Subunit-specific inhibition of inward-rectifier K^+ channels by quinidine

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Received 24 August 1995; revised version received 11 September 1995

Abstract Distinct inward-rectifier K^+ channel subunits were expressed in *Xenopus* oocytes and tested for their sensitivity to the channel blocker quinidine. The 'strong' inward-rectifier K^+ channel IRK1 was inhibited by quinidine with an EC_{50} of 0.7 mM, while the 'weak' rectifier channel ROMK1 was only moderately inhibited. ROMK1(N171D)-IRK1$_{C\text{-term}}$ chimeric channels, which carry both sites for strong rectification of IRK1 channels (the negatively charged D171 in the second transmembrane domain and the IRK1-C-terminus including E224), displayed strong rectification like IRK1, but showed weak sensitivity to quinidine-like ROMK1, suggesting independence of quinidine binding and rectification mechanisms. Moreover, BIR10 and BIR11, two strong rectifier subunits originally cloned from rat brain, exerted subunit-specific sensitivity to quinidine, being much higher for BIR11. Quinidine blockade of IRK1 was not voltage-dependent, but strongly dependent on the pH in the superfusate. These results strongly suggest a subunit-specific interaction of inward-rectifier K^+ channels with neutral quinidine within membrane lipid bilayers.

Key words: Quinidine; Inward rectifier K^+ channel; pH

1. Introduction

Inward rectifying K^+ channels determine the resting potential in many excitable and nonexcitable cells [1]. Recently, the first genes encoding inward-rectifying K^+ channels, ROMK1 and IRK1, were successfully cloned [2,3]. These two channels exhibit distinct rectification properties, which may be 'strong' or 'weak' [1]. The strong rectification of IRK1 is due to a high affinity, voltage-dependent block by intracellular polyamines [4–7], while the weak rectification observed in ROMK1 channels corresponds to a much lower affinity of these channels to polyamines [5]. In contrast to physiologically relevant polyamines, specific effects of xenobiotics on distinct inward-rectifier K^+ channels have not been described. Such xenobiotics could be used to distinguish pharmacologically between differentially composed inward-rectifier K^+ channels. Therefore, the aim of the present study was to analyze strong and weak rectifiers for their sensitivity to the antiarrhythmic drug quinidine. For that purpose, the strong rectifiers IRK1, BIR10 and BIR11 [3,8] and the weak rectifier ROMK1 [2] were expressed in *Xenopus* oocytes and tested for their sensitivity to the ion channel blocker quinidine.

2. Materials and methods

Handling and injection of *Xenopus* oocytes and synthesis of cRNA has been described previously in detail [9]. Electrophysiological recordings were performed three to seven days after injection using a two-microelectrode voltage-clamp. Current- and voltage-electrodes were pulled from thick-walled borosilicate glass, had resistances between 0.1 and 0.5 MΩ and were filled with 3 M KCl. Currents were recorded with a TurboTec 01C amplifier (npi, Tamm, Germany), digitized at 0.1 kHz (ITC16, HEKA, Lamprecht, Germany) and stored on harddisk. The bath chamber was made up as a narrow canal to achieve complete solution exchanges in less than 3 s. The basic solution (KFR*) was composed as follows (in MM): KCl 90, NaCl 27.5, $CaCl_2$ 1.8, HEPES 10; pH was adjusted to 7.2. For some experiments this solution was titrated to pH 6.0 and 8.0 using HCl and NaOH, respectively. Quinidine was purchased from Sigma and dissolved in KFR* to yield the final concentrations indicated. All experiments were performed at room temperature (approximately 23°C). Data are presented as mean ± standard error (S.E.M.), n represents the number of experiments performed. A Student's t-test was used to test for statistical significance, which was obtained for $P < 0.05$.

The mutant channel RO-IR$_C$(N171D) was constructed by a three step polymerase chain reaction (PCR) using ROMK1(N171D) and IRK1 as templates: in a first step, a fragment ranging from 5'-UT to amino acid (aa) 171 (last aa of the proposed TM2, according to [3]) of ROMK(N171D), previously prepared according to [10], was amplified using the following primers (5'-GGCAGGGATCCATTGCTTGCTTTG-3', bp −31 to −7; and 5'-GGCACCACACATGAAGGAATTGATG-3', bp 507 to 531).

In a second PCR, a fragment of IRK1 ranging from the first aa C-terminal to TM2 to aa 319 (in the C-terminus) was amplified using the following primers (5'-CATCAATTCCTTCATGTGTGGTGCCGTCATGGCGAAGATGGCAAA-3' corresponding to bp 507 to 531 of ROMK1 + bp 534 to 553 of IRK1; and 5'-CTCTTTCGAAGGTTACCCAC-3', bp 639 to 658 bp of IRK1). Both fragments were then used as templates for the third PCR with the upper primer of the first and the lower primer of the second PCR as primers. The final PCR-product was digested and subcloned into an IRK1 clone previously inserted into a pSP64T-derived vector. The entire construct was sequenced and verified as RO-IR$_C$(N171D).

3. Results

Application of the antiarrhythmic drug quinidine to oocytes previously injected with IRK1-specific cRNA resulted in inhibition of IRK1-mediated currents by 61.3 ± 9.2% (n = 5). The quinidine-mediated inhibition was mostly reversible upon an extended washout (10 min). Subsequently, the effects of quinidine were analyzed in detail on the strong and weak rectifier-channels IRK1 and ROMK1. While IRK1-mediated currents were inhibited with an EC_{50} of 0.71 ± 0.08 mM (Fig. 1A,D; n = 5), ROMK1-mediated currents were decreased by only

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SSDI 0014-5793(95)01182-X

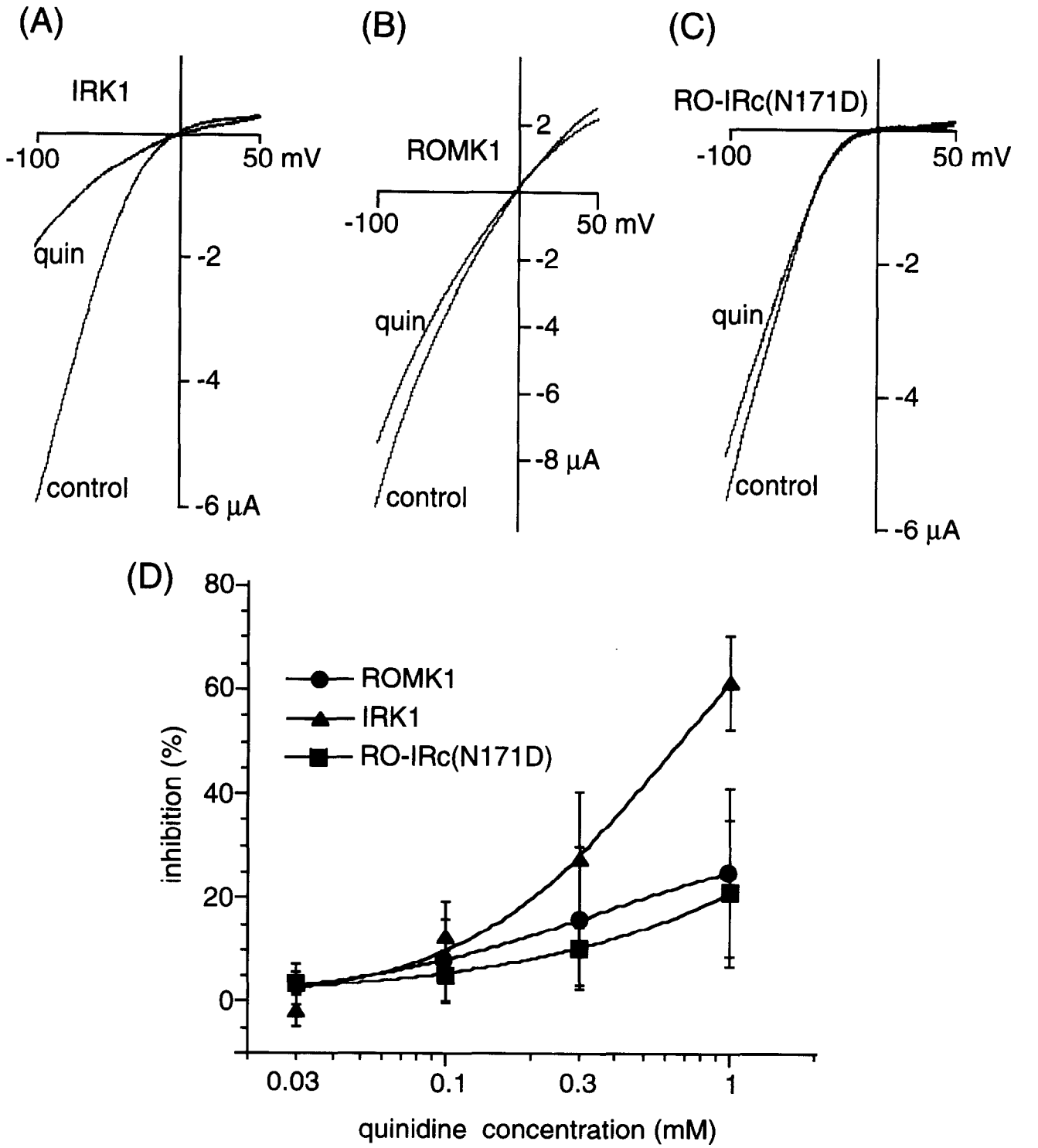

Fig. 1. Quinidine(quin)-mediated inhibition (at 1 mM) of the strong and weak inward-rectifier K^+ channels IRK1 (A), ROMK1 (B) and RO-IR_C(N171D) mutant channels (C). Currents were recorded during voltage ramps from −100 to 50 mV (3.75 s) from. The holding potential was 0 mV. (D) Dose–response curves of quinidine-mediated inhibition of IRK1, ROMK1 and RO-IR_C(N171D) channels, respectively. Data are given as mean ± S.E.M.

25.0 ± 16.0% (Fig. 1B,D; $n = 5$) by application of 1 mM quinidine, suggesting interference between rectification and quinidine-induced current inhibition. To check for such interference a chimeric channel ROMK1(N171D)-$IRK1_{C\text{-term}}$ (RO-IR_C(N171D)) was constructed, which carries both determinants of strong rectification of IRK1: the C-terminus [11] and an aspartate residue at amino acid position 171 [12–14]. As expected, this mutant displayed strong rectification similar to IRK1 (Fig 1C). However, the sensitivity of RO-IR_C(N171D) for quinidine was similar to that observed for ROMK1 chan-

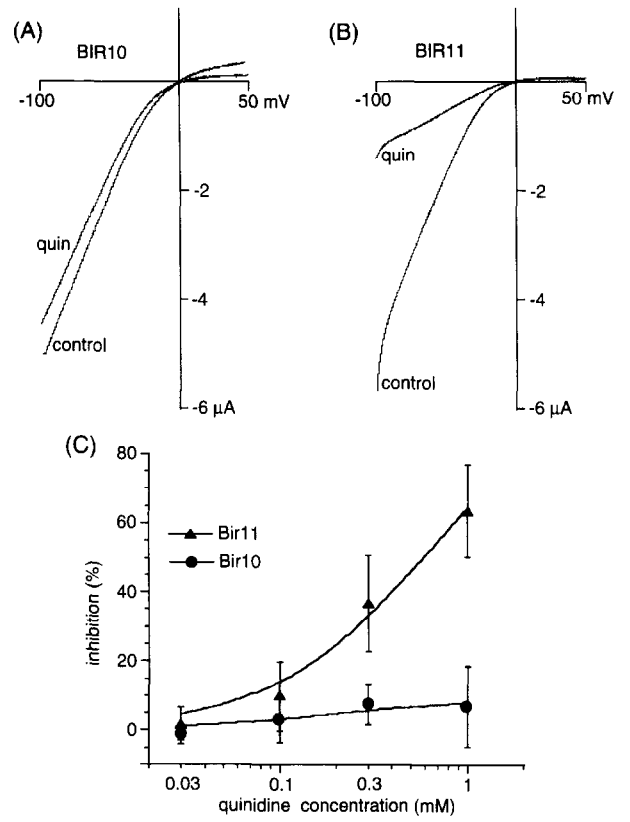

Fig. 2. Quinidine-mediated inhibition (at 1 mM) of the strong rectifier K^+ channels BIR10 (A) and BIR11 (B). (C) Dose–response curve for quinidine-mediated inhibition of BIR10 and BIR11. Data are given as mean ± S.E.M.

nels (Fig. 1D). This suggests independence of quinidine sensitivity and rectification and more likely points to a subunit-specific interaction of the xenobiotic with inward-rectifier K^+ channels.

The inhibitory effect of quinidine was therefore further investigated in the inward-rectifier subunits BIR10 and BIR11, which were originally cloned from rat brain and display strong rectification [8]. BIR11 channels were inhibited by quinidine with an EC_{50} value similar to that obtained for IRK1 (0.58 ± 0.06 mM (Fig. 2A,C; $n = 5$)), while no significant inhibition was observed for BIR10 channels (Fig. 2B,C; $n = 5$), supporting the subunit-specificity of quinidine-channel-interaction.

Subsequently the mechanism of quinidine blockade was further investigated. In a first set of experiments the quinidine inhibition of IRK1 and ROMK1 was analyzed for its voltage-dependence. For both K^+ channel subtypes the extent of quinidine (1 mM) blockade of inward K^+ currents measured at voltage steps to −100 mV, −70 mV and −40 mV (holding potential was 0 mV). Although there was a tendency to a more pronounced block at more depolarized potentials, the observed blockade was not significantly different at these voltages (data not shown; $n = 6$ for both IRK1 and ROMK1).

In another set of experiments, the effects of quinidine on IRK1 and ROMK1 were compared with quinine, a stereoisomer of quinidine. Quinine (1 mM) inhibited both channels with a smaller potency compared to quinidine (Fig. 3A; $n = 5$).

The quinidine molecule comprises two basic groups with pK_as of 5.4 and 10, respectively. Therefore, the effects of extracellular pH on quinidine-mediated inhibition of IRK1 was analyzed at pH 6 and 8. At pH 8 approximately 1% of quinidine is in its neutral form and 99% carry a single positive charge,

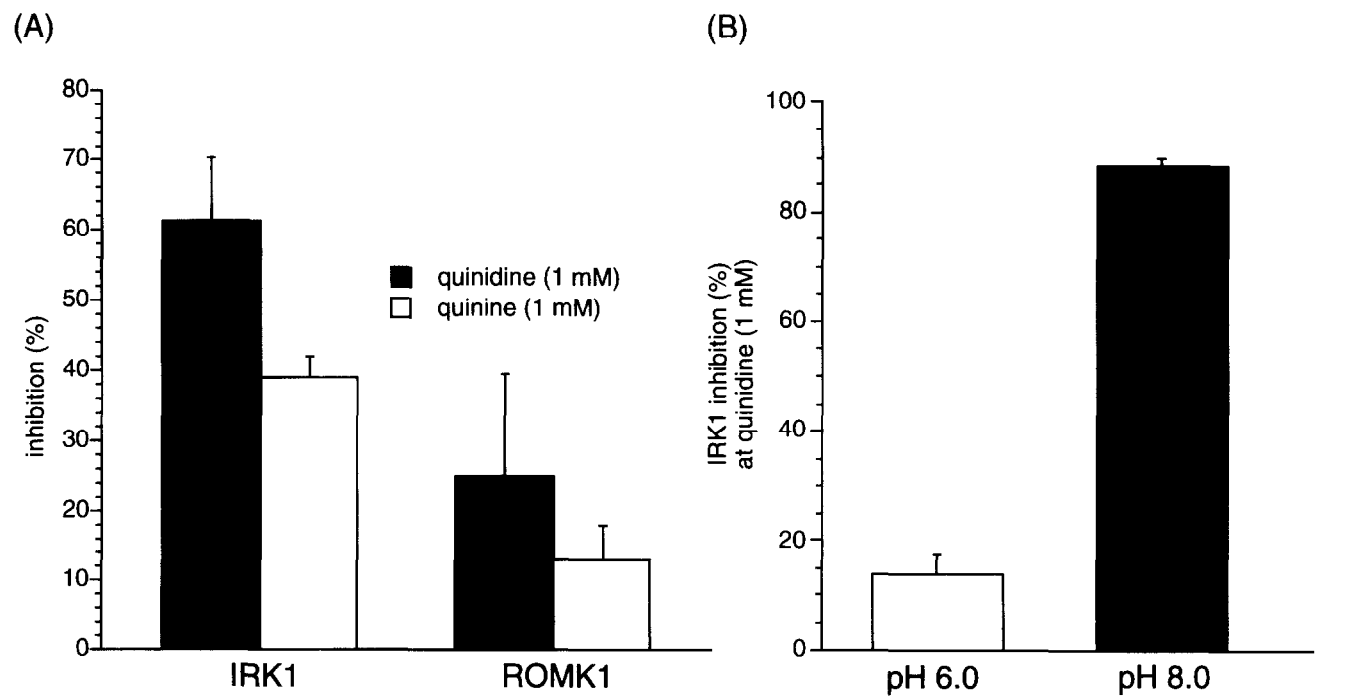

Fig. 3. (A) Comparison of inhibition mediated by quinidine (black columns) and quinine (white columns) at a concentration of 1 mM on IRK1 and ROMK1 channels. (B) pH-dependence of quinidine effects on IRK1. Data are given as mean ± S.E.M.

while at pH 6.0 there is virtually no neutral quinidine, 80% carry a single positive charge and 20% are twofold positively charged. As shown in Fig. 3B, at pH 8.0 quinidine (1 mM) inhibited IRK1 channels much stronger (88.3 ± 1.5%; $n = 5$) than at pH 6 (13.6 ± 3.7%; $n = 5$).

4. Discussion

The aim of the present study was to search for pharmacological agents, which can distinguish between distinct inward-rectifier K^+ channels. Indeed, a subunit-specific sensitivity of inward-rectifier K^+ channels was found for the antiarrhythmic quinidine. Quinidine at a concentration of 1 mM strongly inhibited IRK1 and BIR11 channels, while ROMK1, BIR10 and RO-IR_C(N171D) were almost unaffected.

All these subunits display either weak or strong rectification properties according to their differential sensitivity to intracellular polyamines [5], and an involvement of the binding site for polyamines in quinidine blockade was first hypothesized. However, a number of results refute such a mechanism. First, mutations found to increase sensitivity of ROMK1 channels to polyamines indeed increased its rectification, however, they failed to increase sensitivity to quinidine. Second, binding of quinidine molecules in their protonated form to a binding site within the transmembrane electrical field would be expected to be voltage-dependent, as found for the polyamine block. However, quinidine-mediated inhibition of inward currents through IRK1 and ROMK1 was not significantly different between −100 and −40 mV. Finally, the three strong rectifiers IRK1, BIR10 and BIR11 displayed a distinct quinidine sensitivity, although their sensitivity to intracellular polyamines is almost identical. Thus it may be concluded, that quinidine binds to a site of the channel which is different from the polyamine binding site.

Instead, two results indicate a hydrophobic interaction of quinidine with the channel protein. First, quinidine blockade is virtually voltage-independent. Second and of greater significance, the strong pH-dependence of quinidine supports the hypothesis that the neutral form of quinidine predominantly interacts with the channel protein via hydrophobic binding. It is also possible that quinidine reaches its binding site at the channel protein via a two-step membrane pathway, as has been reported for dihydropyridine binding to L-type calcium channels [15]. In this event, the neutral form of quinidine is more likely to enter the bulk lipid phase and to reach the binding site on the channel protein via lateral diffusion.

In summary, quinidine inhibits inward-rectifier K^+ channels in a subunit-specific manner, which is unrelated to the mechanisms involved in inward-rectification. Thus, quinidine sensitivity may serve as an independent tool to discriminate between inward-rectifier subtypes. However, the specific mechanism of inhibition and site of quinidine binding remains elusive.

Acknowledgements: A.E.B. is a Heisenberg Fellow. The work was supported by grants from the Deutsche Forschungsgemeinschaft (Bu 704/3-1 to A.E.B. and Klinische Forschergruppe Hörforschung). The authors are indebted to Dr. J.P. Adelman for providing the BIR10 and BIR11 clones, to Dr. T. Yamashita and Dr. T. Kumazawa for supporting T.D. and to Drs. P. Hausen and G.L. Busch for the discussion of the manuscript.

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