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Phospholipase A$_2$ and Signaling Pathways in Pheochromocytoma PC12 Cells

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1. Introduction

Phospholipases A$_2$ (PLA2s) (phosphatidylcholine 2-acylhydrolases, EC 3.1.1.4) are ubiquitous lipolytic enzymes. PLA2s are widely spread in nature; they have been found in many organisms including mammals, reptiles, invertebrates, plants, fungi, ameba, bacteria, and viruses as well as in snake and bee venom. The PLA2s include five distinct types of enzymes: the secreted PLA2s (sPLA2), the cytosolic PLA2s, the Ca^{2+} independent PLA2s, the platelet-activating factor acetylhydrolases, and the lysosomal PLA2s (Schaloske & Dennis 2006); a new type, so-called adipose-specific PLA2, has been described recently (Duncan et al., 2008). PLA2s form a numerous protein superfamily, which is divided into 15 groups and many subgroups basing on their amino acid sequences, molecular masses, origin, number of disulphide bonds, Ca^{2+}-dependence and so on.

"Conventional" sPLA2s belonging to groups I/II/V/X are closely related, 13–19-kDa secreted enzymes with a highly conserved Ca^{2+}-binding loop, a catalytic site with a His–Asp dyad, six absolutely conserved disulfide bonds and up to two additional unique disulfide bonds, which contribute to the high degree of stability of these enzymes (Murakami et al., 2011). They require calcium at a level of extracellular environment for the lipolytic activity. For examples, PLA2s of group I are monomers of 13-15 kDa with 7 disulfide bridges. Among these, there are PLA2s of group IA found in Elapidae snake venom and those of group IB found mostly in mammalian pancreas. Group II of PLA2s comprises 6 sub-groups: sub-groups IIA and IIB are typical for Viperidae snake venom whereas sub-groups IIC-IIF are typical for mammalian tissues. Group III comprises sPLA2s from mammals, lizards, and bee venom (Schaloske & Dennis 2006).

Cytosolic PLA2s have no apparent homology to sPLA2s and differ from the latter in molecular mass, which is in the range from 60 to 115 kDa, stability to thiol reagents and requirement in calcium at a cytosolic level which, in contrast to the sPLA2s, is required rather for translocation of the enzyme to intracellular membranes than for catalysis. Cytosolic PLA2s utilize the Asp-Ser catalytic dyad; phosphorylation of the Ser residue enhances lipolytic activity (Schaloske & Dennis 2006). These PLA2s are found only in vertebrates (Murakami et al., 2011).

Ca^{2+}-independent PLA2s are referred to so-called patatin-like phospholipase domain-containing lipases (Murakami et al., 2011). Like cytosolic PLA2, they utilize a serine for catalysis (Schaloske & Dennis 2006).

The main effect of PLA2 is the hydrolysis of the sn-2 position of glycerophospholipids to release fatty acid (as a rule, unsaturated one, especially arachidonic acid, 20:4 *n*–6) and 2-lysophospholipids. The down-stream enzymes of arachidonate methabolism are cyclooxygenases and lipoxygenases (COXs and LOXs). Arachidonic acid is a key precursor of various eicosanoids including prostaglandins, thromboxanes and leukotrienes with their own biological activities. In addition to (or along with) lipolytic activity, PLA2s may exert a broad spectrum of biological effects. So, they may impact different cell functions as well as display pro-inflammatory, platelet-activating, cytotoxic and other stress response-inducing properties (see reviews (Boyanovsky & Webb, 2009; Lambeau & Gelb, 2008)) and even anti-bacterial action (mainly of group IIA, (Buckland & Wilton, 2000; Nevalainen et al., ,2008)). sPLA2s from venoms may also possess anticoagulant activity, presynaptic neurotoxicity, myo- and cytotoxicity. The main biological activities of PLA2s from different groups along with their effects on PC12 cells are summarized in table 1. Some of these diverse effects depend on the enzymatic activity while others do not. In certain cases, e.g., affecting blood coagulation, some of sPLA2s need to hydrolyze phospholipids to gain an anti-coagulant effect while others show the anti-coagulant activity *per se*, without such hydrolysis (Kini, 2005). The non-enzymatic effects may involve different mechanisms, for example, protein-protein interactions (Hanasaki, 2004; Lambeau & Lazdunsky, 1999; Valentin & Lambeau, 2000), which will be discussed below.

None of known PLA2s exerts all variety of the above biological effects. To explain snake venom sPLA2 functional specificity, Kini and Evans (Kini, 2005; Kini & Evans, 1989) have proposed a so-called "target model". According to this model, surface of a target cell or tissue have a "target site" ("target protein") which is recognized by a complementary "pharmacological site" in the PLA2 molecule. The affinity between PLA2s and target proteins lies in the nanomolar range, whereas the affinity between PLA2s and phospholipids covers the micromolar range. Such a difference may explain why the interaction of venom PLA2 with target protein governs the pharmacological specificity (Kini, 2005). The "pharmacological site" is independent of, but sometimes overlapping with, the active enzymatic site. Of course, neighboring phospholipids may also contribute to the interaction (Kini, 2005).

A variety of membrane and soluble proteins strongly bind sPLA2s, suggesting that the sPLA2 enzymes could also function as high affinity ligands (Hanasaki, 2004; Valentin & Lambeau, 2000). Most of the binding data have been accumulated with venom sPLA2s and group IB and IIA mammalian sPLA2s. In general, venom sPLA2s have been shown to bind to membrane and soluble mammalian proteins of the C-type lectin superfamily (M-type sPLA2 receptor and lung surfactant proteins), to N-type receptors, to pentraxin and reticulocalbin proteins, and to factor Xa (see review (Valentin & Lambeau, 2000)). M-type PLA2 receptor has been found in muscle first (therefore – M) and then identified in other organs; the PLA2 binding to this receptor is calcium-insensitive. N-type PLA2 receptor has been found in brain (N – neuronal); more recently N-type-like PLA2 receptors have been found in other organs. The PLA2 binding to this receptor is calcium-dependent. These receptors selectively bind certain sPLA2s with picomolar affinity. So, bee venom PLA2 binds to N-type and M-type receptors with IC_{50} of 80 pM and >0.3 μM, respectively. The affinity of OS1, a PLA2 from taipan snake venom, for these two receptors is quite different: 34 pM for M-type and 1.5 μM for N-type, while OS2 from taipan binds these receptors with almost equal affinity (6 and 10 pM for M- and N-type, respectively) (Lambeau & Lazdunski, 1999). Hence, some additional mechanism(s) besides phospholipide hydrolysis should be considered in

PLA2 group	Distribution	Main activity(s)	Effect(s) on PC12
IA	Elapidae snake venom	Acute toxicity, cytotoxicity; platelet aggregation, anticoagulant.	Differentiation, cytotoxicity.
IB	Pancreatic secretions, lung, liver, spleen, kidney, brain	Digestion of dietary PLs; antibacterial; eicosanoid formation; cell contraction, proliferation, migration, pyknosis.	
	Elapidae snake venom		Differentiation
IIA	Acute phase serum, intestinal mucosa, lacrimal gland cells, prostatic epithelial cells	Inflammation, acute phase protein; antibacterial; atherogenic; anti-tumor or pro-tumorigenic; cell proliferation, migration, apoptosis; exocytosis and neurotransmitter release.	Apoptosis; exocytosis and neurotransmitter release.
	Viperidae snake venom	Acute toxicity, cytotoxicity, miotoxicity, neurotoxicity; platelet aggregation, anticoagulant.	Differentiation; cytotoxicity; exocytosis and neurotransmitter release (blockage at internal application).
III	Kidney, heart, liver, skeletal muscle, epididymus, placenta, leukocytes	Sperm maturation; pro-tumorigenic; antiviral.	Differentiation; cell survival.
	Bee venom	Inflammation; cytotoxicity, pyknosis; antibacterial.	Differentiation, cytotoxicity.
V	Heart, eye, lung, pancreas, macrophages, neutrophils, mastocytes	Antibacterial, antifungal, antiviral; atherogenic; eicosanoid generation; phagocytosis.	Differentiation.
X	Gastrointestinal tract, stomach, lung, testis, spleen, thymus, neutrophils, macrophages	Antibacterial, antiviral, atherogenic, pro-tumorigenic.	Differentiation.
XIII	Bacteria/fungi	Modulation of host inflammatory response.	Differentiation.
Cytosolic PLA2s	Kidney, brain, heart, spleen, thyroid	Inflammation, anaphylaxis; ulceration; acute lung injury, brain injury; parturition; nociception; junction proteins through or from the Golgi.	Apoptosis, hypoxic or ischemic cell death; partition in exocytosis.
Ca-independent PLA2s	Different cell types	Cell activation, proliferation, migration, or apoptosis, pro-tumorigenic; secretion; control of energy metabolism in adipocytes.	Apoptosis, hypoxic or ischemic cell death; regulation of exocytosis.

Table 1. The main biological activities of PLA2s and their effects on PC12 cells. The table includes only those PLA2 groups that have been shown to act on/in PC12 cells. The data have been compiled mainly from (Boyanovsky & Webb, 2009; Farooqui, 2009; Murakami et al., 2011; Nakashima et al., 2003; Schaloske & Dennis 2006).

signaling pathways in which PLA2s take part. For example, it has been suggested that "neuronal" effects of sPLA2s derived from animal venoms may be mediated by their specific binding to N-type receptor on neuronal membranes (Hanasaki, 2004). It should be noted, that to date there is no direct evidence on presence of a sPLA2 receptor in PC12 cells (neither of C-lectin-like nor of any other type).

There is a lot of data that PLA2s may display neurotoxic properties. They may affect also some important neuronal functions, such as neurotransmitter release and neuronal survival as well as neuritogenesis. Thus, there is no coincidence that PLA2s are found in nerve growth cones in PC12 cells. We do not intend to discuss here all the effects of PLA2 on various types of neuronal cells as it has already done in overall by others (for instance, see respective chapters in (Farooqui, 2009) and other reviews). In accordance with the topic of the present book, we shall consider in details the PLA2 effects mainly on PC12 cell line.

2. PLA2 and neurite outgrowth in PC12 cells

PC12 cell line has been derived from rat pheochromocytoma which is a tumor of neuronal origin. This cell line serves sometimes as a convenient model for studying some "neuronal" effects of different compounds. It is known for many years that PC12 cells are also capable to exhibit neurite outgrowth upon appropriate external stimulation. This process may be evoked by application of different biochemical agents: polypeptides (e.g., neurotrophins: nerve growth factor (NGF) or fibroblast growth factors, and some neuropeptides of secretin superfamily (e.g., PACAP) (Ravni et al., 2006; Vaudry et al., 2002)), cAMP, which seems to be a messenger of the latters (Gerdin & Eiden, 2007), and its certain derivatives (Gunning et al., 1981), sialoglycosides (Ledden et al., 1990) and sialic acid precursors (Kontou et al., 2009), lectins (Wu et al., 2004), some phospholipide metabolites (discussed below), calcium ions (by membrane depolarization), etc. Biochemical pathways converting signals from such diverse molecules to the neurite outgrowth may be either very different or almost identical, may overlap, or supplement, or amplify each other. For example, the differentiation pathways in PC12 are summarized in (Eiden et al., 2011). In all cases, the neurite outgrowth in PC12 is accompanied by shifting from a chromaffin cell-like phenotype to a neurite-bearing sympathetic neuron-like phenotype and results in termination of proliferation. The sum of these events is considered as an indicator of cell differentiation (Nakashima et al., 2004), the process opposed to malignant growth. Thus, PC12 is an excellent model to study at a cellular level the neuronal differentiation and antiproliferative effects of different compounds. In this paper we undertake the first attempt to systematize the data about effects of PLA2s on PC12 and to consider the possible mechanism(s) involved in these processes. However, we should say that PC12 cells in non-differentiated and differentiated forms have been documented to display at certain circumstances their own endogenous secretory, cytosolic, and calcium-independent PLA2 activity.

There is a number of papers discussing a role of activation of intracellular phospoholipase C or phospholipase D in NGF-induced PC12 differentiation (as well as in some other cellular events). The effects of these enzymes will not be considered in details in the present paper.

At the same time, increased activity of endogenous PLA2 has been found in nerve growth cones in differentiating PC12 and much of this PLA2 activity has been shown to be calcium-independent and secretory rather than cytosolic (Martin, 1998) or solely secretory (Ferrini et al., 2010). One cannot assert, however, that the activation of endogenous PLA2 is indeed an initial cause of neuritogenesis.

Along with the above mentioned substances, exogenous sPLA2s derived from different sources have been also found to induce the neurite outgrowth as well as produce some other effects in PC12 cells. Such a "neuritogenic" property has been already reported for sPLA2s of groups IA and IB from cobra venom (Makarova et al., 2006; Osipov et al., 2010), IIA from viper venom (Makarova et al., 2006), III from bee venom (Nakashima et al., 2003) and from human neuronal cells (Masuda et al., 2008), V from mouse (Nakashima et al., 2003), X from mouse (Nakashima et al., 2003) and human (Ikeno et al., 2005; Masuda et al., 2005), as well as XIII from bacteria and fungi (Nakashima et al., 2003). According to the early report (Hanada et al., 1996), fungal PLA2 p15 promotes only NGF-induced neuritogenesis but itself alone fails to induce neurite outgrowth in PC12; however, under some conditions p15 can displays neuritogenic properties (Wakatsuki et al, 1999). The concentrations at which sPLA2s begin to exert the neuritogenic effect are quite different. So, sPLA2 from bee venom being the most active among studied sPLA2s induces neurite outgrowth at concentration as low as 0.1 nM. sPLA2 of group XIII is effective starting from 1 nM (Nakashima et al., 2003), and snake venom sPLA2s of groups IA, IB, and IIA are effective at concentrations of 1-10 μM (Makarova et al., 2006; Osipov et al., 2010). Not all of sPLA2s can induce neuritogenesis: for example, mammalian sPLA2s of groups I and II are unable to produce this effect (Nakashima et al., 2003), while PLA2s of these groups from snake venom and mammalian PLA2s of groups V and X are effective in inducing neurite outgrowth .

It is assumed now that neurite outgrowth stimulated by PLA2s in PC12 cells is induced by the products of phospholipids hydrolysis and/or by their metabolites. Numerous data argue for this assumption. For instance, lysophosphatidylcholine (LPC) but not other lysophospholipids or arachidonic acid has been shown to promote the neurite outgrowth (Ikeno et al., 2005; Masuda et al., 2008). Overproduction or suppression of G2A, a G-protein-coupled receptor involved in LPC signaling, results in the enhancement or reduction, respectively, of neuritogenesis induced by sPLA2 treatment (Ikeno et al., 2005). LPC (either exogenously added or generated in situ by sPLA2-catalysed phosphatidyl choline hydrolysis) acts through an L-type Ca^{2+} channel-dependent mechanism. No synergistic enhancement of both sPLA2-promoted and LPC-induced neuritogenesis is produced by the co-addition of 100 μM arachidonic acid (Nakashima et al., 2003). Another way for LPC signaling is an activation of cytosolic phospholipase D2 which is involved in the regulation of depolarization-induced PC12 cell differentiation through activation of tyrosine kinases Pyk2(Y881) and ERK (extracellular signal-regulated kinase). However, action of different phospholipid products on PC12 is multilateral and dependent on certain extra factors. For example, similarly to PLA2 docosahexaenoic acid (22:6 n–3) is present in neurite growth cones. This acid decreases time-course activity of cytosolic PLA2 (Martin, 1998) and attenuates (Kim et al., 2001) or accelerates (Schonfeld et al., 2007) apoptotic death in PC12 cells. On the other hand arachidonic acid suppresses neurite outgrowth induced by nerve growth factor (Ikemoto et al., 1997) and also may be both anti-apoptotic (Kim et al., 2001) and pro-apoptotic at deprivation of NGF and serum (Atsumi et al., 1997) in PC12 (see also section 4). The above mentioned phospholipase D2 hydrolyzes the lysophospholipids with the formation of lysophosphatidic acid, which enhances cell proliferation. In differentiated PC12 cells lysophosphatidic acid provokes a rapid withdrawal of neurites (Moolenaar et al., 2004).

The direct relationship between the PLA2 enzymatic and differentiating activities may not be always evident. A number of papers (Ikeno et al., 2005; Nakashima et al., 2003; Nakashima et al., 2004) argues that anti-proliferative activity of PLA2s in PC12 is directly

related to enzymatic activity. It has been suggested (Ikeno et al., 2005) that the binding of bee venom sPLA2 to the putative neuronal N-type receptor (see above) unlikely to be involved in PLA2-induced neurite outgrowth in PC12 cells. However, the data of (Nakashima et al., 2003) are contradictory to these conclusions: it has been shown that in PC12 cells catalytically inactive mutants of sPLA2s from groups V and X in which the active site His has been replaced by Ala exhibit neuritogenic activity that is only two times smaller than that of the native forms. Therefore, the His residue in the active site only promotes but does not determine the neuritogenic activity of sPLA2s in PC12. Another example showing that phospholipid hydrolysis is not the single requirement for the neuritogenic activity is a sPLA2 from Egyptian cobra venom: it possesses very weak enzymatic activity but induces neurite outgrowth in PC12 even more efficiently than cobra venom sPLA2 with strong enzymatic activity (Osipov et al., 2010).

Tumor cells of definite types can change their shape and stop proliferation being separated from a substrate. In whole, this is true for PC12 cells too. The possible role of extracellular matrix in the PLA2-induced neuritogenesis becomes evident from the following data. When PC12 cells are grown on polyethyleneimine used as a plate coat, fungal PLA2 p15 is unable to induce neurites but can only enhance the effect of NGF (Hanada et al., 1996). On the other hand it is known that collagen is the most effective purified extracellular matrix component supporting PC12 cell adhesion and NGF-induced neurite outgrowth. If the PC12 cells are grown on collagen-coated surface, p15 alone induces neurite outgrowth at concentration as low as 1 nM and does not require the addition of NGF for this effect (Wakatsuki et al, 1999). Moreover, the effect of p15 is faster than that of NGF: small neurites appear after 6 h of p15 treatment versus 24 h in the case of NGF (Wakatsuki et al, 1999).

3. Interdependent action of PLA2 and other agents

PC12 cells express sodium, potassium, and calcium ion channels and receptors for a lot of different ligands (grouth factors, neurotransmitters, adenosine and so on); effects of all these membrane proteins are in strong interdependence. At the first place, there are receptors for growth factors. There are two classes of receptors for NGF on cell surface: high affinity TrkA, a receptor tyrosine kinase which is highly specific for NGF, and a low affinity p75 neurotrophin receptor which binds all members of the neurotrophin family with a similar affinity (Skaper, 2008). Activation of p75 results in apoptosis of PC12 cells while activation of TrkA results in their differentiation. There is one intriguing and so far unexplained observation that NGF and several other agents are mitogens for normal chromaffin cells, but are anti-proliferative agents and induce neuronal differentiation of PC12 cells (Tischler et al., 2004). It should be mentioned that NGF induces an activation of the Na^+, K^+-pump in PC12 cells and increases sodium influx. Moreover, some researchers have seen small changes in cAMP level and in calcium currents evoked by NGF while others have not.

In PC12 cells NGF increases mRNA level for cytosolic PLA2 after treatment for 4h and cytosolic PLA2 protein level at 24h after beginning of treatment via the ERK1/2, p38 MAPK and PKC pathways (Akiyama et al, 2004). The following data support synergism in PLA2 and NGF action (Masuda et al., 2008). Adenoviral expression of human sPLA2 of group III (PLA2-III) in PC12 cells or dorsal root ganglion explants facilitates neurite outgrowth, whereas expression of a catalytically inactive PLA2-III mutant or use of PLA2-III-directed small interfering RNA (siRNA) reduces NGF-induced neuritogenesis. The results of the experiment with siRNA suggest that endogenous sPLA2-III profoundly affects

neuritogenesis of PC12 cells. PLA2-III also suppresses neuronal death induced by NGF deprivation. In principle, this work (Masuda et al., 2008) demonstrates that the effects of NGF and PLA2 are different, however they complement each other. Thus, the overexpression of PLA2 replaces NGF, while NGF is not effective if PLA2 is switch off.

The data on blockage of PC12 differentiation evoked either by NGF or by PLA2 indicate that different pathways are involved in effects of these two agents. Thus, tyrosine kinase inhibitor K-252a blocks differentiation induced by NGF. Unlike NGF, neuritogenesis induced by sPLA2s is insensitive to K-252a. In contrary, inhibition of L-type calcium channel or depletion of extracellular calcium, which are ineffective in blocking NGF-induced neuritogenesis, may inhibit sPLA2-induced neurite outgrowth (Wakatsuki et al., 1999).

On the other hand, NGF activates not only tyrosine kinase cascade but also phospholipases C and A$_2$ which in turn results in enhance of arachidonate release from the cell lipids (Tsukada et al., 1994). Phosphorylation *in vitro* and *in vivo* of cytosolic PLA2 by mitogen-activated protein kinase at Ser505 increases its intrinsic enzyme activity several fold (Lin et al., 1993). After NGF treatment, the PLA2 activity in the PC12 cell lysate increases 4-fold on the third day (Ferrini et al., 2010) and during 7 days increases approximately 1.5-fold (Matsuzawa et al., 1996) i.e., effects of NGF and PLA2 on PC12 cells are mediated by different but somehow related mechanism(s). NGF changes the subcellular localization of group IIa endogenous sPLA2 in PC12 cells. In untreated cells, this PLA2 is mainly cytosolic and mitochondrial in localization. As soon as 6 h after NGF stimulation, it is no longer associated with mitochondria being found diffusely in the cytoplasm and associated with the plasma membrane as well as in growth cones at specific membrane domains. After 24 h, IIA PLA2 is detected within neurites and, at longer periods, the enzyme is mostly localized at neurite tips of neuron-like differentiated cells, as determined by confocal laser immunofluorescence microscopy analysis using anti-rat group IIA PLA2 monoclonal antibody (Ferrini et al., 2010). After treatment with NGF under identical conditions, group V PLA2 retains its cytoplasmic and nuclear localization and is scarcely present within neurites and neurite tips.

PC12 cells themselves comprise enzymes for synthesis of both catecholamines and acetylcholine. At the same time they bear acetylcholine receptors of both nicotinic and muscarinic types; stimulation of the formers results in the release of catecholamines while stimulation of the latter results in calcium influx and phosphoinositide hydrolysis (Fujita et al., 1989).

Calcium ion influx through voltage-gated channels triggers a variety of cellular events leading to neurite outgrowth via the concerted action of various calcium-binding proteins. Calcium influx mediates induction of gene expression in response to membrane depolarization. In PC12 cells, L-type channels are the primary carrier of voltage-sensitive calcium currents and have been shown to be required for sPLA2-induced neuritogenesis (Nakashima et al., 2003). The calcium signal transduction pathway promoting neurite outgrowth causes the rapid activation of protein tyrosine kinases, which include MEK1 and Src. They, in turn, further activate Ras (small guanine nucleotide-binding protein) and MAP kinase. A set of stimuli evokes the calcium influx; simultaneously cytosolic PLA2s are activated which results in release of arachidonic acid and other products of phospholipid hydrolysis. Interestingly, such a calcium-dependent activation of the cytosolic PLA2 does not need the extracellular calcium, and calcium from intracellular stores would be enough. Activation of an exogenous sPLA2 (either applied externally or expressed in PC12 cells) requires an influx of extracellular calcium. Thus, sub-millimolar and sub-micromolar Ca^{2+}

concentrations are necessary for activation of secretory and cytosolic PLA2s, respectively (Matsuzawa et al., 1996). Similarly to calcium as well as NGF pathways (Rusanescu et al., 1995; Vaudry et al., 2002), the sPLA2-induced neuritogenesis in PC12 involves the activation of Src and Ras proteins and is accompanied by the activation of MAPK cascade (Wakatsuki et al., 1999). The existing data indicate that PLA2, NGF, and calcium ions can act independently but synergistically in PC12 cells. However, as all of them activate the Ras/MAPK cascade, it is reasonable to suggest that their signaling pathways partially overlap.

Endogenous PLA2s in PC12 can be activated by different damaging agents (cyanides, reactive oxygen species, some cytolysins, etc.) that results in release arachidonic acid and its metabolites. Arachidonic acid being released by diacylglycerol hydrolysis promotes the opening of L- and/or N-type calcium channels that may lead to neurite outgrowth. Furthermore, the direct addition of arachidonic acid to the culture medium stimulates neurite outgrowth when PC12 cells grow on a fibroblast monolayer. However, arachidonic acid does not increase the steady-state calcium levels in neuronal growth cones (Nakashima et al., 2003, as cited in Walsh & Doherty, 1997). From existing data it is still not obvious, whether one of the activation pathway induced by either PLA2 or calcium channels begins first and then evokes other one.

Besides NGF receptor, PC12 express also two classes of epidermal growth factor receptors (activation of which in contrast to NGF receptor results in enhancement of PC12 proliferation, (Vaudry et al., 2002)) and receptors for fibroblast growth factor. To our knowledge, there are no data published on any cross-interaction of these receptors and PLA2.

Thus, PLA2s induce different cellular responses via very complex biochemical pathways in which PLA2s themselves or products of their enzymatic activity interact with various substances. These interactions are shown in a simplified form in Fig.1.

4. PLA2 in apoptosis and cytotoxicity

There are a lot of reports on the cytotoxic property of sPLA2s. This property is mostly attributable to PLA2s from animal venom. Concerning cytotoxicity of PLA2 in respect to PC12, it should be mentioned that sPLA2s from bee venom at concentrations higher than 10 nM (Nakashima et al., 2003), and from cobra and viper venoms at concentrations 10 μM and higher (Makarova et al., 2006) are toxic for these cells (i.e., induce the necrotic changes). Thus, cytotoxicity of many venom PLA2s attenuates strongly their "therapeutic" potential for differentiating action (section 2). However, non-cytotoxic sPLA2s (e.g., mammalian sPLA2s of groups V, X, and fungal/bacterial XIII (Nakashima et al., 2003), as well as a non-cytotoxic variant of venom sPLA2 (Osipov et al., 2010)) still preserve this potential and can be regarded antiproliferative agents.

Some toxic agents (for examples, reactive oxygen species produced by cyanides (Kanthasamy et al., 1997), by hydrogen peroxide (Akiyama et al., 2005), and so on) require cytosolic PLA2 activity for their damaging action.

A mechanism of endogenous sPLA2 cytotoxicity remains unknown yet. Sometimes, researchers assign it to enzymatic activity and to released arachidonate. In several cases such correlation may be traced, indeed. For instances, arachidonic acid itself being added to PC12 in concentrations above 10 μM kills the cells within 1-2 hours and does not require extracellular Ca^{2+} for the toxic effect. The removal of extracellular Ca^{2+} dramatically
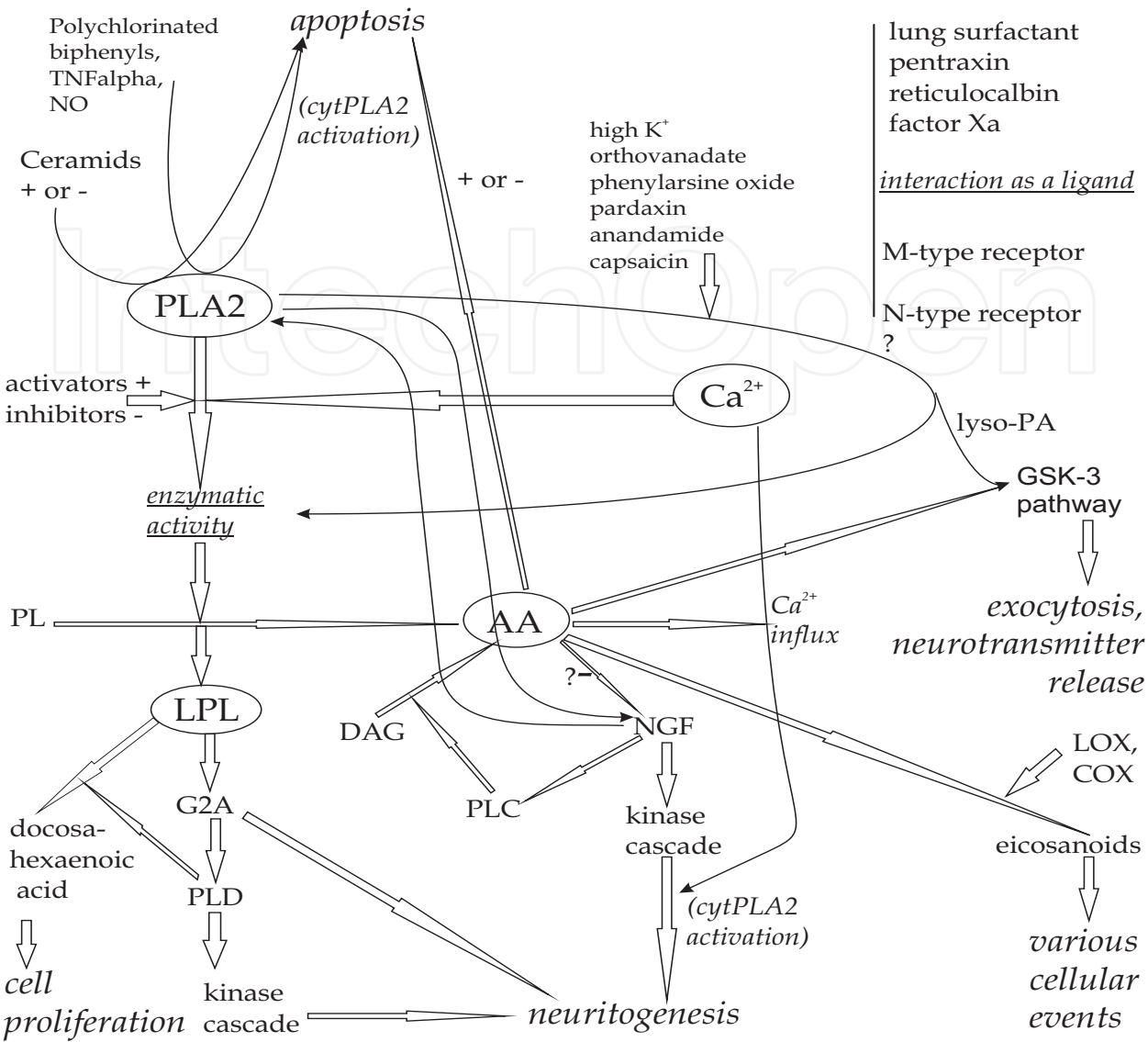
Fig. 1. Scheme of PLA2 interactions with the various substances mentioned in the text. The key players are in the ellipses; the processes considered are italicized. Inhibiting effects are indicated by minus, stimulating effects – by plus. DAG is diacylglycerol, PL is a phospholipide, lyso-PA is lyso-phosphatidic acid, and PLC and PLD are phospholipases C and D, respectively.

accelerates the acute cell death (Doroshenko & Doroshenko, 2004). Cytolysins impair ionic permeability of cell membrane, leading to cell death. Several different cytolysins have been tested on PC12 cells and found to enhance PLA2 activity as well as levels both of arachidonic acid and certain its metabolites (Raya et al., 1993). Peroxynitrite-dependent release of arachidonate by sPLA2 is supposed to be casually linked to peroxynitrite-dependent induction of DNA cleavage and toxicity (Guidarelli et al., 2000). However, there are enough reports (including cited in this paper) on PLA2s which possess high enzymatic activity and devoid of apparent cytotoxicity. Moreover, several papers describe the role of arachidonate in programmed cell death (apoptosis) but not in acute cytotoxicity.

PC12 may undergo apoptotic cell death under some stimuli (for example, under NGF deprivation or by activating p75 NGF receptor). PLA2s have been shown to take part in this

process and extracellular Ca^{2+}-dependent release of arachidonic acid by sPLA2 is considered to mediate their action (Atsumi et al., 1997). Association of sPLA2 with cell surface proteoglycan, which has been shown to be a prerequisite for endogenous sPLA2-dependent arachidonic acid release from the plasma membranes of live cells, is not essential for sPLA2-mediated hydrolysis of apoptotic cell membranes. The authors give evidences that the apoptotic cell membrane is a potential target for extracellular type II sPLA2 (Atsumi et al., 1997). Arachidonic acid, a signaling lipid potentially associated with tumor necrosis factor receptor-I signal cascade, induces apoptosis in PC12 cells through inhibition of both protein kinase C zeta and nuclear factor kappaB activity. Apoptosis induced by arachidonate cannot be prevented by NGF (Macdonald et al., 1999).

Controversially, arachidonic acid has been reported to prevent neuronal apoptosis during serum starvation and COX and LOX do not participate in this process (Kim et al., 2001). Another research group has reported that arachidonic acid shows normal survival of NGF-differentiated PC12 whereas stearic acid and palmitic acid induce apoptotic cell death (Ulloth et al., 2003).

Several compounds have been reported to induce apoptosis in PC12 cells and in many cases PLA2 activity appears to be essential. For examples, short- and long-chain sphingosines are mediators of many cellular events including apoptosis in both naïve and NGF-differentiated PC12 (Posse de Chaves, 2006). C2-Ceramide (N-acetyl-D-erythro-sphingosine) alone stimulates arachidonic acid release and enhances the ionomycin-induced release. In contract, some sphingosines show an opposite effect and directly inhibit cytosolic phospholipase A2alpha activity (Nakamura et al., 2004). Nitric oxide can induce apoptosis. Nitric oxide is involved in the regulation of cytosolic PLA2, its phosphorylation and activity, arachidonic acid release and as consequence in up-regulation of expression and activity of COXs and LOXs; blockage of their activity may rescue cell death (Pytlowany et al., 2008). In neuronal PC12 cells, TNF-alpha induces moderate apoptosis. Application of TNF-alpha to the PC12 cells results in p38 MAPK phosphorylation and activation. Phosphorylation of p38 MAPK is regulated by cytosolic PLA2, which produces arachidonic acid. The results present one possible mechanism for enhancing the neuronal cell death by arachidonate through the regulation of p38 MAPK. However, how arachidonate attenuates the phosphorylation of p38 MAPK is unknown (Park et al., 2002).

Ortho-substituted polychlorinated biphenyls induce apoptosis in PC12. These compounds evoke PLA2-mediated Ca^{2+} influx. However, in the presence of extracellular Ca^{2+}, PLA2 activation is inhibited by neither an extracellular nor an intracellular Ca^{2+} chelator but is depressed by inhibitors of calcium-independent PLA2 (Shin et al., 2002).

Along apoptosis, PC12 cells may undergo hypoxic or ischemic caspase-independent cell death characterized by nuclear shrinkage (pyknosis). This process comes with participation of calcium-independent PLA2 and, to a lesser extension, cytosolic PLA2. Interestingly, bee venom PLA2 and porcine pancreatic sPLA2 also can cause nuclear shrinkage in another cell type. Neither arachidonate nor other fatty acids (stearic, linoleic, palmitic, and oleic acids) induces the shrinkage suggesting that PLA2 induces nuclear shrinkage itself and that its metabolites are ineffective (Shinzawa & Tsujimoto, 2003).

Thus, calcium-independent PLA2 seems to play an important role in programmed cell death in PC12 that is consistent with the data on participation of group VIA calcium-independent PLA2 in apoptosis in cell types other than PC12 (Balsinde et al., 2006; Lei et al., 2010). Cytosolic PLA2 also seems to participate in apoptosis.

Nevertheless, it is not quite understood yet why a PLA2 activity in PC12 results in cell necrosis in several cases, in apoptosis in some cases, and in differentiation in other.

5. Exocytosis and secretion in PC12 cells and PLA2

As PC12 cells are tumor scions of neuroendocrine chromaffin cells, it is relevant to mention the PLA2 action on catecholamine secretion in the chromaffin cells. Pre-synaptic neurotoxic snake venom sPLA2, taipoxin, enhances exocytosis in bovine chromaffin cells in response to depolarizing stimuli. It entries into the cytosol, that is already detectable after 5 min and is independent on extracellular calcium, i.e., the toxin does not use calcium-dependent endocytosis to reach the chromaffin cell cytoplasm. After 1 h, a large portion of the toxin has redistributed to the plasma membrane and fragmentation of the F-actin cytoskeleton is observed; an increased number of events of granule fusion takes place during the initial phase of secretion with an enhancement of the initial rate of release, and after 1 day, cell death becomes evident (Neco et al., 2003). Authors (Giner et al., 2007) propose mechanism according to which, in neuroendocrine chromaffin cells, neurotoxic sPLA2s bind to surface membrane receptors and hydrolyze phospholipids that results in release of lysophosphatidic acid among other products. This product activates intracellular pathways involving the glycogen synthase kinase 3 (GSK-3), an important modulator of many physiological processes, such as neurodevelopment, the cell cycle, apoptosis and oncogenesis (either as a suppressor or as a promoter) (Mishra, 2010), causing cytoskeleton alterations with neurite retraction. Alterations in actin cytoskeleton result in the loosening of the peripheral cortex barrier thus facilitating the access of secretory granules to the release sites of the plasmalemma (Giner et al., 2007).

There is a lot of indications on the participation of PLA2s in exocytosis in PC12. On the one hand, PLA2 may participate in regulation of cell secretion; on the other hand, sPLA2 itself may be released by exocytosis. sPLA2 of group II is involved in the regulation of neurotransmitter secretion by PC12 cells. The neurotransmitters released from PC12 cells by PLA2 are catecholamines and acetylcholine. PLA2 seems to be involved also in the degranulation process in neuronal cells and itself may influence exocytosis in PC12 cells.

External application of group IIA sPLA2 (purified crotoxin subunit B from snake venom or purified human synovial sPLA2) causes an immediate increase in exocytosis and neurotransmitter release in PC12 cells, detected by carbon fiber electrodes placed near the cells, or by changes in membrane capacitance of the cells. There is an indication that the effect of sPLA2 is dependent on calcium and sPLA2 enzymatic activity (Wei et al., 2003). When type II sPLA2 purified from rat platelets is added to NGF-treated PC12 cells, there is a dose-dependent release of both noradrenaline and dopamine, reaching a maximum release of ~10% at 20-40 μg/ml sPLA2 after 10-30 min of incubation. Release of dopamine by exogenous sPLA2 is dependent on extracellular calcium. Interestingly, thielocin A1, a type II sPLA2-specific inhibitor, or neutralizing antibody against type II sPLA2 suppress noradrenaline release in a dose-dependent manner. These results indicate that endogenous sPLA2 may be involved in catecholamine secretion from PC12 cells. The concentration of exogenous type II sPLA2 required for catecholamine secretion from PC12 cells is higher than that which can be released from activated PC12 cells (Matsuzawa et al., 1996). Secretion of dopamine from PC12 cells that is stimulated by glutamate is suppressed by type II sPLA2 inhibitors. Exogenous type II sPLA2 added alone directly elicites release of dopamine from PC12 cells (Kudo et al., 1996). Because the antibody could not pass through the plasma

membrane without permeabilization, it is likely that sPLA2 might act from outside the cells, becoming accessible to the antibody after cell activation. This is supported by the fact that exogenously added sPLA2 directly elicites dopamine release from PC12 cells in an extracellular Ca^{2+}-dependent manner (Matsuzawa et al., 1996).

In contrast to external application, internal application of sPLA2 to PC12 cells produces blockade of neurotransmitter release (Wei et al., 2003).

It was suggested (Matsuzawa et al., 1996) that arachidonic acid metabolism elicited by neurotransmitters or by depolarization, which mobilizes intracellular Ca^{2+}, is more likely to be regulated by cytosolic PLA2. However, the authors do not rule out the possibility for hydrolysis of membrane phospholipids by sPLA2 to liberate arachidonic acid, which leads to catecholamine secretion. Arachidonic acid may be released from PC12 cells in response to different stimuli. Thus, it is released from undifferentiated PC12 under NGF action (Tsukada et al., 1994). PLA2 also promotes release of arachidonate from NGF-differentiated PC12 cells which are stimulated, for examples, by staphylococcal alpha-toxin at subcytotoxic concentrations (Fink et al., 1989), peroxynitrite (by activation of endogenous sPLA2) (Guidarelli et al., 2000), high K^+ or via direct stimulation of phospholipide hydrolysis by mastoparan, a tetradecapeptide PLA2 activator from bee venom (Ray et al., 1999). Several other compounds including orthovanadate, phenylarsine oxide, pardaxin, anandamide and capsaicin have been reported to induce the arachidonate releasing by PLA2 from PC12 treated with NGF, with subsequent prostaglandin F2alpha formation. Orthovanadate activates cytosolic PLA2 to release arachidonate, stimulates tyrosine phosphorylation in proteins and enhances Ca^{2+}-induced noradrenaline release (Mori et al., 2001). Phenylarsine oxide acts probably via activation of endogenous secretory PLA2; it may act synergistically with exogenous sPLA2 from bee venom (Ohsawa et al., 2002). Pardaxin, an α-helical cytolysin from the fish *Pardachirus marmoratus*, aggregates in the plasma membrane to form ionic channels, followed by calcium influx. By this way it activates calcium-dependent PLA2, but it also can activate calcium-independent PLA2 in PC12 (Abu-Raya et al., 1998). Anandamide and capsaicin stimulate arachidonic acid release even in the absence of extracellular calcium. The effects of anandamide and capsaicin are inhibited by PLA2 inhibitors, but not by an antagonist for vanilloid VR1 receptor (Someya et al., 2002). Exocytosis in PC12 may be evoked also by lysophosphatidylinositol, another product of phospholipid hydrolysis by sPLA2 (Ma et al., 2010).

Arachidonate release is associated with acetylcholine release in NGF-differentiated PC12 cells. Botulinum neurotoxin type A (BoTx) inhibits this process. K^+-stimulated acetylcholine release is also totally inhibited by pretreatment of cells with BoTx (2 nM). Inclusion of exogenous arachidonate, or the PLA2 activator melittin, or bee venom sPLA2 itself prevents the effect of BoTx (Ray et al., 1993). Treatment of differentiated PC12 cells with mastoparan and high (80 mM) K^+ induces acetylcholine exocytosis. The acetylcholine release depends upon Ca^{2+} influx via the N-type voltage-sensitive Ca^{2+} channels. The release is followed by a rise in intracellular free Ca^{2+} concentration; the increased Ca^{2+} activates PLA2 and, thereby, increases the arachidonate level (Ray et al., 1999). BoTx cleaves SNAP-25, a conserved synaptosomal protein essential for vesicle fusion and docking during neuroexocytosis. Blockage of SNAP-25 function by the antisense oligonucleotides has shown that the neuroexocytosis involving PLA2 activity proceeds without participation of this protein. Moreover, mastoparan prevents the neurotoxic effect of BoTx through induction PLA2 activity that seems not to involve SNAP-25 pathway (Ray et al., 1999).

Differentiated PC12 cells, being stimulated with carbamylcholine, potassium, or glutamate, release sPLA2 into the medium. Thus, sPLA2 of group II is released in response to stimulation by neurotransmitters or depolarization. sPLA2 is released from neuronally differentiated PC12 cells upon stimulation by carbamylcholine via acetylcholine receptors or by high K$^+$ via voltage-dependent Ca^{2+} channels through membrane depolarization (Matsuzawa et al., 1996). When NGF-treated PC12 cells are activated with glutamate, approximately 40% of their group II sPLA2 is released into the extracellular medium. Glutamate-stimulated secretion of dopamine from PC12 cells is PLA2-dependent, as it is suppressed by group II sPLA2 inhibitors (Kudo et al., 1996).

As after activation of PC12 cells by carbamylcholine, the time courses of noradrenaline and sPLA2 release are not parallel (the release of noradrenaline reaches a plateau at 10 min and that of sPLA2 at 15 min), sPLA2 and catecholamine may be stored in different secretory granules (Matsuzawa et al., 1996).

A recent report has appeared concerning a role of calcium independent PLA2 in exocytosis and mitochondrial function in PC12: inhibition of this type of PLA2 results in excessive exocytosis through increased oxidative damage (or failure to repair such damage) and defects in mitochondrial function (Ma et al., 2011).

It is still unclear, however, whether the relation between neurotransmitter signaling/release and the effects of PLA2 plays a significant role in other discussed processes including the PC12 differentiation.

6. Conclusion

In general, PLA2s are involved in different signaling pathways in PC12 cells. Various types of PLA2s and even groups within types may exert diverse effects on the cells. Most of them in one or another way are now associated with phospholipid products. One should note, however, that it would not be correct to attribute such diversity of effects to enzymatic activity of PLA2 only. Understanding the mechanisms involved in PC12 differentiation induced by PLA2s may results in development of new drugs inhibiting tumor cell proliferation.

As it has been discussed in this paper, PLA2 enzymes manifest a number of very diverse biological properties. At the same time, PC12 cell line is a valuable model for study of different signaling mechanisms. The effects of PLA2s on PC12 cells are quite complex and multifaceted. Several striking findings have already been made upon investigation of these effects and one can expect further exciting findings in this area.

7. Acknowledgment

8. References

Abu-Raya, S., Bloch-Shilderman, E., Shohami, E., Trembovler, V., Shai, Y., Weidenfeld, J., Yedgar, S., Gutman, Y. & Lazarovici, P. (1998). Pardaxin, a new pharmacological tool to stimulate the arachidonic acid cascade in PC12 cells. *The Journal of pharmacology and experimental therapeutics,* Vol.287, No.3, (December 1998), pp. 889-896, ISSN 0022-3565

Akiyama, N., Hatori, Y., Takashiro, Y., Hirabayashi, T., Saito, T. & Murayama, T. (2004). Nerve growth factor-induced up-regulation of cytosolic phospholipase A2alpha level in rat PC12 cells. *Neuroscience letters*, Vol. 365, No.3, (July 2004), pp. 218-222. ISSN 0304-3940

Akiyama, N., Shimma, N., Takashiro, Y., Hatori, Y., Hirabayashi, T., Horie, S., Saito, T. & Murayama, T. (2005). Decrease in cytosolic phospholipase A2alpha mRNA levels by reactive oxygen species via MAP kinase pathways in PC12 cells: effects of dopaminergic neurotoxins. *Cellular signalling*, Vol.17, No.5, (May 2005), pp. 597-604, ISSN 0898-6568

Atsumi, G., Murakami, M., Tajima, M., Shimbara, S., Hara, N. & Kudo, I. (1997). The perturbed membrane of cells undergoing apoptosis is susceptible to type II secretory phospholipase A2 to liberate arachidonic acid. *Biochimica et Biophysica Acta*, Vol.1349, No.1, (November 1997), pp. 43-54. ISSN 0006-3002

Balsinde, J., Pérez, R. & Balboa, M.A. (2006). Calcium-independent phospholipase A2 and apoptosis. *Biochimica et Biophysica Acta*, Vol.1761, No.11, (November 2006), pp. 1344-1350, ISSN 0006-3002

Boyanovsky, B.B. & Webb, N.R. (2009). Biology of secretory phospholipase A2. *Cardiovascular drugs and therapy*, Vol.23, No.1, (February 2009), pp 61-72, ISSN 0920-3206

Buckland, A.G. & Wilton, D.C. (2000). The antibacterial properties of secreted phospholipases A(2). *Biochimica et Biophysica Acta*, Vol.1488, No.1-2, (October 2000), pp. 71–82, ISSN 0006-3002

Doroshenko, N. & Doroshenko, P. (2004). Ca^{2+} influx is not involved in acute cytotoxicity of arachidonic acid. *Biochemical pharmacology*, Vol.67, No.5, (March 2004), pp. 903-909, ISSN: 0006-2952

Duncan, R.E., Sarkadi-Nagy, E., Jaworski, K., Ahmadian, M. & Sul, H.S. (2008). Identification and functional characterization of adipose-specific phospholipase A2 (AdPLA). *Journal of biological chemistry*, Vol.283, No.37, (September 2008), pp. 25428-25436, ISSN 0021-9258

Eiden, L., Lazarovici, P., Vaudry, D., Stork, P.J.S. & Samal, B. (no date) Differentiation Pathway in PC12 Cells. In: *Sci. Signal. Connections Map in the Database of Cell Signaling*, 20.07.2011, available at http://stke.sciencemag.org/cgi/cm/stkecm;CMP_8038

Farooqui, A.A. (2009). *Hot Topics in Neural Membrane Lipidology.* Springer Science+Business Media, LLC, ISBN 978-0-387-09692-6, New York, USA.

Ferrini, M., Nardicchi, V., Mannucci, R., Arcuri, C., Nicoletti, I., Donato, R. & Goracci, G. (2010). Effect of NGF on the subcellular localization of group IIA secretory phospholipase A(2) (GIIA) in PC12 cells: role in neuritogenesis. *Neurochemical research*, Vol.35, No.12, (December 2010), pp. 2168-2174, ISSN 0364-3190

Fink, D., Contreras, M.L., Lelkes, P.I. & Lazarovici, P. (1989). Staphylococcus aureus alpha-toxin activates phospholipases and induces a Ca^{2+} influx in PC12 cells. *Cellular signalling*, Vol.1, No.4, pp. 387-393, ISSN 0898-6568

Fujita, K., Lazarovici, P. & Guroff, G. (1989). Regulation of the differentiation of PC12 pheochromocytoma cells. *Environmental health perspectives*, Vol.80, (March 1989), pp. 127-142, ISSN 0091-6765

Gerdin, M.J. & Eiden, L.E. (April 2007). Regulation of PC12 cell differentiation by cAMP signaling to ERK independent of PKA: do all the connections add up? In: *Science's STKE: signal transduction knowledge environment*, 17.04.2007, ISSN 1525-8882, Available from http://stke.sciencemag.org/cgi/content/abstract/sigtrans;2007/382/pe15

Giner, D., López, I., Neco, P., Rossetto, O., Montecucco, C. & Gutiérrez, L.M. (2007). Glycogen synthase kinase 3 activation is essential for the snake phospholipase A2 neurotoxin-induced secretion in chromaffin cells. *The European journal of neuroscience*, Vol.25, No.8, (April 2007), pp. 2341-2348, ISSN 0953-816X

Guidarelli, A., Palomba, L. & Cantoni, O. (2000) Peroxynitrite-mediated release of arachidonic acid from PC12 cells. *British journal of pharmacology*, Vol.129, No.8, (April 2000), pp. 1539-1541, ISSN 0007-1188

Gunning, P. W, Letourneau, P. C., Landreth, G. E., & Shooter, E. M. (1981).The action of nerve growth factor and dibutyryl adenosine cyclic 3', 5'-monophosphate on rat pheochromocytoma reveals distinct stages in the mechanisms underlying neurite outgrowth. *The Journal of neuroscience*, Vol.1, No.10, (October 1981), pp. 1085-1095 ISSN 0270-6474

Hanada, T., Sato, T., Arioka, M., Uramoto, M. & Yamasaki, M. (1996). Purification and characterization of a 15 kDa protein (p15) produced by Helicosporium that exhibits distinct effects on neurite outgrowth from cortical neurons and PC12 cells. *Biochemical and biophysical research communications*, Vol.228, No.1, (November 1996), pp. 209-215, ISSN 0006-291X

Hanasaki, K. (2004). Mammalian Phospholipase A2: Phospholipase A2 Receptor. *Biological & pharmaceutical bulletin*, Vol.27, No.8, pp. 1165-1167, ISSN 0918-6158

Ikemoto, A., Kobayashi, T., Watanabe, S. & Okuyama, H. (1997). Membrane fatty acid modifications of PC12 cells by arachidonate or docosahexaenoate affect neurite outgrowth but not norepinephrine release. *Neurochemical research*, Vol.22, No.6, (June 1997), pp. 671-678, ISSN 0364-3190

Ikeno, Y., Konno, N., Cheon, S.H., Bolchi, A., Ottonello, S., Kitamoto, K., & Arioka, M. (2005) Secretory phospholipases A2 induce neurite outgrowth in PC12 cells through lysophosphatidylcholine generation and activation of G2A receptor. *Journal of biological chemistry*, Vol.280, No.30, (July 2005), pp. 28044-28052, ISSN 0021-9258

Kanthasamy, A.G., Ardelt, B., Malave, A., Mills, E.M., Powley, T.L., Borowitz, J.L. & Isom, G.E. (1997). Reactive oxygen species generated by cyanide mediate toxicity in rat pheochromocytoma cells. *Toxicology letters*, Vol.93, No.1, (September 1997), 47-54, ISSN 0378-4274

Kim, H.Y., Akbar, M. & Kim, K.Y. (2001). Inhibition of neuronal apoptosis by polyunsaturated fatty acids. *Journal of molecular neuroscience*, Vol.16, No.2-3, (April-June 2001), pp. 223-227; discussion 279-284, ISSN 0895-8696

Kini, R.M. (2005). Structure-function relationships and mechanism of anticoagulant phospholipase A2 enzymes from snake venoms. *Toxicon*, Vol.45, No.8, (June 2005), pp. 1147-1161, ISSN 0041-0101

Kini, R.M. & Evans, H.J. (1989). A model to explain the pharmacological effects of snake venom phospholipases A2. *Toxicon*, Vol.27, No.6, pp. 613-635, ISSN 0041-0101

Kontou, M., Weidemann, W., Bork, K. & Horstkorte, R. (2009). Beyond glycosylation: sialic acid precursors act as signaling molecules and are involved in cellular control of differentiation of PC12 cells. *Biological chemistry*, Vol.390, No.7, (July 2009), 575-579, ISSN 1431-6730

Kudo, I., Matsuzawa, A., Imai, K., Murakami, M., & Inoue, K. (1996). Function of type II phospholipase A2 in dopamine secretion by rat neuronal PC12 cells. *Journal of lipid mediators and cell signalling*, Vol.14, No.1-3, (September 1996), 25-31, ISSN 0929-7855

Lambeau, G. & Gelb, M.H. (2008). Biochemistry and physiology of mammalian secreted phospholipases A(2). *Annual review of biochemistry*, Vol.77, pp. 495–520, ISSN 0066-4154

Lambeau, G. & Lazdunski, M. (1999) Receptors for a growing family of secreted phospholipases A2. *Trends in pharmacological sciences*, Vol.20, No.4, (April 1999), pp. 162-170, ISSN 0165-6147

Ledeen, R.W., Wu, G., Cannella, M.S., Oderfeld-Nowak, B. & Cuello, A.C. (1990). Gangliosides as neurotrophic agents: studies on the mechanism of action. *Acta neurobiologiae experimentalis*, Vol.50, No.4-5, pp. 439-449, ISSN 0065-1400

Lei, X., Barbour, S.E. & Ramanadham, S. (2010). Group VIA Ca2+-independent phospholipase A2 (iPLA2beta) and its role in beta-cell programmed cell death. *Biochimie*, Vol.92, No.6, (June 2010), pp. 627-637, ISSN 0300-9084

Lin, L.L., Wartmann, M., Lin, A.Y., Knopf, J.L., Seth, A. & Davis, R.J. (1993) cPLA2 is phosphorylated and activated by MAP kinase. *Cell*, Vol.72, No.2, Jan pp. 269-278, ISSN 0092-8674

Ma, M.T., Yeo, J.F., Farooqui, A.A., Zhang, J., Chen, P. & Ong, W.Y. (2010). Differential effects of lysophospholipids on exocytosis in rat PC12 cells. *Journal of neural transmission*, Vol.117, No.3, (March 2010), pp. 301-308, ISSN 0300-9564

Ma, M.T., Yeo, J.F., Farooqui, A.A. & Ong, W.Y. (2011). Role of calcium independent phospholipase A2 in maintaining mitochondrial membrane potential and preventing excessive exocytosis in PC12 cells. *Neurochemical research*, Vol.36, No.2, (February 2011), pp. 347-354, ISSN 0364-3190

Macdonald, N.J., Perez-Polo, J.R., Bennett, A.D. & Taglialatela, G. (1999). NGF-resistant PC12 cell death induced by arachidonic acid is accompanied by a decrease of active PKC zeta and nuclear factor kappa B. *Journal of neuroscience research*, Vol.57, No.2, (July 1999), pp. 219-226, ISSN 0360-4012

Makarova, Y.V., Osipov, A.V., Tsetlin, V.I. & Utkin, Y.N. (2006). Influence of phospholipases A2 from snake venoms on survival and neurite outgrowth in pheochromocytoma cell line PC12. *Biochemistry (Moscow)*, Vol.71, No.6, (June 2006), pp. 678-684, ISSN: 0006-2979

Martin, R.E. (1998). Docosahexaenoic acid decreases phospholipase A2 activity in the neurites/nerve growth cones of PC12 cells. *Journal of neuroscience research*, Vol.54, No.6, (December 1998), pp. 805-813, ISSN 0360-4012

Masuda, S., Murakami, M., Takanezawa, Y., Aoki, J., Arai, H., Ishikawa, Y., Ishii, T., Arioka, M. & Kudo, I. (2005). Neuronal expression and neuritogenic action of group X secreted phospholipase A2. *Journal of biological chemistry*, Vol.280, No.24, (June 2005), pp. 23203-23214, ISSN 0364-3190

Masuda, S., Yamamoto, K., Hirabayashi, T., Ishikawa, Y., Ishii, T., Kudo, I. & Murakami, M. (2008). Human group III secreted phospholipase A2 promotes neuronal outgrowth and survival. *The Biochemical journal*, Vol.409, No.2, (January 2008), pp. 429-438. ISSN 0264-6021

Matsuzawa, A., Murakami, M., Atsumi, G., Imai, K., Prados, P., Inoue, K. & Kudo, I. (1996). Release of secretory phospholipase A2 from rat neuronal cells and its possible function in the regulation of catecholamine secretion. *The Biochemical journal*, Vol.318, No.Pt 2, (September 1996), pp. 701-709, ISSN 0264-6021

Mishra, R. (June 2010). Glycogen synthase kinase 3 beta: can it be a target for oral cancer. In: *Molecular Cancer*, 11.06.2010, Available from: http://www.molecular-cancer.com/content/9/1/144

Moolenaar, W.H., van Meeteren, L.A. & Giepmans, B.N. (2004). The ins and outs of lysophosphatidic acid signaling. *BioEssays: news and reviews in molecular, cellular and developmental biology*, Vol.26, No.8, pp. 870-881, ISSN 0265-9247

Mori, A., Yasuda, Y., Murayama, T. & Nomura, Y. (2001). Enhancement of arachidonic acid release and prostaglandin F(2alpha) formation by Na$_3$VO$_4$ in PC12 cells and GH3 cells. *European journal of pharmacology*, Vol.417, No.1-2, (April 2001), pp. 19-25, ISSN 0014-2999

Murakami, M., Taketomi, Y., Miki, Y., Sato, H., Hirabayashi, T. & Yamamoto, K. (2011). Recent progress in phospholipase A(2) research: From cells to animals to humans. *Progress in lipid research*, Vol.50, No.2, (April 2011), pp. 152-192, ISSN 0163-7827

Nakamura, H., Hirabayashi, T., Someya, A., Shimizu, M. & Murayama, T. (2004). Inhibition of arachidonic acid release and cytosolic phospholipase A2 alpha activity by D-erythro-sphingosine. *European journal of pharmacology*, Vol.484, No.1, (January 2004), pp. 9-17, ISSN 0014-2999

Nakashima, S., Ikeno, Y., Yokoyama, T., Kuwana, M., Bolchi, A., Ottonello, S., Kitamoto, K., Arioka, M. (2003). Secretory phospholipases A2 induce neurite outgrowth in PC12 cells. *The Biochemical journal*, Vol.376, No.Pt 3, (December 2003), pp. 655-666, ISSN 0264-6021

Nakashima, S., Kitamoto, K. & Arioka, M. (2004). The catalytic activity, but not receptor binding, of sPLA2s plays a critical role for neurite outgrowth induction in PC12 cells. *Brain research*, Vol.1015, No.1-2, (July 2004), pp. 207-211, ISSN: 0006-8993

Neco, P., Rossetto, O., Gil, A., Montecucco, C. & Gutiérrez, L.M. (2003). Taipoxin induces F-actin fragmentation and enhances release of catecholamines in bovine chromaffin cells. J Neurochem. *Journal of neurochemistry*, Vol.85, No.2, (April 2003), pp. 329-337, ISSN 0022-3042

Nevalainen, T.J., Graham, G.G. & Scott, K.F. (2008). Antibacterial actions of secreted phospholipases A2. Review. *Biochimica et Biophysica Acta*, Vol.1781, No.1-2, (January-February 2008), pp. 1-9, ISSN 0006-3002

Ohsawa, K., Mori, A., Horie, S., Saito, T., Okuma, Y., Nomura, Y. & Murayama, T. (2002). Arachidonic acid release and prostaglandin F2alpha formation induced by phenylarsine oxide in PC12 cells: possible involvement of secretory phospholipase A2 activity. *Biochemical pharmacology*, Vol.64, No.1, (July 2002), pp. 117-124, ISSN 0006-2952

Osipov, A.V., Filkin, S.Y., Makarova, Y.V., Tsetlin, V.I. & Utkin, Y.N. (2010). A new type of thrombin inhibitor, noncytotoxic phospholipase A2, from the Naja haje cobra venom. *Toxicon*, Vol.55, No.2-3, (February-March 2010), pp. 186-194, ISSN 0041-0101

Park, J.G., Yuk, Y., Rhim,H., Yi, S.Y. & Yoo, Y.S. (2002). Role of p38 MAPK in the regulation of apoptosis signaling induced by TNF-alpha in differentiated PC12 cells. *Journal of biochemistry and molecular biology*, Vol.35, No.3, (May 2002), pp. 267-272, ISSN 1225-8687

Pytlowany, M., Strosznajder, J.B., Jeśko, H., Cakała, M. & Strosznajder, R.P. (2008). Molecular mechanism of PC12 cell death evoked by sodium nitroprusside, a nitric oxide donor. *Acta biochimica Polonica*, Vol.55, No.2, pp. 339-347. ISSN 0001-527X

Ravni, A., Bourgault, S., Lebon, A., Chan, P., Galas, L., Fournier, A., Vaudry, H., Gonzalez, B., Eiden, L.E. & Vaudry, D. (2006). The neurotrophic effects of PACAP in PC12 cells: control by multiple transduction pathways. *Journal of neurochemistry*, Vol.98, No.2, (July 2006), pp. 321-329, ISSN 0022-3042

Ray, P., Berman, J.D., Middleton, W. & Brendle, J. (1993). Botulinum toxin inhibits arachidonic acid release associated with acetylcholine release from PC12 cells. *Journal of biological chemistry*, Vol.268, No.15, (May 1993), pp. 11057-11064, ISSN 0021-9258

Ray, P., Ishida, H., Millard, C.B., Petrali, J.P. & Ray, R. (1999). Phospholipaise A2 and arachidonic acid-mediated mechanism of neuroexocytosis: a possible target of botidinum neurotoxin A other then SNAP-25. *Journal of applied toxicology*, Vol.19, Suppl 1, (December 1999), pp. S27-28, ISSN 0260-437X

Raya, S.A., Trembovler, V., Shohami, E. & Lazarovici, P. (1993). Cytolysins increase intracellular calcium and induce eicosanoids release by pheochromocytoma PC12 cell cultures. *Natural toxins*, Vol.1, No.5, pp. 263-270, ISSN 1056-9014

Posse de Chaves, E.I. (2006). Sphingolipids in apoptosis, survival and regeneration in the nervous system. *Biochimica et Biophysica Acta*, Vol.1758, No.12, (December 2006), pp. 1995-2015, ISSN 0006-3002

Rusanescu, G., Qi, H., Thomas, S.M., Brugge, J.S. & Halegoua, S. (1995). Calcium influx induces neurite growth through a Src-Ras signaling cassette. *Neuron*, 15, No.6, (December 1995), pp. 1415-1425 ISSN 0896-6273

Schaloske, R.H. & Dennis, E.A. (2006). The phospholipase A2 superfamily and its group numbering system. *Biochimica et Biophysica Acta*, Vol.1761, No.11, (November 2006), pp. 1246-1259, ISSN 0006-3002

Shin, K.J., Chung, C., Hwang, Y.A., Kim, S.H., Han, M.S., Ryu, S.H. & Suh, P.G. (2002). Phospholipase A2-mediated Ca2+ influx by 2,2',4,6-tetrachlorobiphenyl in PC12 cells. *Toxicology and applied pharmacology*, Vol.178, No.1, (January 2002), pp. 37-43. ISSN 0041-008X

Shinzawa, K. & Tsujimoto, Y. (2003). PLA2 activity is required for nuclear shrinkage in caspase-independent cell death. *The Journal of cell biology*, Vol.163, No.6, (December 2003), pp. 1219-1230 ISSN 0021-9525

Schonfeld, E., Yasharel, I., Yavin, E. & Brand, A. (2007). Docosahexaenoic acid enhances iron uptake by modulating iron transporters and accelerates apoptotic death in PC12 cells. *Neurochemical research*, Vol.32, No.10, (October 2007), pp. 1673-84, ISSN 0364-3190

Skaper, S.D. (2008). The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors. *CNS and neurological disorders drug targets*, Vol.7, No.1, (February 2008), pp. 46-62, ISSN 1871-5273

Someya, A., Horie, S. & Murayama, T. (2002). Arachidonic acid release and prostaglandin F(2alpha) formation induced by anandamide and capsaicin in PC12 cells. *European journal of pharmacology*, Vol.450, No.2, (August 2002), pp. 131-139, ISSN 0014-2999

Tischler, A.S., Powers, J.F. & Alroy, J. (2004). Animal models of pheochromocytoma. *Histology and histopathology*, Vol.19, No.3, (July 2004), pp. 883-95, ISSN 0213-3911

Tsukada, Y., Chiba, K., Yamazaki, M. & Mohri, T. (1994). Inhibition of the nerve growth factor-induced neurite outgrowth by specific tyrosine kinase and phospholipase inhibitors. *Biological & pharmaceutical bulletin*, Vol.17, No.3, (March 1994), pp. 370-375, ISSN 0918-6158

Ulloth, J.E., Casiano, C.A. & De Leon, M. (2003). Palmitic and stearic fatty acids induce caspase-dependent and -independent cell death in nerve growth factor differentiated PC12 cells, *Journal of neurochemistry,* Vol.84, No.4, (February 2003), pp. 655-668, ISSN 0022-3042

Valentin, E. & Lambeau, G. (2000) Increasing molecular diversity of secreted phospholipases A2 and their receptors and binding proteins. *Biochimica et Biophysica Acta* Vol.1488, No.1-2, (October 2000), pp. 59–70, ISSN 0006-3002

Vaudry, D., Stork, P.J., Lazarovici, P. & Eiden, L.E. (2002). Signaling pathways for PC12 cell differentiation: making the right connections. *Science*, Vol.296, No.5573, (May 2002), pp. 1648-1649, ISSN 0036-8075

Wakatsuki, S., Arioka, M., Dohmae, N., Takio, K., Yamasaki, M. & Kitamoto, K. (1999). Characterization of a novel fungal protein, p15, which induces neuronal differentiation of PC12 cells. *Journal of biochemistry*, Vol.126, No.6, (December 1999), pp. 1151-1160, ISSN 0021-924X

Wei, S., Ong, W.Y., Thwin, M.M., Fong, C.W., Farooqui, A.A., Gopalakrishnakone, P. & Hong W. (2003). Group IIA secretory phospholipase A2 stimulates exocytosis and neurotransmitter release in pheochromocytoma-12 cells and cultured rat
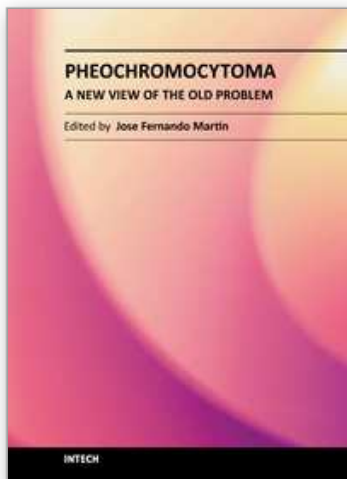
hippocampal neurons. *Neuroscience*, Vol.121, No.4, pp. 891-898, ISSN: 0306-4522

Wu, Y., Sheng, W., Chen, L., Dong, H., Lee, V., Lu, F., Wong, C.S., Lu, W.Y. & Yang, B.B. (2004). Versican V1 isoform induces neuronal differentiation and promotes neurite outgrowth. *Molecular biology of the cell*, Vol.15, No.5, (May 2004), pp. 2093-2104, ISSN: 1059-1524

Pheochromocytoma - A New View of the Old Problem

Edited by Dr. Jose Fernando Martin

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