Vol. 183, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1992 Pages 300-305

PAF-INDUCED ACTIVATION OF POLYPHOSPHOINOSITIDE-HYDROLYZING PHOSPHOLIPASE C IN CEREBRAL CORTEX

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Received January 22, 1992

SUMMARY: The action of platelet-activating factor (PAF) on phosphoinositide hydrolysis was studied in rat brain slices. PAF produced a significant increase of ^{32}P incorporation into phosphoinositides and phosphatidic acid (PA), in a dose- and time-dependent manner. Concomitantly, an increase of inositol phosphates and diacylglycerol (DAG) production was observed. Both inositol bisphosphate (IP_2) and inositol trisphosphate (IP_3) were detected as early as 5 s and they returned immediately to basal levels; concommitantly, formation of inositol monophosphate (IP) was detected. These findings demonstrated that PAF causes a rapid hydrolysis of polyphosphoinositides in cerebral cortex by a phospholipase C-dependent mechanism followed by subsequent resynthesis.

PAF, an alkyl-ether phospholipid (1-O-alkyl-2-acetyl-sn-glycerol-3-phosphocholine) (1) is an active lipid produced by a variety of cells, and released in response to different stimuli (2). Recently, binding sites for PAF have been characterized in the brain (3,4); however, the biochemical mechanism which PAF receptor uses in transmembrane signaling is still uncertain. Several laboratories have recently

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Abbreviations: DAG, diacylglycerol; IP, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; PA, phosphatidic acid; PAF, platelet-activating factor; PI, phosphatidylinositol; PIP, phosphatidylinositol 4 phosphate; PIP_2, phosphatidylinositol 4,5 bisphosphate.

0006-291X/92 $1.50

reported some evidence that PAF is involved in the regulation of neuronal function; thus, it has been reported that the nervous tissue is able to synthesize PAF by the novo pathway (5). On the other hand, there are studies showing increases in PAF levels in the brain during ischemia and convulsions (6). Moreover, PAF antagonists can modify pathological consequences of brain injury (6). Evidence derived from phospholipid metabolism studies, supports that both phospholipases C and A_2 are playing critical role in the signal transduction mechanism for PAF in platelets and other cell types (7). With respect to neuronal tissue, the secondary messenger systems associated with PAF receptors, have not yet been defined fully. In this context, some evidence has been reported in neurohybrid cells (8,9), and in synaptosomal preparation (10). The objective of the present work was to characterize the PAF receptor-mediated phosphoinositide turnover, using a more physiological preparation as brain slices, which preserve the cellular integrity, in order to acquire a more knowledge about the PAF role in central nervous system.

MATERIALS AND METHODS

Materials

[^{32}P]ortophosphate, [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid and myo[2-^{3}H]inositol (14.3 Ci/mmol) were purchased from Amersham. PAF was from Sigma Chemical (St. Louis, MO). PCA-4248, a PAF antagonist, was a generous gift from Alter S.A. Lab. (Madrid, Spain). High performance thin layer chromatography plates were purchased from Merck (Darmstadt, Germany). All other reagents were of highest analytical grade available.

Tissue preparation and ^{32}P incorporation into phospholipids

Slices were prepared from rat cerebral cortex as described previously (11). 50 µl aliquots of gravity-packed slices were pipetted into glass tubes containing 50 µCi ^{32}P orthophosphate in 180 µl of Krebs Henseleit solution, pH 7.4 and shaken at 37^{o} C for 45 min. Thereafter, different concentration of PAF were added. In some experiments 100 µM PAF antagonist was added 1 min prior to PAF treatment. The incubation mixtures were aerated periodically, and incubation was stopped by removing the medium and replacing with 20 ml of ice-cold chlorophorm/methanol/13 M HCl (20:100:0.7, v/v). After 15 min at 4^{o} C, phosphoinositides and PA were extracted essentially as described previously (12). Phospholipid separation was performed by thin layer chromatography as described previously (11).

Diacylglycerol production

For the analysis of DAG, slices were preincubated in the presence of [^{3}H]arachidonic acid (1 µCi/ml) for 120 min at

37° C. After labeling, 100 µl aliquots were treated with PAF. Lipids were then extracted by the method of Bligh and Dyer (13). DAG was separated as described previously (11).

Inositol phosphates accumulation

For the labeling with myo[2-^{3}H]inositol, brain slices were incubated in the presence of 8 µCi/ml isotope for 120 min. Then, slices were washed three times with a Krebs Henseleit solution. After 10 min at 37° C, 1 nM PAF was added. Incubations were stopped after the appropriate times with 10 % cold trichloroacetic acid. After 30 min at 4° C, slices were sonicated and centrifuged at 800 x g for 10 min. Pellets were used for protein measurement, and supernatants were used for inositol phosphates analysis, as described previously (14).

RESULTS AND DISCUSSION

Fig. 1 illustrates the PAF effect on incorporation of ^{32}P into phosphoinositides and PA (time course). Addition of 1 nM PAF resulted in a slight but not significant decrease of ^{32}P-polyphosphoinositides. Then, labeling increased strongly over basal levels, reaching a maximal increase by 15 s; a more rapid increase of ^{32}P incorporation into PA was observed.

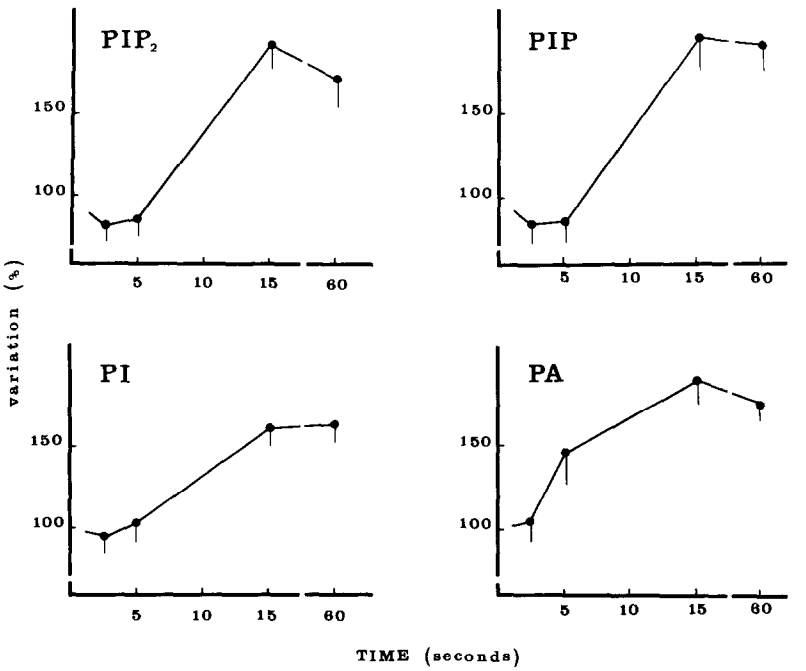

Fig.1. Time-course of PAF effects on ^{32}P incorporation into phosphoinositides and PA. ^{32}P-prelabeled cortical slices were incubated in the absence or the presence of 1 nM PAF. Data represent percentage of variation relative to control value (100 %). Results are mean ± SE of three determinations from three separate experiments.

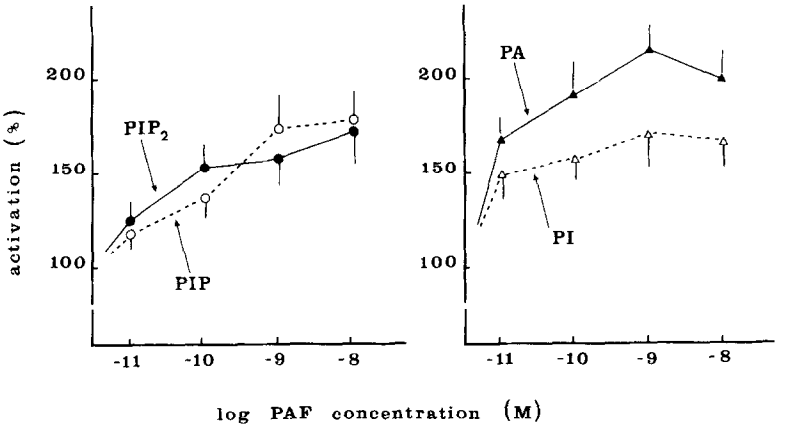

Fig. 2. Dose-response relationship for PAF action on phosphoinositides and PA. The PAF treatment was 15 s. Data represent percentage of variation relative to control value (100 %). Results are mean ± SE of three determinations from three separate experiments.

PAF-induced increase on ^{32}P incorporation into inositol lipids and PA was dependent on the concentration of the agonist (Fig. 2) PAF-induced PA and phosphoinositide formation was totally inhibited by pretreatment with 100 μM PCA-4248, a receptor antagonist described previously (15) (data not shown). This observation indicates that PAF effects on phosphoinositide metabolism in cerebral cortex pass through an interaction with its receptors. In this regard, the Kd described for the PAF high affinity binding sites in rat brain is 3 nM (3) similar to the most effective dose described here.

In addition, 1 nM PAF stimulated a rapid (5 s) and transient (15 s) accumulation of IP_3 (Table 1). The early accumulation of IP_3 was accompanied by an accumulation of IP_2. The accumulation of IP occurred more slowly and was accompanied by a return to basal levels of both IP_3 and IP_2. In the same Table, data concerning to DAG production are also shown. As can be seen, DAG formation is stimulated by PAF concurrently with IP_3 accumulation. However, the slower kinetic of PAF-induced DAG formation compared to that of IP_3 suggests that phospholipids, different from phosphoinositides, may serve as sources for the elevated DAG, as it has been previously observed (16, 17).

The time dependence of appearance of the phosphoinositide metabolites (inositol phosphates and DAG) and the rapid

TABLE 1

Effect of PAF on inositol phosphates and DAG in cerebral cortex

time (s)	IP	IP_2	IP_3	DAG
5	108 ± 14	222 ± 21	230 ± 19	117 ± 12
15	150 ± 22	250 ± 16	140 ± 11	126 ± 10
60	116 ± 9	109 ± 8	97 ± 17	115 ± 18

Cortical slices were incubated with 1 nM PAF for the indicated times. Results are given as percentage respect to control value (100 %). Data, pooled from five experiments, are the mean ± SE from triplicate determinations.

increase of PA indicate that PAF induces PIP_2 or both PIP and PIP_2 hydrolysis, via phospholipase C. In this regard, the PAF stimulating effect on the hydrolysis of PIP_2 or both PIP_2 and PIP has been documented (8, 10, 17-21).

The signal transduction processes, induced by PAF in central nervous system, have not extensively examined. According to previous data (10), addition of PAF to ^{32}P-labeled synaptosomes resulted in a rapid transient decrease in the ^{32}P content of PIP and PIP_2, without changes in both PA and phospatidylinositol (PI) labeling. Since in the experimental conditions used by these authors (stimulation without the presence of ^{32}P) resynthesis cannot be observed, those data are compatible with the idea that PAF stimulates phospholipase C in brain, as reported in the present work. It would be interesting to determine the involvement of other phospholipases (i.e. phospholipase A_2) in the PAF action. In this regard, activation of phospholipase A_2 by PAF has been described in hepatocytes (22). Moreover, it is also likely that both distinct pathways of PAF-induced hydrolysis of phosphoinositides (by phospholipases A_2 and C) coexist in the central nervous system, as described in human skin cells (17) and Kupffer cells (20).

Support for the idea that polyphosphoinositide hydrolysis by phospholipase C is mediating the PAF mechanism of action on neuronal tissue is provided by previous observations, specially those that PAF induces calcium mobilization in two clonal neurohybrid cell lines (8,9).

In summary, we have shown evidence that biologically relevant concentrations of PAF stimulate polyphosphoinositide

hydrolysis via phospholipase C in cerebral cortex. Additional work is now carried out in order to further characterize the signal transduction mechanism evoked by PAF in central nervous system.

ACKNOWLEDGMENTS

The authors are indebted to Miss M. Sanz and Mr. J. Palacin for their valuable assistance. This work was supported in part by DGICIT, CAM, FIS and Fundación Ramón Areces.

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