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ARTICLE

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Cell-type specific depression of neuronal excitability in rat hippocampus by activation of ATP-sensitive potassium channels

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Abstract The contribution of ATP-sensitive potassium (K_{ATP}) channels to neuronal excitability was studied in different types of pyramidal cells and interneurones in hippocampal slices prepared from 9- to 15-day-old rats. The presence of functional K_{ATP} channels in the neurones was detected through the sensitivity of whole-cell currents to diazoxide, a K_{ATP} channel opener, and to tolbutamide, a K_{ATP} channel inhibitor. The percentages of neurones with K_{ATP} channels increase in the sequence: CA1 pyramidal cells (37%) < < CA3 pyramidal cells (86%)≈CA1 interneurones of the stratum radiatum (87%) < CA1 interneurones of the stratum oriens (92%). Activation of K_{ATP} channels by diazoxide strongly hyperpolarized stratum radiatum interneurones by a mean of –18.6 mV and effectively suppressed spontaneous and induced action potentials. Pyramidal cells containing a lower density of K_{ATP} channels were hyperpolarized by diazoxide by only –4.0 mV (CA1) and –7.9 mV (CA3), and the frequencies of spontaneous and induced action potentials decreased less than in interneurones. All effects of diazoxide were reversed by tolbutamide. Our results show that K_{ATP} channels have profound effects on the excitability of hippocampal neurones and imply that channel activation during ischaemia or hypoxia depresses the activities of excitatory pyramidal cells to a much lesser extent than those of inhibitory interneurones. This distinct cell-type specific depression of neuronal excitability could account for the generation of seizures and the selective neuroprotection of interneurones in the hippocampus during periods of energy depletion.

Keywords Action potential · Neuronal excitability · ATP-sensitive potassium channel · Hippocampus · Energy depletion · Neuroprotection

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Introduction

ATP-sensitive potassium (K_{ATP}) channels are expressed in a great variety of cells and regulate numerous physiological processes (for a review on K_{ATP} channels, see Ashcroft and Ashcroft 1990). Of growing interest is the possible function of K_{ATP} channels to preserve the viability of cells or to retard cell death in various states of energy depletion, e.g. during anoxia or hypoxia, ischaemia, hypoglycaemia or metabolic inhibition. Under these conditions, protection of cells with K_{ATP} channels arises from the decline of the intracellular ATP concentration, the concomitant activation of K_{ATP} channels and the resulting membrane hyperpolarization which prevents excessive Ca^{2+} entry into the cytoplasm (Heurteaux et al. 1993) and silences electrically excitable cells (Fujimura et al. 1997). An additional beneficial effect of K_{ATP} channel activation is to preclude an intracellular Na^+ overload and a K^+ loss from the cell since this would stimulate the Na^+/K^+-ATPase and thereby lead to a further consumption of intracellular ATP.

In this study, the link between activation or inhibition of K_{ATP} channels and modulation of neuronal excitability is investigated in more detail. Our experiments were performed on the hippocampus, a brain area which is extremely sensitive to ischaemia (Schmidt-Kastner and Freund 1991), and where K_{ATP} channels are activated during anoxia or hypoxia (Krnjević and Ben-Ari 1989; Fujimura et al. 1997), hypoglycaemia (Tromba et al. 1992; Zawar and Neumcke 2000a) and metabolic inhibition (Riepe et al. 1992; Zawar and Neumcke 2000a). There is a very pronounced regional specific expression of K_{ATP} channels in the hippocampus, as deduced initially from the densities of binding sites for the sulphonylurea glibenclamide, a fairly specific inhibitor of K_{ATP} channels (Mourre et al. 1990). More recently, from whole-cell current measurements and determinations of K_{ATP} channel subunits in single hippocampal cells, the lowest channel densities were found in excitatory CA1 pyramidal cells and the highest densities in inhibitory CA1

interneurones of the stratum radiatum (Zawar et al. 1999). However, it is not yet known whether the distinct distribution of K_{ATP} channels also applies to other pyramidal cells and interneurones. Therefore, we determined and compared the densities of K_{ATP} channels in CA1 and CA3 pyramidal cells and in CA1 interneurones of the stratum radiatum and the stratum oriens.

The main objective of the present work was to demonstrate the contribution of K_{ATP} channels to the excitability of neurones and to the frequencies and shapes of action potentials. These studies were performed on three types of hippocampal neurones (CA1 and CA3 pyramidal cells and CA1 interneurones of the stratum radiatum) with low, intermediate and high densities of K_{ATP} channels and were designed to correlate alterations of the resting membrane potential, the excitation threshold and properties of action potentials with the activation or inhibition of K_{ATP} channels. We suggest a decisive, cell-type specific role of K_{ATP} channels in the initiation of spontaneous and induced action potentials and discuss the inferences for the generation of seizures and protection of neurones by activation of K_{ATP} channels during energy depletion.

Some of the results have been published in abstract form (Zawar and Neumcke 2000b; Griesemer and Neumcke 2001).

Materials and methods

Hippocampal slice preparation

Hippocampal slices were prepared from brains of young (9- to 15-day-old) Wistar rats as described in detail previously (Edwards et al. 1989; Zawar et al. 1999). In short, rats were decapitated and isolated brain hemispheres transferred into ice-cold bicarbonate-buffered standard saline (for composition see below). Hippocampal slices of 200 μm thickness were cut with a vibratome, then kept in standard saline bubbled with 95% O_2 and 5% CO_2 at 34 °C for 1 h, thereafter at 25 °C, before electrophysiological recordings were performed.

To document the structure of the hippocampus (Fig. 1, top), a separate set of slices was incubated in 2.5 μg/mL 4′,6-diamidino-2-phenylindole dihydrochloride hydrate (DAPI, Sigma) for 2 min and the fluorescence detected at 620 nm using a two-photon microscope (for details see Zawar et al. 1999).

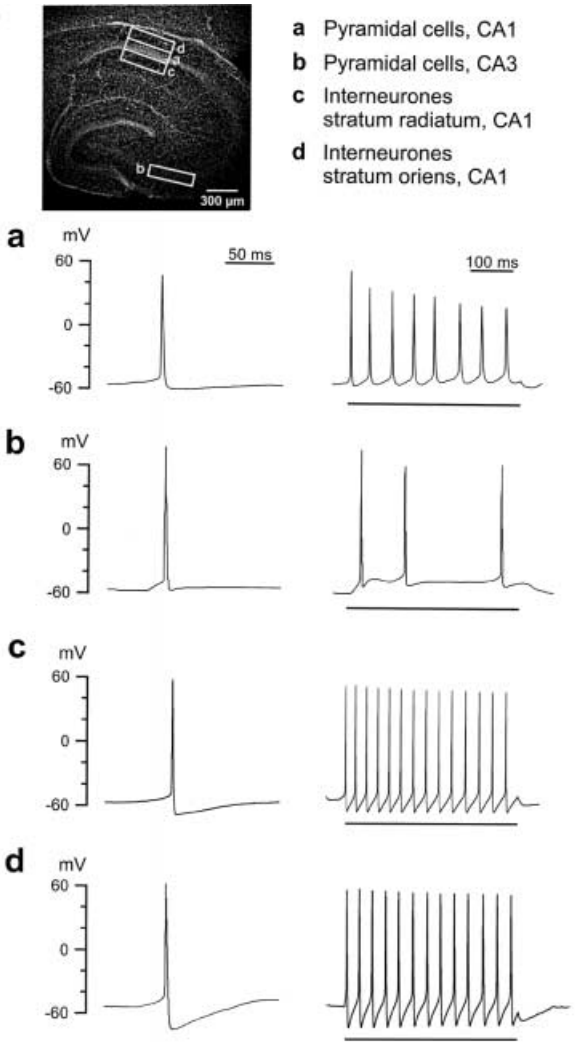

Fig. 1a–d. Action potentials in CA1 and CA3 pyramidal cells and in CA1 interneurones of the stratum radiatum and stratum oriens. *Top*: hippocampal slice stained with DAPI. The *white frames* enclose the regions in which the somata of the cells were located. **a–d** Spontaneous action potentials (*left column*) and trains of induced action potentials (*right column*) recorded in the whole-cell configuration for the four types of neurones. Trains of action potentials were induced by 400-ms current pulses of 50 pA, as indicated by the *bars*

Whole-cell and perforated-patch recordings

Slices were transferred to the recording chamber, where they were continuously perfused with oxygenated standard saline at room temperature (19–23 °C) or with this saline containing diazoxide and/or tolbutamide (see below). The cell types studied (CA1 and CA3 pyramidal cells and CA1 interneurones from the stratum radiatum or from the stratum oriens) were identified visually using a ×40 water immersion objective on an upright microscope (Axioscope FS, Zeiss) with infrared differential interference contrast (IR-DIC) optics.

Patch pipettes were pulled from borosilicate glass (Hilgenberg, Malsfeld, Germany) and had resistances between 2.5 and 4.1 MΩ when filled with the internal solution. Most of the experiments were performed using the tight-seal whole-cell configuration of the patch clamp technique. In some control experiments, perforation of cell membranes by the ionophore gramicidin D was achieved as described previously (Zawar and Neumcke 2000a). Whole-cell and perforated-patch recordings were done with an EPC-9 amplifier and Pulse software (HEKA, Lambrecht, Germany). Slow capacity current transients were compensated and the cell capacitance (C) estimated from this compensation. Currents from individual neurones were normalized to the cell capacitance (see Fig. 5) to obtain a measure of the current density of each cell. The series resistances R_S in whole-cell experiments were within the range between 4.5 and 22.1 MΩ with a mean (±SEM) of 10.6 ± 0.4 MΩ ($n = 107$). Perforated patches were accepted if $R_S < 35$ MΩ; the values were 18.2 ± 1.5 MΩ ($n = 20$).

In the current-clamp mode, the control resting membrane potential of a neuron was determined at the beginning of an experiment in standard saline, and action potentials were recorded which occurred spontaneously at this potential or which were induced by 400-ms current pulses of 50 pA (Fig. 1a–d). In experiments on CA1 and CA3 pyramidal cells and on CA1 interneurones of the stratum radiatum, the K_{ATP} channel modulators diazoxide (0.3 mM) and/or tolbutamide (0.5 mM) were subsequently added to the bath and the actual resting membrane potential registered after approximately

10 min. Spontaneous and induced action potentials starting from this potential level were then recorded (protocol 1). Action potentials were evoked by 400-ms current pulses of 50 or 100 pA (see Fig. 6). In some of the experiments on CA3 pyramidal cells and CA1 interneurones of the stratum radiatum, additional action potentials were recorded after the membrane potential had been shifted by current injection to the original control resting potential (protocol 2). Alterations in the frequency of action potentials measured with protocol (1) indicate effects of K_{ATP} channel activation or inhibition on the excitability of a neuron, which, however, could also be due to the altered membrane potential. Thus the protocol (2) was used to reveal direct effects of the K_{ATP} channel modulators on action potentials starting from the same potential (see Fig. 7).

In the voltage-clamp mode, the holding potential was set to –70 mV and membrane currents were recorded before, during and after 100-ms pulses to –80 and –60 mV (see inset in Fig. 3). The gap between the –80 and –60 mV pulses was 100 ms and the interval between subsequent –80 mV pulses was 15 s. Stationary currents at the end of the –60 and –80 mV test pulses were then measured before, during and after application of the K_{ATP} channel modulators diazoxide and tolbutamide (Figs. 3, 4). Since diazoxide-induced currents exhibit an almost linear voltage dependence between –120 and –60 mV (Zawar et al. 1999; Fig. 5), the –80 and –60 mV test pulse protocol comprises currents through K_{ATP} channels and excludes contributions from all types of channels that are activated at potentials > –60 mV (e.g. voltage-dependent sodium and potassium channels).

Solutions and drugs

The standard saline (bath solution) contained (mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 20 glucose (pH 7.4 when gassed with 95% O_2 and 5% CO_2). Bicuculline-methiodide (10 μM) was added to the solution to block $GABA_A$-mediated postsynaptic currents. Tolbutamide (Sigma) and diazoxide (Schering, Berlin) were added to the standard saline as 100 mM stock solutions dissolved in 0.15 M NaOH, and the pH was adjusted to 7.4 with HCl.

The pipette solution was composed of (mM): 140 KCl, 11 EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid], 1 $CaCl_2$, 1 $MgCl_2$, 0.5 Na_2ATP, 10 HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], and the pH was adjusted to 7.2 with KOH. Stock solutions of gramicidin D (Sigma) were prepared (25 mg/mL dimethyl sulfoxide) and diluted in the pipette solution to a final concentration of 25 μg/mL.

The calculated K^+ and Na^+ equilibrium potentials at 22 °C are –102 and +128 mV, respectively, for the bath and pipette solutions used in our experiments.

Statistics

In our study, no differences between the electrophysiological responses of specific types of neurones from the hippocampus of 9- to 15-day-old rats could be observed. Therefore, the results from different ages were pooled, and cumulative results from *n* cells are given as means ± SEM. Significant differences between data from different types of neurones were determined using Student's unpaired *t* test or the multifactorial analysis of variance (ANOVA) at the 5%, 1% or 0.1% significance levels.

Results

Resting and action potentials of different types of pyramidal cells and interneurones

The top of Fig. 1 shows a hippocampal slice and the locations of the cell types studied in this work: CA1 and CA3 pyramidal cells and CA1 interneurones of the stratum radiatum and the stratum oriens. The resting potentials of these neurones in standard saline were: –55.4 ± 0.5 mV, $n = 16$ (CA1 pyramidal cells), –60.1 ± 0.7 mV, $n = 34$ (CA3 pyramidal cells), –59.9 ± 0.8 mV, $n = 13$ (CA1 interneurones stratum radiatum) (see Table 1) and –57.7 ± 1.5 mV, $n = 13$ (CA1 interneurones stratum oriens).

Spontaneous action potentials and trains of action potentials induced by 50-pA current injection are illustrated in Fig. 1a–d for pyramidal cells and interneurones. The action potentials were registered in the whole-cell configuration of the patch clamp technique and had large positive overshoots due to the low Na^+ concentration (1 mM) of the pipette solution. The amplitudes of spontaneous action potentials were: 89.4 ± 4.7 mV, $n = 10$, CA1 pyramidal cells; 86.8 ± 4.6 mV, $n = 10$, CA3 pyramidal cells; 94.8 ± 3.8 mV, $n = 10$, CA1 interneurones stratum radiatum; and 121.5 ± 3.4 mV, $n = 4$, CA1 interneurones stratum oriens. The shapes and afterpotentials of spontaneous and induced action potentials in the cells were as described (Bilkey and Schwartzkroin 1990; Morin et al. 1996). Thus, spontaneous action potentials in CA1 and CA3 pyramidal cells were broader than those of interneurones (durations of action potentials: 6.7 ± 0.8 ms, $n = 10$, CA1 pyramidal cells; 6.3 ± 0.6 ms, $n = 10$, CA3 pyramidal cells; 3.2 ± 0.3 ms, $n = 10$, CA1 interneurones stratum radiatum; 2.6 ± 0.2 ms, $\underline{n} = 4$, CA1 interneurones stratum oriens) and showed a smaller after-hyperpolarization (AHP) (maximal AHPs: –7.30 ± 0.87 mV, $n = 10$, CA1 pyramidal cells; –6.90 ± 0.95 mV, $n = 10$, CA3 pyramidal cells; –20.50 ± 1.08 mV, $n = 10$, CA1 interneurones stratum radiatum; –18.25 ± 1.89 mV, $n = 4$, CA1 interneurones stratum oriens). Trains of induced action potentials in both types of pyramidal cells were decrementing (Fig. 1a, b), but the rate of accommodation was low in CA1 interneurones (Fig. 1c, d).

The whole-cell registrations of action potentials shown in Fig. 1 were performed with a high concentration (11 mM) of EGTA in the pipette solution. Diffusion of this calcium-chelating agent into the cytoplasm might affect the activity of calcium-activated potassium channels which were suggested to modulate the shapes, bursting characteristics and AHPs of action potentials in hippocampal pyramidal neurones (Storm 1990). To test this possibility, perforated-patch recordings of action potentials were performed on CA1 pyramidal cells ($n = 20$) and compared with standard whole-cell registrations. Perforation of cell membranes was achieved by the ionophore gramicidin D forming pores impermeable to EGTA and thereby preserving the calcium homeostasis of the cell. Figure 2 illustrates trains of induced action potentials for a whole-cell and a perforated-patch clamp experiment. There are no noticeable differences between the action potentials in both patch configurations. The frequencies of action potentials during the 400-ms current pulses (whole-cell: 15.33 ± 1.23 Hz, $n = 16$, see Table 1; perforated-patch: 17.25 ± 0.95 Hz, $n = 20$) were not significantly different ($P > 0.05$). Also, there are no characteristic differences between the fast AHP

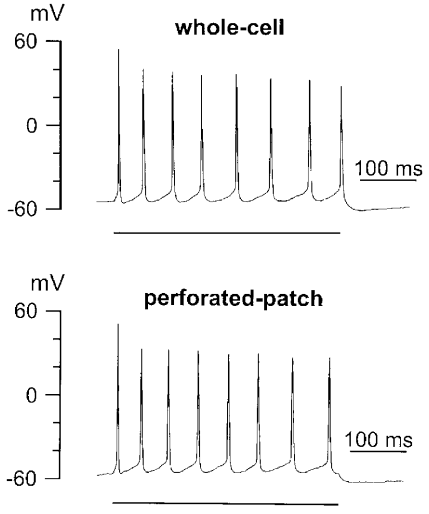

Fig. 2. Trains of action potentials in CA1 pyramidal cells recorded in the whole-cell or the perforated-patch configuration. The action potentials were induced by 400-ms current pulses of 50 pA, as indicated by the *bars*

phases after the trains of action potentials for both patch configurations in Fig. 2 and in the other comparative experiments. The subsequent medium and slow AHP phases (Storm 1990) are beyond the time range of our registrations and were not studied in the present investigation. Since no differences between action potentials in the whole-cell and perforated-patch configurations could be detected under the conditions of our experiments, the excitability of hippocampal neurones was explored with standard whole-cell patch registrations in the following.

K_{ATP} channels in different types of pyramidal cells and interneurones

As in our previous study (Zawar et al. 1999), we used a voltage-pulse protocol and a pharmacological approach to detect K_{ATP} channels and to estimate the channel densities in different types of hippocampal cells. Figures 3 and 4 show the effects of the K_{ATP} channel activator diazoxide and the channel inhibitor tolbutamide on two additional, not yet studied types of neurones (CA3 pyramidal cell, CA1 interneurone of the stratum oriens). In the figures, stationary currents at the end of the –60 and –80 mV test pulses are plotted at various times after establishing the whole-cell mode. The slow spontaneous increase of inward currents observed in all records during the first 5 min is probably related to the exchange of low molecular weight substances between the cell interior and the pipette solution (Pusch and Neher 1988). A possible wash-out of ATP from the cell with concomitant activation of K_{ATP} channels would generate outward currents. As found in experiments with an ATP-free pipette solution (Zawar et al. 1999; Fig. 3), this process begins approximately 5 min after forming the whole-cell mode and is complete after 10 min. In contrast, in the experiments of the present study, cell dialysis was performed with a 0.5 mM ATP

Table 1. Resting membrane potentials and frequencies of spontaneous and induced action potentials in three types of hippocampal neurones[a]

	CA1 pyramidal cells	CA3 pyramidal cells	CA1 interneurones stratum radiatum
Resting membrane potential (mV)			
Control	–55.4 ± 0.5 (16)	–60.1 ± 0.7 (34)	–59.9 ± 0.8 (13)
Diazoxide	–59.4 ± 1.9 (16)	–68.0 ± 1.3 (34)[d]	–78.5 ± 2.0 (13)[d]
Tolbutamide	–48.9 ± 1.1 (16)[b]	–55.2 ± 0.6 (34)[c]	–59.1 ± 1.4 (13)
Spontaneous action potentials (Hz)			
Control	1.54 ± 0.27 (16)	0.51 ± 0.16 (34)	2.83 ± 0.68 (13)
Diazoxide (1)	0.92 ± 0.37 (16)	0.05 ± 0.03 (34)	0 (13)
Diazoxide (2)	–	0 (16)	0 (4)
Tolbutamide (1)	2.16 ± 0.74 (16)	1.08 ± 0.30 (34)	4.45 ± 1.18 (13)
Tolbutamide (2)	–	0.45 ± 0.33 (16)	3.10 ± 1.39 (4)
Induced action potentials (Hz)			
Control	15.33 ± 1.23 (16)	5.73 ± 0.90 (34)	25.78 ± 3.20 (13)
Diazoxide (1)	12.03 ± 1.86 (16)	2.05 ± 1.15 (34)	1.15 ± 0.53 (13)
Diazoxide (2)	–	3.13 ± 1.30 (16)	5.63 ± 4.83 (4)
Tolbutamide (1)	10.49 ± 2.25 (16)	5.08 ± 0.88 (34)	19.63 ± 2.23 (13)
Tolbutamide (2)	–	6.58 ± 1.73 (16)	26.25 ± 5.15 (4)

[a]Resting membrane potentials were measured and action potentials recorded at the beginning of the experiment (control), 10 min after bath application of 0.3 mM diazoxide and 10 min after bath application of 0.5 mM tolbutamide. Induced action potentials were evoked by 400-ms current pulses of 50 pA. In the presence of diazoxide and tolbutamide, spontaneous and induced action potentials were recorded in all neurones at the actual resting potential (1). In some of the experiments on CA3 pyramidal cells and CA1 interneurones of the stratum radiatum, the action potentials were also recorded at the control resting potential (2). The values denote the means ± SEM and the corresponding number of cells

[b]$P < 0.05$

[c]$P < 0.01$

[d]$P < 0.001$ with respect to control (ANOVA)

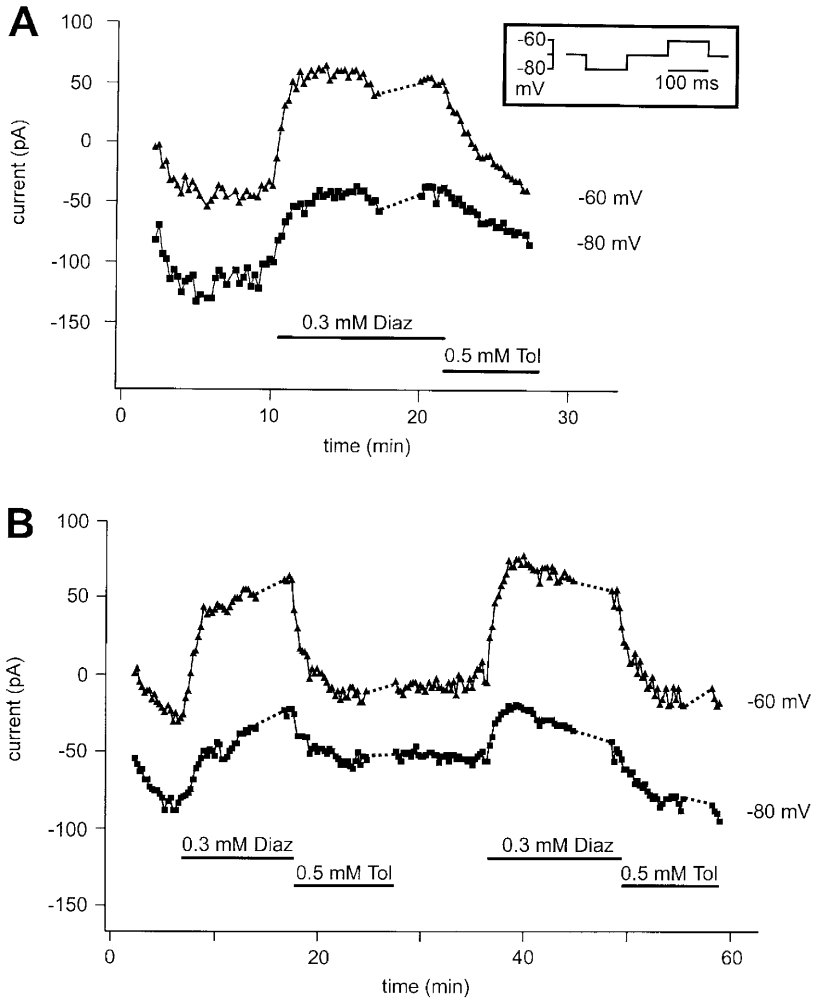

Fig. 3A, B. Responses of two CA3 pyramidal cells to application of 0.3 mM diazoxide (Diaz) and 0.5 mM tolbutamide (Tol), as indicated by the *bars*. The *inset* illustrates the protocol of the voltage pulses to –80 and –60 mV. The figures show stationary currents at the end of the –60 and –80 mV test pulses at various times after forming the whole-cell mode. During the gaps at 17 min (**A**) and 15, 27, 46 and 56 min (**B**) action potentials were registered in the current-clamp mode

pipette solution which did not induce outward currents within 10 min in a CA3 pyramidal cell (Fig. 3A) and within 8 min in a CA1 interneurone of the stratum oriens (Fig. 4). Thus an ATP concentration of 0.5 mM in the pipette solution is sufficient to block essentially all K_{ATP} channels as expected from the known ATP-affinity of neuronal K_{ATP} channels (e.g. see Ohno-Shosaku and Yamamoto 1992; Schwanstecher and Bassen 1997). Bath application of diazoxide (0.3 mM) activated outward currents in CA3 pyramidal cells (Fig. 3A, B) and in the CA1 interneurone of the stratum oriens (Fig. 4), and the activated currents were totally inhibited by tolbutamide (0.5 mM). A second treatment with diazoxide evoked similar outward currents which, again, were sensitive to tolbutamide (Fig. 3B). The effects of the K_{ATP} channel modulators illustrated in Figs. 3 and 4 indicate the presence of functional K_{ATP} channels in the neurones studied.

Further evidence for the existence of potassium channels activated by diazoxide is provided by the reversal potentials of the activated currents which were near the calculated K^+ equilibrium potential of –102 mV. Thus the reversal potentials, as obtained by linear interpolation between the maximally activated currents at –60 and –80 mV, were –96 mV (Fig. 3A), –104 mV (Fig. 3B) and –115 mV (Fig. 4). The reversal potentials from all experiments on the four hippocampal cell types were: -99.7 ± 1.4 mV, $n = 39$ (CA1 pyramidal cells), -103.5 ± 1.6 mV, $n = 24$ (CA3 pyramidal cells), -98.2 ± 1.1 mV, $n = 29$ (CA1 interneurones stratum radiatum) and -102.1 ± 1.9 mV, $n = 12$ (CA1 interneurones stratum oriens).

To compare the effects of diazoxide and tolbutamide on the four hippocampal cell types investigated, we determined for each neurone the maximum current at a potential of –60 mV in the presence of 0.3 mM diazoxide minus the stationary current after a subsequent application of 0.5 mM tolbutamide. This difference protocol comprises the contributions of all K_{ATP} channels which were activated by diazoxide and which possibly opened spontaneously before the application of the channel activator. Cells of each type with current differences larger than 5 pA at –60 mV were distinguished from cells that did not respond. By this criterion, two distinct populations of neurones of each type with and without functional K_{ATP} channels were defined. The lower panel of Fig. 5 gives the proportions of the two populations; the ratios of the cells with K_{ATP} channels

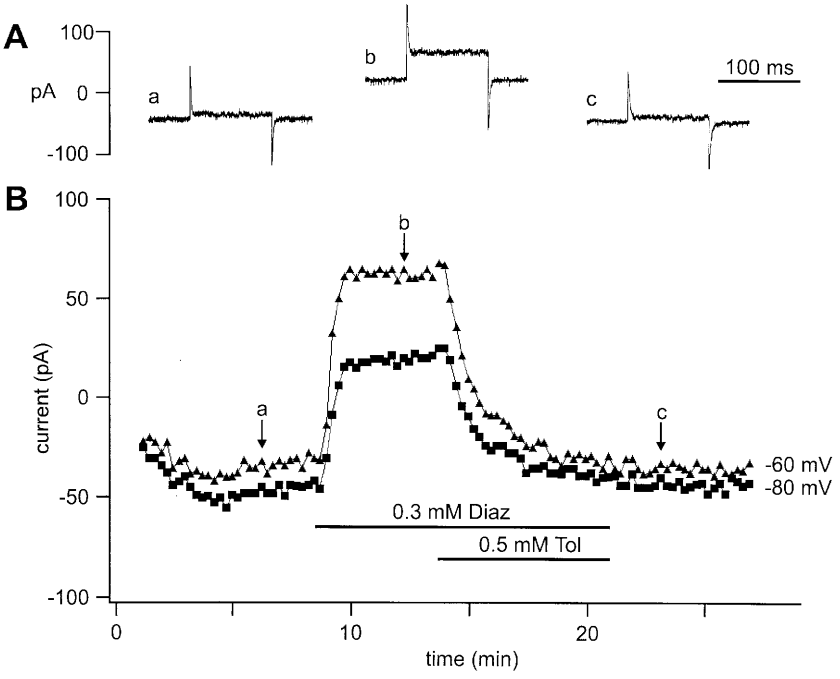

Fig. 4A, B. Response of a CA1 interneurone from stratum oriens to application of 0.3 mM diazoxide (Diaz) and 0.5 mM tolbutamide (Tol). **A** Currents recorded before, during and after 100-ms pulses from –70 to –60 mV at the times indicated by *a–c* in the experiment illustrated in **B**. **B** Stationary currents at –60 and –80 mV at various times after forming the whole-cell mode

increase in the sequence: CA1 pyramidal cells (16/43) << CA3 pyramidal cells (19/22)≈CA1 interneurones of the stratum radiatum (27/31) < CA1 interneurones of the stratum oriens (12/13). A cell-type specific difference was also found for the density of K_{ATP} channels in responding cells. As a quantitative measure of the channel density in these cells, we normalized the differences of the currents in the presence of diazoxide and tolbutamide to the cell capacitance C of each cell ($C = 22.4 \pm 1.4$ pF, $n = 36$, CA1 pyramidal cells; 20.8 ± 0.9 pF, $n = 34$, CA3 pyramidal cells; 13.4 ± 0.9 pF, $n = 25$, CA1 interneurones stratum radiatum and 15.8 ± 1.6 pF, $n = 12$, CA1 interneurones stratum oriens). The results plotted in the upper part of Fig. 5 show that the largest

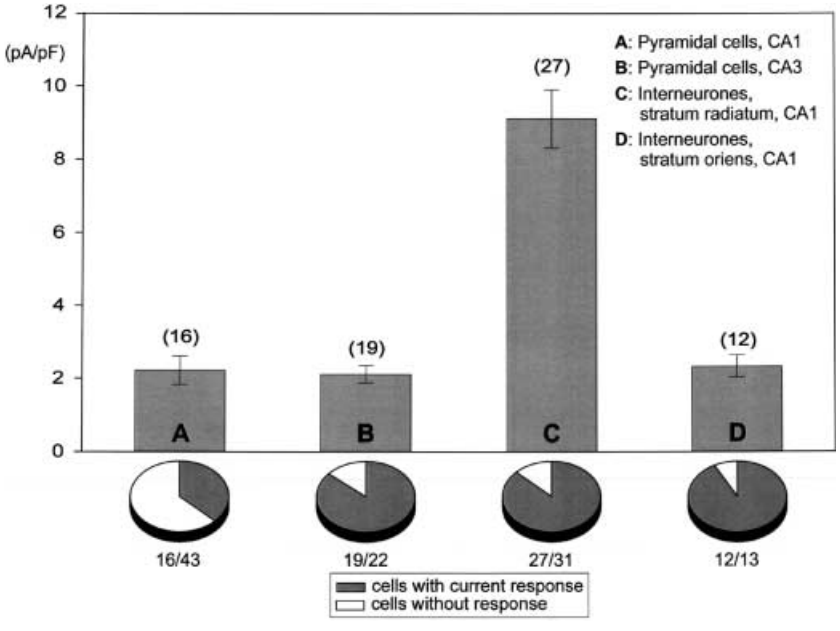

Fig. 5. Diazoxide-induced currents in four types of hippocampal neurones as indicated. The *lower panel* shows the proportions of cells with functional K_{ATP} channels that responded to diazoxide and to tolbutamide. The maximum currents of the responding cells induced by 0.3 mM diazoxide at a potential of –60 mV were normalized to the cell capacitance and are plotted in the *upper panel*. The figure summarizes experiments of this work (16 CA1 pyramidal cells, 22 CA3 pyramidal cells, 13 CA1 interneurones from stratum radiatum, 13 CA1 interneurones from stratum oriens) and from a previous study (Zawar et al. 1999) performed under identical conditions (27 CA1 pyramidal cells, 18 CA1 interneurones from stratum radiatum). The *columns* and *bars* denote the means ± SEM

normalized current responses and thus the highest K_{ATP} channel densities were found in CA1 interneurones of the stratum radiatum. On the other hand, responding CA1 interneurones of the stratum oriens and CA1 and CA3 pyramidal cells had much lower channel densities.

Effects of K_{ATP} channel modulators on resting membrane potential and excitability of neurones

The effects of the K_{ATP} channel opener diazoxide and the inhibitor tolbutamide on resting and action potentials were studied in the current-clamp mode in three types of hippocampal neurones with a low (CA1 pyramidal cells), an intermediate (CA3 pyramidal cells) and a high (CA1 interneurones stratum radiatum) density of K_{ATP} channels. Figure 6 illustrates the effects of the two K_{ATP} channel modulators on action potentials induced in a CA1 interneurone of the stratum radiatum. After activation of K_{ATP} channels by diazoxide, no action potentials could be evoked by a 50-pA current pulse, whereas a single action potential was elicited by a stronger 100-pA pulse. Thus the K_{ATP} channel opener diazoxide effectively depressed the excitability of the

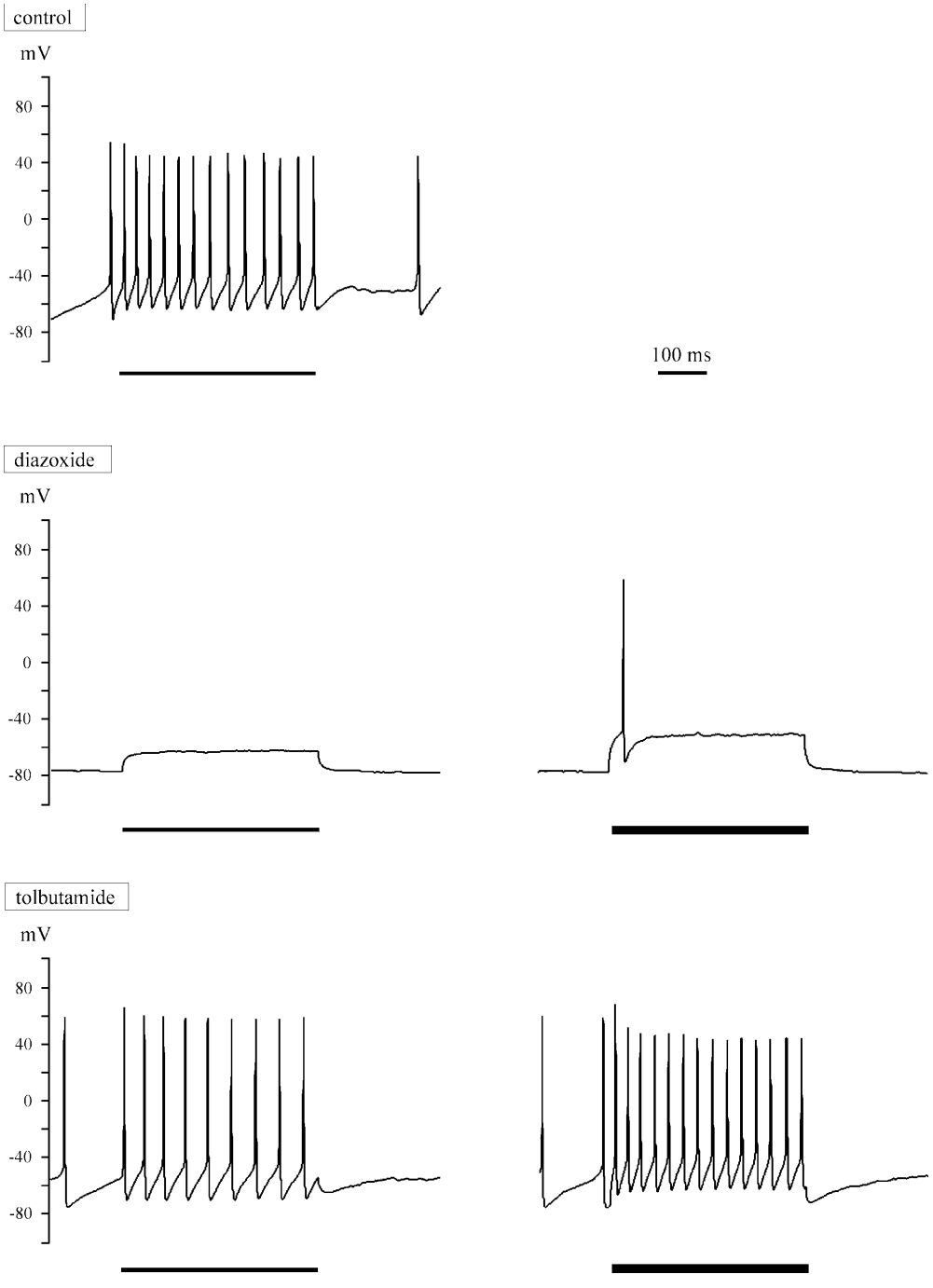

Fig. 6. Trains of action potentials in a CA1 interneurone of the stratum radiatum recorded in the whole-cell configuration in standard saline (control), in the presence of 0.3 mM diazoxide or 0.5 mM tolbutamide. The resting potentials were –60 mV (control), –76 mV (diazoxide) and –60 mV (tolbutamide), and the action potentials started from these potential levels (protocol 1). The trains of action potentials were induced by 400-ms current pulses of 50 pA (*left column*) or 100 pA (*right column*, diazoxide and tolbutamide), as indicated by the *bars*. Before and after the current pulses, spontaneous action potentials occurred in the presence of tolbutamide and in standard saline

interneurone. The subsequent application of the channel inhibitor tolbutamide acted in the opposite direction. The K_{ATP} channel modulators also altered the resting membrane potentials in a similar way: with respect to the initial control value of –60 mV, diazoxide produced a hyperpolarization of –16 mV, and tolbutamide restored the control membrane potential (see legend to Fig. 6).

Table 1 summarizes the effects of diazoxide and tolbutamide on resting potentials and frequencies of action potentials in three types of hippocampal neurones. The mean values of membrane hyperpolarization by diazoxide (–4.0 mV for CA1 pyramidal cells, –7.9 mV for CA3 pyramidal cells, –18.6 mV for CA1 interneurones of the stratum radiatum) were cell-type specific and increased with the density of K_{ATP} channels in the neurones. Upon application of tolbutamide, the membrane potential depolarized beyond the initial resting potential by about 5 mV for both types of pyramidal cells, but not for interneurones. A similar membrane depolarization was not observed in additional control experiments on CA1 pyramidal cells in standard saline during a comparable time. The resting potentials at the beginning of the whole-cell experiments (-54.2 ± 1.4 mV) and after 30 min perfusion with standard saline (-53.3 ± 1.9 mV) were not significantly different ($n = 6$, $P > 0.05$).

As illustrated in Fig. 6 for a CA1 interneurone of the stratum radiatum, diazoxide depressed the excitability of all types of neurones studied. The decline of the frequency of spontaneous and induced action potentials by diazoxide was minimal for CA1 pyramidal cells with a low density of K_{ATP} channels and maximal for CA1 interneurones of the stratum radiatum with the highest channel densities. For pyramidal cells and interneurones, the K_{ATP} channel opener diazoxide and the channel inhibitor tolbutamide altered the membrane potentials and the frequencies of spontaneous and induced action potentials in opposite directions (Table 1).

Diazoxide almost totally suppressed the excitability of CA1 interneurones of the stratum radiatum with the highest densities of K_{ATP} channels (Table 1). Therefore, the effects of the K_{ATP} channel modulators diazoxide and tolbutamide on the shape and afterpotential of an individual action potential were studied in CA3 pyramidal cells with an intermediate density of K_{ATP} channels. Figure 7 shows single action potentials (first action potentials evoked by 400-ms current pulses of 50 pA) in a neuron of this type for which the presence of K_{ATP} channels had been verified by a –20 mV hyperpolarization after application of diazoxide (see legend to Fig. 7). The records illustrate that activation of K_{ATP} channels by diazoxide had no effects on the shape of the action potential, whereas the action potential had a smaller amplitude and a slightly longer duration in the presence of the channel inhibitor tolbutamide. These observations were confirmed in a total of four CA3 pyramidal cells with the following results: AP amplitudes: 102.0 ± 4.3 mV (control), 105.5 ± 4.7 mV (diazoxide), 90.2 ± 8.4 mV (tolbutamide); AP durations: 4.8 ± 0.2 ms (control), 4.6 ± 0.3 ms (diazoxide), 4.9 ± 0.3 ms (tolbutamide); maximal AHPs: -3.4 ± 1.6 mV (control), -3.1 ± 0.7 mV (diazoxide), -2.1 ± 0.8 mV (tolbutamide).

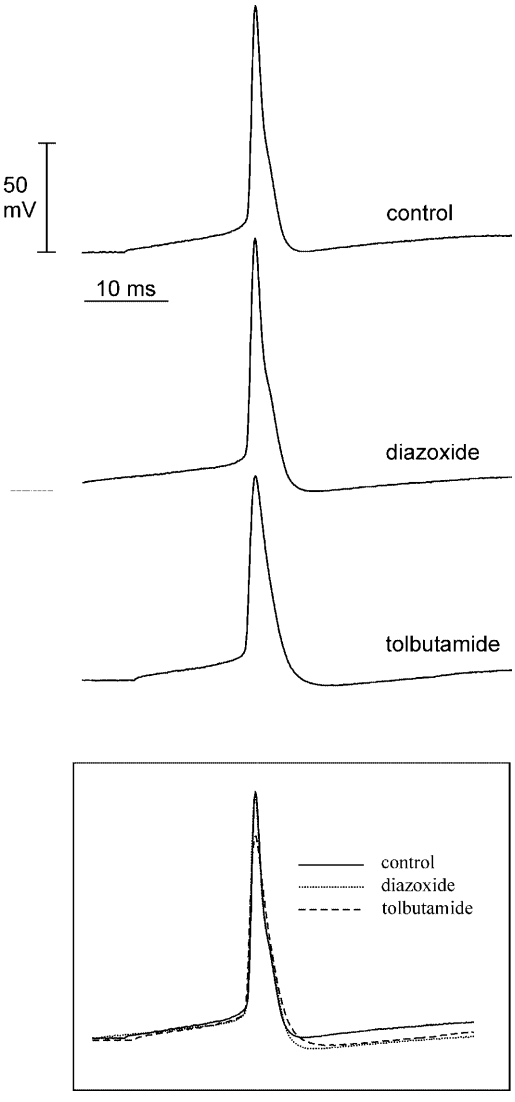

Fig. 7. Single action potentials of a CA3 pyramidal cell recorded in the whole-cell configuration in standard saline (control), in the presence of 0.3 mM diazoxide or 0.5 mM tolbutamide. For better comparison, the three action potentials in the upper part were superimposed at the bottom of the figure. The resting potentials were –54 mV (control), –74 mV (diazoxide) and –50 mV (tolbutamide). Before recording action potentials in the presence of diazoxide and tolbutamide, the membrane potentials were shifted by current injection to the original control resting potential of –54 mV (protocol 2). Shown are the first action potentials evoked by 400-ms current pulses of 50 pA

Discussion

The results of this paper confirm and extend previous studies on the distribution of K_{ATP} channels in the rat hippocampus (Zawar et al. 1999) and their metabolic

sensitivities (Zawar and Neumcke 2000a). In the present work we describe for the first time the existence of K_{ATP} channels in two additional cell types: in CA3 pyramidal cells (Fig. 3) and in CA1 interneurones of the stratum oriens (Fig. 4). Furthermore, we compared action potentials in the whole-cell and perforated-patch configurations (Fig. 2) and analysed the effects of K_{ATP} channel modulators on trains of induced action potentials (Fig. 6) and on the shape of single action potentials (Fig. 7).

The results compiled in Fig. 5 reveal that the percentages of cells with functional K_{ATP} channels and the channel densities differ not only among various cell types (pyramidal cells, interneurones), but are also distinct in cells of the same type located in different areas of the hippocampus (CA1 and CA3 pyramidal cells, CA1 interneurones from the stratum radiatum and the stratum oriens). These results were derived from experiments on hippocampal slices prepared from 9- to 15-day-old rats. During this time the densities of glibenclamide receptors in the CA1 and CA3 fields of the hippocampus increase by approximately 50% (Mourre et al. 1990), but a similar dependence with age could not be observed in this study for the densities of K_{ATP} channels in the hippocampal cell types examined. In general, the results of this study and from our previous investigations (Zawar et al. 1999; Zawar and Neumcke 2000a) were derived from hippocampal cells of immature rats and may not be applicable to fully differentiated neurones of the adult rat hippocampus with a two- to three-times higher density of glibenclamide binding sites (Mourre et al. 1990).

There are numerous types of interneurones in the hippocampus as found by combined electrophysiological and morphological studies (Freund and Buzsáki 1996; Morin et al. 1996). From our patch clamp experiments, only electrophysiological criteria are available to characterize CA1 interneurones from the stratum radiatum and stratum oriens. Thus the short duration and pronounced AHPs of spontaneous action potentials and the high frequency and low rate of accommodation in trains of induced action potentials (Fig. 1c, d) are characteristic for fast-spiking interneurones of the CA1 region (Morin et al. 1996). Obviously, the different densities of K_{ATP} channels in CA1 interneurones of the stratum radiatum and stratum oriens (Fig. 5) are not reflected by major differences between the action potentials in both types of interneurones (Fig. 1c, d) recorded in standard saline. This indicates that K_{ATP} channels are of minor importance for the excitability of neurones under normal conditions but may be operative during periods of energy depletion (see below).

K_{ATP} channels are heteromultimers composed of four inwardly rectifying potassium channel subunits Kir6.x and four sulfonylurea receptors SUR (for a review, see Bryan and Aguilar-Bryan 1997). In CA1 pyramidal cells and interneurones of the stratum radiatum, all subunits Kir6.1, Kir6.2, SUR1 and SUR2 were detected, and the most frequent combination resulting in functional K_{ATP} channels was Kir6.2 together with SUR1 (Zawar et al. 1999). This type of K_{ATP} channel, originally found in β-cells of the pancreas, is characterized by a high tolbutamide affinity and strong metabolic sensitivity, as deduced from experiments on substantia nigra neurones (Liss et al. 1999). A K_{ATP} channel with low tolbutamide affinity and weak metabolic sensitivity, composed of Kir6.2 and the smooth muscle sulfonylurea receptor isoform SUR2B (Liss et al. 1999), may be also expressed in the hippocampus since only SUR2B, not the cardiac isoform SUR2A, was reported to be present in the forebrain of the mouse (Isomoto et al. 1996). Other possible hippocampal K_{ATP} channels might be composed of the Kir6.1 subunit together with SUR1 or SUR2B, because these channels have a low conductance of the order of 30 pS, as observed for single K_{ATP} channels in dentate gyrus granule cells of the rat hippocampus (Pelletier et al. 2000). Thus several types of K_{ATP} channels with different conductances, tolbutamide affinities and metabolic sensitivities may be expressed in the hippocampus, but none of them contains the isoform SUR2A forming diazoxide-insensitive K_{ATP} channels (Inagaki et al. 1996). Therefore, diazoxide was applied in our experiments in a concentration of 0.3 mM to activate all hippocampal K_{ATP} channels effectively.

This study presents a detailed analysis of the effects of K_{ATP} channel activation and inhibition on spontaneous and induced action potentials and shows that channel activation strongly reduces the excitability of hippocampal neurones. One obvious reason for the depressing effects of diazoxide is that neurones have a more negative resting potential in the presence of the K_{ATP} channel opener (Table 1). The membrane hyperpolarization was maximal for CA1 interneurones of the stratum radiatum, still highly significant for CA3 pyramidal cells but less pronounced for CA1 pyramidal cells. This sequence correlates well with the different densities of K_{ATP} channels in these cells (Fig. 5). Interestingly, the membrane potentials of CA1 and CA3 pyramidal cells depolarized beyond the initial control values in the presence of the K_{ATP} channel blocker tolbutamide (Table 1). Since pyramidal cells did not depolarize spontaneously in standard saline during a comparable time (see Results), there remain two possible explanations of this result: pyramidal cells could contain open K_{ATP} channels already in the initial resting state which were finally inhibited by tolbutamide, or this agent is not a specific K_{ATP} channel blocker but inhibits additional types of open resting K^+ channels (Erdemli and Krnjević 1996) or Cl^- channels.

Application of diazoxide had hardly any effect on the frequencies of spontaneous and induced action potentials in CA1 pyramidal cells containing few K_{ATP} channels but markedly reduced the excitability of CA3 pyramidal cells with more K_{ATP} channels and drastically suppressed spontaneous and induced action potentials in CA1 interneurones of the stratum radiatum with the highest channel densities (Fig. 6, Table 1). The depressing effects of diazoxide on induced action potentials

were very pronounced when action potentials started from the actual hyperpolarized resting potential (protocol 1), but were still clearly observed when the membrane potential had been depolarized to the initial resting potential (protocol 2). This indicates that membrane hyperpolarization by the K_{ATP} channel opener is not the sole reason for the suppression of excitability in hippocampal neurones by diazoxide. In addition, K_{ATP} channels activated by diazoxide at the initial resting potential tend to stabilize this potential, thereby increasing the threshold of excitation. Conversely, inhibition of K_{ATP} channels by tolbutamide enhanced the excitability of neurones by membrane depolarization and a decrease of the resting K^+ conductance (Table 1). In contrast, a direct effect of K_{ATP} channel activation by diazoxide on the amplitude and duration of action potentials could not be observed in our experiments, while alterations of the shapes of action potentials by tolbutamide (Fig. 7) may be due to unspecific effects of this agent on other types of ion channels (see above).

As explained before, activation of all K_{ATP} channels in hippocampal neurones was achieved in this study by application of the K_{ATP} channel opener diazoxide. Similarly, opening of K_{ATP} channels is induced by a decline of the intracellular ATP concentration during periods of energy depletion, and inhibition of these channels occurs after resumption of normal cellular metabolism. For example, activation of neuronal K_{ATP} channels by anoxia or hypoxia was reported for the first time in dentate granule cells of the hippocampus (Krnjević and Ben-Ari 1989) and subsequently in CA1 pyramidal cells (Fujimura et al. 1997), in inspiratory (Mironov et al. 1998) and dopaminergic neurones (Murphy and Greenfield 1992; Guatteo et al. 1998) and in neurones of the neocortex (Garcia de Arriba et al. 1999). Alternatively, opening of K_{ATP} channels was stimulated by removal of glucose or application of metabolic inhibitors in hippocampal (Riepe et al. 1992; Tromba et al. 1992; Zawar and Neumcke 2000a), dopaminergic (Murphy and Greenfield 1992; Röper and Ashcroft 1995) and vagal (Ballanyi et al. 1996) neurones. Channel activation by hypoxia and hypoglycaemia could be verified even for single K_{ATP} channels in granule cells of the dentate gyrus (Pelletier et al. 2000).

Activation of neuronal K_{ATP} channels during various states of energy depletion suppresses spontaneous and induced action potentials (Ballanyi et al. 1996; Fujimura et al. 1997), and this was attributed to membrane hyperpolarization caused by an increased K^+ conductance. As shown in this study, excitability in the presence of diazoxide is diminished even when action potentials start from the initial resting potential (Table 1). Thus membrane hyperpolarization and elevation of excitation threshold lead to a very effective silencing of interneurones containing a high density of K_{ATP} channels, while the excitability of pyramidal cells with fewer K_{ATP} channels is hardly affected. As a consequence, hyperexcitability in the hippocampus may occur because the activity of inhibitory interneurones is depressed more strongly than that of excitatory pyramidal cells. This unbalance in the neuronal network could contribute to the generation of acute seizures in the hippocampus during the initial phases of hypoxia (Jensen et al. 1998). On the other hand, Kir6.2-deficient mice have a lower threshold for hypoxia-induced generalized seizures in the substantia nigra pars reticulata than wild-type mice (Yamada et al. 2001), suggesting a dominant role of K_{ATP} channels in excitatory neurones of this brain region.

During longer periods of energy depletion, activation of K_{ATP} channels and depression of neuronal excitability preserves the impaired cellular metabolism and thus is neuroprotective (Heurteaux et al. 1993; Pérez-Pinzón and Born 1999). Conversely, inhibition of K_{ATP} channels diminishes the neuroprotective effects of an ischaemic preconditioning in hippocampal neurones (Plamondon et al. 1999). As expected from the heterogeneous distribution of K_{ATP} channels in the hippocampus, described recently (Zawar et al. 1999) and in this paper (Fig. 5), the neuroprotective effects from K_{ATP} channel activation depend on the channel density and, therefore, differ among various cell types. Thus CA1 pyramidal cells containing few K_{ATP} channels are more vulnerable to ischaemia than CA3 pyramidal cells, CA1 interneurones and granule cells of the dentate gyrus with more functional K_{ATP} channels (Siesjö 1988; Schmidt-Kastner and Freund 1991). In conclusion, the cell-type specific depression of excitability of hippocampal neurones by activation of K_{ATP} channels may have profound effects on the function of the hippocampus and the survival of cells, and K_{ATP} channel modulators are promising agents to diminish or to prevent the generation of seizures and the death of neurones during periods of energy depletion.

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