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Research Report

Two-pore-domain potassium channels contribute to neuronal potassium release and glial potassium buffering in the rat hippocampus

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ABSTRACT

Two-pore-domain potassium (K2P) channels have been suggested to be involved in neuronal K^+ release and glial K^+ uptake. We studied effects of the K2P channel blockers quinine (200 or 500 μM), quinidine (500 μM), and bupivacaine (200 μM) on stimulus-induced and iontophoretically induced transient increases of the extracellular potassium concentration ($[K^+]_o$) in area CA1 of rat hippocampal slices, always in presence of AMPA/kainate and NMDA receptor antagonists. Increases in $[K^+]_o$ evoked by repetitive alvear stimulation (20 Hz) were blocked by quinine and quinidine but amplitudes of population spikes were only modestly reduced. Bupivacaine suppressed both rises in $[K^+]_o$ and population spikes. In contrast, iontophoretically induced rises in $[K^+]_o$ were moderately augmented by quinine and quinidine while bupivacaine had no effect. Barium at concentrations of 2 mM which should block both potassium inward rectifier (Kir) and some K2P channels doubled iontophoretically induced rises in $[K^+]_o$ also in presence of quinine, quinidine, and bupivacaine. The data suggest that quinine/quinidine-sensitive K2P channels mediate K^+ release from neurons and possibly contribute to glial K^+ buffering.

1. Introduction

Neuronal activity in the central nervous system leads to accumulation of potassium ions (K^+) in the extracellular space (Heinemann and Lux, 1975; Krnjevic et al., 1982a; Lux and Neher, 1973). The mechanisms underlying K^+ release from activated neurons are not fully understood. K^+ channel blockers like tetraethylammonium (TEA) and 4-aminopyridine (4-AP) had no effect on increases in extracellular potassium concentration ($[K^+]_o$) evoked by antidromic stimulation in area CA1 (alvear stimulation during blockage of glutamatergic synaptic transmission) (Jones and Heinemann, 1987). This suggests that K^+ currents via leak potassium channels such as two-pore-domain potassium (K2P) channels (Lesage and Lazdunski, 2000) are involved in neuronal K^+ release.

Once released, K^+ diffuses not only through the extracellular space but also throughout the glial syncytium, a process termed spatial K^+ buffering (Orkand et al., 1966). Local accumulation of K^+ depolarizes astrocytes (Ransom and Goldring, 1973). This depolarization spreads to distant compartments resulting in spatially varying gradients between membrane potential and K^+ equilibration potential which

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Abbreviations: 4-AP, 4-aminopyridine; 2-APV, 2-amino-5-phosphonopentanoic acid; Ba^{2+}, barium ions; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; fp, field potential; K^+, potassium ion; $[K^+]_o$, extracellular potassium concentration; K2P, two-pore-domain potassium (channel); Kir, inwardly rectifying potassium (channel); sfp, slow field potential; TEA, tetraethylammonium

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drive K^+ uptake into astrocytes at sites of high $[K^+]_o$ and K^+ release at sites of low $[K^+]_o$. Wallraff et al. (2006) provided evidence that these processes are facilitated by gap junction-dependent and gap junction-independent mechanisms.

K^+ uptake into astrocytes (Ballanyi et al., 1987) is mediated by several mechanisms (Walz, 2000) of which rapid and large effects on $[K^+]_o$ equilibration are likely mediated by barium (Ba^{2+})-sensitive K^+ channels (Jauch et al., 2002) such as inwardly rectifying K^+ channels (Kir) (Ransom and Sontheimer, 1995; Sontheimer and Waxman, 1993), in particular Kir 4.1 (Butt and Kalsi, 2006; Kofuji and Newman, 2004; Olsen et al., 2006). This suggestion is supported by several studies which imply that the glial K^+ buffer capacity is reduced when Kir channels are down-regulated (Binder and Steinhauser, 2006; Hinterkeuser et al., 2000; Kivi et al., 2000; Kucheryavykh et al., 2007).

As recently reported, 100 μM Ba^{2+} enhanced iontophoretically induced rises in $[K^+]_o$ by about 70% (Ivens et al., 2007) while 2 mM Ba^{2+} led to an increase by about 100% (Jauch et al., 2002). At this high concentration, Ba^{2+} also blocks certain K2P channels (Lesage and Lazdunski, 2000; Rajan et al., 2001), suggesting its involvement in spatial K^+ buffering.

The aim of the present study was to find out whether K2P channels contribute to neuronal K^+ release and to spatial K^+ buffering by glial cells. K2P channels are expressed in hippocampal neurons (Talley et al., 2001) and have been found in astrocytes of several brain regions (Ferroni et al., 2003; Gnatenco et al., 2002; Kindler et al., 2000; Skatchkov et al., 2006). They are differently sensitive to quinine, quinidine, and bupivacaine. Quinine blocks TWIK-1 (Lesage et al., 1996), quinidine blocks TWIK-1, TREK-1, and TREK-2 (Lesage et al., 1997; Lesage et al., 2000; Patel et al., 1998), and bupivacaine potently inhibits TASK-1 and -3 (Kim et al., 2000; Kindler et al., 1999).

Here, we studied effects of quinine, quinidine, bupivacaine, and Ba^{2+} on transient increases in $[K^+]_o$ (rises in $[K^+]_o$) which estimate (i) K^+ efflux from discharging neurons and (ii) glial K^+ uptake. Such rises in $[K^+]_o$ were induced (i) by antidromic stimulation and (ii) by K^+ iontophoresis. Both types of rises in $[K^+]_o$ were accompanied by extracellular slow negative field potentials reflecting net moving of positive charges out of the extracellular space into cellular compartments (Dietzel et al., 1989). In order to relate drug-mediated changes in the number of antidromically activated neurons to stimulus-evoked rises in $[K^+]_o$, we also measured fast field potential transients (population spikes) representing neuronal population discharges.

2. Results

In the presence of glutamate-R antagonists, 2-APV and CNQX, electrical stimulation of the alveus with single pulses (0.1 ms duration, 20-s pulse interval) and increasing intensity (2–10 V) induced population spikes getting larger in amplitude up to a maximum value (maximal population spike). The mean maximum value was 7.5±0.48 mV (n=28 slices, 20 animals). Repetitive stimulation of the alveus resulted in rises of $[K^+]_o$ and negative slow field potentials which are dependent on stimulus frequency, intensity and train duration. In this study, we used 10-s stimulus trains with 20 Hz at low and moderate stimulus intensity. Stimulation intensities eliciting 42±1.9% and 67±3.2% of the maximal population spike amplitude revealed rises in $[K^+]_o$ of 1.0±0.06 mM and 2.01±0.14 mM (n=28, p<0.001) and slow field potentials of −0.15±0.02 mV and −0.37±0.04 mV (n=28, p<0.001), respectively. Likewise, iontophoresis current intensities of 169±18 nA and 297±30 nA applied for 20 s induced rises in $[K^+]_o$ of 1.1±0.08 mM and 2.04±0.14 mM (n=28, p<0.001) and slow field potentials of −0.17±0.04 mV and −0.30±0.06 mV (n=15, p=0.001), respectively. As previously reported (Krnjevic and Morris, 1975), rises in $[K^+]_o$ and absolute size of negative slow field potentials were positively correlated (r=0.73, n=56, p<0.001 for alvear stimulation; r=0.65, n=28, p<0.001 for K^+ iontophoresis).

Effects of bath-applied quinine (200 μM, 500 μM), bupivacaine (200 μM) or Ba^{2+} (2 mM) are illustrated in Figs. 1–4, displaying representative sample recordings and summary graphs which follow the course of the experiment. In order to monitor whether drug-induced changes of rises in $[K^+]_o$ may be associated with corresponding changes in number of antidromically activated neurons, we show drug effects on population spikes for each experimental epoch. Tables 1 and 2 display the mean and SEM of absolute control values and their drug-induced changes, given as mean-normalized differences in percent of the control value, also in the text. With respect to the presentation of drug effects on stimulus-induced and iontophoretically induced rises in $[K^+]_o$, it should be noted that changes of low and moderate rises in $[K^+]_o$ and corresponding slow field potentials were indistinguishable (not shown) and pooled. All error probabilities were determined using the Wilcoxon's test for paired comparison of absolute values (value of the respective drug period versus control value), except otherwise noted. Control values were taken after 60 min perfusion with ACSF containing 30 μM 2-APV and 30 μM CNQX. All other drugs have been applied and washed out via the same perfusion medium.

2.1. *200 μM quinine altered neuronal efflux of K^+ and slightly reduced glial K^+ uptake*

As depicted in Figs. 1A–C, quinine at a concentration of 200 μM caused a negligible reduction of the population spike amplitude within 60 min (Fig. 1A), but a clear reduction of stimulus-induced rises in $[K^+]_o$ and slow field potentials (Fig. 1B). In contrast, iontophoretically induced rises in $[K^+]_o$ were slightly augmented, at least after 60 min, while the corresponding slow field potentials became smaller (Fig. 1C). Surprisingly, subsequent combined application of quinine and 2 mM Ba^{2+} resulted in loss of population spikes, disappearance of stimulus-evoked rises in $[K^+]_o$ and slow potential drop but in doubling of iontophoretically induced rises in $[K^+]_o$ accompanied by further reduction of the negative slow field potential. Washout of quinine and Ba^{2+} by changing the perfusion solution to only 2-APV+CNQX-containing ACSF led to partial recovery of all signals within 3 h.

Summary graphs (Fig. 1D) and Table 1a, b, c display the quantitative results for 10 slices. The mean changes of control values at 60-min application of 200 μM quinine were an insignificant reduction of the maximal population spike amplitude (−4%, n=10, p=0.203), a significant reduction of the stimulus-induced rise in $[K^+]_o$ (−66%, n=20, p=0.001) and a