmia; antiarrhythmics; K channel; chromanol

Introduction The I_{sK} (also called minK) protein induces slowly activating, voltage-dependent K^+ channels in *Xenopus* oocytes, previously called I_{minK}, I_{sK} or I_{Ks} channels (Takumi *et al*., 1988). I_{Ks} channels in *Xenopus* oocytes exhibit almost identical biophysical, pharmacological and regulatory properties as described for the K^+ conductance I_{Ks} in cardiac myocytes (reviewed by Busch & Suessbrich, 1997). Recent studies showed that the I_{sK} protein heteropolymerizes with a 'classical' K^+ channel subunit (KvLQT1) to form functional I_{Ks} channels (Sanguinetti *et al*., 1996; Barhanin *et al*., 1996). I_{Ks} channels represent a potentially important target for antiarrhythmic drugs. It is important to elucidate the individual roles of I_{sK} and KvLQT1 subunits for drug binding within the I_{Ks} channel complex. In the present study we therefore analysed the effects of distinct I_{Ks} channel blockers and activators on mouse KvLQT1 subunits expressed either alone or together with the human I_{sK} protein to form heteromultimeric I_{Ks} channels.

Methods Handling of *Xenopus* oocytes, synthetis of cRNA, voltage-clamp experiments and the analysis thereof have been described in detail (Busch *et al*., 1994a). Azimilide was a gift from Procter & Gamble Pharmaceuticals. 17-β-oestradiol, DIDS (4,4′-diisothiocynostilbene-2,2′-disulphonic acid) and mefenamic acid were purchased from Sigma. Concentration-blockade releationships were calculated with the Hill equation. Student's t test was used to test for statistical significance, which was assumed if $P<0.05$.

Results Expression of mouse KvLQT1 in *Xenopus* oocytes alone or together with the I_{sK} protein induced voltage-dependent K^+ channels (Figure 1a) with similar characteristics as previously described (Barhanin *et al*., 1996). The current amplitude after 2 days of I_{sK}/KvLQT1 coexpression (i.e. I_{Ks} channels) was approximately 4 fold larger than the current induced by expression of the I_{sK} protein alone (which forms I_{Ks} channels with endogenous KvLQT1 subunits in *Xenopus* oocytes; Sanguinetti *et al*., 1996). However, the possibility that heteropolymerization of endogenous and exogenous KvLQT1 subunits may occur for a small portion of the KvLQT1 and I_{Ks} channel populations cannot be excluded.

Oocytes expressing KvLQT1 channels or I_{Ks} channels were analysed for their sensitivity to the blockers 293B, azimilide and 17-β-oestradiol (Busch *et al*., 1994b; Busch *et al*., 1996; Waldegger *et al*., 1996). 293B, azimilide and 17-β-oestradiol blocked KvLQT1 with estimated IC_{50} values of 40.9 ± 0.9 μM ($n=5$), 77.4 ± 6.9 μM ($n=5$) and >50 μM ($n=5$), respectively. Coexpression of KvLQT1 and I_{sK} subunits induced I_{Ks} channels which were blocked by 293B, azimilide and 17-β-oestradiol with IC_{50} values of 6.7 ± 0.5 μM ($n=5$), 5.6 ± 0.7 μM and 2.2 ± 1.0 μM (Figure 1b; $n=5$), respectively. Therefore, the coexpression of I_{sK}/KvLQT1 subunits induced I_{Ks} channels with a much higher blocker affinity compared to homopolymeric KvLQT1 channels. Moreover, the I_{Ks} channels induced by the overexpression of both I_{sK} and exogenous KvLQT1 subunits exerted the same sensitivity to the applied blockers as previously described for I_{Ks} channels induced by the expression of the I_{sK} protein alone. This suggests that exogenous and endogenous KvLQT1 subunits possess a similar affinity for these compounds, which is no surprise considering the high homology of the two proteins.

The Cl^- channel blockers mefenamic acid and DIDS are known to have positive modulatory effects on I_{Ks} channels induced by expression of the human I_{sK} protein alone (Busch *et al*., 1994a). As shown in Figure 2a and b, mefenamic acid (0.1 mM) had little effect on KvLQT1 channels ($n=5$). In contrast, the coexpression of I_{sK} and KvLQT1 subunits induced I_{Ks} channels with high sensitivity to mefenamic acid. As shown in Figure 2a and b, superfusion with mefenamic acid increased steady state currents at -10 mV about 5 fold and arrested I_{Ks} channels in an open state, i.e. at the holding potential of -70 mV no significant I_{Ks} channel deactivation was observed ($n=5$). This effect was rapidly reversed upon wash-out. Qualitatively similar results on I_{Ks} deactivation and steady state currents were observed with 0.1 mM DIDS (Figure 2b; $n=5$).

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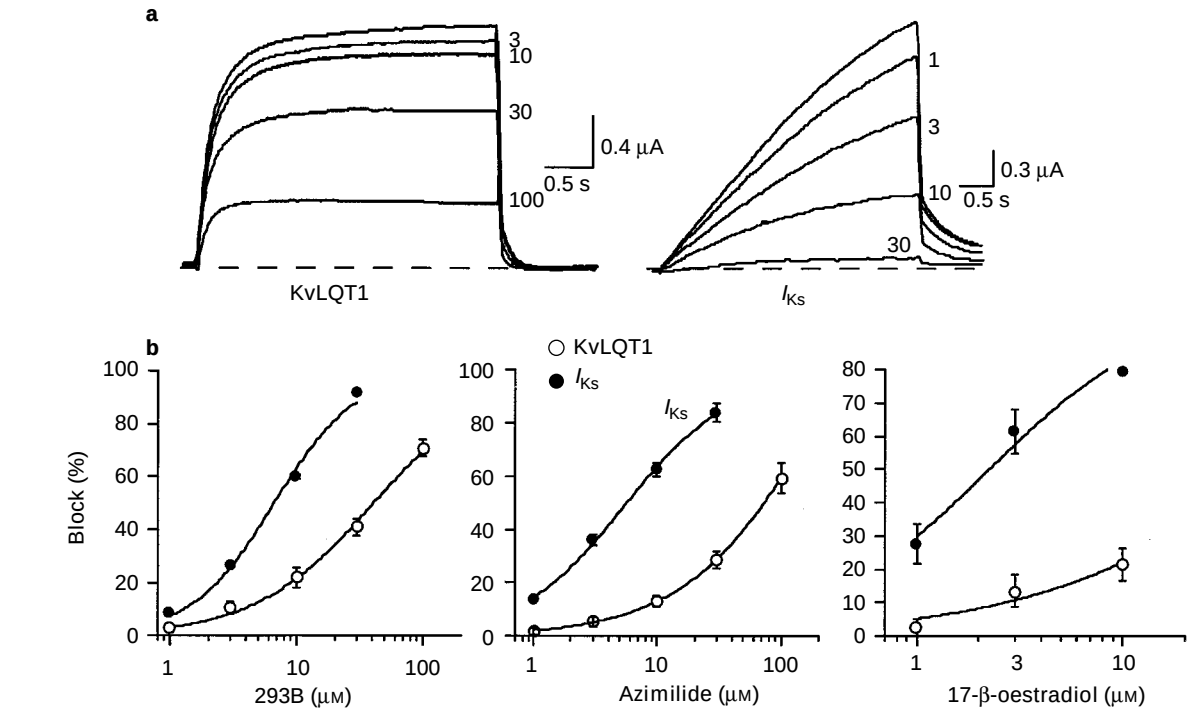

Figure 1 Effects of I_{Ks} blockers on KvLQT1 and I_{Ks} channels in *Xenopus* oocytes. (a) Left: KvLQT1 expression induced fast activating potassium channels (depolarizations to −20 mV from a holding potential of −70 mV) Right: I_{Ks} channels were slower at activating than KvLQT1 channels and were more sensitive to 293B. I_{Ks} channels were activated with depolarizations from −70 mV to −10 mV. (b) Concentration-dependence of inhibition by 239B, azimilide and 17-β-oestradiol of KvLQT1 and I_{Ks}.

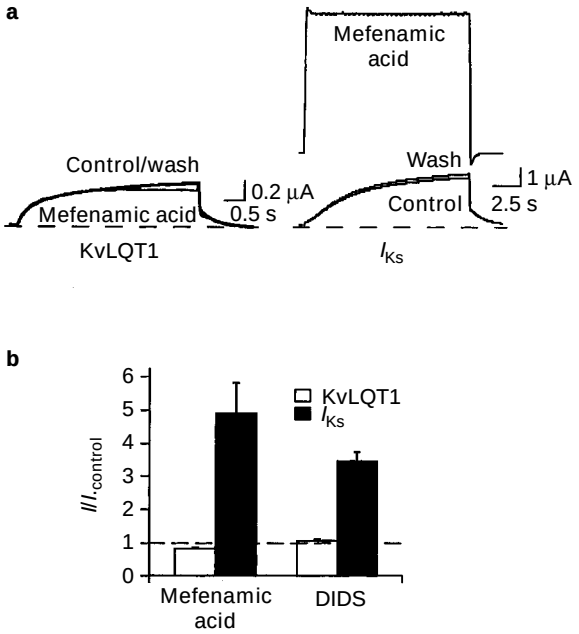

Figure 2 (a) Effects of mefenamic acid (0.1 mM) on KvLQT1 (left) and I_{Ks} channels (right). I_{Ks} channels were repetitively activated with depolarizations from −70 mV to −10 mV. (b) Effects of mefenamic acid and DIDS (0.1 mM) on steady state currents at −10 mV of KvLQT1 and I_{Ks}. The currents were normalized against control currents. The dashed line represents the relative control current.

Discussion The present data demonstrate that the I_{sK} protein plays a crucial role not only for the biophysical properties and regulation of I_{Ks} channels but also for their pharmacology. Upon overexpression it enhances the sensitivity to blockers and even more dramatically to I_{Ks} activators. This suggests that the I_{sK} protein contributes to the binding of these compounds. The binding region for these compounds may also represent a crucial interaction interface between I_{sK} and KvLQT1 subunits. Both I_{sK} and KvLQT1 proteins are expressed in heart and numerous other epithelial tissues (reviewed by Busch & Suessbrich, 1997). Interestingly, a study by Folander *et al.* (1990) suggested that the sex hormone oestrogen upregulates I_{sK} protein expression in uterus, whereas Drici *et al.* (1996) found a downregulation in the heart after oestrogen treatment. A differential expression of the I_{sK} and KvLQT1 subunits may therefore also account for the tissue-specific action of drugs such as those described in the present study.

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References

BARHANIN, J., LESAGE, F., GUILLEMARE, E., FINK, M., LAZDUNSKI, M. & ROMEY, G. (1996). KvLQT1 and I_{sK} (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature*, **384,** 78–80.

BUSCH, A.E., HERZER, T., WAGNER, C.A., SCHMIDT, F., RABER, G., WALDEGGER, S. & LANG, F. (1994a). Positive regulation by chloride channel blockers of I_{sK} channels expressed in *Xenopus* oocytes. *Mol. Pharmacol.*, **46,** 750–753.

BUSCH, A.E., MALLOY, K., GROH, W.J., VARNUM, M.D., ADELMAN, J.P. & MAYLIE, J. (1994b). The novel class III antiarrhythmics NE-10064 and NE-10133 inhibit I_{sK} channels expressed in *Xenopus* oocytes and I_{Ks} in guinea pig cardiac myocytes. *Biochem. Biophys. Res. Commun.*, **202,** 265–270.

BUSCH, A.E. & SUESSBRICH, H. (1997). Role of the I_{sK} protein in the I_{minK} channel complex. *Trends Pharmacol. Sci.*, **18,** 26–29.

BUSCH, A.E., SUESSBRICH, H., WALDEGGER, S., SAILER, E., GREGER, R., LANG, H., LANG, F., GIBSON, K.J. & MAYLIE, J.G. (1996). Inhibition of I_{Ks} in guinea pig cardiac myocytes and guinea pig I_{sK} channels by the chromanol 293B. *Pflugers Archiv - Eur. J. Physiol.*, **432,** 1094–1096.

DRICI, M.D., BURKLOW, T.R., HARIDASSE, V., GLAZER, R.I. & WOOSLEY, R.L. (1996). Sex hormones prolong QT interval and downregulate potassium channel expression in the rabbit heart. *Circulation*, **94,** 1471–1474.

FOLANDER, K., SMITH, J.S., ANTANAVAGE, J., BENNETT, C., STEIN, R.B. & SWANSON, R. (1990). Cloning and expression of the delayed-rectifier I_{sK} channel from neonatal rat heart and diethylstilbestrol-primed rat uterus. *Proc. Nat. Acad. Sci. U.S.A.*, **87,** 2975–2979.

SANGUINETTI, M.C., CURRAN, M.E., ZOU, A., SHEN, J., SPECTOR, P.S., ATKINSON, D.L. & KEATING, M.T. (1996). Coassembly of KvLQT1 and minK (I_{sK}) proteins to form cardiac I_{Ks} potassium channel. *Nature*, **384,** 80–83.

TAKUMI, T., OHKUBO, H. & NAKANISHI, S. (1988). Cloning of a membrane protein that induces a slow voltage-gated potassium current. *Science*, **242,** 1042–1045.

WALDEGGER, S., LANG, U., HERZER, T., SUESSBRICH, H., KIESL, L., LANG, F. & BUSCH, A.E. (1996). Inhibition of minK protein induced K channels in *Xenopus*. oocytes by estrogens. *Naunyn-Schmiedberg's Arch. Pharmacol.*, **354,** 698–702.

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