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POTASSIUM CURRENTS IN ISOLATED CA1 NEURONS OF THE RAT AFTER KINDLING EPILEPTOGENESIS

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Abstract—Daily tetanic stimulation of the Schaffer collaterals generates an epileptogenic focus in area CA1 of the rat hippocampus, ultimately leading to generalized tonic-clonic convulsions (kindling). Potassium currents were measured under voltage-clamp conditions in pyramidal neurons, acutely dissociated from the focus of fully kindled rats, one day and six weeks after the last generalized seizure. Their amplitude, kinetics, voltage dependence and calcium dependence were compared with controls.

With Ca^{2+} influx blocked by 0.5 mM Ni^{2+}, the sustained current (delayed rectifier) and the transient current (A-current) were not different after kindling. Calcium influx evoked an additional fast transient current component. This transient calcium-dependent current component was increased by 154%, but only immediately after the seizure. A second, slow calcium-dependent potassium current component was dependent on the intracellular calcium level, set by the pipette as well as on calcium influx. The peak amplitude of this slow calcium-dependent current was under optimal calcium conditions not different after kindling, but we found indications that either calcium homeostasis or the calcium sensitivity of the potassium channels was affected by the kindling process.

In contrast to the previously described enhancement of calcium current, kindling epileptogenesis did not change the total potassium current amplitude. The minor changes that were observed can be related either to changes in calcium current or to changes in intracellular calcium homeostasis.

In the kindling model of epileptogenesis, daily tetanization of an afferent pathway ultimately creates an epileptic focus in the stimulated area, with enhanced excitability and seizure susceptibility.[5] In area CA1 of the rat hippocampus, about 25 tetanic stimulations of the Schaffer collateral/commissural fibres are sufficient to evoke generalized tonic-clonic seizures.[14] Once the epileptic focus is established, this state of enhanced excitability persists for several months.

Neuronal excitability is determined by the specific set of voltage- and calcium-dependent ion conductances. Potassium currents play an important role in action potential repolarization[9,17,20,21] and frequency adaptation,[2,11] and they determine resting potential[21] and firing rate.[15] Even small changes in potassium conductance will affect neuronal excitability,[2,10,18] and suppression of potassium currents can evoke epileptiform activity, as is shown in several acute models of epilepsy.[16,23] The increased propensity of burst firing of CA1 pyramidal neurons, observed after kindling,[28,30] could result from a reduced calcium-dependent potassium current.[2,27]

In previous studies we have established that kindling epileptogenesis in the CA1 area is associated with an enhancement of high-voltage-activated (HVA) calcium current.[24,25] We now want to assess whether this increase is specific for the calcium current or just part of a general increase in ion conductances. To this end, we firstly investigated the strictly voltage-dependent potassium currents (A-current and delayed rectifier) after kindling epileptogenesis. An increase in calcium current, however, will indirectly affect potassium currents that depend on calcium influx or level. We therefore secondly analysed the calcium-dependent potassium currents (C-currents) after kindling.

CA1 pyramidal neurons were acutely isolated from the focal area of fully kindled rats and compared with cells from matched controls using the whole-cell voltage-clamp technique. In a first attempt to distinguish possible immediate effects of seizures from long-lasting changes, we measured the current one day as well as six weeks after the last of at least seven generalized seizures.

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Abbreviations: 4-AP, 4-aminopyridine; $[Ca^{2+}]_i$, intracellular free calcium concentration; CL, long-term control group; CS, short-term control group; EGTA, ethyleneglycol-bis(β-aminoethylether)-*N,N,N′,N′*-tetra-acetate; HEPES, *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulphonic acid; HVA, high-voltage-activated; I_{KA}, A-current-like potassium current; $I_{K(Ca,f)}$, fast calcium-dependent potassium current; $I_{K(Ca,s)}$, slow calcium-dependent potassium current; I_{KD}, delayed rectifying potassium current; KL, long-term kindled group; KS, short-term kindled group; LVA, low-voltage-activated; PIPES, piperazine-*N,N′*-bis(2-ethanesulphonic acid); TEA, tetraethylammonium.

EXPERIMENTAL PROCEDURES

Kindling procedure

Male Wistar rats (200–225 g, Harlan, The Netherlands) were implanted under pentobarbital anaesthesia (65 mg/kg) as described in detail previously.[7] Stainless steel recording electrodes (60 μm diameter) were placed in the stratum pyramidale and the stratum radiatum of the dorsal hippocampus. A stimulation electrode was positioned in the Schaffer collateral/commissural fibre bundle. Half of the rats received, twice a day, a tetanic stimulation (200–300 μA, 50 Hz, 1 s, biphasic bipolar pulses of 0.1 ms) eliciting afterdischarges, which were monitored by electroencephalographic recordings and increased in duration with successive stimuli. Six rats that had experienced a minimum of seven class V^{14} generalized tonic-clonic seizures were used one day after the last seizure and called the short-term kindled (KS) group. The seven rats in the long-term kindled (KL) group were killed six weeks later. Seven short-term control (CS) rats and nine long-term control (CL) rats were treated identically but did not receive tetanic stimuli.

Cell isolation

Implanted rats were anaesthetized with ether and decapitated. CA1 pyramidal neurons were isolated according to the method developed by Kay and Wong.[8] Briefly, the ipsilateral hippocampus was cut into slices 500 μm thick from which area CA1 was dissected into sections of 1 mm^2. These tissue pieces were incubated for 90 min at 32°C in oxygenated dissociation solution containing (in mM): NaCl 120, PIPES 10, KCl 5, $CaCl_2$ 1, $MgCl_2$ 1, D-glucose 25 and 1 mg/ml bovine trypsin (type XI); pH was set at 7.0. Thereafter, the tissue was washed with the same solution without the enzyme and kept at room temperature. Cells were isolated from CA1 sections by trituration through Pasteur pipettes of decreasing diameter. After settling on the bottom of the recording chamber, fusiform neurons with a bright and smooth appearance and no visible organelles[8] were selected for recording.

Recording of potassium currents

Whole-cell voltage-clamp recordings were carried out using patch pipettes of 1.5–3.0 MΩ resistance filled with one of two intracellular recording solutions differing in free calcium concentration ($[Ca^{2+}]_i$). The low-$[Ca^{2+}]_i$ solution contained in mM: KF 135, EGTA 10, HEPES 10, $MgCl_2$ 2, MgATP 2, $CaCl_2$ 1 and leupeptin 0.1; pH set at 7.3. Estimated $[Ca^{2+}]_i$ was <20 nM. In the high-$[Ca^{2+}]_i$ solution, we used 2 mM EGTA and 2 mM $CaCl_2$, estimating $[Ca^{2+}]_i > 200$ nM. The bath was perfused with a solution containing (in mM): NaCl 140, HEPES 10, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1 and D-glucose 25 (pH 7.4). Recordings were carried out at room temperature (20–22°C). Sodium currents were blocked by 1 μM tetrodotoxin. After the first set of measurements we perfused with a medium in which 0.5 mM Ca^{2+} was replaced by 0.5 mM Ni^{2+} to block the calcium influx through voltage-dependent calcium channels.[25] All chemicals were obtained from Sigma.

Currents were recorded with a computer-controlled List EPC-7 amplifier. Capacitive transients and series resistance were compensated (>85%) and readjusted after perfusing with $NiCl_2$. The membrane capacitance (C_m) as read from the amplifier dial was used as a measure of membrane surface. After seal formation (>0.5 GΩ) and membrane rupturing, the cells were allowed to stabilize for 5 min. Holding potential was kept at −60 mV. The currents were evoked with a minimum interval of 10 s. Because potassium current amplitude, under these experimental conditions, changes slightly over time, we used a strictly controlled time protocol. Data are given as mean ± S.E.M. Statistical comparisons were made with Student's t-test.

RESULTS

Potassium currents in the presence of Ni^{2+}

Potassium currents can be evoked by a depolarizing potential step to 30 mV, following a conditioning voltage step of 300 ms at −120 mV (Fig. 1A; voltage protocol given in the inset). Application of 0.5 mM Ni^{2+} blocks the calcium current[25] and consequently the component of the potassium current that depends on it.

In the presence of Ni^{2+}, depolarizing potential steps of 400 ms from holding potential evoked a slowly activating sustained potassium current (Fig. 1B). The voltage dependence of current activation was determined by measuring its peak current amplitude (A_p) at different levels of depolarization (Fig. 2A, open squares; 12 cells from CS, measured with high-$[Ca^{2+}]_i$ in the pipette). The threshold for activation was around −35 mV. Equimolar substitution of 5 mM extracellular Na^+ by 4-aminopyridine (4-AP) reduced A_p of the current evoked at 30 mV (1.4 ± 0.3 nA for 4-AP vs 1.9 ± 0.2 for control, $n = 11$, paired t-test, $P < 0.05$), while substitution with 10 mM tetraethylammonium (TEA) reversibly reduced A_p (1.4 ± 0.2 nA for TEA vs 2.0 ± 0.3 nA for control, $n = 9$, $P < 0.05$). This current resembles a delayed rectifier[12,13,17] and will be indicated as I_{KD}. When the depolarization was preceded by a short hyperpolarizing pulse (40 ms at −120 mV), an additional fast transient potassium current was evoked (Fig. 1C, voltage protocol C1). It was separated from I_{KD} by subtracting the current evoked by a protocol where hyper- and depolarization were separated by a 50 ms pulse at −50 mV, which allowed almost complete inactivation (voltage protocol C2).[12] The threshold for activation of the isolated transient potassium current was around −40 mV (Fig. 2A, open triangles). It inactivated exponentially with a time constant (τ_i) of ~30 ms at 30 mV. The voltage-dependent removal of inactivation was determined for this current by varying the potential of the conditioning step between −50 and −150 mV Fig. 2B, inset). Normalized amplitude as a function of conditioning potential (Fig. 2B, open triangles) was fitted by a Bolzmann equation: $I(V)/I_{max} = [1 + \exp\{(V_h - V)/V_c\}]^{-1}$, where V_h is the potential of half-maximal inactivation and V_c proportional to the slope at V_h. V_h was -84 ± 4 mV and V_c was -12 ± 3 mV ($n = 12$). This transient current was highly sensitive to 5 mM 4-AP (1.0 ± 0.3 nA for 4-AP vs 3.5 ± 0.5 nA for control, $n = 11$, $P < 0.001$) and was insensitive to 10 mM TEA (3.2 ± 0.5 nA for TEA vs 3.0 ± 0.4 nA for control, $n = 9$, n.s.). This current resembles an A-current[12,15,18] and will be indicated as I_{KA}.

When the depolarizations were preceded by a longer hyperpolarization (300 ms at −120 mV) and separated by a 50 ms pulse to −50 mV, to inactivate I_{KA}, we evoked a slow potassium current (Fig. 1D, voltage protocol D1). Subtraction of I_{KD} (voltage

protocol D2) from this current isolated a potassium current that activates around -50 mV and slowly inactivates ($\tau_i \approx 140$ ms at 30 mV). Inactivation was described by a Bolzmann function with a V_h of -99 ± 4 mV ($n = 12$) and V_c of -12 ± 3 mV (Fig. 2B, open circles). The amplitude of this current was dependent on $[Ca^{2+}]_i$ set by the pipette as CS_h differed from CS_l ($P < 0.01$; see Table 1). The current was highly sensitive to 10 mM TEA (1.7 ± 0.2 nA for TEA vs 6.0 ± 0.7 nA for control, $n = 11$, $P < 0.001$) and insensitive to 5 mM 4-AP (4.6 ± 0.6 nA for 4-AP vs 5.4 ± 0.7 nA for control, $n = 9$, n.s.). This current is a slow calcium-dependent potassium current[1,12] and will be indicated as $I_{K(Ca,s)}$.

Potassium currents after kindling

The three potassium currents in the presence of Ni^{2+}, I_{KD}, I_{KA} and $I_{K(Ca,s)}$, in cells obtained from short-term kindled rats, were compared with those from matched controls (Table 1) with either low (CS_l vs KS_l) or high-$[Ca^{2+}]_i$ set by the pipette solution (CS_h vs KS_h). Membrane capacitance C_m was given as a measure of cell surface. I_{KD} and I_{KA} were not different after kindling epileptogenesis. The A_p of $I_{K(Ca,s)}$ was decreased by 38%, but only when measured under high-$[Ca^{2+}]_i$ conditions. Voltage dependence of activation and inactivation of $I_{K(Ca,s)}$ (Fig. 2) was not affected.

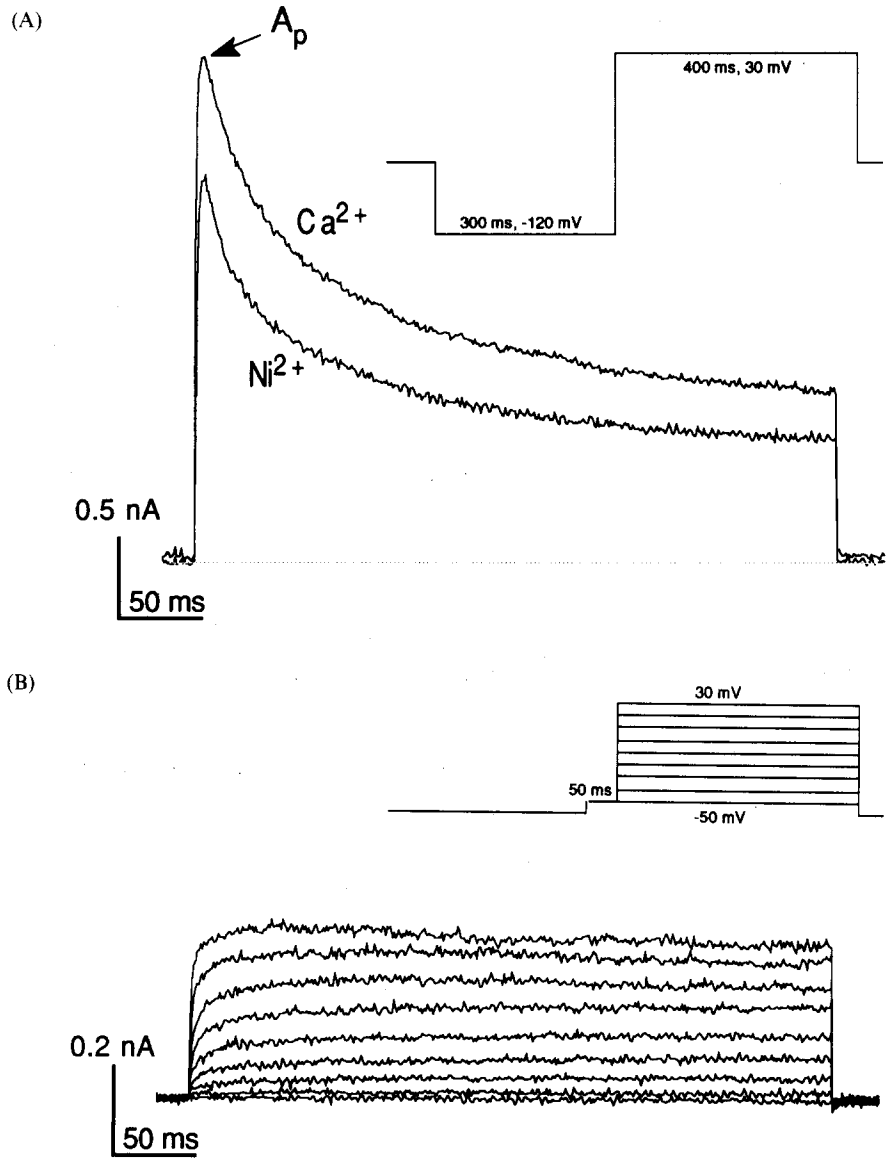

Fig. 1. (A) Potassium currents in a CA1 pyramidal neuron from a control animal, under high-$[Ca^{2+}]_i$ conditions, evoked by a potential step of 400 ms to 30 mV, following a conditioning potential step of 300 ms to -120 mV (voltage protocol is given as an inset). Current evoked either in the absence (Ca^{2+}) or in the presence (Ni^{2+}) of 0.5 mM Ni^{2+} in the bath. B–D give potassium currents in the presence of Ni^{2+}. (B) Sustained potassium currents (I_{KD}) elicited by depolarizations to varying potential levels (voltage protocol is given as an inset).

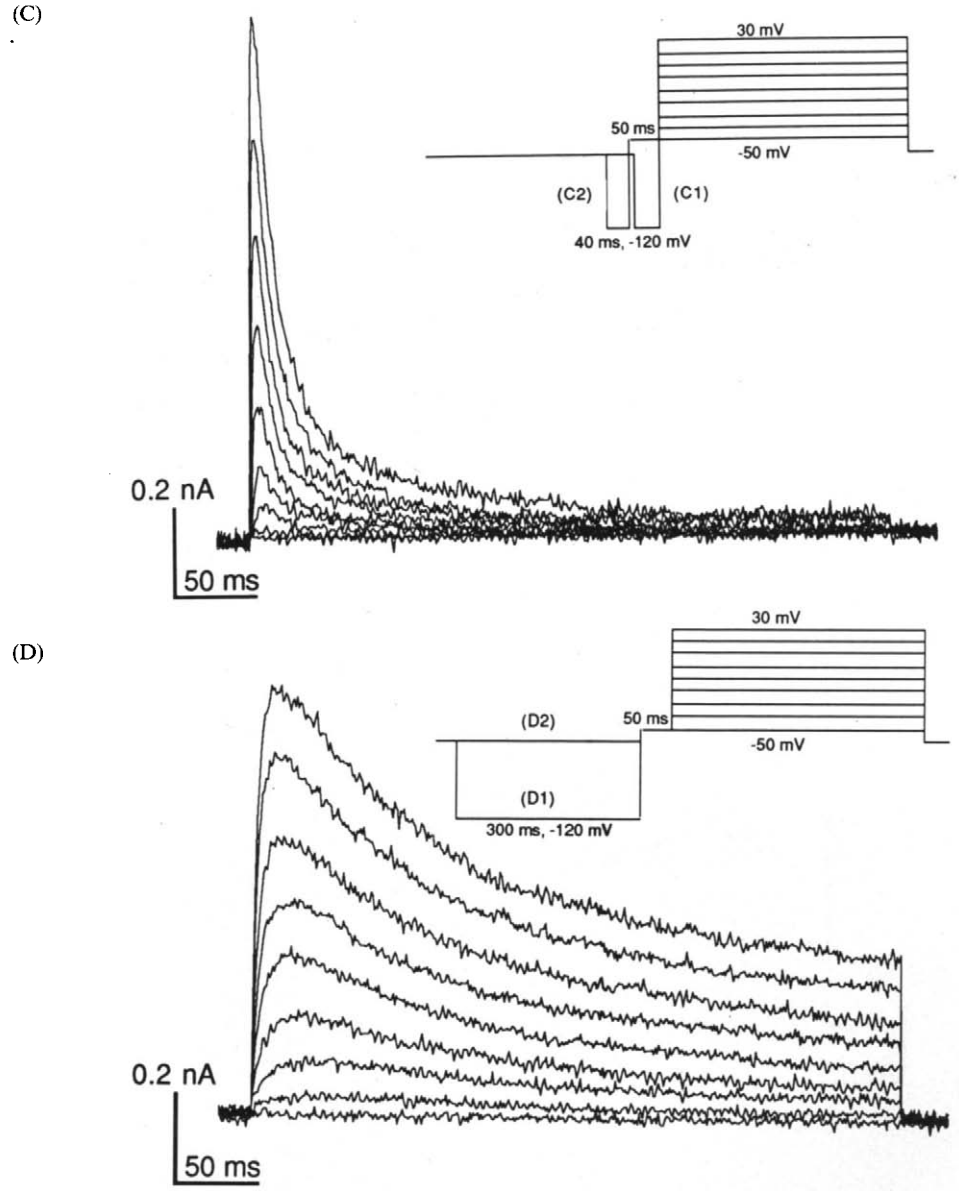

Fig. 1 (C) Transient potassium current (I_{KA}) is isolated by subtraction of the current evoked by voltage protocol C2 from the one evoked by protocol C1. (D) A slowly-inactivating potassium current that was sensitive to $[Ca^{2+}]_i$ ($I_{K(Ca,s)}$) is obtained by subtraction of the current evoked by voltage protocol D2 from the one evoked by protocol D1. Note the long-lasting prepulse necessary to remove inactivation and the interpulse of 50 ms to −50 mV to inactivate I_{KA}.

Calcium influx-dependent potassium currents

Application of Ni^{2+} partially blocked the potassium current (Fig. 1A); subtraction gave the potassium current component that was dependent on calcium influx through voltage-dependent calcium channels.

The calcium influx-dependent component of I_{KD} was negligible and not further quantified.

The additional component of $I_{K(Ca,s)}$ obtained by subtraction is given in Fig. 3A. Its kinetics and voltage dependence of inactivation are indistinguishable from those of $I_{K(Ca,s)}$ evoked in the presence of Ni^{2+}, suggesting that the additional calcium influx has enhanced the amplitude of a current already present (compare Figs 1D and 3A).

The additional calcium influx-dependent current component evoked with the protocol for I_{KA} is given in Fig. 3B. This very fast transient calcium-dependent potassium current will be called $I_{K(Ca,f)}$. Further analysis of its properties was carried out in 10 cells from KS_h that had an amplitude larger than 1 nA. In comparison with I_{KA}, the threshold for activation of $I_{K(Ca,f)}$ was 15 mV lower and activation was three times faster. Inactivation was almost five times faster, the voltage dependence was shifted slightly (V_h: -92 ± 1 mV for $I_{K(Ca,f)}$ vs -85 ± 2 mV for I_{KA},

Table 1. Peak amplitude and inactivation time constant of outward current evoked by a 400 ms potential step to 30 mV, measured with low-$[Ca^{2+}]_i$, set by the pipette (l) or with high-$[Ca^{2+}]_i$ (h), in n cells with membrane capacitance C_m

		Group			
		CS_l	KS_l	CS_h	KS_h
	n	23	14	22	18
	C_m (pF)	8.5 ± 0.5	8.6 ± 0.6	8.7 ± 0.6	8.5 ± 0.4
Potassium currents in the presence of Ni^{2+}					
I_{KD}	A_p (nA)	2.2 ± 0.3	2.2 ± 0.2	2.2 ± 0.2	2.3 ± 0.4
I_{KA}	A_p (nA)	2.2 ± 0.4	1.7 ± 0.3	2.2 ± 0.3	2.4 ± 0.1
	τ_i (ms)	29 ± 3	32 ± 4	30 ± 4	27 ± 4
$I_{K(Ca,s)}$	A_p (nA)	2.9 ± 0.3	2.8 ± 0.3	5.0 ± 0.6†	3.1 ± 0.4**
	τ_i (ms)	144 ± 25	132 ± 14	138 ± 11	140 ± 14
Additional currents in the absence of Ni^{2+}					
$I_{K(Ca,s)}$	A_p (nA)	1.2 ± 0.2	1.3 ± 0.2	0.9 ± 0.1	1.5 ± 0.3*
	τ_i (ms)	173 ± 20	137 ± 22	153 ± 27	180 ± 27
$I_{K(Ca,f)}$	A_p (nA)	1.6 ± 0.3	2.0 ± 0.3	1.1 ± 0.3	2.8 ± 0.3***

Upper half of the table gives the current recorded in the presence of Ni^{2+}, lower half the current blocked by Ni^{2+}. Numbers are mean and S.E.M. for n cells obtained from control (CS) and kindled (KS) rats, one day after kindling. Student's t-test probability is indicated as $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ for comparison between control and kindled, and as † for comparison between high- and low-$[Ca^{2+}]_i$, set by the pipette. A_p, peak amplitude; τ_i, inactivation time constant.

$n = 10$, $P < 0.001$, paired t-test) and was steeper (V_c: -10 ± 1 mV for $I_{K(Ca,f)}$ vs -14 ± 2 mV for I_{KA}, $P < 0.05$). We conclude that $I_{K(Ca,f)}$ is a distinct current, although these numbers have to be interpreted with caution, because the procedure used to isolate $I_{K(Ca,f)}$ cannot exclude the presence of a low-voltage-activated (LVA) calcium current.[22]

Calcium influx-dependent potassium currents after kindling

One day after kindling epileptogenesis, the calcium influx-dependent current component $I_{K(Ca,f)}$ was increased in neurons from kindled rats, in particular with high-$[Ca^{2+}]_i$ in the pipette (lower part of Table 1). Comparison of kinetics and voltage dependence of $I_{K(Ca,f)}$ between control and kindled was hampered by the small amplitude in controls.

The calcium influx-related component of $I_{K(Ca,s)}$ was also larger in the kindled group, but only when high-$[Ca^{2+}]_i$ solution was used in the pipette. No changes could be detected in voltage dependence of inactivation (V_h: -102 ± 5 mV for KS_h ($n = 14$) vs -100 ± 4 mV for CS_h ($n = 12$), n.s.).

Long-term changes

For the interpretation of the kindling-related changes, it is important to know whether they persist and could therefore be related to the kindled state. We recorded potassium currents in animals that were not stimulated for six weeks after their last evoked

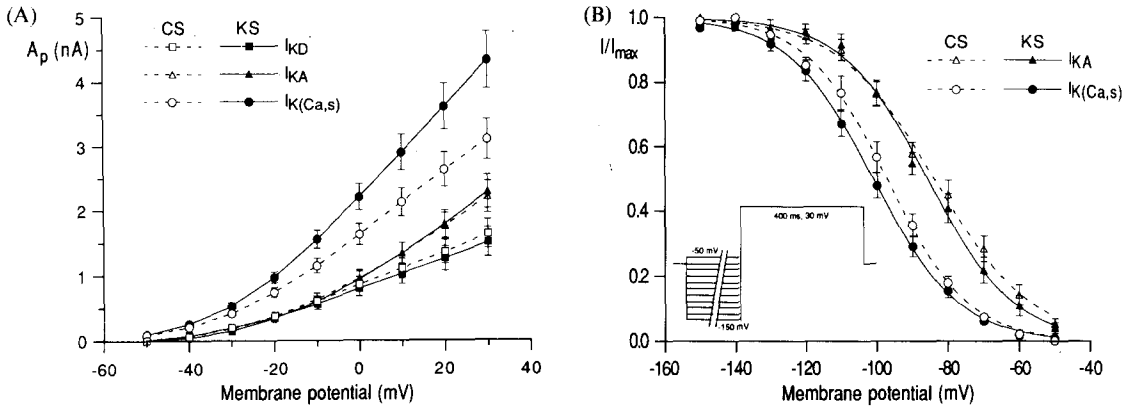

Fig. 2. Voltage-dependent activation and steady-state inactivation of the potassium current measured in the presence of Ni^{2+}, with high-$[Ca^{2+}]_i$ in the pipette. Data points are means of 12 cells from CS (open symbols, dotted lines) and 14 cells from KS (filled symbols, solid lines). Error bars indicate S.E.M. (A) Peak amplitude (A_p) of I_{KD}, I_{KA} and $I_{K(Ca,s)}$ as a function of membrane potential. (B) Normalized A_p of the inactivating potassium currents, I_{KA} and $I_{K(Ca,s)}$, evoked by a depolarizing potential step to 30 mV, as a function of membrane potential during the conditioning pulse (voltage protocol is given as an inset). Lines show the Bolzmann functions describing voltage-dependent removal of inactivation (see text for details).

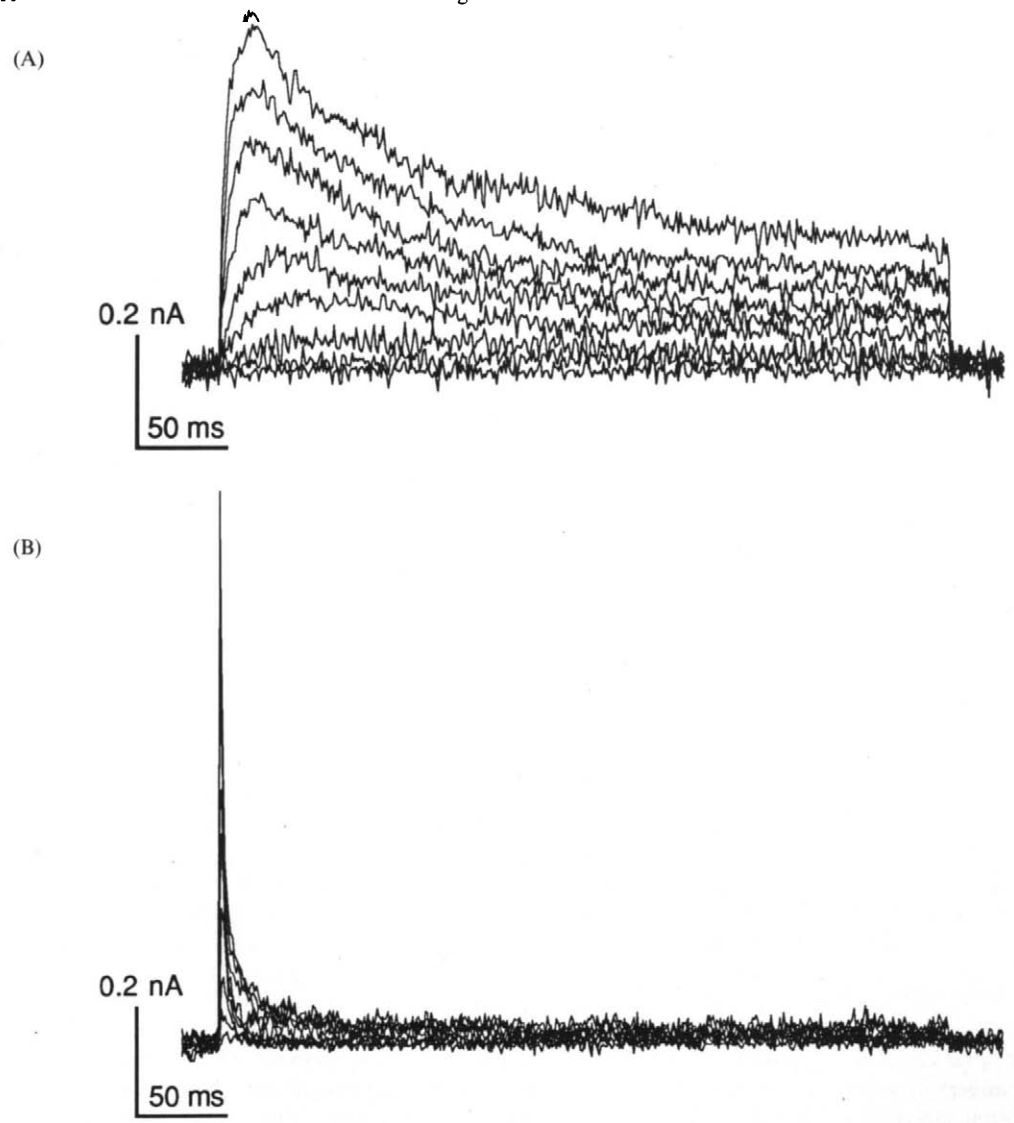

Fig. 3. Current component that is blocked by the application of 0.5 mM Ni^{2+} for the same cell as Fig. 1. Current isolation is described for Fig. 1 using protocols C and D. (A) The additional component of the slow-inactivating current ($I_{K(Ca,s)}$) blocked by the addition of Ni^{2+} (Fig. 1D) from the traces before. (B) Fast transient currents blocked by Ni^{2+} ($I_{K(Ca,f)}$) using the protocols that isolate I_{KA} (as in Fig. 1C).

class V seizure. Since the changes were only observed with high-$[Ca^{2+}]_i$ in the pipette, we restricted the measurements to the condition of high calcium and unobstructed calcium influx. The amplitude of the total potassium current (I_K; voltage protocol given in Fig. 1A) and the properties of the three current components I_{KD}, I_{KA} and $I_{K(Ca,s)}$ are summarized in Table 2. One day (KS_h) and six weeks after kindling epileptogenesis (KL_h), the properties of I_K, I_{KD} and $I_{K(Ca,s)}$ were the same as for controls. One day after kindling, the amplitude and kinetics of the current evoked with the I_{KA} protocol (the sum of I_{KA} and $I_{K(Ca,f)}$) were changed in accordance with an increased proportion of $I_{K(Ca,f)}$ described above. None of the differences could be found in the long-term group, suggesting that they were transient and probably directly related to the evoked seizures, although no further information about the time course is available.

DISCUSSION

Voltage-dependent and calcium-dependent potassium currents were compared between cells acutely isolated from the hippocampal CA1 area in control rats and from fully kindled rats, one day and six weeks after the last evoked seizure. The main question to answer was whether these currents are changed after kindling epileptogenesis and whether the previously described enhancement of calcium current[24] results in changes in calcium-dependent potassium currents.

Potassium current separation

The potassium currents were separated into four components on the basis of their specific voltage dependence of inactivation and their dependence on calcium influx.

The sustained component that could be evoked

Table 2. Peak amplitude, time to peak and inactivation time constant of outward current evoked by a 400 ms potential step to 30 mV, measured in the absence of Ni^{2+} with high-$[Ca^{2+}]_i$, set by the pipette, in n cells with membrane capacitance C_m

		Group			
		CS_h	KS_h	CL_h	KL_h
	n	24	24	26	27
	C_m (pF)	8.7 ± 0.5	8.5 ± 0.3	9.1 ± 0.4	9.3 ± 0.4
I_K total	A_p (nA)	8.3 ± 0.7	8.5 ± 0.7	9.2 ± 0.7	9.2 ± 0.6
I_{KD}	A_p (nA)	2.1 ± 0.2	2.2 ± 0.2	1.9 ± 0.2	2.0 ± 0.4
	t_p (ms)	42 ± 11	39 ± 6	53 ± 8	46 ± 7
I_{KA}	A_p (nA)	2.4 ± 0.3	3.9 ± 0.4**	2.8 ± 0.3	2.9 ± 0.3
and	t_p (ms)	3.5 ± 0.2	2.6 ± 0.3*	3.4 ± 0.3	3.5 ± 0.2
$I_{K(Ca,f)}$	τ_i (ms)	31 ± 3	21 ± 2*	30 ± 3	29 ± 4
	A_p (nA)	5.4 ± 0.6	4.6 ± 0.7	6.3 ± 0.6	6.1 ± 0.5
$I_{K(Ca,s)}$	t_p (ms)	16 ± 2	19 ± 2	17 ± 2	21 ± 3
	τ_i (ms)	150 ± 25	143 ± 14	178 ± 11	176 ± 14

The total potassium current (I_K) was separated into three components; the sustained (I_{KD}), the transient (I_{KA} and $I_{K(Ca,f)}$) and the slow-inactivating component ($I_{K(Ca,s)}$). Numbers and symbols as in Table 1. A_p, peak amplitude; τ_i, inactivation time constant; τ_p, time to peak.

from holding potential was identified as a delayed rectifier (I_{KD}), based on the activation threshold,[17] the very slow kinetics,[12,13,17] the moderate sensitivity to TEA[11] and 4-AP,[13] and the insensitivity to $[Ca^{2+}]_i$.[11,21] The delayed rectifier is the major sustained conductance at depolarized levels[17] and is important for spike repolarization.[17,21]

The transient component activated in the presence of Ni^{2+}, which required a brief hyperpolarization to remove inactivation, was identified as an A-current (I_{KA}), based on the voltage-dependent and kinetic properties,[3,12,18] the sensitivity to 4-AP[12,18,29] and the insensitivity to $[Ca^{2+}]_i$.[21,29] The A-current plays a role in spike repolarization,[3,18,20] modulation of firing rate[15,18] and suppression of burst firing.[10]

A fast transient calcium influx-dependent potassium current, which like I_{KA} required brief hyperpolarization, was called $I_{K(Ca,f)}$. Its sensitivity to Ni^{2+} and very fast activation and inactivation kinetics[9,21,29] suggest resemblance to the transient C-current, which is involved in the early action potential repolarization.[9,29] The low activation threshold of $I_{K(Ca,f)}$ suggests a functional coupling to the LVA calcium current.[22]

The slow potassium current, which requires a long hyperpolarization to remove inactivation ($I_{K(Ca,s)}$) was identified as a C-current, probably conducted by big potassium (BK) channels,[1,20,21] based on its voltage dependence,[12] its sensitivity to TEA,[1,2,20] to $[Ca^{2+}]_i$, set by the pipette solution[1,4] and to calcium influx.[6,12,21] However, some contamination with a slowly inactivating I_{KD} cannot be excluded.[11,12] The slow C-current plays a role in late spike repolarization,[20] fast afterhyperpolarization,[2,20] and in prevention, shaping and termination of somatic burst firing.[2,19,27]

Changes in potassium currents after kindling epileptogenesis

The total potassium current, I_K, evoked in the absence of Ni^{2+} and the calcium-independent potassium currents, I_{KA} and I_{KD}, were not different after kindling epileptogenesis, which confirms the conclusions derived from the unchanged profiles of activity-dependent increases in extracellular potassium concentration[26] and the unchanged action potential repolarization.[28,30] It also implies that the kindling-induced HVA calcium current enhancement described in previous work[24] was specific and not part of a general change of ion conductances. As a consequence of the calcium current enhancement, we expected an enhancement of calcium influx-related potassium currents after kindling. The fast transient calcium influx-related potassium current $I_{K(Ca,f)}$ was increased one day after kindling, particularly when high-$[Ca^{2+}]_i$ was used in the pipette. Since this change was not detected six weeks after kindling, it is most likely a consequence of the occurrence of afterdischarges and cannot underlie the enhanced excitability in the epileptic state. The difference in time course of the increase in $I_{K(Ca,f)}$ and the long-lasting increase of the HVA calcium current[24] made a direct relation unlikely and suggests the involvement of other factors related to calcium homeostasis. Alternatively, the transient increase in $I_{K(Ca,f)}$ may be related to a transient increase in LVA calcium current amplitude observed after kindling (Faas G. C. *et al.*, unpublished observations).

The proportion of the slow calcium-dependent potassium current $I_{K(Ca,s)}$ that was related to calcium influx was increased after kindling epileptogenesis. However, the voltage-dependent and kinetic properties and the amplitude of $I_{K(Ca,s)}$, measured under maximal Ca^{2+} conditions (Table 2), as well as under minimal Ca^{2+} conditions (low-$[Ca^{2+}]_i$, Ni^{2+} present), were not affected by kindling epileptogenesis. This makes a reduction of maximal number of available channels unlikely, but rather suggests a change in the modulation of the potassium channels, either due to a change in their sensitivity to calcium, and/or to the calcium level they detect.[6] Detailed experiments

investigating calcium homeostasis are necessary to clarify this point.

CONCLUSIONS

Long-lasting enhancement of cellular excitability, in concert with modulation of synaptic strength, could underlie the enhanced excitability of the local neuronal network,[7] characteristic of the epileptic state induced by kindling.

The intrinsic neuronal excitability is determined by the balance between depolarizing and hyperpolarizing ion currents. We have now demonstrated that the long-lasting enhancement of calcium current amplitude after kindling epileptogenesis[24] is rather specific and not compensated by changes in the potassium current. This may contribute to the enhanced intrinsic excitability and the increase in burst firing propensity of CA1 pyramidal neurons observed after kindling epileptogenesis.[28,30] Part of the kindling-induced changes in calcium-dependent potassium currents can be attributed to the enhanced calcium influx, but we also obtained indications that either calcium homeostasis or the calcium sensitivity of the potassium channels are affected by kindling. A more detailed analysis of the time course of the changes in calcium-dependent potassium currents after kindling is necessary to elucidate their precise relation to the changes in calcium currents.

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