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Nitric Oxide Mediates the PAF-Stimulated Cyclic GMP Production in Hippocampal Slices

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Platelet activating factor (PAF) treatment caused a transient rise in cyclic GMP levels in rat hippocampal slices. The stimulation of cyclic GMP synthesis induced by PAF was dose-dependent and was suppressed after treatment with PCA-4248, a PAF antagonist, a fact that could suggest the involvement of specific PAF receptors. In addition, when slices were incubated in the presence of N-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor, PAF-stimulated cyclic GMP generation was abolished. Therefore, PAF activates guanylyl cyclase most probably via formation of NO. PAF also induced a time-dependent increase of NO synthase activity in hippocampal slices in correlation with the increase observed in cyclic GMP levels.
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Platelet-activating factor (PAF), a potent mediator of the inflammatory and immune response (1), is generated by neurons and by other cells of the nervous system (2). The presence of PAF and PAF receptors in the central nervous system indicates its neural actions as intercellular (3) and intracellular messenger molecule (4).

In the hippocampus, PAF specifically enhanced excitatory neurotransmitter release (5), mobilized intracellular Ca^{2+} (6) and activated expression of immediate early genes (7). These findings and other indicating that PAF participates in long-term potentiation (8) demonstrate that it plays an important role in the hippocampal function.

Nitric oxide (NO) has emerged as an ubiquitous molecular mediator involved in a wide variety of biological processes in several organ systems (9) which has been widely accepted as a novel neuronal messenger in the brain (10,11). Neuronal excitation leads to elevation in cyclic GMP levels in numerous different brain areas including hippocampus through Ca^{2+}-dependent NO formation and the subsequent activation of the soluble form of guanylyl cyclase (12). NO-dependent cyclic GMP efflux into hippocampus has been reported (13) aside from having been postulated as a component of PAF stimulated microvascular responses (14,15). Moreover, NO synthase inhibitors reduced the PAF-stimulated increase in vasopermeability (16). These findings support the hypothesis that NO generation is a step in the biochemical signaling pathway of some cellular responses to PAF.

In this paper, the effect of PAF on cyclic GMP production in hippocampal slices and the involvement of NO synthase has been studied.

MATERIALS AND METHODS

L-[2,3,4,5-^{3}H]Arginine monohydrochloride (specific activity 11.1 GBq/mmol, 64 Ci/mmol) and [^{3}H]cyclic GMP radioimmunoassay kit were obtained from Amersham. Dowex AG50W-X8 resin was from Bio-Rad. PCA-4248 was a gift by laboratories ALTER, S.A. All other chemicals were obtained from Sigma.

Male Wistar rats, weighing 200-250 g, were sacrificed and the hippocampi were rapidly dissected and placed in

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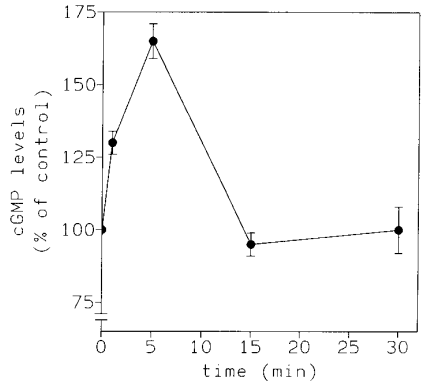

FIG. 1. Time-course of PAF effect on cyclic GMP levels. Hippocampal slices were treated with 10^{-7}M PAF for the times indicated followed by measuring cyclic GMP. Data represent percentage of variation respect to control value (100%). Results are the mean ± S.E. of three determinations from three separate experiments.

ice-cold Krebs buffer containing (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11.7 glucose and 1.2 $CaCl_2$, which has been equilibrated with 95% O_2/5% CO_2. Transverse slices (500×500 μm) were obtained using a McIlwain tissue chopper and were preincubated in fresh Krebs solution in a shaking water bath for 60 min at 37°C.

To determine cyclic GMP, fifty μl aliquots of gravity-packed slices were transferred to minivials and incubated in 950 μl Krebs solution containing 1 mM IBMX at 37°C for 30 min before the addition of specific drugs. At this point PAF or differents inhibitors were added, the tubes were regassed and the slices were incubated for different times. The incubation was terminated by adding 1.5 ml of cold 10% trichloroacetic acid. After homogenization, centrifugation and extraction of trichloroacetic acid by water-saturated diethyleter the levels of cyclic GMP in hippocampal slices were determined by using a [^{3}H]-cyclic GMP radioimmunoassay kit.

To determine the NO synthase activity, hippocampal slices were incubated with PAF at 37°C for different times. Slices were then homogenized with a Polytron-type homogenizer in 750 μl of 50 mM Tris-NaOH buffer pH 7.4 containing 0.1 mM EGTA, 0.1 mM EDTA, proteinase inhibitors (10 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin-A and 1 mM PMSF) and 0.1% β-mercaptoethanol. Homogenates were incubated and centrifuged as described previously (17). Supernatants were immediately removed and frozen at −70°C until use. NO synthase activity was determined by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline (18). Supernatant (100 μl) was incubated with 100 μl of NO synthase reaction buffer (10 μM L-arginine, 1 μCi L-[^{3}H]arginine, 1 mM NADPH, 3 μM BH_4, 3 μM FAD, 2 mM $CaCl_2$, 10 mM HEPES, pH 7.4, 100 U calmodulin) for 30 min at 37°C. The reaction was terminated by the addition of 1 ml stop buffer (20 mM HEPES, 2 mM EGTA, pH 5.5). Samples were passed through DOWEX AG50W-X8 columns (Na^+ form) equilibrated with stop buffer and the effluent was then collected. This was followed by washing the columns with 2 ml of stop buffer and collecting the effluent. L-[^{3}H]citrulline was counted by using liquid scintillation spectrometry. The level of L-[^{3}H]citrulline was defined after subtracting the blank value, which was performed in the absence of cofactors and NO synthase inhibitors.

Protein concentration was determined by the method of Lowry et al. (19).

RESULTS

The effect of PAF on cyclic GMP levels was determined in rat hippocampal slices. PAF, in a concentration of 10^{-7}M, caused a rapid and transient increase of the cyclic nucleotide levels after 15 s of treatment (Fig. 1). This effect resulted to be concentration-dependent, with the most effective dose obtained at 10^{-7}M (Fig. 2). The results presented in Table 1, demonstrate that PAF-stimulated cyclic GMP levels were reversed in the presence of 10 μM N-nitro-L-arginine, a NO synthase inhibitor (20), a fact that might suggest that the NO synthase activation is required. If the slices were incubated in the presence of a PAF antagonist, PCA-4248 (21), the cyclic GMP levels increased by PAF were inhibited, suggesting the involvement of hippocampal PAF receptors (Table 1). In unstimulated slices, no significant variations on cyclic GMP levels were observed after N-nitro-L-arginine or PCA-4248 treatments (not illustrated).

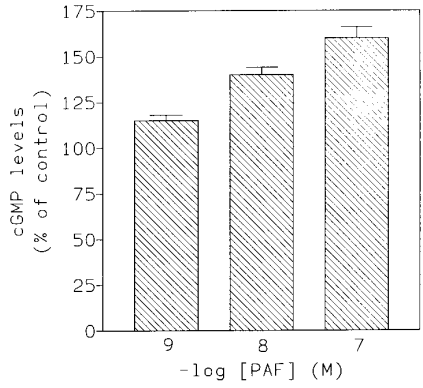

FIG. 2. Dose-response relationship for PAF action on cyclic GMP formation. Slices were treated with different PAF concentration for 5 min. Then cyclic GMP was measured. Data represent percentage of variation respect to control value (100%). Results are the mean ± S.E. from three separate experiments.

NO synthase activity was determined after PAF treatment, by measuring L-[^{3}H]citrulline generated from L-[^{3}H]arginine. Thus, in the presence of 10^{-7}M PAF, L-[^{3}H]citrulline production was increased at all times tested (Fig.3). Maximal response was obtained after 1 min of PAF treatment.

DISCUSSION

There is an evidence that PAF exerts a direct effect on the cells of the central nervous system (4). In this regard, we have reported some actions in the brain. Thus, PAF, acting through its specific receptors, increased (Na^+,K^+)ATPase activity (22) and phosphoinositide turnover in cerebral cortex (23). To characterize further the mechanism of the action of PAF in central nervous system, the effect of PAF on the production of cyclic GMP and NO in the hippocampus has been studied. It is demonstrated here that PAF caused a rapid and transient increase in cyclic GMP in hippocampal slices which results correlate well with the activation of NO synthase observed after PAF treatment. In regard to the results obtained by using the PAF antagonist, PCA-4248, it can be suggested that the PAF effects appear to require the specific PAF receptors. Concerning the basal cyclic GMP content, no significant modulation related to NO production could be detected by using N-nitro-L-arginine, in comparison with

TABLE 1
Effects of L-NNA or PCA-4248 on PAF-Stimulated Cyclic GMP Accumulation

Treatment	Cyclic GMP formation (% of control)
Control (PAF vehicle)	100 ± 13
PAF (10^{-7} M)	152 ± 16*
N-nitro-L-arginine + PAF	85 ± 10
PAF + PCA-4248	101 ± 8

The concentrations of N-nitro-L-arginine and PCA-4248 were 10 μM. Data represent percentage of variation relative to control value (100%). Results are the mean ± S.E. from three separate experiments. *$P < 0.05$.

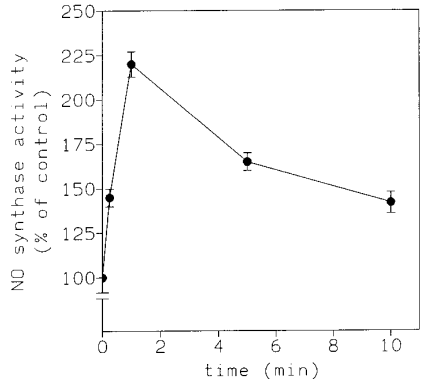

FIG. 3. PAF effect on NO synthase activity. Slices were treated with 10^{-7}M PAF for the times indicated, and NO synthase activity was measured. Data represent percentage of variation respect to control value (100%). Results are the mean ± S.E. from three separate experiments.

control condition. Since PAF is able to increase significantly cyclic GMP content in hippocampus, it is likely that although NO/cyclic GMP pathway can be stimulated, in basal conditions the low cyclic nucleotide level is not sufficient to detect a modulation of this pathway. Similar results have been reported previously in bovine aortic endothelial cells (24).

Nitric oxide formed by NO synthase may act either intracellularly or diffuse out and act extracellularly at the heme moeity of soluble guanylyl cyclase to produce an increase in the content of cyclic GMP (12). It has been reported that levels of cyclic GMP were increased following NMDA receptors activation in rat cerebellar and hippocampal slices (25,26). In the presence of NO synthase inhibitors the level of cyclic GMP following NMDA receptors stimulation was reduced. These data support a regulatory role for NO transduction system, subsequent to the activation of a subtype of glutamate receptors (NMDA), as an intra- and extraneural messenger in hippocampus. It is interesting to note that PAF appears to mediate release of glutamate in brain (5). Besides, an increased release of glutamate and aspartate in the hippocampus during ischemia has been reported (27) and its neurotoxicity attributed to NMDA receptors in neurons (28). Although the endogenous concentration of PAF in brain is low, it increases dramatically after different neuronal damage, including ischemia (29). It is possible that PAF, by interacting with presinaptic binding sites, stimulates glutamate/NMDA neurotransmission leading to neuronal disfunctions (4).

The formation of cyclic GMP in different brain areas, including hyppocampus, has been well documented (12). However, the function of cyclic GMP in the central nervous system is still unclear, although some findings are now beginning to shed new light on this respect. Thus, the existence of cyclic GMP-operated channels and phosphorylation of specific protein by a cyclic GMP-dependent protein kinase have been recently reported (12).

In summary, the present results suggest that PAF, after interacting with its specific receptors, stimulates production of NO from L-arginine, which in turn activates soluble guanylyl cyclase in hippocampus, thus causing elevation of cyclic GMP levels.

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