European Journal of Neuroscience, Vol. 13, pp. 2319–2323, 2001

Phospholipase A_2 mediates ischemic injury in the hippocampus: a regional difference of neuronal vulnerability

Ken Arai,[1] Yuji Ikegaya,[1] Yoshihito Nakatani,[2] Ichiro Kudo,[2] Nobuyoshi Nishiyama[1] and Norio Matsuki[1]
[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The Universe of Tokyo, 7–3-1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan
[2]Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University, Tokyo 142–8555, Japan

Abstract

Although it is well known that the hippocampal CA1 subfield is highly vulnerable to ischemic injury, cellular mechanisms leading to this neuronal degeneration are not fully understood. Using organotypic cultures of rat hippocampal slices, we determined whether phospholipase A_2 (PLA_2) is activated in response to ischemic conditions (OGD; oxygen and glucose deprivation). The PLA_2 activity in the pyramidal cell layer increased immediately following a 35-min exposure to OGD, which was likely to be mediated by selective activation of cytosolic Ca^{2+}-dependent PLA_2 subtype ($cPLA_2$). This enhancement lasted for at least 24 h. Interestingly, no apparent increase was detected in the dentate gyrus. Twenty-four hours after the OGD exposure, neuronal death was detected mainly in the CA1 region of hippocampal slices. To examine whether the PLA_2 activation is causally or protectively involved in the ischemic injury, we investigated the effect of pharmacological blockade of PLA_2 on the OGD-induced neuronal death. The PLA_2 inhibitor bromophenacyl bromide efficiently prevented the cell death in a concentration-dependent manner. Similar results were obtained for the selective $cPLA_2$ inhibitor $AACOCF_3$. However, the Ca^{2+}-independent PLA_2 inhibitor bromoenol lactone and the secretory PLA_2 inhibitor LY311727 were virtually ineffective. These results suggest that $cPLA_2$ plays a causative role in the neuronal death following OGD exposure. Thus, the present study may provide novel therapeutic targets for the development of neuroprotective agents.

Keywords: arachidonic acid, hippocampus, organotypic slice cultures, oxygen/ glucose deprivation, phospholipase A_2

Introduction

In cerebral ischemia, a reduction in the supply of glucose and oxygen leads to a complex cascade of cellular events, eventually resulting in neuronal death (Siesjo, 1992a, 1992b). Both clinical and experimental studies on transient forebrain ischemia revealed that the hippocampus, particularly the CA1 subfield, is highly vulnerable to ischemic injury (Schmidt-Kastner & Freund, 1991; Diemer *et al*., 1993). However, mechanisms underlying this regional specificity in vulnerability remain to be determined.

Arachidonic acid and its metabolites, e.g. prostaglandins, leukotrienes and other eicosanoids, exert a variety of neuromodulatory actions in the central nervous system such as fever generation, sleep–wakefulness regulation, pain and olfactory sensation and neuroendocrine regulation (Shimizu & Wolfe, 1990; Katsuki & Okuda, 1995; Matsumura *et al*., 1998). The initial step of the arachidonate cascade is mediated by phospholipase A_2 (PLA_2) that hydrolyses the *sn*-2 fatty acyl ester bond of glycerophospholipids to release free arachidonic acid and lysophospholipids. At present, mainly three types of PLA_2s are identified, i.e. cytosolic Ca^{2+}-dependent PLA_2 ($cPLA_2$), cytosolic Ca^{2+}-independent PLA_2 ($iPLA_2$), and the secretory form of PLA_2 ($sPLA_2$) (Dennis, 1997).

Correspondence: Yuji Ikegaya, as above.
E-mail: ikegaya@tk.airnet.ne.jp

Received 2 February 2001, revised 1 May 2001, accepted 3 May 2001

The hippocampus is a prominent site of expression of PLA_2 (Molloy *et al*., 1998; Kishimoto *et al*., 1999), and the release of arachidonic acid from membrane phospholipids and subsequent initiation of the arachidonic acid cascade are a well-documented observation following transient global ischemia (Rehncrona *et al*., 1982; Abe *et al*., 1987; Hsu *et al*., 1989). However, it remains unclear how these PLA_2 metabolites contributes to ischemic injury. We have focused the present study on the role of PLA_2 in ischemia-induced neuronal death. Using organotypic cultures of hippocampal slices, this work shows for the first time that ischemic conditions cause an increase in hippocampal PLA_2 activity, and also that PLA_2 inhibitors attenuated the ischemic injury in the hippocampus.

Materials and methods

Hippocampal slice cultures were prepared from 9-day-old Wistar/ST rat (SLC, Shizuoka, Japan), essentially as described (Ikegaya, 1999). Animals were deeply anaesthetized by hypothermia, and their brains were aseptically removed and cut into transverse slices (300 μm thick) in aerated ice-cold Gey's balanced salt solution supplemented with 6.5 mg/mL glucose using a vibratome (DTK-1500; Dosaka EM, Kyoto, Japan). The entorhino-hippocampi were dissected out under stereomicroscopic control. Then, selected slices were cultured using the membrane interface techniques (Stoppini *et al*., 1991). Cultures were fed with 1 mL of culture medium consisting of 50% minimal essential medium (Life Technologies, Grand Island, NY, USA), 25% horse serum (Cell Culture Laboratory, Cleveland, OH, USA), and 25% Hanks' balanced salt solution (HBSS), and were maintained in a humidified incubator at 37 °C in 5% CO_2, and the medium was changed every 3 days. The cultures were used at 12–13 days *in vitro*.

Combined oxygen-glucose deprivation (OGD/ischemia) experiments were performed in a chamber containing an anaerobic gas mixture (95% N_2 and 5% CO_2). The culture medium was replaced with deoxygenated glucose-free HBSS. During the deprivation procedure, cells were placed in a humidified 37 °C incubator for 35 min. Deprivation was terminated by replacing the exposure medium with normal medium containing 0.5 µg/mL propidium iodide (PI) (Molecular Probes, Eugene, OR, USA). The cultures were then incubated in a CO_2 incubator at 37 °C for 24 h. Fluorescence images of PI were obtained with the Bio-Rad MRC-600 confocal imaging system (Bio-Rad Microscience Division, Cambridge, MA, USA). Pixel fluorescence intensity of 8-bit resolution was measured at three different areas of the slice, i.e. the CA1 and CA3 stratum pyramidale and the stratum granulosum of the dentate gyrus (DG). Average intensity (F_t) was estimated for each slice by acquiring values in five different areas ($5 \times 82.5\ \mu m^2$) within each hippocampal subregion. Simultaneously, the background intensity (F_0) was obtained outside the slices. Forty-eight hours after OGD insult, all cells were killed by 24-h incubation at a low temperature (4 °C), and then the final PI fluorescence (F_{fin}) was measured. PI uptake was determined as $(F_t - F_0)/(F_{fin} - F_0) \times 100\%$.

PLA_2 activity was assayed by measuring the amounts of free radiolabelled fatty acids released from the substrates 1-palmitoyl-2-[^{14}C]arachidonoyl-sn-glycero-3-phosphoethanolamine (Amersham Pharmacia, Arlington Heights, IL, USA) (Horigome *et al*., 1987). The pyramidal cell layer and the granule cell layer were dissected out immediately and 24 h after OGD, and lysed. Reaction mixture consisted of an aliquot of the required sample, 100 mM Tris-HCl, pH 7.4, 4 mM $CaCl_2$,and 2 µM substrate. After 30 min incubation in the absence or presence of PLA_2 inhibitors at 37 °C, the ^{14}C-fatty acids released were extracted by Dole's method (Horigome *et al*., 1987), and the radioactivity was counted.

p-Bromophenacyl bromide (BPB), bromoenol lactone (BEL), ibuprofen and nordihydroguaiaretic acid (NDGA) were purchased from Wako Pure Chemicals (Osaka, Japan). Arachidonyl trifluoromethyl ketone ($AACOCF_3$) was purchased from Biomol Res. Laboratory (Plymouth Meeting, PA, USA). LY311727 was obtained as a gift from E. Mihelich (Eli Lilly and Company, Indianapolis, IN, USA). These drugs were added to culture medium from 40 min before until 40 min after the OGD.

All data are expressed as means ± SEM. Statistical significance was evaluated by Tukey's multiple range test following one-way ANOVA. Differences were considered significant if $P < 0.05$.

Results

Although a number of studies have shown the release of arachidonic acid following transient global ischemia (Rehncrona *et al*., 1982; Abe *et al*., 1987; Hsu *et al*., 1989), there has been no indication that ischemic conditions actually activate PLA_2 in the hippocampus. Therefore, we first evaluated OGD-induced changes in PLA_2 activity in organotypic cultures of hippocampal slices (Fig. 1).

Baseline PLA_2 activity was not equal in the pyramidal cell layer of Ammon's Horn and the granule cell layer of the DG. The activity in the DG was approximately twice as high as in Ammon's Horn.

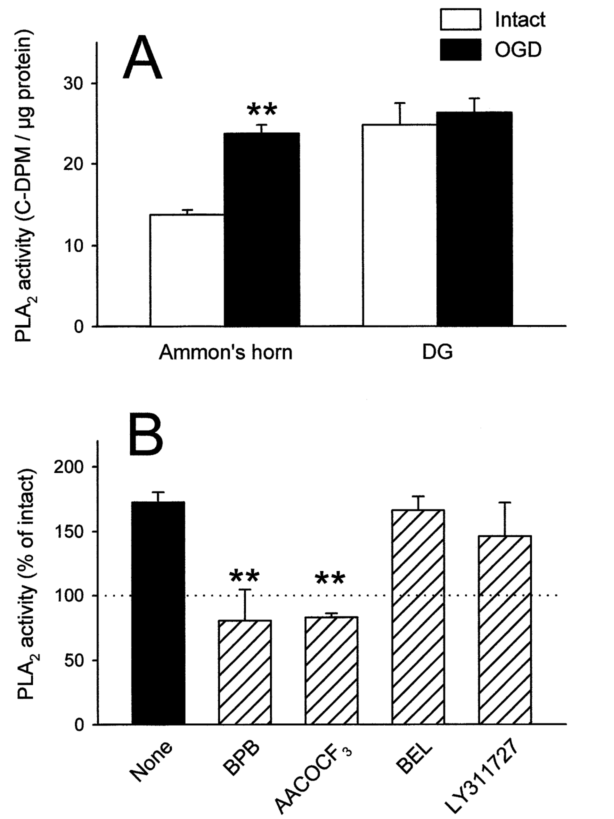

FIG. 1. OGD-induced $cPLA_2$ activation in hippocampal slice cultures. (A) PLA_2 activity was assessed immediately after hippocampal slices were exposed to OGD for 35 min. OGD caused a 2-fold increase in PLA_2 activity in the pyramidal cell layer of the Ammon's horn, but no change was observed in the activity in the granule cell layer of the DG. $^{**}P < 0.01$ vs. Intact. (B) PLA_2 activity was measured in the presence of diverse inhibitors of PLA_2, i.e. 10 µM BPB, 10 µM $AACOCF_3$, 10 µM BEL and 10 µM LY311727. BPB and $AACOCF_3$ effectively blocked OGD-increased PLA_2 activity. The ordinate is expressed as a percentage of baseline PLA_2 activity obtained from intact slices. Data represent means ± SEM (12 slices). $^{**}P < 0.01$ vs. None.

Exposure to OGD induced an ≈ 2-fold increase in the PLA_2 activity of Ammon's Horn whilst it did not affect that of the DG in the same cultures. The PLA_2 activity in Ammon's Horn remained enhanced until at least 24 h after OGD exposure (data not shown). This increase in PLA_2 activity was blocked by BPB, a broad-spectrum inhibitor of PLA_2 (Fig. 1B). To determine which subtype of PLA_2 was activated by OGD exposure, we examined the effect of three selective inhibitors of PLA_2. The OGD-increased activity was prevented by the selective $cPLA_2$ inhibitor $AACOCF_3$, but not by the selective $iPLA_2$ inhibitor BEL or the selective $sPLA_2$ inhibitor LY311727. The data suggest that PLA_2 activation following OGD exposure is almost fully attributable to changes in $cPLA_2$ activity.

The cell death in cultured hippocampal slices was assessed by PI fluorescence (Macklis & Madison, 1990) (Fig. 2). Although faint PI uptake was detected in intact slices, evident PI fluorescent signals appeared 24 h after OGD exposure. A significant increase in PI uptake was observed in the pyramidal cell layer of the CA1 and CA3 subregions but the neurons in the DG were relatively resistant to the OGD insult. The order of hippocampal vulnerability was the CA1 region, the CA3 region and the DG, which closely resembles a rank of vulnerability in cerebral ischemia *in vivo*. Therefore, we investigated the involvement of PLA_2 in the OGD-induced neuronal death.

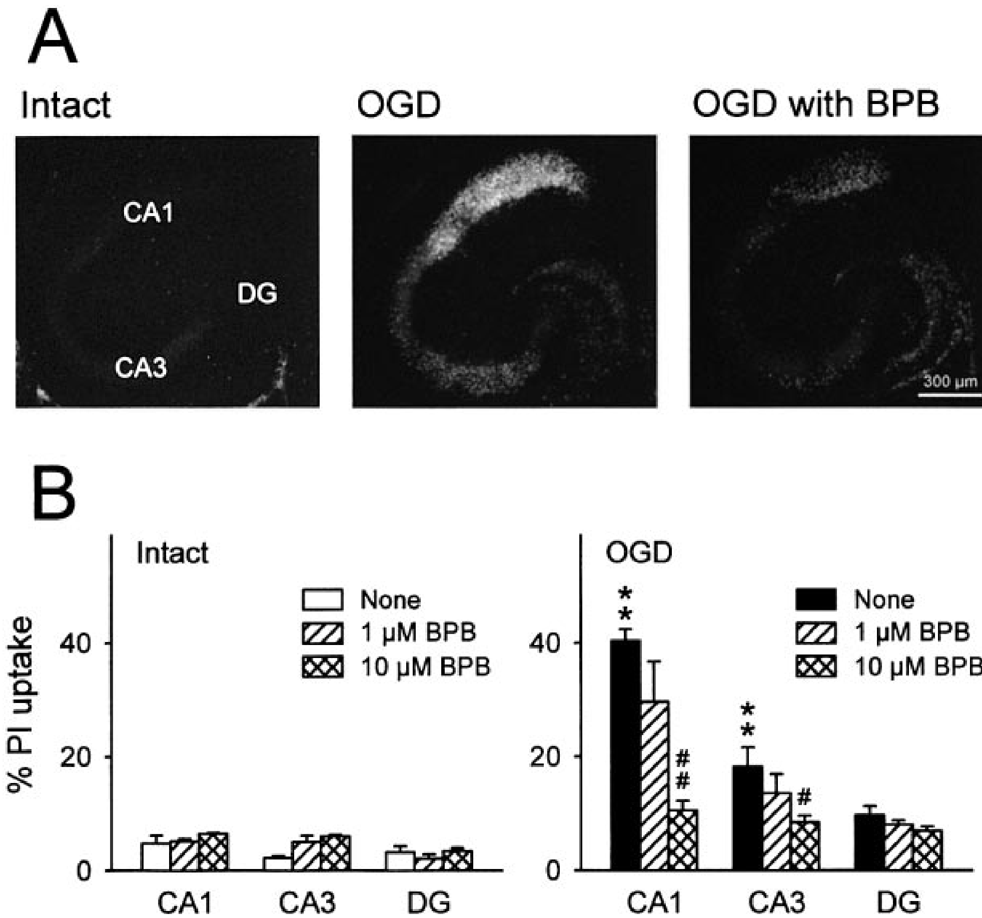

FIG. 2. PLA_2 activity mediates OGD-induced neuronal damage in hippocampal slice cultures. (A) Representative confocal images of PI fluorescence show the untreated slice (intact) and the slices that received OGD exposure in the absence (OGD) or presence (OGD with BPB) of BPB. (B) Inhibitory effects of BPB on OGD-induced cell death. PI uptake was quantified in the pyramidal cell layer of the CA1 and CA3 region, and the granule cell layer in the DG. Data were expressed as means ± SEM of eight slices. $^{**}P < 0.01$ vs. Intact/None, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. OGD/None.

The cell death was efficiently attenuated by BPB in a concentration-dependent manner, whilst BPB *per se* had no apparent effect on the cell survival in intact slices (Fig. 2). This result indicates that PLA_2 activity contributes causally to hippocampal ischemic injury. Thus, we examined which subtype of PLA_2 mediates the OGD-induced neuronal death. Pharmacological blockade of $cPLA_2$ by $AACOCF_3$ significantly inhibited the neuronal death in the CA1 region (Fig. 3A). BEL or LY311727 was virtually ineffective. Similar results were obtained for OGD-induced neuronal death in the CA3 region (data not shown). Incidentally, none of these inhibitors alone affected PI uptake in intact slices (data not shown). Therefore, $cPLA_2$ is likely to play a pivotal role in hippocampal ischemic injury.

Although arachidonic acid is released from membrane phospholipids in response to ischemic stimulus, it rapidly decreases during postischemic period, as compared with other PLA_2 products such as free fatty acids and lysophospholipids (Yoshida *et al*., 1986), which suggests that the ischemic stimulus also facilitates arachidonate metabolisms, probably via cyclooxygenase and lipoxygenase. We therefore addressed the possible involvement of cyclooxygenase and lipoxygenase in the OGD insult. The cyclooxygenase inhibitor ibuprofen did not protect against the neuronal death, whereas the lipoxygenase inhibitor NDGA significantly reduced the OGD-elicited neuronal damage (Fig. 3B).

Discussion

Although the involvement of PLA_2 in ischemic injury of the hippocampus has received much attention in recent years (Shimizu & Wolfe, 1990; Katsuki & Okuda, 1995), there has been no direct evidence for the correlation between PLA_2 and neuronal degeneration. Using organotypic cultures, we have shown for the first time that OGD exposure causes an increase in hippocampal PLA_2 activity, and also that blockade of PLA_2 activity during OGD exposure improves survival of hippocampal neurons.

Because our organotypic cultures were essentially tiny in size, we were unable to precisely assess the PLA_2 activity when the CA1 and CA3 regions were isolated. Therefore, we measured the total PLA_2 activity in the whole Ammon's horn. Thus, we could not determine whether the OGD-induced PLA_2 activation reflects a change in either one or both of the CA1 and CA3 regions. Actually, the CA1 region is more vulnerable to OGD injury than is the CA3 region, but the PLA_2 inhibitors prevented neuronal damages equally in the CA1 and CA3 regions. Therefore, we consider that the PLA_2 activation is involved in neuronal death of the CA3 region as well as the CA1 regions. For the same reason, we could not examine whether PLA_2 plays a similar role in the hilus of the DG, although this part of the DG is known to be a region showing the highest ischemic vulnerability. Whether or

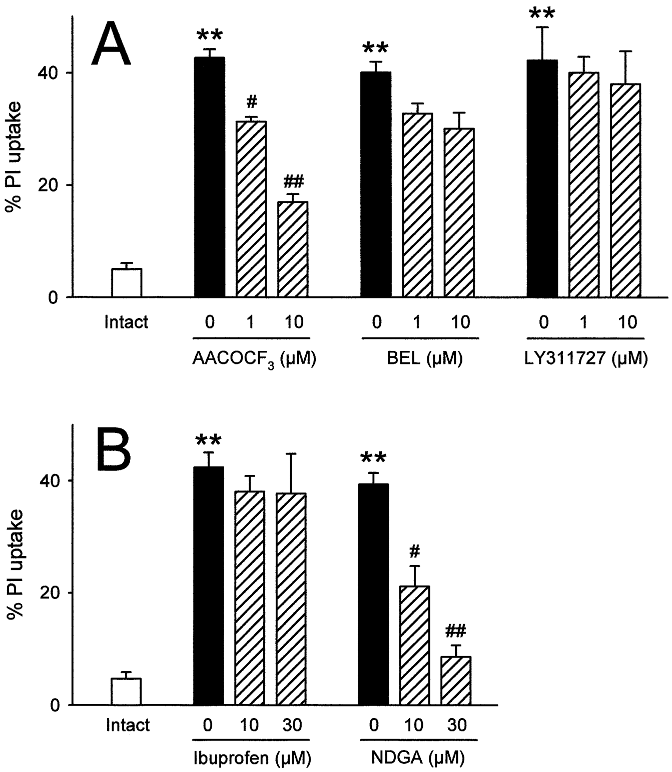

FIG. 3. Effects of various inhibitors on OGD-induced neuronal death in hippocampal slice cultures. PI uptake was assessed in the CA1 pyramidal cell layer 24 h after OGD insult. Open columns represent intact slices. Closed columns and hatched columns represent the slices exposed to OGD alone or in the presence of inhibitors, respectively. (A) Effects of PLA_2 inhibitors on the OGD injury. OGD-induced neuronal death was significantly attenuated by $AACOCF_3$. (B) Effects of inhibitors of arachidonate cascades on the OGD injury. OGD-induced neuronal death was effectively prevented by NDGA. Data were means ± SEM of eight slices. $^{**}P < 0.01$ vs. Intact; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. 0 μM of corresponding inhibitors.

not PLA_2 mediates ischemia-induced neuronal death in the hilus requires further investigation.

Several reports showed a dramatic increase in expression of PLA_2 mRNA after transient forebrain ischemia (Lauritzen *et al*., 1994; Owada *et al*., 1994); therefore it is likely that the increases in PLA_2 activity participates in the later stage of ischemic injury. However, we showed that the PLA_2 activity is enhanced even immediately after OGD insult. The result suggests a post-translational modification of pre-existing PLA_2 proteins. $cPLA_2$ activity depends on the concentration of Ca^{2+} and the phosphorylation (Clark *et al*., 1991; Lin *et al*., 1993; Muthalif *et al*., 1996). Such modulations of the enzyme activity may be involved in the rapid activation of PLA_2 following ischemia. On the other hand, glutamate receptor activation induces a rapid increase in release of arachidonic acid, which is assumed to contribute to the induction of hippocampal synaptic plasticity (Dumuis *et al*., 1988; Drapeau *et al*., 1990; Bramham *et al*., 1994). Thus, it is possible that the PLA_2 activation under ischemic conditions involves a common molecular mechanism for synaptic plasticity.

A number of studies addressed the contribution of arachidonic acid and its metabolites to neuronal death or survival. In primary cultures of hippocampal neurons, arachidonic acid at a low concentration (1 μM) exerts its trophic effect but shows profound severe toxicity at concentrations over 5 μM (Okuda *et al*., 1994). Recently, McGinty *et al*. (2000) indicated that cyclooxygenase-2-derived prostaglandins probably serve as a protective mediator in PC12 cells. In contrast, Nakayama *et al*. (1998) showed harmful effects of cyclooxygenase-2 and prostaglandin E_2 on hippocampal neuron survival. Furthermore, Murphy *et al*. (1989) suggested that lipoxygenase-derived metabolites mediate glutamate-induced toxicity in N18-RE-105 neuronal cell line. These complicated observations make it difficult to conclude whether PLA_2 activation contributes to cell death or survival. However, most important is the eventual consequence at tissue levels rather than the effect of each mediator on individual cells. Here we have shown that blockade of PLA_2 prevents neuronal death in hippocampal organotypic cultures, suggesting that, at least from a macroscopic viewpoint (tissue levels), PLA_2 activation exerts aversive effects on neuron survival under ischemic conditions. In this context, the result that the PLA_2 activity was unchanged in the DG following OGD exposure may explain the minimal ischemic damage to DG neurons. On the other hand, considering the high level of baseline PLA_2 activity in the DG, the DG neurons may be intrinsically equipped with protective machineries against the toxic effects of PLA_2 metabolites. Thus, elucidating mechanisms underlying the survival of DG neurons even under high levels of PLA_2 activity may provide a new therapeutic target for the development of neuroprotective agents.

Because arachidonic acid is known to modulate glutamate transporter activity in the central nervous system (Zerangue *et al*., 1995), it is possible that arachidonic acid itself directly triggers ischemic injury. However, our data showed that OGD-induced neuronal death was efficiently prevented by pharmacological blockade of lipoxygenase, but not of cyclooxygenase. Very recently, we also reported that 12-lipoxygenase may play a pivotal role in hippocampal ischemic injury (Arai *et al*., 2001). Indeed, the receptor for 12-HETE, one of the major 12-lipoxygenase metabolites, is shown to interact with steroid receptor coactivator-1 (Kurahashi *et al*., 2000), which may in turn be recruited by several nuclear receptors, e.g. oestrogen, glucocorticoid, progesterone, thyroid hormone, and the 9-*cis* retinoic acid receptor (Oñate *et al*., 1995). Importantly, all these steroid hormones are potent regulators of apoptosis in various types of cells (Kiess & Gallaher, 1998). Such steroid receptor recruitment may be involved in the PLA_2-mediated ischemic injury.

Abbreviations

$AACOCF_3$, arachidonyl trifluoromethyl ketone; BEL, bromoenol lactone; BPB, bromophenacyl bromide; $cPLA_2$, cytosolic Ca^{2+}-dependent cytosolic phospholipase A_2; DG, dentate gyrus; HBSS, Hanks' balanced salt solution; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; NDGA, nordihydroguaiaretic acid; OGD, oxygen-glucose deprivation; PI, propidium iodide; PLA_2, phospholipase A_2; $sPLA_2$, secretory form of phospholipase A_2

References

Abe, K., Kogure, K., Yamamoto, H., Imazawa, M. & Miyamoto, K. (1987) Mechanisms of arachidonic acid liberation during ischemia in gerbil cerebral cortex. *J. Neurochem.*, **48**, 503–509.

Arai, K., Nishiyama, N., Matsuki, N. & Ikegaya, Y. (2001) Neuroprotective effects of lipoxygenase inhibitors against ischemic injury in rat hippocampal slice cultures. *Brain Res.*, in press.

Bramham, C.R., Alkon, D.L. & Lester, D.S. (1994) Arachidonic acid and diacylglycerol act synergistically through protein kinase C to persistently enhance synaptic transmission in the hippocampus. *Neuroscience*, **3**, 737–743.

Clark, J.D., Lin, L.L., Kriz, R.M., Ramesha, C.S., Sultzman, L.A., Lin, A.Y., Milona, N. & Knopf, J.L. (1991) A novel arachidonic acid-selective cytosolic PLA_2 contains a Ca^{2+}-dependent translocation domain with homology to PKC and Gap. *Cell*, **65**, 1043–1051.

Dennis, E.A. (1997) The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem. Sci.*, **22**, 1–2.

Diemer, N.H., Johansen, F.F., Benveniste, H., Bruhn, T., Berg, M., Valente, E. & Jorgensen, M.B. (1993) Ischemia as an excitotoxic lesion: protection against hippocampal nerve cell loss by denervation. *Acta Neurochir. (Suppl.) (Wien)*, **57**, 94–101.

Drapeau, C., Pellerin, L., Wolfe. L.S. & Avoli, M. (1990) Long-term changes in synaptic transmission induced by arachidonic acid in the CA1 subfield of the rat hippocampus. *Neurosci. Lett.*, **115**, 286–292.

Dumuis, A., Sebben, M., Haynes, L., Pin, J.P. & Bockaert, J. (1988) NMDA receptors activate the arachidonic acid cascade system in striatal neurons. *Nature*, **336**, 69–70.

Horigome, K., Hayakawa, M., Inoue, K. & Nojima, S. (1987) Purification and characterization of phospholipase A2 released from rat platelets. *J. Biochem. (Tokyo)*, **101**, 625–631.

Hsu, C.Y., Liu, T.H., Xu, J., Hogan, E.L., Chao, J., Sun, G., Tai, H.H., Beckman, J.S. & Freeman. B.A. (1989) Arachidonic acid and its metabolites in cerebral ischemia. *Ann. N.Y. Acad. Sci.*, **559**, 282–295.

Ikegaya, Y. (1999) Abnormal targeting of developing hippocampal mossy fibers after epileptiform activities via L-type Ca^{2+} channel activation in vitro. *J. Neurosci.*, **19**, 802–812.

Katsuki, H. & Okuda, S. (1995) Arachidonic acid as a neurotoxic and neurotrophic substance. *Prog. Neurobiol.*, **46**, 607–636.

Kiess, W. & Gallaher, B. (1998) Hormonal control of programmed cell death/ apoptosis. *Eur. J. Endocrinol.*, **138**, 482–491.

Kishimoto, K., Matsumura, K., Kataoka, Y., Morii, H. & Watanabe, Y. (1999) Localization of cytosolic phospholipase A_2 messenger RNA mainly in neurons in the rat brain. *Neuroscience*, **92**, 1061–1077.

Kurahashi, Y., Herbertsson, H., Söderström, M., Rosenfeld, M.G. & Hammarström, S. (2000) A 12 (S) -hydroxyeicosatetraenoic acid receptor interacts with steroid receptor coactivator-1. *Proc. Natl. Acad. Sci. USA*, **97**, 5779–5783.

Lauritzen, I., Heurteaux, C. & Lazdunski, M. (1994) Expression of group II phospholipase A_2 in rat brain after severe forebrain ischemia and in endotoxic shock. *Brain Res.*, **651**, 353–356.

Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A. & Davis, R.J. (1993) $cPLA_2$ is phosphorylated and activated by MAP kinase. *Cell*, **72**, 269–278.

Macklis, J.D. & Madison, R.D. (1990) Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. *J. Neurosci. Meth.*, **31**, 43–46.

Matsumura, K., Cao, C., Watanabe, Y. & Watanabe, Y. (1998) Prostaglandin system in the brain: sites of biosynthesis and sites of action under normal and hyperthermic states. *Prog. Brain Res.*, **115**, 275–295.

McGinty, A., Chang, Y.W., Sorokin, A., Bokemeyer, D. & Dunn, M.J. (2000) Cyclooxygenase-2 expression inhibits trophic withdrawal apoptosis in nerve growth factor-differentiated PC12 cells. *J. Biol. Chem.*, **275**, 12095–12101.

Molloy, G.Y., Rattray, M. & Williams, R.J. (1998) Genes encoding multiple forms of phospholipase A_2 are expressed in rat brain. *Neurosci. Lett.*, **258**, 139–142.

Murphy, T., Parikh, A., Schnaar, R. & Coyle, J. (1989) Arachidonic acid metabolism in glutamate neurotoxicity. *Ann. NY Acad. Sci.*, **559**, 474–477.

Muthalif, M.M., Benter, I.F., Uddin, M.R. & Malik, K.U. (1996) Calcium/ calmodulin-dependent protein kinase IIα mediates activation of mitogen-activated protein kinase and cytosolic phospholipase A_2 in norepinephrine-induced arachidonic acid release in rabbit aortic smooth muscle cells. *J. Biol. Chem.*, **271**, 30149–30157.

Nakayama, M., Uchimura, K., Zhu, R.L., Nagayama, T., Rose, M.E., Stetler, R.A., Isakson, P.C., Chen, J. & Graham, S.H. (1998) Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. *Proc. Natl. Acad. Sci. USA*, **95**, 10954–10959.

Okuda, S., Saito, H. & Katsuki, H. (1994) Arachidonic acid: toxic and trophic effects on cultured hippocampal neurons. *Neuroscience*, **63**, 691–699.

Oñate, S.A., Tsai, S.Y., Tsai, M.J. & O'Malley, B.W. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science*, **270**, 1354–1357.

Owada, Y., Tominaga, T., Yoshimoto, T. & Kondo, H. (1994) Molecular cloning of rat cDNA for cytosolic phospholipase A_2 and the increased gene expression in the dentate gyrus following transient forebrain ischemia. *Mol. Brain Res.*, **25**, 364–368.

Rehncrona, S., Westerberg, E., Akesson, B. & Siesjo, B.K. (1982) Brain cortical fatty acids and phospholipids during and following complete and severe incomplete ischemia. *J. Neurochem.*, **38**, 84–93.

Schmidt-Kastner, R. & Freund, T.F. (1991) Selective vulnerability of the hippocampus in brain ischemia. *Neuroscience*, **40**, 599–636.

Shimizu, T. & Wolfe. L.S. (1990) Arachidonic acid cascade and signal transduction. *J. Neurochem.*, **55**, 1–15.

Siesjo, B.K. (1992a) Pathophysiology and treatment of focal cerebral ischemia. Part 1: Pathophysiology. *J. Neurosurg.*, **77**, 169–184.

Siesjo, B.K. (1992b) Pathophysiology and treatment of focal cerebral ischemia. Part 2: Mechanisms of damage and treatment. *J. Neurosurg.*, **77**, 337–354.

Stoppini, L., Buchs, P.A. & Muller, D. (1991) A simple method for organotypic cultures of nervous tissue. *J. Neurosci. Meth.*, **37**, 173–182.

Yoshida, S., Ikeda, M., Busto, R., Santiso, M., Martinez, E. & Ginsberg, M.D. (1986) Cerebral phosphoinositide, triacylglycerol and energy metabolism in reversible ischemia: origin and fate of free acids. *J. Neurochem.*, **47**, 744–757.

Zerangue, N., Arriza, J.L., Amara, S.G. & Kavanaugh, M.P. (1995) Differential modulation of human glutamate transporter subtypes by arachidonic acid. *J. Biol. Chem.*, **270**, 6433–6435.