In Vitro Effects of Anandamide and Prostamide E2 on Normal and Transformed Nerve Cells

E. L. Andrianova*, E. E. Genrikhs*, M. Yu. Bobrov*,, A. A. Lizhin*, N. M. Gretskaya**, L. E. Frumkina*, L. G. Khaspekov*, and V. V. Bezuglov****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 1, pp. 36-38, January, 2011
Original article submitted January 25, 2010

We studied the effects of endocannabinoid anandamide and its cyclooxygenase derivative prostamide E2 on cultured cerebellar granular cells and C6 glioma cells from rats. Prostamide E2 prevented apoptosis in cerebellar neurons induced by potassium deprivation of cultures, while anandamide had no neuroprotective properties. Prostamide E2 did not modulate the survival rate of glioma cells, while anandamide produced a cytotoxic effect. Our results indicate that cyclooxygenase transformation of anandamide is followed by the loss of antitumor activity of this agent. By contrast, prostamide E2 exhibited strong neuroprotective properties.

Key Words: *prostamide E2; anandamide; neuroprotection; endocannabinoids; gliomas*

Fatty acid-acylated derivatives of ethanolamine belong to a recently discovered family of endogenous neurolipins with a wide range of properties. They serve as modulators of the brain endocannabinoid and endovaniloid systems [7]. Much attention was paid to endocannabinoid N-arachidonoyl ethanolamine (anandamide, AA-EA), which belongs to this family. This substance is synthesized and released from neurons in response to membrane depolarization. AA-EA serves as a neuronal messenger [5]. Under the effect of fatty acid amide hydrolase, AA-EA is degraded with the formation of arachidonic acid and ethanolamine [11]. Another possible pathway of AA-EA metabolism is oxidation by cyclooxygenases with the formation of prostaglandin ethanolamides, or prostamides [12]. It cannot be excluded that oxidized derivatives exhibit a specific range of biological properties.

Endocannabinoids regulate processes determining cell viability. They can produce either cytoprotective or cytotoxic effect depending on environmental conditions and stage of differentiation. Published data show that endocannabinoid production is increased in the damaged brain [9]. Moreover, the cells not carrying cannabinoid receptors are more sensitive to injury [8]. It was hypothesized that endocannabinoids play a role in the neuroprotective mechanisms. However, endocannabinoids produce the cytotoxic and antiproliferative effect on some tumor cells (including nerve cells) [6]. The involvement of AA-EA cyclooxygenase metabolites in these processes received little attention.

Here we studied the protective and toxic properties of AA-EA and prostamide E2 (PGE2-EA) in cultures of normal and transformed nerve cells.

MATERIALS AND METHODS

Ethanolamides of arachidonic acid and prostaglandin E2 (AA-EA and PGE2-EA; Fig. 1) were synthesized and characterized at the Laboratory of Oxylipins (M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry) [3].

Primary cultures of rat cerebellar granular cells were obtained as described elsewhere [4]. The neuro-

*Laboratory of Experimental Neurocytology, Neurology Research Center, Russian Academy of Medical Sciences; **Laboratory of Oxylipins, M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** mbobr@mail.ru. M. Yu. Bobrov

0007-4888/11/1511-0030

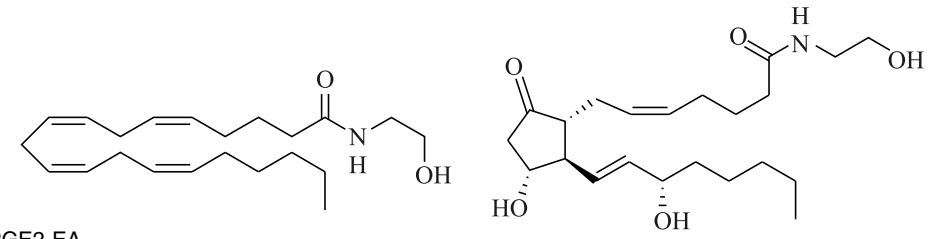

Fig. 1. Structural formulas of AA-EA and PGE2-EA.

protective effect in cultured cerebellar granular cells from 7-day-old rats was studied on the model of K^+ deprivation. To this end, K^+ concentration in the nutrient medium was reduced from 25 to 9 mM on day 7-8 of culturing, which induced apoptosis of neurons. The survival of neurons in the presence of AA-EA or PGE2-EA was evaluated by the reduction of dimethylthiazole diphenyl tetrazolium bromide to water-insoluble formazan (MTT test) after 24 h. The survival was expressed in percentage (relative to the control cultures not exposed to K^+ deprivation).

C6 glioma cells were cultured as described previously [1]. The cells were put in wells of a 96-well plate (2×10^4 cells per well). The test substances were added after 24-h culturing (1 concentration in 4 wells). Matrix solutions were prepared in the nutrient medium with 10% fetal bovine serum, which improved solubility of lipid compounds. Cell survival was estimated in the MTT test after 24-h incubation. The cells were dissolved in DMSO. Formazan absorption (A) was measured on a Packard plate photometer at 570 and 620 nm. The relative number of viable cells was calculated as follows: A_{570}-A_{620}. The effect of the test compounds was evaluated in 3 independent experiments. The percentage of survived cells was calculated (relative to the intact control).

The significance of differences was estimated by the analysis of variance and Dunnet's test.

RESULTS

The presence of AA-EA or PGE2-EA (0.1-10 μM) in the control nutrient medium with 25 mM K^+ for 24 h had no effect on survival of neurons (data not shown). Under conditions of K^+ deprivation, AA-EA in concentrations of 0.1-1 μM had no neuroprotective effect (Table 1). The survival of neurons increased by 20% with increasing AA-EA concentration to 10 μM. PGE2-EA in the same concentrations produced a potent protective effect, which reached 77% at a concentration of 1 μM (Table 1).

Neurodegenerative processes are often accompanied by inflammation. Published data show that activity of induced cyclooxygenase 2 (COX-2) in astrocytes, neurons, neutrophils, and capillary endothelial cells increases under these conditions [10]. Increased synthesis of N-acyl ethanolamines in damaged nerve tissues probably contributes to the improvement of neuronal survival [9]. Our results indicate that transformation of N-acyl ethanolamines into the corresponding prostamides can be a protective mechanism realized in the ischemic area.

Opposite results were obtained in experiments on rat C6 glioma cells. AA-EA in concentrations of 1-10 μM caused a moderate decrease in the viability of cells, while PGE2-EA in concentrations of 0.1-5 μM had no toxic effect (Table 2). Published data show that the content of N-acyl ethanolamines and expression of COX-2 increase in some primary tumors and metastases [2]. These data suggest that transformation of unsaturated N-acyl ethanolamines into prostamides in tumors serves as a pathway for inactivation of these compounds.

We conclude that AA-EA and PGE2-EA (cyclooxygenase derivative of this compound) produce various effects on normal and transformed nerve cells. It

TABLE 1. Survival of Neurons after K^+ Deprivation-Induced Apoptosis in the Presence of AA-EA and PGE2-EA (% of intact control; *M*±*m*)

Compound	Concentration, μM		
	0.1	0.5	1
AA-EA	0.20±1.07	0.00±0.65	2.4±2.3
PGE2-EA	21.1±8.6*	59.3±4.9*	77.40±2.87*

Note. Here and in Table 2: analysis of variance, $p<0.05$. *$p<0.01$ compared to the intact control (Dunnet's test).

can be hypothesized that activation of cyclooxygenases in tumors is a protective mechanism reducing AA-EA activity. The formation of PGE2-EA in normal tissues prevents apoptosis of neurons, which increases their resistance to adverse factors.

This work was supported by the Russian Foundation for Basic Research (grants No. 09-04-00317-a and 08-04-12213-ofi).

REFERENCES

1. E. L. Andrianova, M. Yu. Bobrov, N. M. Gretskaya, *et al*., *Neirokhimiya*, **27**, No. 1, 1-10 (2010).
2. V. V. Bezuglov and S. S. Konovalov, *Lipids and Cancer. Essays on Lipidology of the Oncology Process* [in Russian], St. Petersburg (2009).
3. V. V. Bezuglov, E. M. Manevich, A. V. Archakov, *et al*., *Bioorgan. Khimiya*, **23**, No. 3, 938-942 (1997).
4. N. Andreeva, B. Khodorov, E. Stelmashook, *et al*., *Brain Res*., **548**, Nos. 1-2, 322-325 (1991).
5. V. Di Marzo, A. Fontana, H. Cadas, *et al*., *Nature*, **372**, 686-691 (1994).
6. C. J. Fowler, K. O. Jonsson, A. Andersson, *et al*., *Biochem. Pharmacol*., **66**, No. 5, 757-767 (2003).
7. E. Fride, *Prostaglandins Leukot. Essent. Fatty Acids*, **66**, Nos. 2-3, 221-233 (2002).

TABLE 2. Survival of C6 Glioma Cells in the Presence of AA-EA and PGE2-EA (% of intact control; *M±m*)

Compound, μM		Survival rate, %
AA-EA, μM	1	102.1±2.4
	2.5	79.1±6.2*
	5	74.9±5.8*
	10	65.4±6.1*
PGE2-EA, μM	0.1	99.2±7.2
	0.5	92.8±4.2
	1	102.3±7.4
	5	106.7±6.7

8. G. Marsicano, S. Goodenough, K. Monory, *et al*., *Science*, **302**, 84-88 (2003).
9. N. Stella, P. Schweitzer, and D. Piomelli, *Nature*, **388**, 773-778 (1997).
10. T. Takemiya, K. Matsumura, and K. Yamagata, *Neurochem. Int*., **51**, Nos. 2-4, 112-120 (2007).
11. N. Ueda, R. A. Puffenbarger, S. Yamamoto, and D. G. Deutsch, *Chem. Phys. Lipids*, **108**, No. 1-2, 107-121 (2000).
12. M. Yu, D. Ives, and C. S. Ramesha, *J. Biol. Chem*., **272**, No. 34, 21,181-21,186 (1997).