Brain Research, 385 (1986) 411–414
Elsevier

BRE 21856

Kainate reduces two voltage-dependent potassium conductances in rat hippocampal neurons in vitro

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(Accepted 8 July 1986)

Key words: Kainate — Voltage-clamp — Hippocampal slice — Q-current — M-current — C-current

The mechanisms of action of kainate were studied in CA_1 hippocampal neurons using the single electrode voltage-clamp technique in vitro. Kainate (100–200 nM) reduced the potassium current which is responsible for the anomalous rectification (I_Q). In 30% of the cells the drug reduced the calcium-dependent potassium current (I_C) which is responsible for the afterhyperpolarization that follows calcium action potentials. The reduction of I_C will contribute to the enhancement of the neural excitability by this drug.

Kainic acid (KA), a conformationally rigid analogue of glutamic acid, has a particularly powerful excitatory action on several brain areas. Systemic or intracerebral injection of KA produces a paroxysmal activity which preferentially involves the hippocampus and limbic structures, followed by neuropathological lesions reminiscent of those observed in human with temporal lobe epilepsy[2,13].

Intracellular studies from CA_1 hippocampal cells in the in vitro slice preparation have shown that KA induces membrane depolarization and increases firing activity[15]. Several mechanisms have been suggested to explain these effects: either an enhancement of synaptic excitation[5] or a failure of GABA-mediated inhibition[6]. In the course of current-clamp experiments we have noticed that KA affects some intrinsic properties of hippocampal neurons[4], notably the calcium-dependent afterhyperpolarization (AHP) and the anomalous rectification. The present experiments were undertaken to study the effects of KA on the voltage-dependent potassium conductances responsible for the AHP and anomalous rectification using a single electrode voltage-clamp technique. An abstract of some of this work has been published[7].

The experiments were performed using rat hippocampal slice preparations. Male Wistar rats (150–200 g) were killed by a heavy blow to the chest. Hippocampi were removed and transverse slices (350 μm thick) were cut using a McIlwain tissue chopper. The slices were incubated in artificial cerebrospinal fluid (CSF) at 21–23 °C for at least 60 min and transferred to a submerged recording chamber. The slices were superfused (2 ml/min) with artificial CSF (in mM): NaCl 126, KCl 3.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2, glucose 11, $NaHCO_3$ 25, at 21–23 °C, gassed with 95% O_2-5% CO_2 (pH 7.3). Intracellular recordings were made with 3 M KCl-filled electrode (DC resistance 40–50 MΩ). The neurons were voltage-clamped using a single electrode voltage clamp amplifier (Axoclamp-2) at a sampling frequency of 3 kHz, 30% duty cycle. To ensure correct operation of the clamp, the unsampled voltage across the microelectrode tip was monitored on a separate oscilloscope. Sampled membrane currents and voltage were directly recorded on a Gould Brush pen recorder. No corrections for leakage or capacitative currents were made. Sodium spikes were routinely suppressed by tetrodotoxin (TTX, 1 μM). Drugs were bath applied via a 3-way tap system; the equilibrium was apparently reached within 3 min. Drugs used were: kainate (Sigma), carbachol (Sigma), TTX

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(Sigma) and tetraethylammonium chloride (TEA, Sigma).

A total of 14 neurons were voltage-clamped and in 5 of these applications of KA (200 nM) activated a steady inward current (130–270 pA) which underlies the KA-induced depolarization observed under current clamp conditions.

In control conditions the voltage-current relationship of CA_1 neurons shows a marked reduction in its slope for values of membrane potential 10–20 mV more negative than the resting membrane potential (anomalous rectification[14]). Kainate in low doses (100–200 nM) reduced this rectification making the voltage–current relationship more linear (n = 3) (Fig. 1A). In 3 other neurons the effect of KA was associated with a small (20%) reduction of input resistance and the effect of this toxin on the anomalous rectification was less clear. The anomalous rectification appears to be due to the activation of a K^+ current reminiscent of I_Q described by Halliwell and Adams (1982)[9] which is sensitive to extracellular cesium and insensitive to TTX and barium. This current is inwardly directed because it is activated by voltage steps values below the equilibrium potential for K^+.

In two neurons we studied the effects of KA on the Q-current. In control conditions hyperpolarizing voltage steps from a holding potential of −75 mV activated an inward current followed at the end of the voltage pulse by an inward relaxation. Kainate (100 nM) reduced this current by 30% (Fig. 1B) without any change of the leak current. This is shown in the plot of Fig. 1C where instantaneous values (see insert) corresponding to the leak current did not change while the steady-state values corresponding to I_Q were diminished with KA.

In current-clamp experiment we observed that KA reduced the duration and the amplitude of the AHP following calcium spikes (Fig. 2A). We studied in voltage-clamp conditions the effects of KA on the calcium-dependent potassium current responsible for AHP (n = 12). Depolarizing voltage command steps (20 mV) from a holding potential of −50 mV activated an initial inward current followed by an outward current. An outward tail current was recorded at the end of the pulse (Fig. 2B). The outward current is comprised of two currents: I_C and I_M. The former is a calcium-dependent K^+ current, activated at membrane potentials positive to −45 mV and abolish-

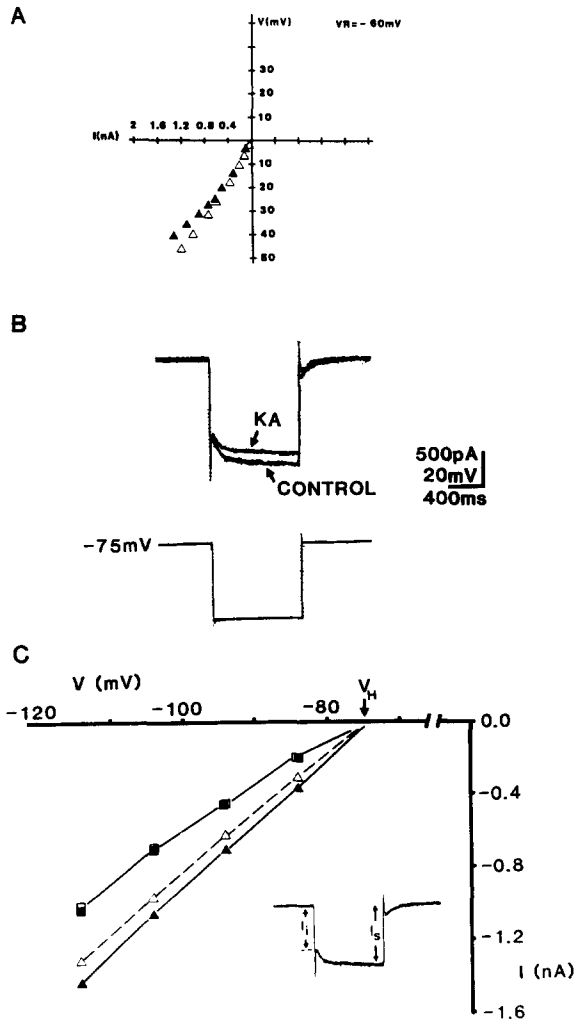

Fig. 1. Kainate reduces the anomalous rectification and the Q-current. A: V/I curve for a CA_1 neuron before (▲) and during superfusion of Kainate (△). Resting potential in control and in kainate solution was −60 mV (corresponding to 0 mV on the ordinate). Note that the curve is more linear after kainate. B: lower trace: voltage recordings; upper trace: current recordings. The inward current (I_Q) activated by a hyperpolarizing command step of −40 mV from a holding potential of −75 mV was reduced by superfusion of KA (100 nM). C: I/V relation is plotted for the neuron shown in B. Instantaneous currents (I_i) in control conditions (■) and during kainate (□) and steady-state currents (I_s) before (▲) and during kainate (△). Note that the leak current corresponding to I_i is not changed by kainate.

ed by Ca-channel blockers[3]. The latter is a potassium current insensitive to Ca^{2+} and abolished by muscarinic agonists[9]. In agreement with these earlier studies, superfusion of La^{3+} (1 mM) or Mn^{2+} (5 mM) depressed the outward current (by 40%), carbachol

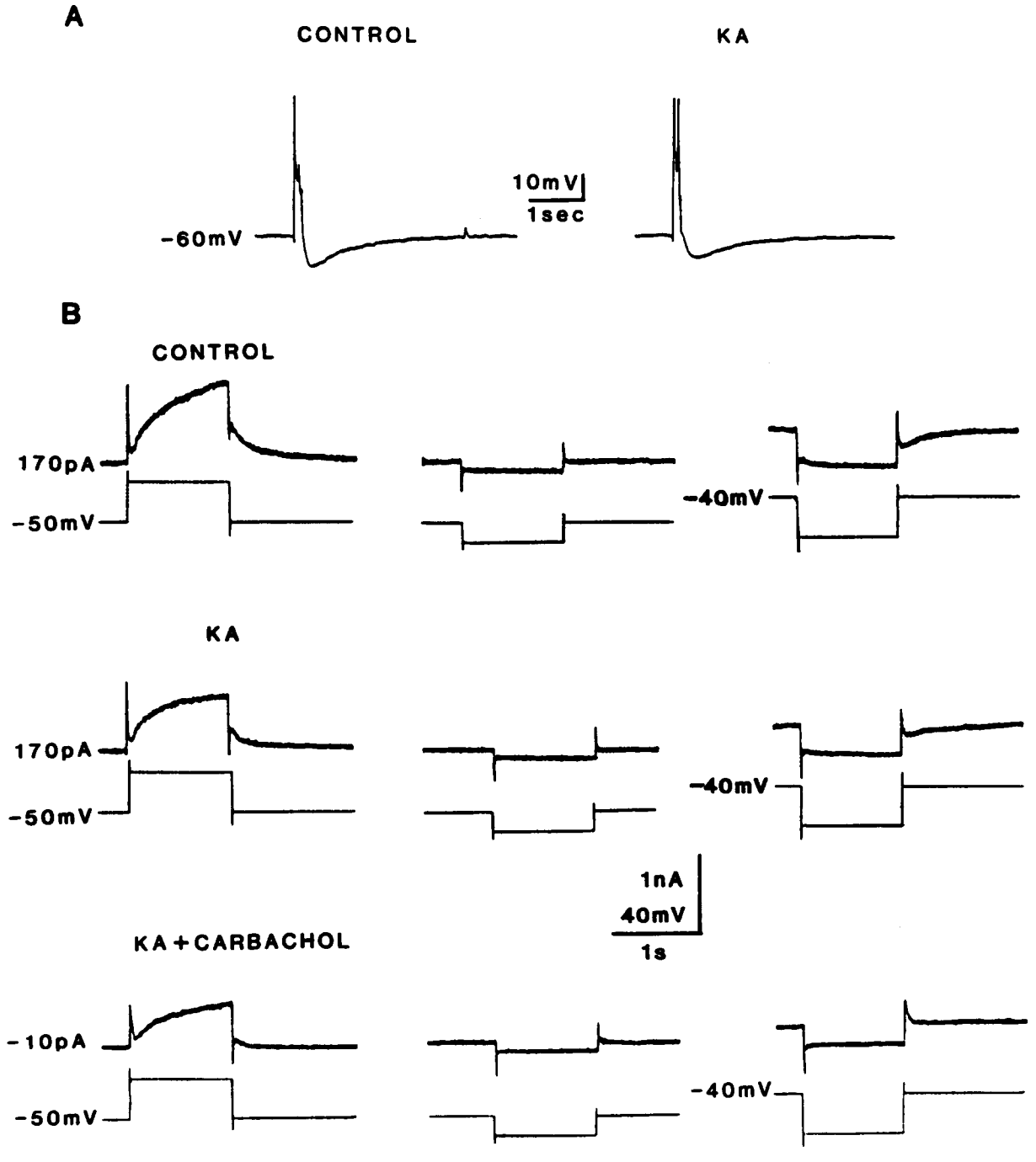

Fig. 2. Kainate reduces the AHP following calcium action potentials and the C-current. A: calcium action potentials evoked in the presence of TTX (1 μM) and TEA (3 mM), before (left) and during superfusion of kainate (right) 100 nM. Note that the AHP is reduced in spite of the fact that the number of spikes is enhanced. B: voltage-clamp experiment in another neuron. In each couple of traces the lower one corresponds to the voltage recordings and the upper one to the current recordings. Note that kainate (200 nM) reduced the outward current activated by a depolarizing voltage step of 20 mV without changing the leak current (middle column). The outward current (I_M) activated at the end of a hyperpolarizing command step of 20 mV (right column) was only slightly reduced. Carbachol (50 μM) further reduced the outward current and completely blocked I_M. Note that during superfusion of carbachol a steady inward current of 180 pA developed.

also depressed it by a same percentage (not shown).

In 4 neurons, KA (200 nM) reduced the outward current by 40 ± 17% (mean ± S.E.M.) without any change of the leak current Fig. 2B; the maximum effect was achieved within 3–5 min and full recovery was never obtained. To examine whether KA reduces I_C, I_M or both, we have first studied the effects of KA on the isolated I_M. In this case, a hyperpolarizing command step of 20 mV from a holding potential of −40 mV induced a current relaxation followed at the end of the pulse by an outward current corresponding, respectively, to the inactivation and activation of the M-current[9]. KA reduced the outward current with little change in the M-current; application of carbachol (50 μM) after kainate completely blocked I_M (Fig. 2B). It seems, therefore, that the effects of KA are not due to a reduction of I_M.

In this study two main effects of KA on the intrinsic membrane properties of hippocampal CA_1 neurons have been observed: a reduction of I_Q responsible for the anomalous rectification and I_C responsible for the AHP. A similar effect on a potassium current reminiscent of I_Q is produced by glutamate on retinal horizontal cells[11] acting on kainate type receptors[10]. Since in the hippocampus this current is activated at values of membrane potential below the equilibrium potential for K^+, it is unclear whether I_Q has a functional significance in normal operating conditions[14] and it seems unlikely that the reduction of I_Q by KA contributes significantly to the greatly enhanced cell excitability produced by this toxin.

The finding that KA reduced I_C (a current activated at more depolarizing potentials) in a third of the neurons tested confirms and extends previous observations in current-clamp studies where kainate reduced the AHP following calcium spikes in a similar proportion of neurons[4]. This action of KA, in keeping with that of several neuroactive substances also known to inhibit the calcium-dependent afterhyperpolarization[1,8,12], will contribute to an enhancement of cell excitability.

This work was supported in part by a twinning grant from the European Science Foundation. M.G. is a fellow of the Fondation Fyssen.

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