Pharmacological Reports
2008, 60, 345–352
ISSN 1734-1140

Effects of 3-aminopyridine-induced seizures on platelet eicosanoid synthesis

Gábor Csányi[1], Béla Kis[1], Árpád Gecse[1], Gyula Telegdy[1], Zoltán Szupera[2], László Vécsei[2,4], Magdolna Szente[3], István Leprán[5], Zsófia Mezei[1]

[1]Department of Pathophysiology, Faculty of Medicine, University of Szeged, Semmelweis 1, 6725 Szeged, Hungary

[2]Department of Neurology, Faculty of Medicine, University of Szeged, Semmelweis 6, 6725 Szeged, Hungary

[3]Department of Comparative Physiology, University of Szeged, Középfasor 52, 6726 Szeged, Hungary

[4]Neurology Research Group of the Hungarian Academy of Sciences, Semmelweis 6, 6725 Szeged, Hungary

[5]Department of Pharmacology and Pharmacotherapy, University of Szeged, Dom square 12, 6720 Szeged, Hungary

Correspondence: Zsófia Mezei, e-mail: mezeizsofia@gmail.com

Abstract:
We investigated the influence of recurrent epileptic seizures on the arachidonic acid (AA) cascade in platelets and brain microvessels, using [^{14}C]AA as a tracer substrate and chromatographic determination. The recurrent epileptic seizures of male Wistar rats were induced every second day with 3-aminopyridine (3-AP, 25 mg/kg *ip*) for two weeks.
In the chronic 3-AP model, the earlier epileptic insults resulted in a decreased incidence of limbic seizures and higher survival rate at later administration of 3-AP. After 3-AP treatment, the formation of lipoxygenase products was unchanged, but the total amount of cyclooxygenase (COX) metabolites was decreased both in platelets and brain microvessels. The reduction in COX-mediated eicosanoid synthesis after recurrent seizures was due to the decreased synthesis of vasodilator and vasoconstrictor COX metabolites. In platelets, the 3-AP-treatment reduced the synthesis of vasodilator prostacyclin (PGI_2), prostaglandin E_2 (PGE_2) and 12-L-hydroxy-5,8,10-heptadecatrienoic acid (12-HHT), while the synthesis of prostaglandin D_2 (PGD_2) remained unchanged. In isolated brain capillaries, the PGD_2, PGE_2 and 12-HHT synthesis was decreased after recurrent seizures. As for the vasoconstrictor COX metabolites, both platelets and brain microvessels synthesized significantly lesser amount of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane A_2 (TxA_2) upon 3-AP administration.
Our results indicate that platelets and isolated brain capillaries synthesize significantly lesser amount of COX metabolites after chronic 3-AP treatment. The decreased conversion of AA into different COX products may play a role in the neuroprotective/preconditional adaptation of the brain against subsequent seizures.

Key words:
seizure, platelets, eicosanoids, 3-aminopyridine, prostaglandins, brain microvessels

Abbreviations: AA – arachidonic acid, 3-AP – 3-aminopyridine, CON – amount of vasoconstrictor cyclooxygenase metabolites, COX – cyclooxygenase, DIL – amount of vasodilator cyclooxygenase metabolites, dpm – disintegrations per minute, GABA – γ-aminobutyric acid, 12-HHT – 12-L-hydroxy-5,8,10-heptadecatrienoic acid, 6-keto-$PGF_{1\alpha}$ – 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin), LOX – lipoxygenase, PGD_2 – prostaglandin D_2, PGE_2 – prostaglandin E_2, $PGF_{2\alpha}$ – prostaglandin $F_{2\alpha}$, PUFA – polyunsaturated fatty acid, TxB_2 – thromboxane B_2 (stable metabolite of TxA_2)

Introduction

Prostaglandins (PG) are produced in mammalian brain tissues and play a significant role in the central nervous system, e.g. in the regulation of the cerebral blood flow, sleep-wakefulness cycle and body temperature [19, 32, 37, 48]. There are convincing evidences supporting the activation of arachidonate cascade in the brain during experimental epilepsy [5, 17]. Indeed, the levels of PGD_2, PGE_2 and $PGF_{2\alpha}$ have been found to be high in different brain regions such as the hippocampus and cerebral cortex after chemically or electrically induced seizures [6, 34]. It has been also reported that the PG profile is differentially altered in the rat brain during the early and the late phase after seizures [34].

The possible role of PGs and their inhibitors in modulation of seizure activity is still inconclusive. Some reports pointed out that PGs (e.g. PGD_2, PGE_2, and $PGF_{2\alpha}$) might have anticonvulsant properties [16, 20]. In contrast to this, another author demonstrated that cyclooxygenase (COX)-inhibitors decreased the number of epileptic convulsions [45]. Moreover, Rosenkranz et al. [41] reported that pretreatment with $PGF_{2\alpha}$ potentiated the pentylenetetrazole- and electroshock-induced seizures.

On the other hand, it was previously shown that chronic administration of the conventional antiepileptic drug valproate reduced the arachidonic acid (AA) turnover within brain phospholipids [8, 11] and decreased the concentrations of vasoactive COX and lipoxygenase (LOX) products in rat platelets [44].

Some metabolites of the AA cascade are important second messengers in neurons [27] and in platelets [25, 35]. Blood platelets have been frequently used as a model to investigate the imbalance between the uptake of the inhibitory amino acid transmitter γ-aminobutyrate (GABA) and excitatory amino acid glutamate in epileptic patients [39, 40, 49]. Increased glutamate [40] and decreased GABA uptake [39] by platelets in epileptic patients has led to the hypothesis that high seizure activity may induce compensatory alteration in platelet function [40]. Previous studies using the platelet model in other disorders of the central nervous system also reported that glutamate uptake was decreased in platelets from patients with Alzheimer's [14] and Parkinson's [15] disease. These findings suggest that platelets are useful as a peripheral marker of glutamatergic involvement and as an adjunctive diagnostic tool in these disorders.

Endothelial cells of brain microvasculature contribute to the regulation of blood flow by releasing vasodilator (DIL; PGI_2, PGE_2, PGD_2 and 12-HHT) and vasoconstrictor (CON; TxA_2 and $PGF_{2\alpha}$) COX metabolites [1, 18]. PGs influence the cerebral blood flow in various pathologic conditions [3, 36, 43] and may result in pial arteriolar dilatation in epileptic seizures [9]. In addition to the vasoactive effects of PGs, it has been reported that PGE_2 increases the Ca^{2+} influx into hippocampal CA1 pyramidal neurons, leading to increased membrane excitability and increased frequency of firing [12]. It is also known that endothelial cells and platelets may influence each other's function *via* synthesizing platelet inhibitory and aggregatory PGs (PGI_2 and TxA_2, respectively) as well as intercellular exchange of AA and endoperoxides [10]. Therefore, a significant communication between the endothelium and platelets may occur both during physiologic and pathologic conditions.

We hypothesized that repeated 3-AP-treatment, inducing chronic epilepsy, might influence the PG synthesis in platelets and/or brain microvessels, and these changes may influence epileptogenesis and seizure susceptibility. The aim of the present work was to examine the effects of repeated 3-AP treatment on the behavior of rats and to investigate the effects of chronic (repeated) seizures on the arachidonate cascade in isolated platelets and brain capillaries in the animals 24 h after the recovery from seizure.

Materials and Methods

Animals

This study was performed on adult male Wistar rats weighing 150–170 g. The animals were maintained on 12-h dark/12-h light cycles in a room maintained at constant temperature (23 ± 1°C) with access to standard laboratory food and water *ad libitum*. Animal experiments were performed according to a protocol accepted by the Ethical Committee for the Protection of Animals in Research, University of Szeged, Hungary. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Chemicals

AA (grade I), 12-HHT and 12-hydroxy-5,8,10,14-eicosatetraenoic acid unlabeled standards, serum-free tissue culture Medium 199 and 3-AP were purchased from Sigma (St. Louis, MI, USA). [^{14}C]AA (specific activity: 2035 MBq/mM) was obtained from Amersham (England). Silica gel thin-layer plates (0.25 mm) were obtained from Merck AG (Darmstadt, Germany). PGE_2, PGD_2, TxB_2 (the stable metabolite of TxA_2), $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin) were generously provided by Upjohn Co. (Kalamazoo, USA). Pentobarbitone was purchased from Ceva-Phylaxia (Budapest, Hungary). Chloroform and methanol were purchased from Reanal (Hungary).

3-AP treatment

Animals (n = 16) were treated every second day with 3-aminopyridine (3-AP; 25 mg/kg *ip*, dissolved in 1 ml of isotonic saline solution) for two weeks (altogether seven 3-AP treatments). Age-matched control rats (n = 9) were treated with the same volume of saline alone. After the administration of 3-AP the animals experienced local and generalized seizures, observed and graded according to Zhang et al. [50]. Surviving rats, showing typical features of epileptic seizures were used for the experiments. Platelets and brain microvessels were isolated one day after the last treatment.

Isolation of platelets

Isolation of platelets was carried out by using the method described previously [31]. Briefly, under pentobarbitone anesthesia (60 mg/kg *ip*) blood was drawn from the abdominal aorta and diluted (1:2) with phosphate buffer (pH 7.4), containing EDTA (5.8 mM) and glucose (5.55 mM). Platelet-rich plasma was collected after the whole blood had been centrifuged at 200 × g for 10 min at room temperature. Platelets were separated from the supernatant by centrifugation at 2,000 × g for 10 min. The contaminating red blood cells could metabolize arachidonic acid *via* the LOX pathway and release leukotrienes [29], therefore erythrocytes were lysed with hyposmotic ammonium chloride (0.83%, 9 parts), containing EDTA (0.02%, 1 part) at 4°C for 15 min. Platelets were then washed with phosphate buffer (pH 7.4, containing 5.8 mM EDTA and 5.55 mM glucose) and centrifuged at 2,000 × g for 10 min at room temperature. During the separation procedure, the activation of platelets was inhibited by applying Ca^{2+}-free medium and siliconized glassware. The washed platelet suspension was free from other cellular elements of the blood (red blood cells, leukocytes, etc.) and plasma proteins. After the last centrifugation, platelets were resuspended (10^8 platelets/ml) in serum-free tissue culture Medium 199.

Isolation of brain microvessels

Rats were anesthetized as described above and the brains were perfused with 0.9% NaCl (containing 5.8 mM EDTA) to eliminate blood from the capillaries. After decapitation the brain was quickly removed and blood-free brain microvessels were isolated from 300 mg wet weight of brain cortex by the modified [44] centrifugation method [22]. Microvessels were resuspended in 1 ml of Medium 199 and an aliquot was examined for purity under light and phase-contrast microscope. The viability of capillaries was determined by trypan blue exclusion method and was found to be over 90% at the end of the separation procedure [28].

Analysis of eicosanoids

The separation and measurment of radiolabeled eicosanoids were described elsewhere [31]. Briefly, platelets (10^8 cells/ml in each sample) and brain microvessels (300 mg wet weight of brain cortex/ml in each sample) were preincubated at 37°C for 5 and 10 min, respectively. The enzyme reaction was initiated by the introduction of the tracer substrate, [^{14}C]AA (3.7 kBq, 0.172 pmol), into the incubation mixture. Ten and 30 min later the enzyme reaction was stopped by bringing the pH to 3 with formic acid in mixtures containing platelets and microvessels. Samples were then extracted with ethyl acetate (2 × 3 ml) and the organic phases were pooled and evaporated to dryness under nitrogen. The residues were reconstituted in 2 × 100 µl of ethyl acetate and quantitatively applied to silica gel G thin-layer plates. The plates were developed to a distance of 15 cm in the organic phase of ethyl acetate : acetic acid : 2,2,4-trimethylpentane : water (110:20:30:100 v/v/v/v) by means of overpressure thin-layer chromatography (Chrompres 25, Labor MIM, Hungary) [1, 13]. Each 3-mm band of the chromatograms was then scraped off and the radioactivity

was determined by liquid scintillation analyzer (TRI-CARB 2100TR, Canberra Packard, USA) using 5 ml of toluene containing 0.44% (w/v) of 2,5-diphenyloxazole, 0.02% (w/v) of 1,4-di-[2-(5-phenyl) oxazoyl] benzene and 10% (v/v) of ethanol. Radioactivity was expressed in disintegrations per minute (dpm). The radiolabeled products of AA were identified with unlabeled authentic standards, i.e., 6-keto-$PGF_{1\alpha}$ (Rf = 0.19), $PGF_{2\alpha}$ (Rf = 0.29), PGE_2 (Rf = 0.48), PGD_2 (Rf = 0.58), TxB_2 (Rf = 0.39), 12-HHT (Rf = 0.69) and 12-HETE (Rf = 0.79) [30] which were detected by anisaldehyde reagent [26]. Assuming that the exogenously administered [^{14}C]AA, as a tracer is converted in the same way as the endogenous source, our method allows to measure the relative amount of various prostanoids.

Statistical analysis

The results are expressed as the means ± SEM. Significance of the differences between eicosanoid synthesis of 3-AP-treated and control rats were evaluated by F-test, followed by modified Student's *t*-test [46]; $p < 0.05$ was considered to be statistically significant.

Results

Behavioral analysis

The 3-AP-induced epileptic behavioral profile was monitored for 3 h post-injection. Control rats (vehicle-injected animals) did not show abnormal behavior. Epileptic rats, immediately after the first 3-AP injection, presented behavioral changes, including immobilization, staring, head nodding, tremor and wet-dog shakes, that persisted for up to 15 min. These initial changes were followed by increased forelimb jerks, salivation and then progressed into motor limbic seizures in 69% of animals (11/16 animals). Limbic convulsive behavior persisted for 50–60 min and led to death in 13% of the animals (2/16 animals). The remaining rats (87%) returned back to normal behavior. Following the second 3-AP treatment, the animals presented the same initial behavior and 36% of animals (5/14 animals) had limbic seizures, which progressed to death in 21% of animals (3/14 animals). The remaining rats (n = 11) survived the last five 3-AP treatment and the incidence of limbic seizures reduced to 10–20%.

Effects of 3-AP treatment on eicosanoid synthesis

Platelets

Chronic 3-AP treatment significantly reduced the total amount of AA metabolites in platelets (18.6%; Fig. 1). The conversion of AA to COX products was significantly decreased (33.9%; Fig. 2), while the amount of LOX products remained unchanged after 3-AP administration (in 1,000 dpm:) 72.98 ± 4.5 and

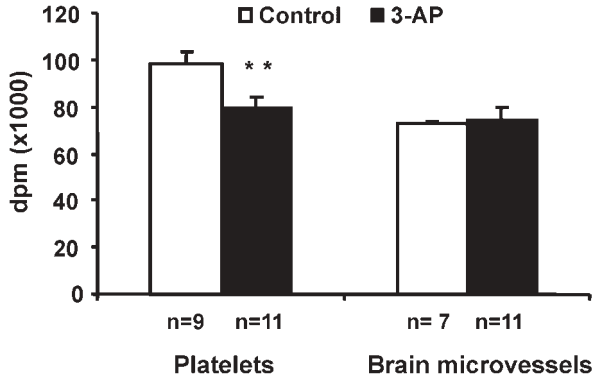

Fig. 1. Total production of arachidonic acid metabolites synthesized by platelets and brain microvessels in control (□) and 3-AP-treated (■) rats. Data represent the means ± SEM; ** $p < 0.01$ when control rats were compared with 3-AP-treated animals

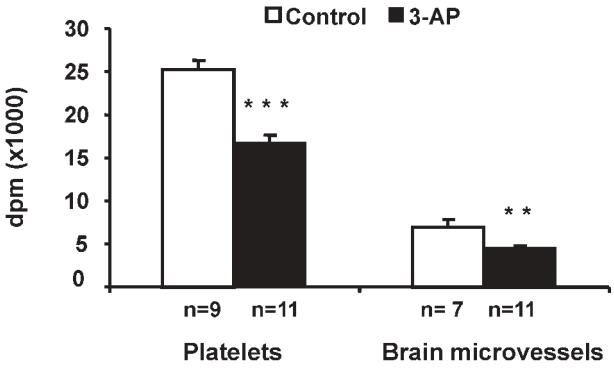

Fig. 2. Comparison of COX metabolites synthesized by platelet and brain microvessels in control (□) and 3-AP-treated (■) rats. Data represent the means ± SEM; ** $p < 0.01$ and *** $p < 0.001$ when control rats were compared with 3-AP-treated animals

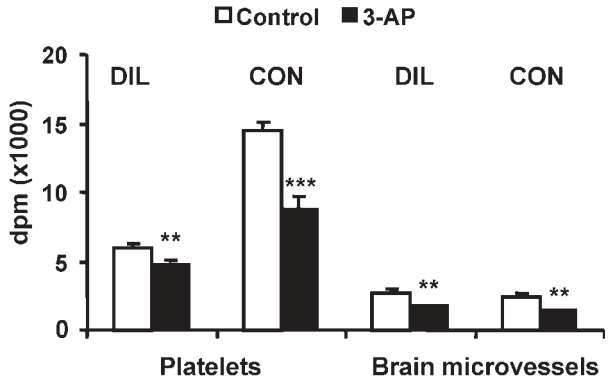

Fig. 3. The amount of vasodilator (DIL) and vasoconstrictor (CON) COX metabolites in control (□) (n = 9 and n = 7 for platelet and brain microvessels, respectively) and 3-AP-treated (■) (n = 11) rats. Data represent the means ± SEM ; ** $p < 0.01$ and *** $p < 0.001$ when control rats were compared with 3-AP-treated animals

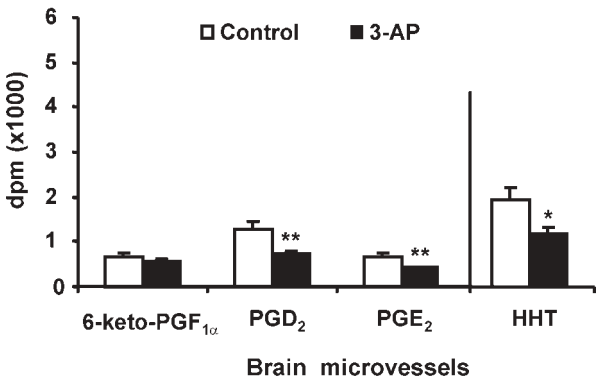

Fig. 5. Comparison of 6-keto-$PGF_{1\alpha}$, PGD_2, PGE_2 and 12-HHT synthesized by brain microvessel in control (□) (n = 7) and 3-AP-treated (■) (n = 11) rats. Data represent the means ± SEM; * indicates $p < 0.05$ and ** $p < 0.01$ when control rats were compared with 3-AP-treated animals

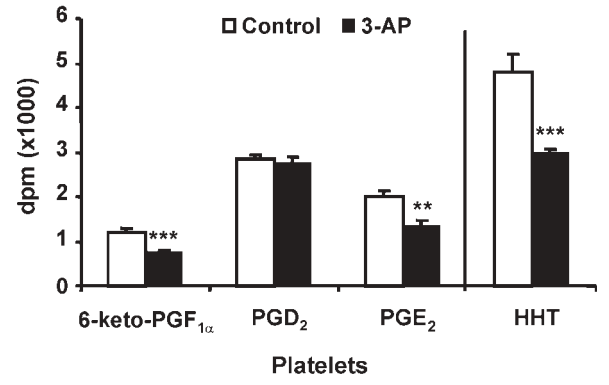

Fig. 4. Comparison of platelet 6-keto-$PGF_{1\alpha}$, PGD_2, PGE_2 and 12-HHT synthesis in control (□) (n = 9) and 3-AP-treated (■) (n = 11) rats. Data represent the means ± SEM; ** $p < 0.01$ and *** $p < 0.001$ when control rats were compared with 3-AP-treated animals

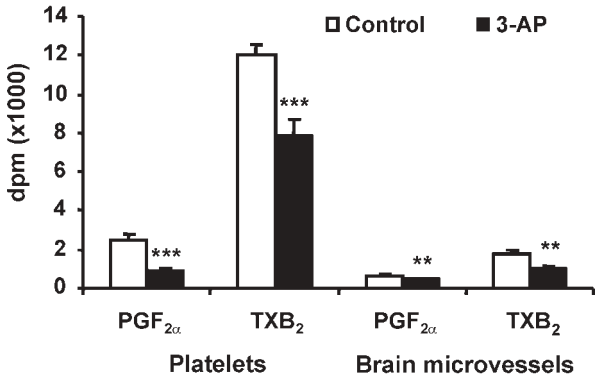

Fig. 6. Comparison of platelet and brain microvessel $PGF_{2\alpha}$ and TxB_2 synthesis in control (□) (n = 9 and n = 7 for platelet and brain microvessels, respectively) and 3-AP-treated (■) (n = 11) rats. Data represent the means ± SEM; ** $p < 0.01$ and *** $p < 0.001$ when control rats were compared with 3-AP-treated animals

63.28 ± 4.1 for control and 3-AP-treated rats, respectively). The reduction in COX-mediated eicosanoid synthesis after chronic 3-AP treatment was due to decreased synthesis of both the DIL (20.0%) and the CON metabolites (38.5%) (Fig. 3). Indeed, 3-AP treatment reduced the production of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) (37.0%), PGE_2 (33.3%) and 12-HHT (37.4%) synthesis. Interestingly, recurrent seizures did not induce alteration in platelet PGD_2 production (in 1,000 dpm: 2.84 ± 0.11 and 2.74 ± 0.14 for control and 3-AP-treated rats, respectively) (Fig. 4). The amount of $PGF_{2\alpha}$ (60.6%) and TxB_2 (the stable metabolite of TxA_2) (34.0%) was also decreased upon 3-AP administration (Fig. 6).

Cerebral microvessels

In isolated brain capillaries, chronic 3-AP treatment did not change the total amount of AA metabolites (in 1,000 dpm: 72.77 ± 1.23 and 74.39 ± 5.54 for control and 3-AP-treated rats, respectively) (Fig. 1). Similarly to platelets, the synthesis of COX products (Fig. 2) was reduced (35.7%), but no significant change in LOX products (in 1,000 dpm: 65.78 ± 1.2 and 69.89 ±

5.45 for control and 3-AP-treated rats, respectively) was observed. The reduction in COX-mediated eicosanoid synthesis after chronic 3-AP treatment was due to decreased synthesis of both the DIL (32.2%) and the CON (36.9%) metabolites (Fig. 3). Indeed, 3-AP treatment reduced PGD_2 (40.2%), PGE_2, (34.3%) and 12-HHT (38.1%) synthesis while the synthesis of 6-keto-$PGF_{1\alpha}$ remained unchanged (in 1,000 dpm: 0.67 ± 0.07 and 0.57 ± 0.03 for control and 3-AP-treated rats, respectively) (Fig. 5). Finally, the amount of $PGF_{2\alpha}$ (27.7%) and TxB_2 (40.9%) was also decreased upon 3-AP administration in brain capillaries (Fig. 6).

Discussion

Sufficient evidence has accumulated on the biological actions of platelets to consider them as important players in the disorders of the central nervous system, including epilepsy [39, 40, 49]. Indeed, it was hypothesized that high seizure activity induced compensatory alterations in platelet function, that may play a beneficial role in epileptogenesis [40]. It is also known that differences between the effect of single and recurrent seizures on neuronal function and presumably on PG synthesis may exist [4]. Since frequent seizures were suggested to induce changes in platelet function, we used the chronic 3-AP model to investigate the PG synthesis in platelets. The AA cascade was investigated one day after the last treatment, thus, any alteration in PG production was not due to acute convulsions, but was caused by changes in platelet function after recurrent seizures.

In the chronic 3-AP model, the earlier epileptic insults may offer a neuroprotective adaptation of the brain against subsequent seizures [24, 42]. In our experimental settings, the development of epileptic tolerance was manifested as a decreased incidence of limbic seizures and higher survival rate at later administration of 3-AP. After the first and second 3-AP treatment 69% and 36% of animals had limbic seizures, which led to the death of 13% and 21% of animals, respectively. During the last five 3-AP treatments, only 10–20% of animals had limbic seizures, and the seizures were not severe enough to induce the death of the animals. Although, the exact molecular and cellular mechanisms involved in the development of epileptic tolerance are not yet fully understood, the role of ATP-sensitive K^+ channels [21, 38] and heat shock proteins [47] is presumed. It was demonstrated that pretreatment with polyunsaturated fatty acids (PUFA) could provide a potent neuroprotective adaptation of the brain against epileptic seizures [7]. Considering this finding and our present results, one may suggest that decreased conversion of PUFAs to PGs may contribute to the mechanisms of epileptic tolerance.

Our results demonstrate that chronic 3-AP treatment alters the formation of AA metabolites in rats. In platelets, recurrent seizures reduced the total production of AA metabolites and this reduction derived from a decreased synthesis of DIL and CON COX products. In isolated brain capillaries, despite the similar reduction of DIL and CON COX production by chronic 3-AP treatment**,** the total amount of AA metabolites remained unchanged. In platelets among the vasodilator and platelet antiaggregatory COX products, the formation of PGI_2, PGE_2 and 12-HHT was decreased, whereas the amount of PGD_2 remained unchanged after 3-AP treatment. In brain microvessels, recurrent seizures induced a decreased synthesis of PGD_2, PGE_2 and 12-HHT. As for the individual CON COX metabolites, both the platelets and the brain microvessels synthesized significantly lesser amount of $PGF_{2\alpha}$ and TxA_2 after chronic administration of 3-AP. In contrast, chronic 3-AP treatment did not alter the total amount of LOX metabolites either in platelets or in brain microvessels. However, further investigations are needed to elucidate whether 3-AP-induced seizures influence the availability of AA in the membrane, or changes the activity of phospholipase A_2 and COX.

Previously, it was found that primary cultures of rat cerebral neurons lack the ability to desaturate fatty acids [33], therefore, AA and other PUFAs must be provided by the bloodstream, cerebromicrovascular endothelial cells or astroglial cells [23]. Accordingly, one might suggest that any alterations in the AA cascade of platelets and brain microvessels, through the alteration of extracellular release of AA or other PUFAs into the bloodstream and interstitial space, may influence the AA supply to the neurons and have an impact on neuronal PG synthesis and excitability. In the present study, we demonstrated that conversion of AA into different COX metabolites was decreased both in platelets and brain capillaries 24 h after the last induction of seizures. However, the question

whether the development of epileptic tolerance, induced by recurrent seizures, is due to a decreased conversion of PUFAs to PG or to an increased intercellular exchange of PUFAs, remains to be elucidated.

Here, we demonstrate for the first time that the 3-AP-induced reduction in the total amount of CON PGs was similar in platelets (38.5%) and in brain microvessels (37.1%). In contrast, the decrease in the synthesis of DIL PGs induced by recurrent seizures was different in platelets (19.9%) and in brain capillaries (32.3%). This difference between platelet and brain microvessels was due to the fact that chronic 3-AP treatment did not reduce PGD_2 production in platelets (3.6%), while it decreased it in brain capillaries (40.4%). Interestingly, PGD_2 is the most abundant PG during seizures [16, 34], and the most promising candidate for a possible anticonvulsant effect of prostanoids [2]. Since preserved platelet PGD_2 production may improve oxygen supply to the neurons and decrease neuronal excitability, it is conceivable that platelets may play a compensatory role in recurrent seizures.

The present results indicate that recurrent seizures did not modify the LOX pathway in platelets and brain microvessels, but decreased the total amount of DIL as well as CON COX products. The decreased conversion of AA into different COX products may play a role in the neuroprotective/preconditional adaptation of the brain against the subsequent seizures.

Acknowledgments:
This work was supported by grants from OTKA 017 724, ETT 48/2000, RET (0814OMFB 066/2005) and Öveges (HEF 06 1-MEZEI ZS).

References:

1. Abdel-Halim MS, Lundén I, Cseh G, Anggard E: Prostaglandin profiles in nervous tissue and blood vessels of the brain of various animals. Prostaglandins, 1980, 19, 249–258.
2. Akarsu ES, Mamuk S, Comert A: Inhibition of pentylenetetrazol-induced seizures in rats by prostaglandin D_2. Epilepsy Res, 1998, 30, 63–68.
3. Aranda JV, Beharry K, Sasyniuk B, Chemtob S: The role of prostanoids in neonatal cerebral blood flow autoregulation. J Lipid Mediat, 1993, 6, 493–501.
4. Baram TZ: Long-term neuroplasticity and functional consequences of single versus recurrent early-life seizures. Ann Neurol, 2003, 54, 701–705.
5. Bazan NG, Birkle DL, Tang W, Reddy TS: The accumulation of free arachidonic acid, diacylglycerols, prostaglandins, and lipoxygenase reaction products in the brain during experimental epilepsy. Adv Neurol, 1986, 44, 879–902.
6. Berchtold-Kanz E, Anhut H, Heldt R, Neufang B, Hertting G: Regional distribution of arachidonic acid metabolites in rat brain following convulsive stimuli. Prostaglandins, 1981, 22, 65–79.
7. Blondeau N, Widmann C, Lazdunski M, Heurteaux C: Polyunsaturated fatty acids induce ischemic and epileptic tolerance. Neurosci, 2002, 109, 231–241.
8. Bosetti F, Weerasinghe GR, Rosenberger TA, Rapoport SI: Valproic acid down-regulates the conversion of arachidonic acid to eicosanoids via cyclooxygenase-1 and -2 in rat brain. J Neurochem, 2003, 85, 690–696.
9. Busija DW, Leffler CW: Role of prostanoids in cerebrovascular responses during seizures in piglets. Am J Physiol, 1989, 256, 120–125.
10. Camacho M, Vila L: Transcellular formation of thromboxane A(2) in mixed incubations of endothelial cells and aspirin-treated platelets strongly depends on the prostaglandin I-synthase activity. Thromb Res, 2000, 99, 155–164.
11. Chang MC, Contreras MA, Rosenberger TA, Rintala JJ, Bell JM, Rapoport SI: Chronic valproate treatment decreases the *in vivo* turnover of arachidonic acid in brain phospholipids: a possible common effect of mood stabilizers. J Neurochem, 2001, 77, 796–803.
12. Chen C, Bazan NG: Endogenous PGE_2 regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. J Neurophysiol, 2005, 93, 929–941.
13. Dahl ML, Uotila P: Verapamil decreases the formation of thromboxane from exogenous ^{14}C-arachidonic acid in human platelets *in vitro*. Prostaglandins Leukot Med, 1985, 17, 191–198.
14. Ferrarese C, Begni B, Canevari C, Zoia C, Piolti R, Frigo M, Appollonio I et al.: Glutamate uptake is decreased in platelets from Alzheimer's disease patients. Ann Neurol, 2000, 47, 641–643.
15. Ferrarese C, Zoia C, Pecora N, Piolti R, Frigo M, Bianchi G, Sala G et al.: Reduced platelet glutamate uptake in Parkinson's disease. J Neural Transm, 1999, 106, 685–692.
16. Förstermann U, Heldt R, Hertting G: Effects of intracerebroventricular administration of prostaglandin D_2 on behaviour, blood pressure and body temperature as compared to prostaglandins E_2 and $F_{2\alpha}$. Psychopharmacology, 1983, 80, 365–370.
17. Forstermann U, Heldt R, Hertting G: Increase in brain prostaglandins during convulsions is due to increased neuronal activity and not to hypoxia. Arch Int Pharmacodyn Ther, 1983, 263, 180–188.
18. Gecse A, Ottlecz A, Mezei Z, Telegdy G, Joó F, Dux E, Karnushina I: Prostacyclin and prostaglandin synthesis in isolated brain capillaries. Prostaglandins, 1982, 23, 287–297.
19. Hayaishi O: Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D_2 and E_2. FASEB J, 1991, 5, 2575–2581.

20. Hertting G, Seregi A, Forstermann U: Formation and functions of prostaglandins in the central nervous system in rodents. Adv Prostaglandin Thromboxane Leukot Res, 1985, 15, 573–576.
21. Heurteaux C, Lauritzen I, Widmann C, Lazdunski M: Essential role of adenosine, adenosine A_1 receptors and KATP channels in cerebral ischemic preconditioning. Proc Natl Acad Sci USA, 1995, 92, 4666–4670.
22. Hwang MS, Weiss S, Segal S: Uptake of L-(35S) cystein by isolated rat brain capillaries. J Neurochem, 1980, 35, 417–424.
23. Katsuki H, Okuda S: Arachidonic acid as a neurotoxic and neurotrophic substance. Prog Neurobiol, 1995, 46, 607–636.
24. Kelly ME, McIntyre DC: Hippocampal kindling protects several structures from the neuronal damage from kainic acid-induced status epilepticus. Brain Res, 1994, 634, 245–256.
25. Khan WA, Blobe GC, Hannun YA: Activation of protein kinase C by oleic acid. Determination and analysis of inhibition by detergent micelles and physiologic membranes: requirement for free oleate. J Biol Chem, 1992, 267, 3605–3612.
26. Kiefer HC, Johson CR, Arora KL: Colorimetric identification of prostaglandins in subnanomole amounts. Anal Biochem, 1975, 68, 336–340.
27. Kim EJ, Kwon KJ, Park JY, Lee SH, Moon CH, Baik EJ: Neuroprotective effects of prostaglandin E_2 or cAMP against microglial and neuronal free radical mediated toxicity associated with inflammation. J Neurosci Res, 2002, 70, 97–107.
28. Kis B, Gáspár T, Mezei Z, Gecse A, Telegdy G: Pituitary adenylate cyclase-activating polypeptide inhibits the cyclooxygenase pathway of rat cerebral microvessels. Acta Physiol Scand, 1999, 167, 43–47.
29. Kobayashi T, Levine L: Arachidonic acid metabolism by erythrocytes. J Biol Chem, 1983, 258, 9116–9121.
30. Mezei Zs, Kis B, Gecse Á, Tajti J, Boda B, Telegdy Gy, Vécsei L: Platelet arachidonate cascade of migraineurs in the interictal phase. Platelets, 2000, 11, 222–225.
31. Mezei Z, Kis B, Gecse Á, Telegdy Gy, Ábrahám Gy, Sonkodi S: Platelet eicosanoids and the effect of captopril in blood pressure regulation. Eur J Pharmacol, 1997, 340, 67–73.
32. Młodzikowska-Albrecht J, Steinborn B, Żarowski M: Cytokines, epilepsy and antiepileptic drugs – is there a mutual influence? Pharmacol Rep, 2007, 59, 129–138.
33. Moore SA, Yoder E, Murphy S, Dutton GR, Spector AA: Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). J Neurochem, 1991, 56, 518–524.
34. Naffah-Mazzacoratti MG, Bellissimo MI, Cavalheiro EA: Profile of prostaglandin levels in the rat hippocampus in pilocarpine model of epilepsy. Neurochem Int, 1995, 27, 461–466.
35. Nozawa Y, Nakashima S, Nagata K: Phospholipid-mediated signaling in receptor activation of human platelets. Biochim Biophys Acta, 1991, 1082, 219–238.
36. Parfenova H, Shibata M, Zuckerman S, Leffler CW: CO_2 and cerebral circulation in newborn pigs: cyclic nucleotides and prostanoids in vascular regulation. Am J Physiol, 1994, 266, 1494–1501.
37. Phillis JW, Horrocks LA, Farooqui AA: Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: Their role and involvement in neurological disorders. Brain Res Rev, 2006, 52, 201–243.
38. Plamondon H, Blondeau N, Heurteaux C, Lazdunski M: Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A_1 receptors and KATP channels. J Cereb Blood Flow Metab, 1999, 19, 1296–1308.
39. Rainesalo S, Eriksson K, Saransaari P, Keranen T: Uptake of GABA and activity of GABA transaminase in blood platelets from children with absence epilepsy. Neurochem Res, 2004, 29, 1873–1877.
40. Rainesalo S, Keranen T, Peltola J, Saransaari P: Glutamate uptake in blood platelets from epileptic patients. Neurochem Int, 2003, 43, 389–392.
41. Rosenkranz RP, Killam KF Jr: Potentiation of pentylenetetrazole and electroshock induced seizures by prostaglandin $F_{2\alpha}$. Proc West Pharmacol Soc, 1979, 22, 43–45.
42. Sasahira M, Lowry T, Simon RP, Greenberg DA: Epileptic tolerance: prior seizures protect against seizure-induced neuronal injury. Neurosci Lett, 1995, 185, 95–98.
43. Siesjö BK: Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanisms of damage and treatment. J Neurosurg, 1992, 77, 337–354.
44. Szupera Z, Mezei Z, Kis B, Gecse A, Vécsei L, Telegdy G: The effects of valproate on the arachidonic acid metabolism of rat brain microvessels and of platelets. Eur J Pharmacol, 2000, 387, 205–210.
45. Wallenstein MC: Attenuation of penicillin models of epilepsy by nonsteroidal anti-inflammatory drugs. Exp Neurol, 1987, 98, 152–160.
46. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. Circ Res, 1980, 47, 1–9.
47. Welch WJ: Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. Physiol Rev, 1992, 72, 1063–1081.
48. Wolfe LS: Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. J Neurochem, 1982, 38, 1–14.
49. Yogeeswari P, Sriram D, Vaigundaragavendran J: The GABA shunt: an attractive and potential therapeutic target in the treatment of epileptic disorders. Curr Drug Metab, 2005, 6, 127–139.
50. Zhang X, Gelowitz DL, Lai CT, Boulton AA, Yu PH: Gradation of kainic acid-induced rat limbic seizures and expression of hippocampal heat shock protein-70. Eur J Neurosci, 1997, 9, 760–769.

Received:
June 26, 2007; in revised form: January 13, 2008.