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PHYSIOLOGY

Comparative Analysis of Changes in Membrane Currents in Neurons and Astrocytes in Rat Hippocampal Slices after Stimulation of Glutamatergic Transmission

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One of important functions of astrocytes in the central nervous system (CNS) is the uptake of neurotransmitters released by neurons into the synaptic cleft. This uptake is mediated by specific transporters [1, 2]. In glutamatergic synapses, astrocytes return the transmitter back to neurons in the glutamate–glutamine cycle [3], and glutamate clearance determines the rate of synaptic response [4]. The amplitude of transport current in astrocytes during synaptic transmission allows the estimation of the amount of the transmitter taken up by astrocytes [2, 4], and this current substantially grows after stimulation of neurons by paired impulses of current. In response to synaptic stimulation, in astrocytes, inward current with slow dynamics (several seconds) can be recorded [5, 6]. It has been supposed [5] that slow synaptic current is induced in astrocytes by potassium release from presynaptic neurons.

The purpose of the present study was to examine the nature of slow astrocytic response to synaptic stimulation of neighboring neurons. For this purpose, in rat hippocampal slices, we simultaneously recorded membrane currents in a postsynaptic neuron and astrocyte connected to it in the CA1 area after stimulation of Schaffer collaterals.

MATERIALS AND METHODS

The experiments were performed in 350-μm hippocampal slices from 16- to 24-day-old rats. The slices were incubated at 36°C for 30 min in a saturated carbogen solution consisting of (in mM): 127 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 25 D-glucose; pH 7.4. For whole cell recording, we used glass electrodes with $R = 6–7$ MΩ filled with the solution consisting of (in mM): 127 K gluconate, 4 KCl, 2 Mg-ATP, 0.3 Tris–GTP, 0.2 EGTA, 10 HEPES, and 10 phosphocreatine; pH 7.3, 295 mΩ cm. Neuronal membrane currents or potentials and astrocyte currents were recorded using a two-channel MultiClamp 700A patch-clamp amplifier (Axon Instruments, United States). Schaffer collaterals, processes of CA3 pyramidal neurons forming glutamatergic synapses on the dendrites of CA1 pyramidal neurons, were stimulated using a coaxial steel electrode with paired stimuli 0.1 ms in duration applied at intervals of 20 ms, with 20 s between the tests. Astrocytes for recording were selected in the stratum radiatum using interference–contrast optics in such a way that the bodies of astrocytes were located as close as possible to a visible process of the pyramidal neuron the body of which was selected for simultaneous recording of synaptic currents in response to stimulation (Fig. 1a). Current recording was performed in so-called "mature" astrocytes with a low input impedance (5.2 ± 4.0 MΩ) and linear current–voltage membrane characteristic, which, in rats older than 15 days, account for up to 91% of the total number of astroglial cells [7]. Group data were compared using Student's t-test at $p < 0.05$. The data are presented as m S.E.M.

RESULTS

Input currents were recorded in astrocytes ($n = 43$) in response to stimulation of Schaffer collaterals and represented a sum of components. Paired stimulation resulted in higher amplitudes of the currents recorded.

A fast current component with an amplitude of 4–40 pA appeared in response to each stimulus with a latency of 1 ms and continued for 5–7 ms (Fig. 1b). Data in Fig. 1b demonstrate that this current component disappeared immediately prior to the start of action potential in the neuron. The value of the fast current component was constant for each astrocyte, and its amplitude did not change during 1-h recording.

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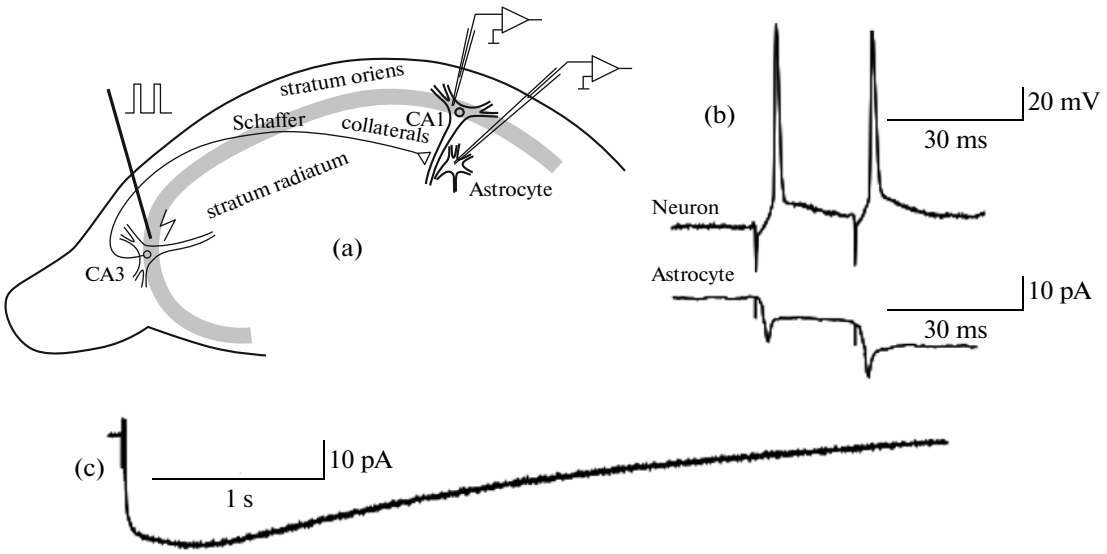

Fig. 1. Paired recording of responses of neuron and astrocyte induced by stimulation of Schaffer collaterals. (a) Location of electrodes; (b) action potential in neuron and "fast" transporter current in astrocytes at the level of membrane potential of –90 mV; (c) "slow" current component in astrocyte.

Addition of 400 μM dihydrokainate, which is not carried by the astrocytic glutamate transporter GLT-1 or EAAT2, inhibited the fast response in astrocytes by $53 \pm 12\%$ ($n = 6$; $p < 0.05$). The blocker of ionotropic glutamate receptor 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) at a concentration of 10 μM did not influence the fast component of the astrocytic response. Thus, the fast current component reflected only the functioning of astrocytic electrogenic glutamate transporters, as it was supposed previously [5, 8]. Blockage of GLT-1 prolonged the duration of the glutamate presence in the synaptic cleft and may increase the amplitude of synaptic currents in interneurons by a factor of more than 15 [4]. This indicates the crucial role of GLT-1 in normal synaptic transmission. Dihydrokainate at the concentration used neither completely inhibited GLT-1 nor influenced the neuronal glunamate transporter EAAT3, which indicates substantially lesser involvement of the neuron in glutamate clearance from the synaptic cleft as compared to astrocyte.

In addition to the fast response, we observed in astrocytes of the stratum radiatum a slowly developing inward current, which also appeared in response to synaptic stimulation of pyramidal neurons of the hippocampus (Fig. 1c). The "slow" current reached the maximum amplitude of 10–50 pA during 0.4–0.5 s after stimulation and gradually decreased during 4–8 s.

Application of 10 μM NBQX decreased the amplitude of the slow response by $61 \pm 21\%$ ($n = 6$; $p < 0.05$), probably, due to blockage of potassium outflow from neurons through AMPA-channels. Inhibition of the GLT-1 functioning with 400 μM dihydrokainate also decreased the amplitude of the slow inward current in astrocytes by $43 \pm 13\%$ ($n = 5$; $p < 0.05$) because the GLT-1 functioning is associated with K^+ outflow from astrocytes. Thus, blockage of both AMPA-channels and GLT-1 decreased the K^+ outflow to the extracellular medium and attenuated the amplitude of the slow current component recorded in astrocytes.

Simultaneous voltage clamp in neurons and astrocytes at the levels of –70 and –90 mV, respectively, practically prevented the potassium outflow from both types of cells via ionic channels. Since astrocytes form a syncytium, being connected with one another through gap junctions permeable for K^+, we can record exchange potassium current between neighboring astrocytes; however, diffusion of potassium ions through the syncytium is much more slow as than the K^+ inflow or outflow via channels of the plasma membrane. In Fig. 2a, a substantial latency of the start of the "slow" current component is shown, which began to develop only after the end of the second stimulus, whereas the peaks representing the fast current through GLT-1 appeared immediately after each stimulus. In the given case, K^+ did not outflew from the neuron closest to the astrocyte studied; therefore, K^+ slowly entered the astrocyte from the syncytium via gap junction, rather than from the synaptic cleft.

Voltage clamp at the level of +40 mV upon the opening of ionotropic glutamate receptors resulted in active K^+ outflow from the neuron; this current is presented in Fig. 2b. The current in the astrocyte was inverted and appeared without any delay, immediately after the first stimulus, and enhanced in response to the second stimulus. In this case, depolarization of the neuron resulted in an active release of K^+ into the synaptic cleft and its fast millisecond clearance by the astrocyte [8]. Excess of K^+ was released through gap junctions into the syncytium and resulted in inversion of the slow current component (Fig. 2b).

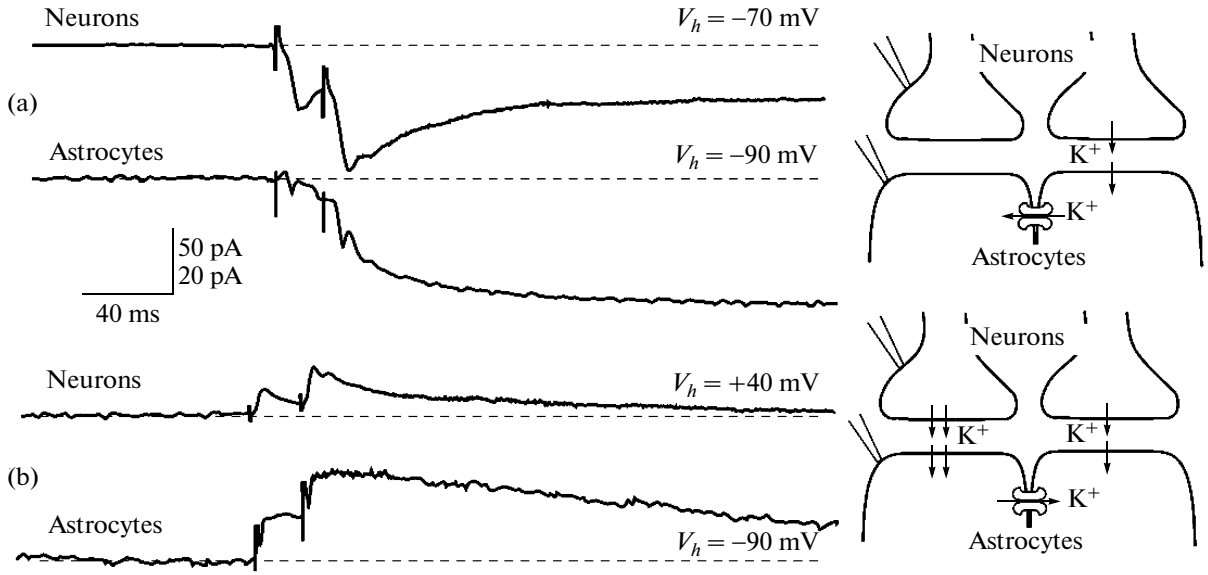

Fig. 2. Simultaneous recording of current responses to paired stimulation of Schaffer collaterals in neuron and astrocyte. (a) Upper and lower records represent synaptic currents in pyramidal cell and astrocyte response consisting of the fast and slow components, respectively; (b) upper and lower records represent synaptic currents in pyramidal cell and astrocyte response consisting of the fast and slow components, respectively when neuron is clamped at +40 mV. Inversion of the "slow" current component is observed in astrocyte when the direction of "fast" transporter current remains unchanged. Right panels, arrows indicate directions of potassium currents between neurons and astrocytes in each experimental condition.

We suppose that, in both cases, long-term decrease in the slow current component depended on the K^+ diffusion rate between astrocytes through gap junctions.

Noteworthy, we did not observe any inversion of the fast current component related to GLT-1 function during depolarization of neuron to the level of +40 mV, because this transporter functions due to the Na^+ but no K^+ gradient. According to the literature data [5], blockage of the action potential in neurons with tetrodotoxin inhibited the slow current component, which may be explained by the absence of depolarization-induced K^+ outflow from neurons.

The efficiency of K^+ clearance by astrocytes strongly depends on the membrane potential maintained by Na/K-ATPase. Specifically, blockage of Na/K-ATPase with 100 μM ouabain for 2 min resulted in a substantial decrease in the amplitude of slow inward current by $67.2 \pm 23\%$ ($n = 5$; $p < 0.05$) in an astrocyte in response to stimulation. However, recording from astrocyte without simultaneous patching of the neuron under the conditions of voltage clamp from −180 to +100 mV did not lead to reversion of the slow current component. This may be related to the inability to clamp the membrane potential in the astrocyte in the area of processes due to a high leakage current via gap junctions.

Thus, we have demonstrated that the low current component that appears in astrocytes in response to stimulation of Schaffer collaterals depends on the rate of potassium redistribution between astrocytes via gap junctions. The local shift in potassium concentration in astrocytes prolonged to several seconds may underlie the mechanism of neuronal facilitation after stimulation with a series of impulses, i.e., after frequent generation of action potentials that occurs in epileptic foci. The parameters of the slow current component in astrocytes studied here show the ability of the astrocytic network to utilize and transport potassium, which may be a factor regulating neuronal facilitation.

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