Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2466–2470, March 1992
Neurobiology

Extracellular K^+ specifically modulates a rat brain K^+ channel

(ion-channel molecular-biology/cellular excitability)

L. A. PARDO*, S. H. HEINEMANN*, H. TERLAU*, U. LUDEWIG*, C. LORRA†, O. PONGS†, AND W. STÜHMER*

*Max-Planck-Institut für biophysikalische Chemie, W-3400 Göttingen, Federal Republic of Germany; and †Zentrum für Molekulare Neurobiologie, W-2000 Hamburg 20, Federal Republic of Germany

Communicated by Erwin Neher, December 20, 1991 (received for review December 2, 1991)

ABSTRACT **Extracellular potassium concentration is actively maintained within narrow limits in all higher organisms. Slight variations in extracellular potassium levels can induce major alterations of essential physiological functions in excitable tissues. Here we describe that superfusion of cultured rat hippocampal neurones with potassium-free medium leads to a decrease of a specific outward potassium current, probably carried by RCK4-type channels (RCK4 are potassium channels found in rat brain). This is confirmed by heterologous expression of these channels in *Xenopus* oocytes. In this system, variations of extracellular potassium in the physiological concentration range induce significant differences in current amplitude. Moreover, the current is completely suppressed in the absence of extracellular potassium. The potassium dependence of macroscopic conductance in RCK4 channels was related by site-directed mutagenesis to that lysine residue in the extracellular loop between the transmembrane segments S5 and S6 of RCK4 protein that confers resistance to extracellular blockage by tetraethylammonium. It is shown that extracellular potassium affects the number of available RCK4 channels, but not the single-channel conductance, the mean open time, or the gating charge displacement upon depolarization.**

K^+ currents are involved in the generation of the action potential, opposing the current flow through Na^+ and Ca^{2+} channels and thus decreasing excitability (1). The delayed rectifier channels are important for repolarization and, thus, the duration of the action potential. However, rapidly inactivating K^+ currents (A-type currents) contribute to the regulation of the firing frequency, since they modulate the rate of recovery from the refractory period (2).

Variations in extracellular K^+ concentration ($[K^+]_o$) have been implicated in the pathogeny of several disorders, such as epilepsy (3) and electrical instability of the heart following acute ischemia (e.g., see ref. 4). Some of the effects of changes in $[K^+]_o$ can be attributed to the shift in the K^+ equilibrium potential, which modifies both the resting potential of the cells and the driving force for K^+. However, in many cases an increase in K^+ membrane conductance is required to explain the observed alterations (5). Indeed, a potassium-dependent K^+ conductance has been described for many cell types (5), but the distinct channels that are affected by $[K^+]_o$ under physiological conditions are currently unknown.

Since the alterations underlying pathological modifications due to $[K^+]_o$ are mainly attributable to alterations on firing frequency, an A-type current may be modulated by $[K^+]_o$. In this paper, we provide evidence for the existence of a specific A-type current that is highly sensitive to $[K^+]_o$ in hippocampal neurones, and we describe this effect on channels expressed in a heterologous system.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Biological Preparations. Rat hippocampal cultures were obtained as in ref. 6. RNA preparation and oocyte injection was performed as described (7).

Electrophysiological Measurements. Whole-cell currents in cultured hippocampal neurones were measured by using Kimax glass pipettes having resistances of 4–5 MΩ and filled with a solution of the following composition: 100 mM KCl, 10 mM NaCl, 20 mM phosphocreatine, 5 units of creatine phosphokinase per ml, 4 mM MgATP, 10 mM EGTA, and 10 mM Hepes·KOH (pH 7.2). The bath solution contained 140 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 200 nM tetrodotoxin, 5 mM tetraethylammonium, 10 nM charybdotoxin, and 10 mM Hepes·NaOH (pH 7.2), with or without 2.8 mM KCl.

For heterologous expression of RCK4 channels (K^+ channels found in rat brain cortex), the specific cDNA-derived mRNA (cRNA) was injected into oocytes (average amount 25 pg per oocyte) and the currents were recorded 2–5 days after injection. The electrophysiological recordings on whole oocytes were performed under conventional voltage-clamp conditions by using a Turbo-TEC amplifier (NPI Electronics, Tamm, F.R.G.) and intracellular electrodes with resistances of 0.6–0.8 MΩ when filled with 2 M KCl. All pulse protocols were designed with 20-s intervals between pulses to allow complete recovery from inactivation of the channel. Leak and capacitive currents were subtracted on-line by using a P/6 method. During the recording, the oocyte was continuously superfused with the test solution at a flow rate of 10 ml/min; the chamber volume was about 0.3 ml.

The current induced by depolarizing steps was also measured in outside-out membrane patches under constant flow of a bathing solution containing various concentrations of cations. For this purpose, we used aluminium-silicate pipettes with resistances of 0.8–1.2 MΩ, filled with 100 mM KCl/10 mM EGTA/10 mM Hepes·KOH, pH 7.2. The bath solution contained 120 mM Tris, 1.8 mM $CaCl_2$, and 10 mM Hepes (pH 7.2 adjusted with HCl). The cations tested were added to this solution as chloride salts, always maintaining the total concentration of monovalent cations to 120 mM.

The gating charge measurement was performed in outside-out patches as described (8), with 120 mM Tris chloride, pH 7.2/10 mM EGTA as pipette solution.

All electrophysiological experiments were performed at 19–21°C. Pulse patterns were generated, and currents were sampled with a VME-bus-based computer system.

RESULTS

Reduction of an Inactivating Outward Current in Rat Hippocampal Neurones by Decreasing $[K^+]_o$. We investigated the dependence of $[K^+]_o$ on a K^+ current present in primary cultured hippocampal neurones. Hippocampal cells only significantly express RCK1 and RCK4 transcripts out of all

Abbreviation: $[K^+]_o$, extracellular potassium concentration; cRNA, cDNA-derived mRNA.

the RCK-type K^+ channels cloned so far (9). RCK4 shows peculiar kinetics, with relatively fast inactivation and an extremely slow recovery from inactivation (with a time constant in the range of seconds) (10), and is the only fast-inactivating K^+ current known to be resistant to charybdotoxin and external (but not internal) tetraethylammonium (11, 12). These properties allow the separation of RCK4-type currents from other inactivating K^+ currents. Experimental conditions were designed to selectively record rapidly inactivating and slowly reactivating currents that are resistant to tetraethylammonium and charybdotoxin. We applied two identical and consecutive depolarizing pulses to 0 mV from a holding potential of −80 mV and then subtracted the second from the first one (Fig. 1 *Inset*). This not only removes leak and capacitive components but also eliminates the currents that do not inactivate over a 200-ms pulse, such as those flowing through RCK1 channels, because these currents will be present also during the second depolarization. In addition, these recordings were made in the presence of 5 mM extracellular tetraethylammonium and 10 nM charybdotoxin, reducing to a minimum the outflow of K^+ through other K^+ channels during the depolarizations (although 59 ± 17% of the outward peak current remained). Under these conditions, all neurones tested (n = 18) showed an inactivating outward current that presumably is carried by RCK4-type channels, given the kinetic and pharmacological properties of these channels. Internal tetraethylammonium (20 mM) blocked any remaining currents obtained with the identical protocol (n = 8). The amplitude of this current was greatly reduced while perfusing the experimental chamber with K^+-free solution, and complete recovery was always achieved on returning to the normal perfusion solution containing 2.8 mM K^+ (Fig. 1). Intermediate concentrations in the range of 1–3 mM $[K^+]_o$ resulted in a graded modulation of the inactivating outward K^+ current.

Heterologously Expressed RCK4 Channels Show Dependence on $[K^+]_o$. The current described in Fig. 1 is most likely mediated by RCK4-type channels. This prompted us to investigate the effect of $[K^+]_o$ on *Xenopus* oocytes expressing cloned RCK4 K^+ channels (11). Reduction of $[K^+]_o$ reduced the outward current by a factor of two or more in whole oocytes under voltage-clamp, and this reduction was fully reversible after supplementing the bathing solution with K^+, even in the absence of Na^+ and Ca^{2+} and despite the decrease in driving force induced by high K^+ concentrations (not shown). In the voltage-clamp experiments, it was difficult to obtain zero $[K^+]_o$. This is probably due to both K^+ leakage from the oocyte and K^+ accumulation in the microvilli on the oocyte surface. Therefore, we performed patch clamp experiments in the outside-out configuration under fast flow of the external solution after removing the oocyte from the chamber, allowing a better control of $[K^+]_o$ in the immediate neighborhood of the membrane. Under these conditions, the absence of potassium in the bathing solution abolished the current (Fig. 2*a*). Na^+ or Li^+ were not able to recover the current (Fig. 2*a*). On the other hand, the current was still present when the patch was perfused with Rb^+, Cs^+, or NH^+_4 (Fig. 2*b*), although these cations were less effective than K^+ in maintaining K^+ currents. The fact that the nonpermeant ion Cs^+ is able to maintain the current shows that the effect does not require ion permeation through the channel, suggesting that an external site is responsible for this effect.

Of all the cloned K^+ channels tested [RCK1 (13), RCK2 (14), RCK3, RCK4, RCK5 (11), Raw3 (15), and ShA2 (16–18)], only RCK3 and RCK4 showed modulation by $[K^+]_o$. In oocytes injected with RCK3 cRNA, we detected a reduction in current density by 60% when $[K^+]_o$ was reduced from 10 to 0.1 mM, but the current did not disappear during perfusion with K^+-free medium. The RCK3 channel is sensitive to charybdotoxin [IC_{50} = 1 nM (11)], does not inactivate within 200 ms, and is not significantly expressed in hippocampus (9). Therefore, it seems reasonable to suppose that the modulatory effects of $[K^+]_o$ on the inactivating K^+ currents seen in hippocampal neurones are due to an action on RCK4-type K^+ channels.

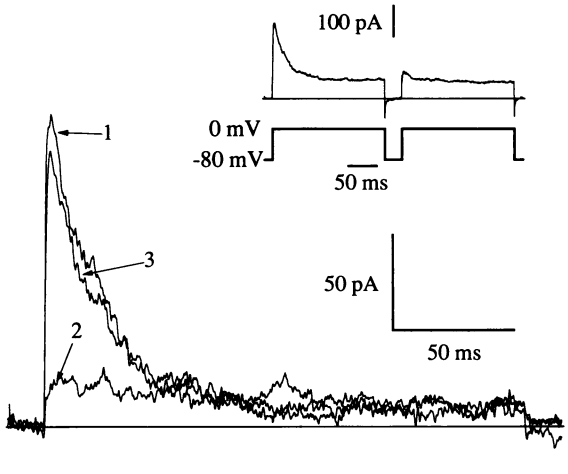

FIG. 1. Whole-cell currents elicited by depolarizations to 0 mV in hippocampal neurones. The peak current recorded at the physiological $[K^+]_o$ of 2.8 mM (trace 1) is reduced when the cell is superfused with K^+-free medium (trace 2). Trace 3 shows the recovery. The amplitude of the current is fully recovered when the cell is perfused again with 2.8 mM $[K^+]_o$. (*Inset*) Pulse protocol used and raw currents. Traces 1, 2, and 3 were obtained by subtracting the second from the first trace in records like the one shown in *Inset* (which corresponds to trace 1). The holding potential was −80 mV.

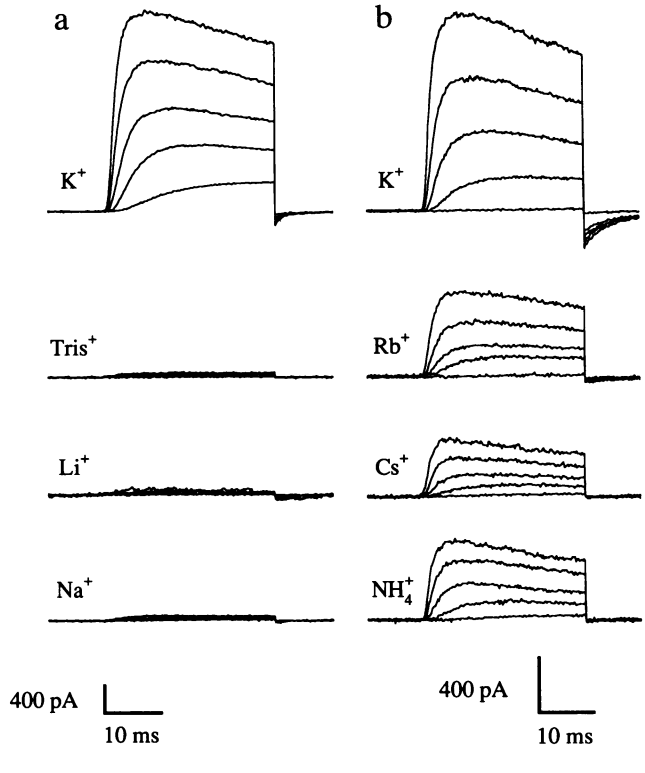

FIG. 2. Outward current measured in outside-out patches obtained from oocytes injected with cRNA coding for RCK4 during depolarizing steps to −40, −20, 0, 20, and 40 mV from a holding potential of −100 mV. *a* and *b* were obtained from two different patches. In addition to 110 mM Tris chloride, the bath solution contained 10 mM of the indicated test cation as the chloride salt. The sequence shown corresponds to the chronological order in which the solutions were changed.

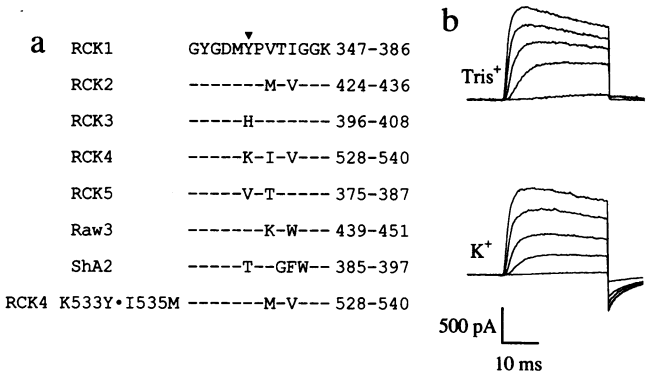

FIG. 3. (*a*) Alignment of amino acid sequences in the region responsible for the K^+ and tetraethylammonium sensitivities of the various K^+ channels. Those residues identical to RCK1 have been substituted by dashes. The numbers to the right indicate the respective positions in the sequences. All of the sequences end at the beginning of the putative S6 segment. (*b*) Families of current responses obtained under the same conditions as those described for Fig. 2 in membrane patches from mutant K533Y·I535M in the presence of 0 or 10 mM $[K^+]_o$.

Localization of the Region Responsible for the K^+ Effect on RCK4. Fig. 3*a* shows the amino acid sequence of the K^+ channels studied in this work in the vicinity of the site that is thought to confer tetraethylammonium sensitivity (19). Those channels sensitive to relatively low concentrations of extracellular tetraethylammonium carry an uncharged residue in the equivalent position to residue 533 of RCK4, while the latter shows a positively charged residue (lysine). RCK3, which shows partial sensitivity, carries a histidine residue in the equivalent position, which should be partially charged under physiological conditions. Because of the competition between $[K^+]_o$ and the external tetraethylammonium-induced block (20) we assumed that Lys-533 in RCK4 not only confers tetraethylammonium insensitivity but also might be involved in the modulatory effect of $[K^+]_o$ on current density.

To test this hypothesis, the RCK4 double mutant K533Y·I535M (Lys-533 → Tyr and Ile-535 → Met) (12) was investigated. This mutant shares 100% homology with RCK2 in the region responsible for tetraethylammonium sensitivity. As reported previously (12), the mutant was sensitive to tetraethylammonium, but most interestingly, the current density was not modified by $[K^+]_o$ (Fig. 3*b*). These results strongly suggest an inverse relation between external tetraethylammonium sensitivity and modulation of K^+ outward current by $[K^+]_o$.

Dose-Dependence of K^+ Modulation. The dose-dependent modulation of K^+ current density in outside-out patches from oocytes injected with RCK4 cRNA as a function of $[K^+]_o$ and $[Cs^+]_o$ is shown in Fig. 4*a*. The $[K^+]_o$ at which half of the maximal amplitude was obtained is 2.20 ± 0.61 mM (mean ± SD in four independent experiments). This value was obtained by fitting the experimental data points with a dose–response equation, and no correction was made for variations in reversal potential caused by the various $[K^+]_o$. Therefore, the value of 2.20 mM for $[K^+]_o$ is the concentration at which the effective K^+ current density is half maximal under physiological conditions. Under the same conditions, the half-maximal activation of ionic currents by Cs^+ was obtained at 4.10 mM (±1.03, n = 4).

$[K^+]_o$ Modifies the Number of Available Channels. The mechanism for the increase in current density on increasing $[K^+]_o$ (despite a concomitant decrease in driving force) is not yet clear. In general terms, the measured current amplitude can be described as the product of the number of available channels multiplied by the current flowing through a single channel and by the probability of the channel to be open, which is related to the mean open time of the channel. No

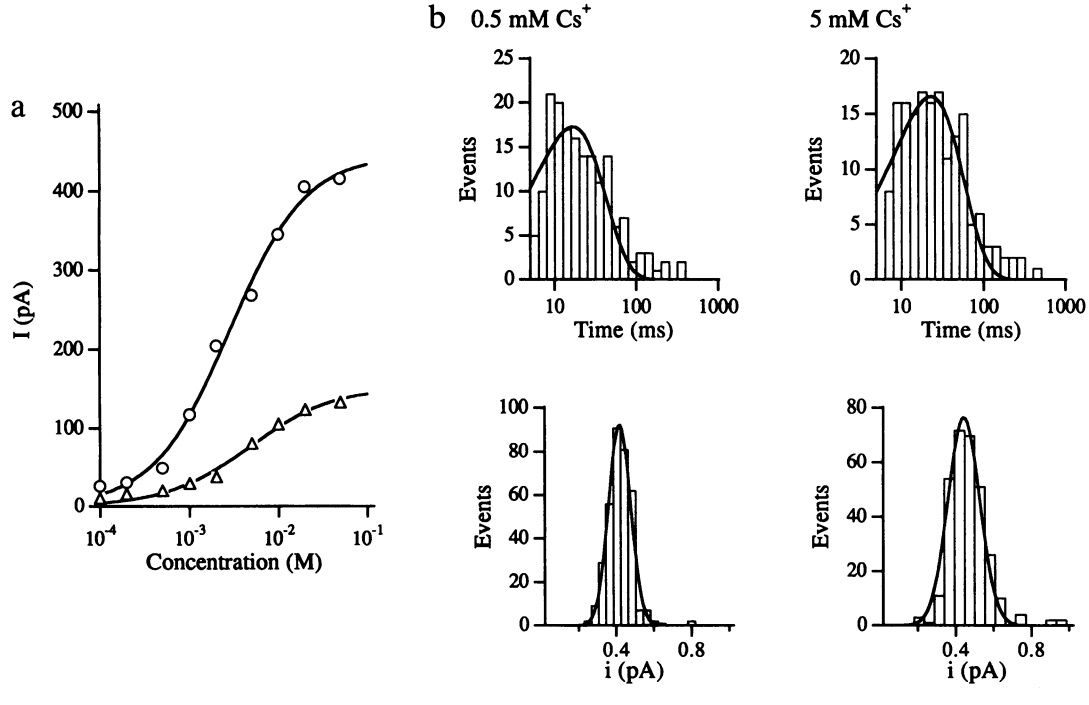

FIG. 4. (*a*) Dose-dependence of the K^+ current evoked by pulse depolarizations to +40 mV from a holding potential of −100 mV, plotted as function of $[K^+]_o$ (○) and $[Cs^+]_o$ (△). All currents were measured in the same outside-out patch with 100 mM KCl/1.8 mM $CaCl_2$/10 mM Hepes·KOH, pH 7.2, in the pipette. The fits through the data points are Bolzmann distributions with following parameters: K_d = 2.8 mM, slope = 1.00 for K^+: K_d = 4.6 mM, slope = 0.92 for Cs^+. (*b*) Duration and amplitude histograms of spontaneous channel openings in an outside-out patch held at +40 mV in the presence of 0.5 or 5 mM Cs^+ in the external solution. The experimental data were fitted by using the following parameters: 0.5 mM Cs^+, mean duration of 47 ms, and mean I = 401 ± 59 fA; 5 mM Cs^+, mean duration of 45 ms, and mean I = 425 ± 84 fA.

significant difference either in single-channel conductance or in mean open time was detected upon changing the Cs^+ concentration from 0.5 mM to 5 mM in the external solution of outside-out patches (Fig. 4*b*). Therefore, the observed $[K^+]_o$-dependent increase in macroscopic RCK4 current is most likely due to an increase in the number of channels available for activation. This was confirmed by means of nonstationary noise analysis (21, 22), which revealed an invariant single-channel current of about 0.6 pA at 80 mV for either 0.5 mM or 5 mM Cs^+ in the extracellular solution (not shown).

A more direct indication for a change in the number of channels able to conduct was obtained by comparing gating currents at various $[K^+]_o$ (Fig. 5*b*). The figure shows that the gating charge displaced is apparently invariant for four different $[K^+]_o$, which would give rise to ionic currents differing by orders of magnitude, indicating that the channels are still able to gate but do not conduct current in the absence of extracellular K^+. Therefore, when $[K^+]_o$ is reduced to nominally zero (which requires a fast and constant flow of K^+-free solution), the ionic current is not detectable. However, it is recovered completely after returning to a K^+-containing solution.

Another possibility for explaining the dependence of the number of available channels by $[K^+]_o$ would be if the inactivated state were affected by $[K^+]_o$. The latter possibility was checked for RCK4. The traces in Fig. 5*a* show that the time constant of inactivation is not changed in these channels by varying the $[K^+]_o$, as determined by fitting an exponential to the decaying phase of the currents during a depolarization. However, the time constant for recovery from inactivation is much slower in the absence of extracellular K^+ (Fig. 5*a*). We do not think that this is the main effect responsible for the variations in current density that we observe, since it is also detectable in other channels whose current densities are insensitive to $[K^+]_o$. In addition, the variations in the slow recovery from inactivation are not likely to play a crucial role during the brief interpulse duration of Fig. 1. Therefore, we favor a direct effect of $[K^+]_o$ on the number of available channels, particularly because inactivated channels would be immobilized and not give rise to gating currents, but we observe similar amounts of charge moved at various $[K^+]_o$ (Fig. 5*b*).

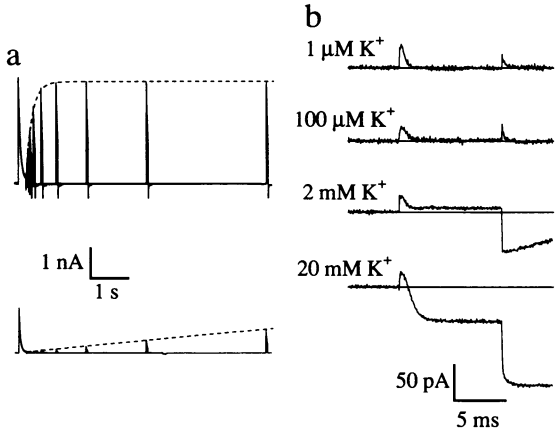

FIG. 5. (*a*) Recovery from inactivation of RCK4 currents at different $[K^+]_o$, measured in cell-attached patches. (*a Upper*) Trace with 50 mM $[K^+]_o$. (*a Lower*) Trace with 1 mM $[K^+]_o$. The dashed lines are single-exponential fits to the peak currents. (*b*) Gating charge displacement at 1 μM, 100 μM, 2 mM, and 20 mM $[K^+]_o$ measured in outside-out patch. The depolarization pulse is to +40 mV from a holding potential of −100 mV; a standard P/4 protocol is used to correct for linear leakage. The pipette contained 120 mM Tris/1.8 mM $CaCl_2$/10 mM Hepes, pH 7.2.

The effect of $[K^+]_o$ on current densities is also present in heterooligomeric channels formed by chimeric dimers constructed by linking RCK1 and RCK4 open reading frames (23). It has been shown before that this chimera forms hybrid channels that inactivate (unlike RCK1), but with inactivation rates significantly slower than homooligomeric RCK4 channels (time constant for inactivation is 200 ms) (23). The absolute reduction of ionic currents in going from a solution with $[K^+]_o$ = 20 to $[K^+]_o$ = 0.2 mM is 30%. The time constant for the recovery from inactivation and the current density are also sensitive to $[K^+]_o$, but the current does not disappear under rapid flow of K^+-free medium, unlike in RCK4 channels.

DISCUSSION

We have shown the modulation of a K^+ current by $[K^+]_o$ in hippocampal neurones and suggested it to be a RCK4-type current because of the match in properties between the currents measured in neurones and in oocytes expressing RCK4 channels. Heterologously expressed channels show a requirement for $[K^+]_o$ to allow permeation, since the current disappears without it. In neurones, we never managed to completely abolish the RCK4-type currents at zero $[K^+]_o$; we believe that the reason for that is the technical difficulty in effectively obtaining a $[K^+]_o$ of zero at the external surface of a neuron in culture, combined with the possible presence of heteromeric channels, which are less sensitive.

All other cloned channels tested lacked a comparable effect. The effect is most pronounced in the range of physiological K^+ concentrations present in mammals, so that small variations in $[K^+]_o$ can induce significant changes in the K^+ current amplitude. Therefore, RCK4-type channels are likely to play an important regulatory role and may induce the response to changes in $[K^+]_o$. Since the $[K^+]_o$ can increase by 0.1–0.8 mM during normal neuronal activity (3), spike frequency may be modulated in an activity-dependent manner, and this might play a role in epilepsy and spreading depression (3, 24, 25). In addition, RCK4 channels are most abundant in the atrium (26), and therefore these channels could play a decisive role in producing arrythmias upon changes in $[K^+]_o$.

The data presented here identify a region mediating the potassium sensitivity. It is the same region that affects tetraethylammonium sensitivity (19) and slow (*n*-type) inactivation (27). The latter process is thought to be due to extracellular Ca^{2+} binding, which matches the hypothesis of a conserved cation binding site in the outer mouth of the channel. We have found that the double mutant K533Y·I535M is sensitive to externally applied tetraethylammonium and insensitive to K^+, while it keeps the kinetics and conductance of the wild-type channel. One of the residues mutated is the one responsible for tetraethylammonium sensitivity and slow inactivation (and therefore Ca^{2+} binding), while the other mutation is a conservative change in terms of charge (isoleucine to methionine). Since all other channels that are insensitive to $[K^+]_o$ carry an uncharged residue in the position equivalent to 533 (except RCK3, which is slightly sensitive), we believe that Lys-533 is the crucial residue for regulation by $[K^+]_o$. This is also supported by the previous finding that increasing $[K^+]_o$ leads to a lower tetraethylammonium sensitivity, which suggests that they might be competing for the same site. Our results strongly support the requirement for K^+ bound to RCK4 channels as a prerequisite for ion permeation, similar to the need for K^+ ions to ensure survival of K^+ currents in squid giant axons (28).

Another A-type channel cloned from mammalian brain, mShal1, shows properties very similar to RCK4, in terms of reactivation kinetics and tetraethylammonium-insensitivity (29). At the moment, it is not possible to discard an impli-

cation of this channel in the generation of the effect that we observe in neurons. However, mShal1 shows a valine residue at the position equivalent to 533 in RCK4, and this residue is the same for RCK5, which lacks both tetraethylammonium-sensitivity and modulation by $[K^+]_o$.

The mechanism responsible for the enhancement of the macroscopic current seems to be an all-or-none effect on permeation because no changes in single-channel conductance or mean open time are detected. Moreover, we observe that the current carried through RCK4 channels is entirely abolished when $[K^+]_o$ is 0. The channels seem to be blocked in the closed or open state, or both, rather than in an inactivated one, since the inactivation mechanisms are not modified. It is important to point out that the acceleration of the recovery from inactivation, which is also induced by $[K^+]_o$ (and might also contribute to modulation of the channel), is not responsible for the increase on macroscopic amplitude, as shown by the following observations. First, all our measurements have been carried out with a pulse interval long enough to achieve complete recovery of the initial amplitude. Second, it is known for RCK4 channels that inactivation is only conserved in the cell-attached configuration and that the kinetics of inactivation are variable from oocyte to oocyte because the oxidation of a specific residue in this channel removes inactivation (10). We have not observed any differences in sensitivity to $[K^+]_o$ due to excision (and therefore oxidation) of the patch in which we observe a loss of inactivation. Third, the double mutant whose current density does not depend on $[K^+]_o$ also shows K^+-dependent recovery from inactivation, and RCK3, which does not show fast inactivation, is also affected by $[K^+]_o$. The effect of K^+ on the recovery from inactivation can be explained by a "knock-off" of the "ball peptide" (30) induced by the inwardly flowing K^+.

Since A-type currents regulate excitability of cells like neurones or cardiomyocytes, this phenomenon might have physiological implications, such as modulation of firing frequency in neurones, whose clarification requires further studies.

We thank Dr. Michael Koenen and Elke Stein for providing some of the wild-type RCK4 cRNA, Monika Papke for technical assistance, Dr. Chris Miller for the gift of charybdotoxin, Barbara Scheufler in the laboratory of Dr. Wilfried Seifert for preparing the hippocampal cultures, and Dr. Anant Parekh for critical reading of the manuscript. L.A.P. is recipient of a fellowship from the Fundación para el Desarrollo en Asturias de la Investigación Científica Aplicada y la Tecnología. This research was supported by a grant from the European Community to W.S. and O.P.

1. Rudy, B. (1988) *Neuroscience* **25**, 729–750.
2. Connor, J. A. & Stevens, C. F. (1971) *J. Physiol. (London)* **213**, 21–30.
3. Heinemann, U., Konnerth, A., Pumain, R. & Wadman, W. J. (1986) *Adv. Neurol.* **44**, 641–661.
4. Fozzard, H. A. & Makielski, J. C. (1985) *Annu. Rev. Med.* **36**, 275–284.
5. Carmeliet, E. (1989) *Pflügers Arch.* **414**, S88–S92.
6. Seifert, W., Ranscht, B., Finck, H. J., Förster, F., Beckh, S. & Müller, H. W. (1983) in *Neurobiology of the Hippocampus*, ed. Seifert, W. (Academic, London), pp. 109–135.
7. Methfessel, C., Witzemann, V., Takahashi, T., Mishina, M., Numa, S. & Sakmann, B. (1986) *Pflügers Arch.* **407**, 577–588.
8. Stühmer, W., Conti, F., Stocker, M., Pongs, O. & Heinemann, S. H. (1991) *Pflügers Arch.* **418**, 423–429.
9. Beckh, S. & Pongs, O. (1990) *EMBO J.* **9**, 777–782.
10. Ruppersberg, J. P., Stocker, M., Pongs, O., Heinemann, S. H., Frank, R. & Koennen, M. (1991) *Nature (London)* **352**, 711–714.
11. Stühmer, W., Ruppersberg, J. P., Schrötter, K.-H., Sakmann, B., Stocker, M., Giese, K. P., Perschke, A., Baumann, A. & Pongs, O. (1989) *EMBO J.* **8**, 3235–3244.
12. Stocker, M., Pongs, O., Hoth, M., Heinemann, S. H., Stühmer, W., Schrötter, K.-H. & Ruppersberg, J. P. (1991) *Proc. R. Soc. London Ser. B* **245**, 101–107.
13. Stühmer, W., Stocker, M., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A. & Pongs, O. (1988) *FEBS Lett.* **242**, 199–206.
14. Grupe, A., Schröter, K.-H., Ruppersberg, J. P., Stocker, M., Drewes, T., Beckh, S. & Pongs, O. (1990) *EMBO J.* **9**, 1749–1756.
15. Schröter, K.-H., Ruppersberg, J. P., Wunder, F., Rettig, J., Stocker, M. & Pongs, O. (1991) *FEBS Lett.* **278**, 211–216.
16. Kamb, A., Tseng-Crank, J. & Tanouye, M. A. (1988) *Neuron* **1**, 421–430.
17. Timpe, L. C., Schwarz, T. L., Tempel, B. L., Papazian, D. M., Jan, Y. N. & Jan, L. Y. (1988) *Nature (London)* **331**, 143–145.
18. Pongs, O., Kecskemethy, N., Müller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H. H., Canal, I., Llamazares, S. & Ferrus, A. (1988) *EMBO J.* **7**, 1087–1096.
19. MacKinnon, R. & Yellen, G. (1990) *Science* **250**, 276–279.
20. Armstrong, C. M. (1971) *J. Physiol. (London)* **58**, 413–437.
21. Sigworth, F. J. (1980) *J. Physiol. (London)* **307**, 97–129.
22. Heinemann, S. H. & Conti, F. (1992) *Methods Enzymol.* **207**, 131–148.
23. Ruppersberg, J. P., Schröter, K.-H., Sakmann, B., Stocker, M., Sewing, S. & Pongs, O. (1990) *Nature (London)* **345**, 535–537.
24. Rice, M. E. & Nicholson, C. (1988) *Brain Res.* **461**, 328–334.
25. Lautritzen, M., Rice, M. E., Okada, Y. & Nicholson, C. (1988) *Brain Res.* **475**, 317–327.
26. Roberds, S. L. & Tamkun, M. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1798–1802.
27. Busch, A. E., Hurst, R. S., North, R. A., Adelman, J. P. & Kavanaugh, M. P. (1991) *Biochem. Biophys. Res. Commun.* **179**, 1384–1390.
28. Almers, W. & Armstrong, C. M. (1980) *J. Gen. Physiol.* **75**, 61–78.
29. Pak, M. D., Baker, K., Covarrubias, M., Butler, A., Ratcliffe, A. & Salkoff, L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4386–4390.
30. Hoshi, T., Zagotta, W. N. & Aldrich, R. W. (1990) *Science* **250**, 533–538.