PHYSIOLOGY

Glutamate-Induced Disturbances in Neurotransmission and Ionic Homeostasis of Extracellular Ca^{2+} and K^+ in CA1 Hippocampal Area

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The effect of various concentrations of L-glutamate on neurotransmission in the CA1 hippocampal area was studied using hippocampal slices. Three intervals of L-glutamate concentration were established: ≤1 mM (all studied parameters are completely reversible upon washout, transmission being preserved), from 1 to 10 mM (both responses to frequency stimulation and single population spikes remain partially suppressed after washout), and above 10 mM (more than 50% suppression of transmission persists after washout).

Key Words: *hippocampus; calcium; potassium; CA1; ion-selective electrodes*

With the advent of specific antagonists of excitatory amino acids, the hypothesis on the neurotoxic role of glutamate (Glu) in brain ischemia was successfully confirmed [4]. This hypothesis asserts that rapid accumulation of endogenous excitatory amino acids in the synaptic gap induces hyperactivation of postsynaptic receptors. This hyperactivation triggers massive inward current of Ca^{2+} and Na^+ and outward K^+ current. Calcium binds to cell organelles and induces enzyme degradation and, finally, neuron degeneration [5].

The aim of the present study was to elucidate parameters of ionic homeostasis, a component of the exotoxic hypothesis, in particular extracellular Ca^{2+}, K^+, and evoked electric activity in the CA1 hippocampal area exposed to increasing concentrations of Glu.

MATERIALS AND METHODS

Hippocampal slices from Wistar rats (150-200 g, n=30) were used. The rats were decapitated under ether anesthesia, the hippocampus was isolated, and 350-400 μ thick slices were prepared and placed into a nonimmersion chamber [3]. They were perfused at 35±0.5°C and a rate of 2 ml/min with ACSF solution (pH 7.4) containing (in mM): 126 NaCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 3 KCl. After a 1-h preliminary perfusion with ACSF solution, the slices were incubated for 1 h with Glu in concentrations of 1, 5, 10, 20, and 50 mM followed by a 2-h washout.

Ortho- and antidromic electric stimulation was performed with bipolar platinum electrodes placed in the Schaffer collateral and alveus areas in a single-pulse mode: 0.1 msec duration, 3-8 V current, and 20 Hz frequency.

Potassium level was recorded by using dual-channel K^+-selective glass microelectrodes filled with Corning 477317 ionophore (Fluka) as described previously [1].

Calcium level was recorded using dual-channel Ca^{2+}-selective glass microelectrodes filled with Calcium Cocktail 21048 (Fluka). One channel of the microelectrode was siliconized and its tip was filled

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with the respective ionophore to 300-400 μ, while the other space was filled with 100 mM $CaCl_2$. The second channel filled with 150 mM NaCl served as the indifferent electrode and was also used for recording the population response. Potential on the Ca^{2+}-selective electrode was measured by differentiation amplifier with high-resistance input channels (10^{12} Ω). The electrodes were calibrated using standard solutions: 1.5, 1.2, 1.0, 0.8, and 0.5 mM $CaCl_2$ and 150 mM NaCl.

RESULTS

The initial basal level of Ca^{2+} and K^+ in the extracellular space of CA1 hippocampal area at a depth of 8-100 μ from the brain surface was 1.2±0.05 and 3±0.1 mM, respectively (n=5).

Rhythmic electrical orthodromic stimulation with a frequency of 20 Hz shifted $[K^+]_0$ to 3.2±0.7 mM, while antidromic stimulation elevated it to 9±0.5 mM; $[Ca^{2+}]_0$ decreased to 0.2±0.002 mM in response to orthodromic stimulation and to 0.2±0.001 mM in response to antidromic stimulation (n=25).

When the incubation solutions were replaced with those containing 1, 5, 10, 20, and 50 mM L-Glu, $[Ca^{2+}]_0$ in the CA1 area decreased in a dose-dependent manner attaining the minimum during the first 10-min exposure. As seen from Fig. 1, the maximum drop in extracellular calcium concentration was achieved with an L-Glu concentration of 50 mM, while the minimum decrease was noted at a concentration of 1 mM. During the subsequent 50-min incubation, the extracellular calcium concentration progressively increased but did not attain the initial level. Washout restored the initial level of $[Ca^{2+}]_0$ during 15-20-min period, regardless the applied concentration of L-Glu.

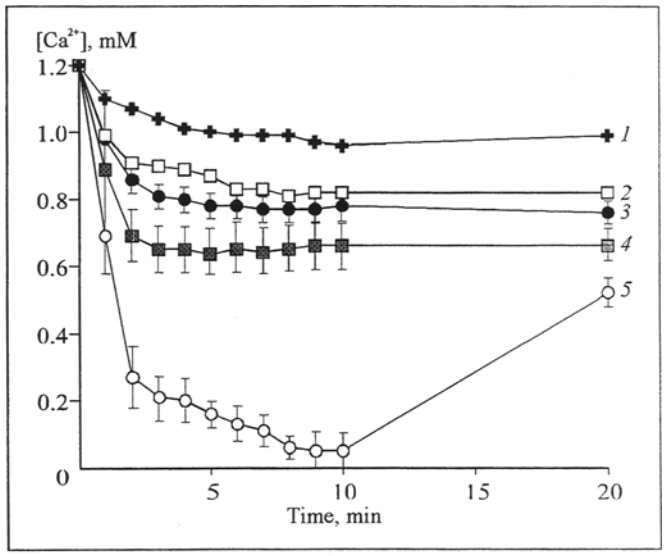

Fig. 1. Integral plot of basal extracellular calcium concentration during 20-min period after application of L-Glu in concentrations of 1 (*1*), 5 (*2*), 10 (*3*), 20 (*4*) and 50 mM (*5*).

The dynamics of the basal $[K^+]_0$ level was somewhat different: $[K^+]_0$ attained the maximum on 2-3 min after addition of L-Glu. On the 10th min, $[K^+]_0$ returned to the initial value and during the subsequent 50-min exposure decreased by 1.5±0.5 mM below the initial level. Washout restored the initial level of $[K^+]_0$ during 10-15 min.

Application of 1 mM L-Glu suppressed the anti- and orthodromic responses to 20-Hz frequency stimulation by only 5-10% of the initial level, the responses being completely restored during a 5-7-min washout. Similar changes were observed for single population responses (Table 1, Figs. 2 and 3).

Application of 5 and 10 mM L-Glu had a more pronounced suppressive effect on $[K^+]_0$ and $[Ca^{2+}]_0$ responses to 20-Hz stimulation, and their initial level

TABLE 1. Concentration of K^+ and Ca^{2+} in Response to Electrical Stimulation (20 Hz) in the Presence of L-Glu

Concentration of L-Glu, mM	Ion	Suppression during exposure, %		Restoration upon washout, %	
		antidromic response	orthodromic response	antidromic response	orthodromic response
1	Ca^{2+}	20.0±2.0	10.0±3.0	100	100
	K^+	30.0±3.0	5.0±2.0	100	100
5	Ca^{2+}	87.5±5.1	78.3±3.7	51.1±4.1	87.7±6.3
	K^+	97.1±3.2	76.2±2.8	75.4±5.2	86.1±12.1
10	Ca^2	85.3±3.2	85.0±4.3	46.0±7.5	76.1±6.4
	K^+	66.4±4.4	86.0±7.1	73.0±3.6	85.3±7.5
20	Ca^{2+}	95.0±2.0	96.0±0	36.1±2.5	43.0±9.1
	K^+	90.0±3.0	87.0±2.0	48.2±3.5	48.0±7.3
50	Ca^{2+}	100	100	5.1±1.1	4.3±2.3
	K^+	100	100	9.3±4.2	7.6±3.6

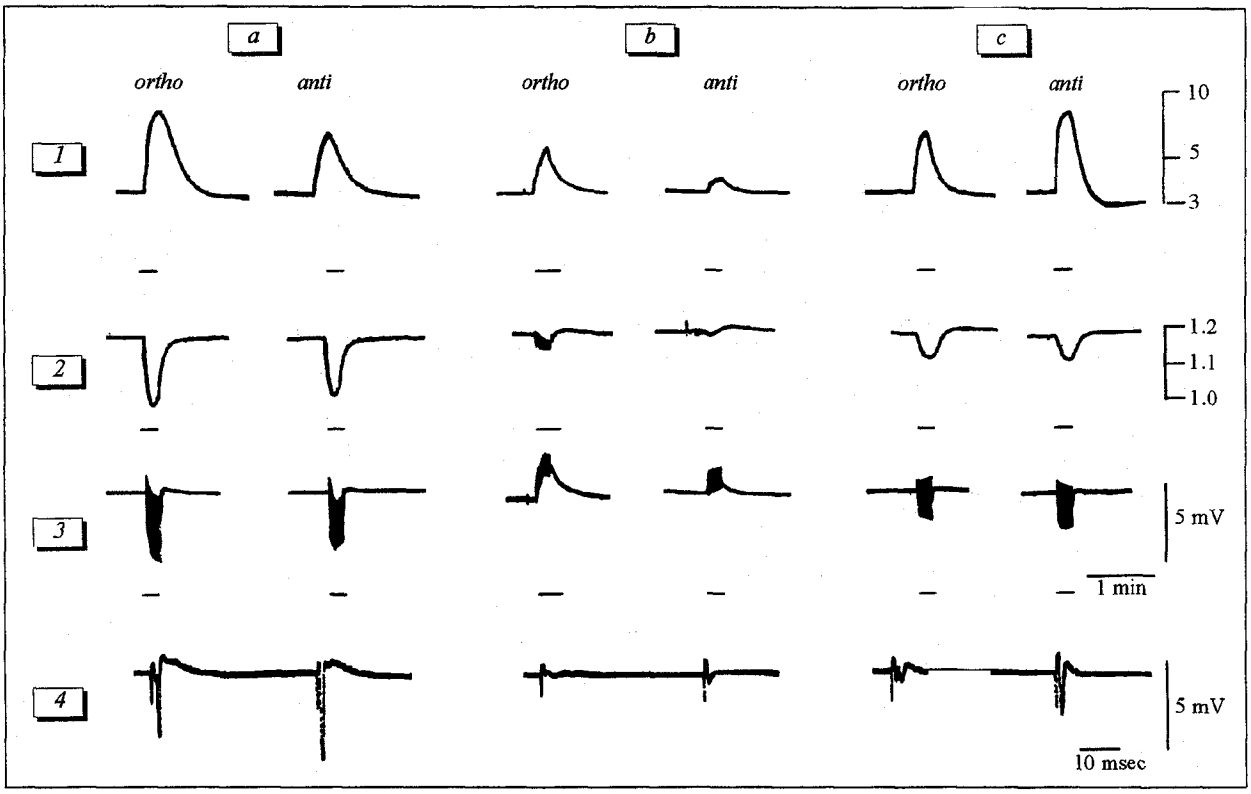

Fig. 2. Experiment with simultaneous registration of $[K^+]_0$ (mM, *1*), $[Ca^{2+}]_0$ (mM, 2), integral profile of a response to 20 Hz stimulation (*3*), and paired population responses in CA1 hippocampal area (*4*). *a*) control: electrical stimulation of Schaffer collaterals (ortho) and alveus (anti) with a frequency of 20 Hz before exposure to L-Glu (10 mM); *b*) 60th min of perfusion; *c*) after 120 min washout.

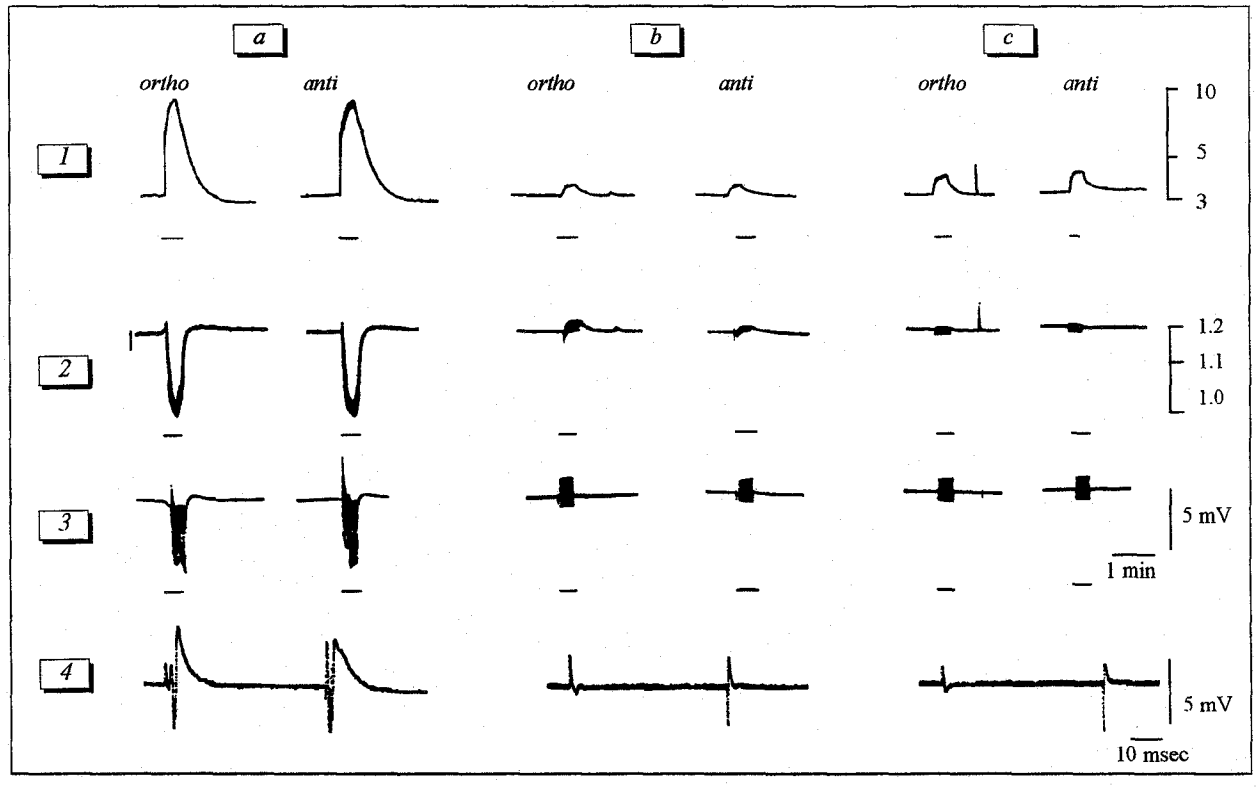

Fig. 3. Experiment with simultaneous registration of $[K^+]_0$ (mM, *1*), $[Ca^{2+}]_0$ (mM, 2), integral profile of response to 20 Hz stimulation (3), and paired population responses in CA1 hippocampal area (*4*). *a*) control: electrical stimulation of Schaffer collaterals (ortho) and alveus (anti) with a frequency of 20 Hz before exposure to L-Glu (50 mM); *b*) 60th min of perfusion; *c*) after 120 min washout.

was only partially restored upon washout. By the end of application of 5 and 10 mM L-Glu, the single population responses were suppressed by 100% and were only partially restored during washout (Fig. 2).

In concentrations of 20 and 50 mM, L-Glu completely inhibited $[K^+]_0$ and $[Ca^{2+}]_0$ responses to 20-Hz stimulation, which were restored by no more than 50 and 10% for these two concentrations, respectively (Fig. 3).

L-Glutamate in concentrations of 20 and 50 mM completely inhibited the single population responses during both 60-min exposure and 120-min washout.

Thus, the dynamics of both the basal $[K^+]_0$ and $[Ca^{2+}]_0$ levels and $[K^+]_0$ and $[Ca^{2+}]_0$ responses to electrical stimulation depends on the applied dose of L-Glu.

As seen from the integral plot of Glu-induced changes in $[Ca^{2+}]_0$, the basal calcium concentration decreases with increasing concentration of L-Glu; however, basal $[Ca^{2+}]_0$ curves are unequally distributed within a L-Glu concentration range of 5-10 mM.

This distribution of basal $[Ca^{2+}]_0$ curves and the peculiarities of suppression and restoration of $[K^+]_0$ and $[Ca^{2+}]_0$ responses to 20-Hz stimulation in cells exposed to the same concentrations of L-Glu suggest the existence of three concentration intervals in transmission disturbances in the CA1 hippocampal area. Interval I (equal or below 1 mM) — all studied parameters are completely reversible upon washout and transmission is preserved. Interval II (from 1 to 10 mM) — both responses to frequency stimulation and single population spikes remain partially suppressed upon washout. Interval III (above 10 mM) — more than 50% suppression of transmission persists after washout; single population responses are completely inhibited.

It can be hypothesized that there is a boundary of neurotoxicity in the concentration interval of 1-5 mM, and application of L-Glu in concentrations surpassing this limit induces Ca^{2+} entry through NMDA and nonNMDA ion channels [2], which impairs the cell ability to restore neurotransmission.

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