Effect of Prostaglandins E_2 and D_2 on Presynaptic NMDA Receptors in Rat Cerebral Cortex

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We studied the effect of prostaglandins on presynaptic NMDA receptors. Prostaglandin E_2 inhibited NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes in low concentrations (IC_{50} ~10 μM), but potentiated it in higher concentrations. Prostaglandin D_2 increased $^{45}Ca^{2+}$ uptake by synaptosomes during stimulation of NMDA receptors. Our results indicate that prostaglandins D_2 and E_2 modulate function of presynaptic NMDA receptors.

Key Words: *prostaglandins; presynaptic NMDA receptors; synaptosomes; $^{45}Ca^{2+}$ uptake*

Astrocytes are the main source of prostaglandins (PG) in the brain. Synaptic release of glutamate is followed by a transient increase in $[Ca^{2+}]_{in}$ in astrocytes. $[Ca^{2+}]_{in}$ variations in astrocytes depend on neuronal activity and regulate PG formation from arachidonic acid (AA). Glutamate release, increase in $[Ca^{2+}]_{in}$, and PG release are observed during activation of ionotropic and metabotropic glutamate receptors on astrocytes [4,13,14]. PG are also produced in neurons. Activation of NMDA receptors in neurons of the brain cortex *in vitro* is accompanied by stimulation of Ca^{2+} entry through NMDA receptors, activation of phospholipase A_2, AA release, and formation of PGE_2 and PGF_{2a} [7]. PG produce various effects on the central nervous system (CNS). They are involved in neuronal transmission and development of neurodegenerative diseases (*e.g.*, Alzheimer's disease). PGE_2 abolishes the neurotoxic effect of β-amyloid by suppressing Ca^{2+} current through L-type Ca^{2+} channels [12]. PGE_2, PGF_{2a}, and PGD_2 modulate activity of postsynaptic GABA receptors, increase the inhibitory influence of GABA and taurine, and potentiate the excitatory effect of glutamate and aspartate on guinea pig Purkinje cells. Purkinje cells carry a considerable number of PGD_2-binding sites. The content of PGD_2 in the brain is much higher compared to other PG. PGD_2 induces sleep, causes membrane depolarization in NE-115 neuroblastoma cells, and modulates Ca^{2+} current. PGF_{2a} binds to its specific receptor via G protein and potentiates activation of postsynaptic NMDA receptors by increasing $[Ca^{2+}]_{in}$ [8]. PGE_2 stimulates Ca^{2+}-dependent secondary release of endogenous glutamate and aspartate in the presynapse, protects neurons in rat cerebral cortex from glutamate toxicity, and plays a role in the regulation of sleep via EP4 receptors [2,3,5,9].

Thus, the modulatory effect of PG can include pre- and postsynaptic centers in CNS. NMDA receptors play an important role in glutamate excitotoxicity in cortical and hippocampal neurons. Hyperactivation of NMDA receptors is accompanied by massive Ca^{2+} entry into the cell and increase in $[Ca^{2+}]_{in}$. These changes are followed by activation of intracellular enzyme systems initiating cell degeneration and lysis.

Here we studied the effects of PGD_2 and PGE_2 on presynaptic NMDA receptors in rat cerebral cortex using the method of $^{45}Ca^{2+}$ uptake by synaptosomes in rat cerebral cortex during NMDA stimulation.

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MATERIALS AND METHODS

Synaptosomes were isolated from the cerebral cortex of newborn Wistar rats (9-10 days) by the method of F. Hajos [6]. The brain was homogenized in a 10-fold volume of cold 0.32 M sucrose (900 rpm) using a glass-Teflon homogenizer. The homogenate was centrifuged at 1500*g* for 10 min. The supernatant was centrifuged at 10,000*g* for 20 min. For accumulation of the radioactive label, the P_2 synaptosomal fraction was suspended in incubation buffer A containing 132 mM NaCl, 5 mM KCl, and 5 mM HEPES (pH 7.4, final protein concentration 1.5-2.0 mg/ml). Ca^{2+} concentration in the final solution was 1.25 mM (1.4 μCi/ml). NMDA (200 μM NMDA and 5 μM glycine) was used to stimulate $^{45}Ca^{2+}$ uptake by synaptosomes. After 3-min incubation with NMDA at 37°C, $^{45}Ca^{2+}$ uptake was stopped by filtering of the mixture through GF/B fiberglass filters (Whatman). The samples were washed 3 times with cold buffer solution B containing 145 mM HEPES, 10 mM Tris, and 54 mM Trilon B (pH 7.4). The measurements were performed in 3-4 parallel samples (3-4 independent experiments). The radioactivity was measured on a liquid scintillation β-counter. The amount of $^{45}Ca^{2+}$ accumulated in synaptosomes was calculated as the difference between label concentrations in the presence and absence of uptake stimulator (NMDA) and expressed in percents from the control.

Specific Ca^{2+} uptake was calculated as follows:

$$K_{(43/21)}=[(Ca_4-Ca_3)/(Ca_2-Ca_1)]\times 100\%,$$

where Ca_1 is $^{45}Ca^{2+}$ uptake in the control (in the absence of agonist and test compounds); Ca_2 is $^{45}Ca^{2+}$ uptake in the presence of agonist (NMDA); Ca_3 is $^{45}Ca^{2+}$ uptake in the presence of PG (without NMDA); and Ca_4 is $^{45}Ca^{2+}$ uptake in the presence of NMDA and PG.

The concentrations of PGE_2 and PGD_2 were 10^{-13}-10^{-6} M.

The results were analyzed by Student's *t* test.

RESULTS

$^{45}Ca^{2+}$ uptake by synaptosomes during stimulation with NMDA is associated with activation of NMDA glutamate receptors. NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes decreases after addition of the following NMDA receptor antagonists: MK-801 (IC_{50} ~1 μM), CPP (IC_{50} ~100 μM), memantine (IC_{50} ~0.4 μM), and Mg^{2+} (IC_{50} ~100 μM). Our results support the data that NMDA activates NMDA receptors in the P_2 synaptosomal fraction of rat cerebral cortex [1]. PGE_2 in low concentrations (10^{-11}-10^{-10} M) inhibited NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes. The maximum inhibition of $^{45}Ca^{2+}$ uptake by synaptosomes was observed in the presence of 10^{-10} M PGE_2 (IC_{50} 10 μM). PGE_2 in concentrations of 10^{-8}-10^{-6} M potentiated $^{45}Ca^{2+}$ uptake by synaptosomes from rat cerebral cortex during NMDA stimulation. The maximum stimulation of $^{45}Ca^{2+}$ uptake by synaptosomes was observed in the presence of 10^{-8} M PGE_2 (Fig. 1).

PGE_2 dose-dependently stimulates glutamate release in the presynapse, thus increasing NMDA-induced cytotoxicity [9]. PGE_2 increases cytotoxicity induced by NMDA via activation of EP2 receptors on cultured rat cortical neurons, activates adenylate cyclase, and increases intracellular cAMP concentration [10]. We found that PGE_2 in concentrations of 10^{-8}-10^{-6} M increases $^{45}Ca^{2+}$ uptake by synaptosomes during stimulation with NMDA. It can be suggested that PGE_2 in the specified concentration range stimulates glutamate release in synaptosomes. These changes result in additional activation of NMDA glutamate receptors and increase in $^{45}Ca^{2+}$ uptake by synaptosomes. Our suggestion is consistent with published data that PGE_2 increases NMDA toxicity via AMPA/kainate receptors, NMDA receptors, and metabotropic receptors [11]. It should be emphasized that PGE_2 has a protective effect on NMDA-induced cytotoxicity. PGE_2 inhibits the intracellular mechanisms associated with glutamate toxicity. PGE_2 stimulates EP2 receptors, which contributes the decrease in NO production [3,5]. The protective effect of PGE_2 is related to activation of EP2 and EP4 receptors, which results in the increase in cAMP concentration and activation of protein kinase. This discrepancy probably

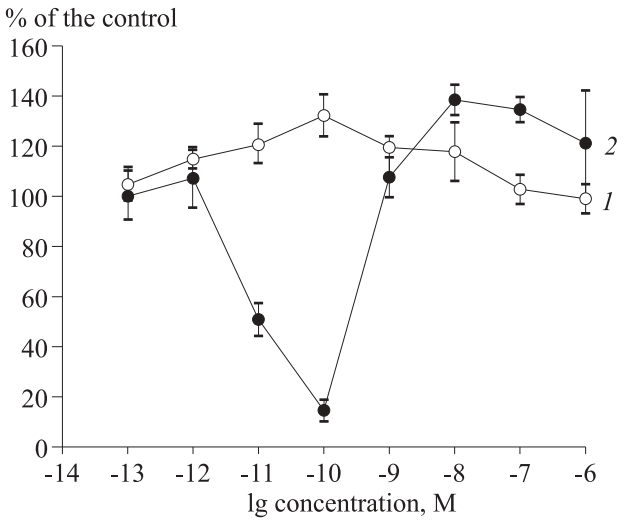

Fig. 1. Effects of PGD_2 (*1*) and PGE_2 (*2*) on $^{45}Ca^{2+}$ uptake by synaptosomes of rat cerebral cortex during stimulation with NMDA.

arises from the fact that PGE_2 affects various subtypes of PGE_2 receptors. It was hypothesized that the protective effect of PGE_2 is mediated by EP2 and EP4 receptors and results from the increase in cAMP concentration. PGE_2 decreases cAMP concentration via EP3 receptors. PGE_2 does not have protective activity under these conditions [2]. Further studies are required to evaluate the mechanism underlying the opposite effects of PGE_2 on CNS via NMDA receptors. We showed that PGE_2 in low concentrations (10^{-11}-10^{-10} M) inhibits NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes of rat cerebral cortex. PGE_2 in the specified concentration range probably protects NMDA receptors by inhibiting $^{45}Ca^{2+}$ uptake by synaptosomes. PGE_2 can produce inhibitory or stimulatory effect on NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes of rat cerebral cortex depending on the concentration of this compound.

PGD_2 increased $^{45}Ca^{2+}$ uptake by synaptosomes. $^{45}Ca^{2+}$ uptake by synaptosomes increased most significantly under the influence of PGD_2 in a concentration of 10^{-10} M (132.2% of the control). Similarly to PGE_2, PGD_2 in concentrations of 10^{-8}-10^{-6} M can potentiate NMDA receptors, which leads to stimulation of glutamate release and increased $^{45}Ca^{2+}$ uptake by synaptosomes.

Our results indicate that PGE_2 protects NMDA receptors due to inhibition of $^{45}Ca^{2+}$ uptake by synaptosomes, but in high concentrations it potentiates the toxic effect of NMDA. The effect of PGE_2 is probably associated with expression of various subtypes of receptors (EP2, EP3, and EP4). PGD_2 always potentiates NMDA receptors. We conclude that PGE_2 and PGD_2 modulate synaptic transmission in CNS via presynaptic NMDA receptors.

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REFERENCES

1. L. N. Petrova and S. O. Bachurin, *Byull. Eksp. Biol. Med.*, **142**, No. 7, 51-54 (2006).
2. A. S. Ahmad, M. Ahmad, A. J. de Brum-Fernandes, and S. Dore, *Brain Res.*, **1066**, No. 1-2, 71-77 (2005).
3. A. Akaike, S. Kaneko, Y. Tamura, *et al.*, *Ibid.*, **663**, No. 2, 237-243 (1994).
4. P. Bezzi, G. Carmignoto, L. Pasti, *et al.*, *Nature*, **391**, 281-285 (1998).
5. C. Cazevieille, A. Muller, F. Meynier, *et al.*, *Neurochem. Int.*, **24**, No. 4, 395-398 (1994).
6. F. Hajos, *Brain Res.*, **93**, No. 3, 485-489 (1975).
7. L. S. Lerea, N. G. Carlson, M. Simonato, *et al.*, *J. Neurosci.*, **17**, No. 1, 117-124 (1997).
8. T. Muratani, M. Nishizawa, S. Matsumura, *et al.*, *J. Neurochem.*, **86**, No. 2, 374-382 (2003).
9. I. Nishihara, T. Minami, Y. Watanabe, *et al.*, *Neurosci. Lett.*, **196**, No. 1-2, 57-70 (1995).
10. T. Takadera and T. Ohyashiki, *Life Sci.*, **78**, No. 16, 1878-1883 (2006).
11. M. E. Turnbach and A. Randich, *Pain*, **97**, No. 1-2, 127-137 (2002).
12. T. Yagami, H. Nakazato, K. Ueda, *et al.*, *Brain Res.*, **959**, No. 2, 328-335 (2003).
13. L. C. Yang, M. Marsala, T. L. Yaksh, *Neuroscience*, **75**, No. 2, 453-461 (1996).
14. M. Zonta, A. Sebelin, S. Gobbo, *et al.*, *J. Physiol.*, **553**, No. 2, 404-414 (2003).