Gangliosides Stabilize Ionic Homeostasis in Rat Cortical Synaptosomes Exposed to Toxic Concentrations of Glutamate

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Glutamate significantly increased intracellular calcium concentration, enhanced $^{45}Ca^{2+}$ entry, and activated Na^+/Ca^{2+} exchanger in synaptosomes from rat cerebral cortex. Preincubation with GM_1 ganglioside reduced the glutamate-induced increase in intracellular calcium and $^{45}Ca^{2+}$ entry. Gangliosides and glutamate stimulated Na^+/Ca^{2+} exchanger showing additive effects.

Key Words: *GM_1 ganglioside; glutamate; calcium; cerebral cortex synaptosomes*

Neuronal damage in ischemia, brain trauma, and some neurological diseases is largely determined by toxic effects of high concentrations of glutamate released into the intercellular space [2,3,12]. Excitotoxin-induced disturbance in intracellular calcium ($[Ca^{2+}]_i$) homeostasis is considered to be the major cause of neuronal death under these conditions [4].

Activation of N-methyl-D-aspartate receptors triggering this process is a necessary but not sufficient condition for sustained elevation of $[Ca^{2+}]_i$. An important role in the development of pathological processes initiated by excitotoxins is played by activation of free radical reactions [1,13] and other processes stimulating Ca^{2+} entry and inactivating the system stabilizing calcium homeostasis.

Gangliosides, the most complex animal glicosphingolipids, improve viability of cultured nervous cells exposed to glutamate [3,6] and significantly decrease $[Ca^{2+}]_i$, which seems to underlie their neuroprotective activity [12]. However, the mechanism of this effect remains unclear. We found no reports on the effects of gangliosides on glutamate-induced activation of Ca^{2+} entry or activity of Na^+/Ca^{2+} exchanger.

Our aim was to study the mechanism of stabilizing effect of gangliosides on Ca^{2+} homeostasis disturbed by excessive glutamate by investigating the effects of glutamate and gangliosides on $[Ca^{2+}]_i$, $^{45}Ca^{2+}$ entry, and activity of Na^+/Ca^{2+} exchanger in synaptosomes from rat cerebral cortex.

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MATERIALS AND METHODS

Synaptosomes isolated from the brain of male Wistar rats (150-180 g) as described elsewhere [9] were resuspended in buffer A containing (in mM): 135 NaCl, 1.2 $MgSO_4$, 2.0 KCl, 10 glucose, and 20 tris-HCl (pH=7.4), centrifuged at 15,000*g* for 15 min, and resuspended again in buffer A (4-5 mg/ml protein concentration).

$[Ca^{2+}]_i$ was measured with Fura-2M (Sigma), a fluorescent calcium probe. Synaptosomes (1 ml) were mixed with 1 ml buffer A containing 0.5% BSA (free from fatty acids) and 8 μM Fura-2M dissolved in 0.4% DMSO and incubated for 40 min at 30°C [16]. Preincubation with 50 nM GM_1 ganglioside was performed simultaneously with Fura-2M loading, which was stopped by adding 20-fold volume of buffer A followed by 15-min centrifugation at 12,000*g*. The sediment was resuspended in $MgSO_4$-free buffer B containing (in mM): 135 NaCl, 2.0 KCl, 1.0 $CaCl_2$, 10 glucose, and 20 tris-HCl (pH=7.4) and incubated with glutamate and other test compounds for 15 min at 30°C. Fluorescence was measured on a MPF-2A fluorimeter (Hitachi) at 340 and 380 nm excitation and 510 nm

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emission wavelength. $[Ca^{2+}]_i$ was calculated according to the formula:

$$[Ca^{2+}]_i = K_d \times (R - R_{min}/R_{max} - R) \times Sf_2/Sb_2), \text{ where}$$

R is the ratio of Fura-2 fluorescences at 340 and 380 nm; R_{min} and R_{max} are the same ratios in Ca^{2+}-free medium and after saturation with Ca^{2+}, respectively. Sf_2 is fluorescence at 380 nm in Ca^{2+}-free medium and Sb_2 is the same after saturation with Ca^{2+}. R_{min} and Sf_2 were measured in the presence of 2 mM EGTA and 0.1% Triton X-100; R_{max} and Sb_2 were measured after addition of 5 mM Ca^{2+} to saturate both EGTA and Fura-2. K_d was taken as 184 nm at 30°C [8].

Calcium accumulation in the control and glutamate-exposed synaptosomes was measured after 30-sec incubation in a medium containing (in mM): 135 NaCl, 1.2 K_2HPO_4, 3.0 KCl, 1.0 $CaCl_2$, 10 glucose, 20 tris-HCl (pH=7.4), and $^{45}Ca^{2+}$ (54 μCi/mmol) . The protein content was about 200 μg/ml. Preincubation with various concentrations of GM_1 ganglioside was performed for 1 h at 37°C. Activity of Na^+/Ca^{2+} exchanger was determined by the difference in $^{45}Ca^{2+}$ accumulation in NaCl- and choline chloride-containing media [5]. Radioactivity was measured on an LKB scintillation counter.

GM_1 ganglioside was isolated on silica gel columns [15]. Gangliosides were isolated from bovine brain as described elsewhere [7]. The isolated GM_1 was of 98-99% purity as shown by high-performance thin layer chromatography on Merck plates.

Synaptosomal protein concentration was measured [10] using bovine serum albumin as the standard. The data were analyzed statistically using Student's t test.

RESULTS

Glutamate (1 mM) significantly elevated synaptosomal $[Ca^{2+}]_i$ by on average 43.6±8.6% ($p<0.05$, Table 1). This finding agrees with published data showing that exposure to glutamate increases $[Ca^{2+}]_i$ in cultured cortical, hippocampal, and other neurons as well as in rat hippocampal synaptosomes [11,13,14]. Preincubation with 50 nM GM_1 ganglioside prevented the increase in $[Ca^{2+}]_i$ induced by glutamate alone or in combination with gangliosides ($p<0.05$, Table 1).

Glutamate significantly increased $^{45}Ca^{2+}$ entry into rat brain synaptosomes ($p<0.01$). Preincubation with GM_1 ganglioside inhibited this effect (Table 2). This inhibition was dose-dependent within the concentration range from 1 pM to 10 nM. The most pronounced inhibition of calcium response to glutamate was attained with 10 nM GM_1 ($p<0.01$), when $^{45}Ca^{2+}$ entry in the presence of glutamate did not differ from the control. GM_1 also exerted a significant, but less pronounced inhibitory effect in a concentration of 10 μM corresponding to a micellar form of the ganglioside. The effects of glutamate and gangliosides on Na^+/Ca^{2+} exchanger were similar and additive: significant activation of Na^+/Ca^{2+} exchange (0.64±0.03 nmol Ca^{2+}/mg protein/30 sec) was observed only after combined exposure to glutamate and GM_1 (Table 3).

These data suggest that the ability of ganglioside to inhibit Ca^{2+} entry induced by glutamate and other excitotoxins plays an important role in the stabiliza-

TABLE 1. Effect of Glutamate and GM_1 Ganglioside on $[Ca^{2+}]_i$ in Cortical Synaptosomes from Rat Brain ($M±m$)

Experiment	$[Ca^{2+}]_i$, nM
Control (n=14)	501.6±64.1
Glutamate, 1 mM (n=14)	720.2±103.7*
GM_1, 50 nM (n=5)	574.4±103.9
Glutamate, 1 mM and GM_1, 10 nM (n=5)	1074.6±117.3*
GM_1, 50 nM+glutamate, 1 mM, and GM_1, 10 nM (n=5)	635.0±133.9**

Note. *$p<0.05$ in comparison with the control; **$p<0.05$ in comparison with glutamate alone.

TABLE 2. Effect of Glutamate and GM_1 Ganglioside on $^{45}Ca^{2+}$ Entry into Cortical Synaptosomes from Rat Brain ($M±m$)

Experiment	$^{45}Ca^{2+}$ entry, nmol/mg protein/30 sec
Control	2.63±0.27
Glutamate, 1 mM	7.87±1.54*
GM_1, 1 pM+glutamate	7.19±2.06
GM_1, 10 pM+glutamate	6.91±1.93
GM_1, 1 nM+glutamate	5.18±0.61
GM_1, 10 nM+glutamate	2.32±0.16+
GM_1, 100 nM+glutamate	4.54±0.52++
GM_1, 1 μM+glutamate	4.24±0.19
GM_1, 10 μM+glutamate	4.52±0.39++

Note. *$p<0.01$ in comparison with the control; +$p<0.01$, ++$p<0.05$ in comparison with the effect of glutamate alone (Student's t test).

TABLE 3. Effect of Glutamate and GM_1 Ganglioside on the Activity of Na^+/Ca^{2+} Exchanger in Rat Brain Synaptosomes

Experiment	Activity of Na^+/Ca^{2+} exchanger, %
Control	100
Glutamate, 1 mM	192.9±42.8
GM_1, 40 pM	183.5±41.3
GM_1, 40 pM+glutamate	224.9±39.4*

Note. *$p<0.05$ in comparison with the control.

tion of intracellular Ca^{2+} homeostasis. As previously shown [6,12] gangliosides do not interfere with glutamate-induced activation of N-methyl-D-aspartate receptors and exert their effect at later stages. The increase in $[Ca^{2+}]_i$ after exposure to toxic glutamate concentrations can result from increased membrane permeability due to accumulation of lipid peroxidation products [14]. Glutamate-induced stimulation of $^{45}Ca^{2+}$ entry is significantly inhibited by α-tocopherol and SOD [1], therefore the effect of gangliosides can be associated with inhibition of free radical reactions activated by glutamate [2,15]. Activation of Na^+/Ca^{2+} exchanger in rat brain synaptosomes by glutamate and GM_1 can also have a protective effect, since its inactivation significantly promotes neuronal death in culture [3].

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