Role of Platelet-Activating Factor in Long-Term Potentiation of the Rat Medial Vestibular Nuclei

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Grassi, Silvarosa, Ermelinda Francescangeli, Gianfrancesco Goracci, and Vito E. Pettorossi. Role of platelet-activating factor in long-term potentiation of the rat medial vestibular nuclei. J. Neurophysiol. 79: 3266–3271, 1998. In rat brain stem slices, we investigated the role of platelet activating factor (PAF) in long-term potentiation (LTP) induced in the ventral part of the medial vestibular nuclei (MVN) by high-frequency stimulation (HFS) of the primary vestibular afferent. The synaptosomal PAF receptor antagonist, BN-52021 was administered before and after HFS. BN-52021 did not modify the vestibular potentials under basal conditions, but it reduced the magnitude of potentiation induced by HFS, which completely developed after the drug wash-out. The same effect was obtained by using CV-62091, a more potent PAF antagonist at microsomal binding sites, but with concentrations higher than those of BN-52021. By contrast both BN-52021 and CV-6209 had no effect on the potentiation once induced. This demonstrates that PAF is involved in the induction but not in the maintenance of vestibular long-term effect through activation of synaptosomal PAF receptors. In addition, we analyzed the effect of the PAF analogue, 1-O-hexadecyl-2-O-(methylcarbamyl)-sn-glycero-3-phosphocholine (MC-PAF) and the inactive PAF metabolite, 1-O-hexadecyl-sn-glycero-3-phosphoglycoline (Lyso-PAF) on vestibular responses. Our results show that MC-PAF, but not Lyso-PAF induced potentiation. This potentiation was prevented by d,l-2-amino 5-phosphonopentanoic acid, suggesting an involvement of N-methyl-D-aspartate receptors. Furthermore, under BN-52021 and CV-6209, the MC-PAF potentiation was reduced or abolished. The dose-effect curve of MC-PAF showed a shift to the right greater under BN-52021 than under CV-6209, confirming the main dependence of MC-PAF potentiation on the activation of synaptosomal PAF receptors. Our results suggest that PAF can be released in the MVN after the activation of postsynaptic mechanisms triggering LTP, and it may act as a retrograde messenger which activates the presynaptic mechanisms facilitating synaptic plasticity.

INTRODUCTION

In rat brain stem slices, long-term potentiation (LTP) can be induced in the medial vestibular nuclei (MVN) by high-frequency stimulation (HFS) of the primary vestibular afferents, through the activation of postsynaptic glutamate NMDA receptors (Capocchi et al. 1992; Grassi et al. 1996). It has been shown that in the hippocampus LTP is triggered postsynaptically, but its expression may depend on a presynaptic increase of transmitter release (Bliss and Collingridge 1993; Bliss et al. 1986). We suggest that presynaptic mechanisms also might be involved in vestibular LTP. Our recent finding showing that the expression of this potentiation requires the activation of metabotropic glutamate receptors (mGluRs) (Grassi et al. 1997) supports a presynaptic mechanism, as mGluRs are greatly implied in increasing the glutamate release (Pin and Duvoisin 1995). The possible existence of presynaptic mechanisms at vestibular level leads us to ascertain whether mediators, which are reported to play a role in coupling the postsynaptic and presynaptic phenomena (Davis and Murphey 1994; Medina and Izquierdo 1995; Williams et al. 1993), are involved in the vestibular LTP.

In particular, it has been demonstrated that platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), is involved in the induction of LTP in the hippocampus as a retrograde messenger, which mediates the glutamate increase accompanying LTP (Clark et al. 1992; Kato et al. 1994; Kornecki et al. 1996; Wierasko et al. 1993). In fact, PAF receptor antagonist, BN-52021, prevents LTP induction (Arai and Lynch 1992; Kato et al. 1994) and application of carbamyl-PAF (c-PAF), a nonhydrolyzable PAF analogue, induces LTP (Izquierdo et al. 1995; Kato et al. 1994; Packard et al. 1996; Wierasko et al. 1993). Furthermore, BN-52021 has been reported recently to affect the firing rate of MVN neurons (MacIennan et al. 1996), suggesting the existence of PAF receptors at this level.

In the present study, we investigated whether PAF might play a role in vestibular LTP. Toward this aim we analyzed the effect of PAF receptor blockade on the induction and maintenance of HFS-LTP and the possibility of inducing LTP by exogenous PAF administration.

METHODS

Brain stem slices (400–500 μm), obtained from 34 Wistar rats (150–250 g), were prepared and maintained as reported previously (Capocchi et al. 1992; Grassi et al. 1995). The field potentials (86 cases) and the unitary potentials (5 cases), evoked by vestibular afferent stimulation, were recorded in the ventral portion (Vp) of MVN (Fig. 1), with 2 M sodium chloride-filled micropipettes (3–10 MΩ). The recorded field potentials consisted of two waves: a positive-wave, representing the primary vestibular fiber activation, and a negative wave (N1), representing the monosynaptic activation of the secondary vestibular neurons (Fig. 1). A second polysynaptic negative wave (N2), was observed rarely in our recording sites.

The presynaptic nature of the N1 wave was verified by a 3-ms interval paired-pulse test, which only caused the disappearance of the N1 wave (Fig. 1), and by using a Ca²⁺-free solution (Grassi et al. 1996) (Fig. 1). All recordings were amplified, filtered with a wideband filter (0–10 kHz) and stored in a computer (486) equipped with a data acquisition card (AT-MIO-16E-2, National Instruments, Austin, TX). In most experiments, bipolar NiIr stimulating electrodes were placed at the point where the vestibular afferents enter the MVN (Fig. 1). Stimulus test parameters were 40- to
PAF IN SYNAPTIC PLASTICITY OF MVN

FIG. 1. Recording sites (●) in the ventral portion of the medial vestibular nuclei (MVN) and medial and lateral stimulating zones (shaded areas) are plotted on the diagrams of 0.5-mm-spaced brain stem slices. Right, top: field potential recorded in normal and Ca\(^{2+}\)-free solution; bottom: single pulse response is superimposed onto a 3-ms paired-pulse stimulation to show how the first negative wave (N1) peak negative voltage was calculated compared with baseline (vertical line). D, descending vestibular nucleus; Md, Mv, medial vestibular nucleus: dorsal and ventral portions; L, lateral vestibular nucleus; S, superior vestibular nucleus.

100-μA intensity, 0.07-ms duration, and 0.06-Hz frequency. HFS consisted of four bursts at 100 Hz applied with alternated polarity for 2 s with a 5-s interval. Moreover, to avoid possible activation of fibers mediating interneuronal interactions and to restrict stimulation to vestibular afferents only, in 14 slices, we stimulated the vestibular root before entering the vestibular nuclear complex (Fig. 1). We pooled together the data from both stimulation zones, as no different results were obtained. The localization of recording and stimulating sites was verified by histological analysis.

We used two PAF receptor antagonists, ginkgolide B (BN-52021, Biomol, Plymouth, PA; 0.5–2 μM), a potent inhibitor of synaptosomal binding sites and CV-6209 (Biomol; 0.5–2 μM), which is a more potent antagonist for microsomal binding sites (Marcheselli et al. 1990). We also used the NMDA receptor antagonist, D,L-2-amino 5-phosphonopentanoic acid (AP-5, Sigma, St. Louis, MO; 100 μM), the nonhydrolyzable PAF analogue, 1-O-hexadecyl-2-O-(methylcarbamyl)-sn-glycero-3-phospholine (MC-PAF, Cayman Chemical, Ann Arbor, MI; 0.004–1 μM), and the inactive PAF metabolite, 1-O-hexadecyl-sn-glycero-3-phospholine (Lyso-PAF, Biomol; 0.1 μM). Stock solution of BN-52021 (1 mM) was dissolved in 25% dimethylsulfoxide (DMSO) and that of MC-PAF (1 mM) in a buffer containing bovine serum albumin. Working drug solutions were prepared freshly in artificial cerebrospinal fluid and perfused at a rate of 2 ml/min.

We analyzed the influence of HFS and drug application by measuring the amplitude of the field potential N1 wave as the difference between the wave peak negative voltage and a baseline influenced by the electrical stimulus decay (Fig. 1) and by measuring the peak latency of evoked unitary potentials.

To show the time course of the effects, the wave amplitudes were measured every 15 s and expressed as a percentage of the baseline (the mean of the 1st 20 responses). To compare the effects within a single experiment and among different ones, we considered the average value within a 5-min interval at the steady state of each experimental condition. Statistical significance was evaluated using paired t-tests and established at \( P < 0.05 \).

RESULTS

Effect of BN-52021 on the induction and maintenance of HFS-LTP in the MVN

BN-52021 (0.5–2 μM) did not modify the amplitude of field potentials under basal conditions, and under drug infusion, HFS could still enhance the N1 wave with the same frequency (71% of the examined cases) as that after HFS alone (see Grassi et al. 1996). However, the magnitude of potentiation was significantly reduced (114.03 ± 2.34%, mean ± SD; \( n = 7 \), Fig. 2, A, C, and D), compared with that induced by HFS alone (135.34 ± 5.22%, Fig. 2, B–D). We pooled together the data from different BN-52021 concentrations, as no different reduction values were obtained. Interestingly, after the drug wash-out, the potentiation...
fully developed to reach 136.88 ± 6.66% (n = 7; Fig. 2, A, C, and D), and HFS applied again did not further enhance it. In contrast, under CV-6209 (0.5 and 1 μM), HFS induced a full potentiation (134.56 ± 6.54, n = 4, Fig. 2C). Only 2 μM CV-6209 could affect the potentiation, which was reduced (122.23 ± 1.56%, n = 3) when HFS was applied under the drug and enhanced to 135.56 ± 3.22%, n = 3, after the drug wash-out.

As it has been reported that DMSO can influence the firing rate of MVN neuron (Maclellan et al. 1996), we tested the effect of this substance (DMSO 0.05%, 4 cases), given alone, on the induction of LTP. In this condition, HFS induced a full potentiation of the N1 wave (133.66 ± 5.08%, n = 4), which remained at the same level at DMSO wash-out (Fig. 2C).

The possible effect on the maintenance of the N1 potentiation of PAF receptor block was assayed by administering BN-52021 (2 μM) and CV-6209 (2 μM) after the establishment of HFS potentiation. No significant change in the N1 wave was observed after drug infusion (Fig. 2, B and C).

**Effect of MC-PAF on the field potentials in the MVN**

MC-PAF at different concentrations induced a potentiation of the N1 wave. Although 0.004, 0.005, and 0.006 μM MC-PAF (3 cases) could not provoke any effect, the potentiation could be induced by using MC-PAF at 0.008 μM (120.19 ± 6.87%, n = 4) (Fig. 3B). Higher potentiation values (~140%) were obtained by using MC-PAF at 0.01 (4 cases) and 0.05 μM (3 cases), whereas lower ones (~122%) were obtained at 0.1 (4 cases), 0.5 (3 cases), and 1 μM (4 cases) (Fig. 3B). The MC-PAF enhancement of N1 began 3–5 min after the drug application, it reached the steady state ~10 min after, and persisted after wash-out throughout the recording period (>60 min; Fig. 3A). HFS applied when this potentiation was established did not enhance it (Fig. 3, A and B).
FIG. 3. A: time course of 1-O-hexadecyl-sn-glycero-3-phosphocholine (Lyso-PAF) and 1-O-hexadecyl-2-O-(methylcarboxymyl)-sn-glycero-3-phosphocholine (MC-PAF) effects in a single experiment. B: mean (column) ± SD (bar) of N1 amplitude under Lyso-PAF (0.1 μM, n = 5), and MC-PAF (0.006 μM, n = 3; 0.008 μM, n = 4; 0.01 μM, n = 4; 0.05 μM, n = 3; 0.1 μM, n = 4; 0.5 μM, n = 3; and 1 μM, n = 4). Drug effects were compared with those induced by HFS after drug wash-out in each experimental condition. *, significant differences (P < 0.05) between the drug and HFS effects. C: time course of the effects of MC-PAF administered during and after D,L-2-amino 5-phosphonopentanoic acid (AP-5; ○ and ▲) and BN-52021 ( ● and ●) infusion, in 2 experiments. D: effect of MC-PAF on the peak latency of evoked unitary potentials (10 superimposed traces). Dotted lines indicate the peak latency shift (mean values). Note the latency reduction and the appearance of doublet discharge after MC-PAF.

MC-PAF (0.05 μM) effect was prevented completely by AP-5 (4 cases), whereas MC-PAF induced potentiation only after AP-5 wash-out (n = 4; Fig. 3C). In addition, the inactive PAF metabolite, Lyso-PAF (4 cases) was quite ineffective (Fig. 3A and B), and the following HFS could induce a clear potentiation (Fig. 3B).

We also examined the effect of MC-PAF (0.05 μM) on the unitary activity elicited in Vp. The peak latency of evoked unitary potentials ranged from 1.2 to 2 ms, and it significantly decreased by ∼0.2 ± 0.03 ms (n = 5) after MC-PAF infusion throughout the recording period (>40 min; Fig. 3D).

Effect of BN-52021 and CV-6209 on the MC-PAF potentiation

N1 wave potentiation by MC-PAF (0.05 μM) was prevented by infusion of BN-52021 (2 μM) (4 cases), and it was induced (135.22 ± 5.22%, n = 4; Fig. 3C) only after drug wash-out. The vehicle alone (DMSO, 0.05%) could not prevent the MC-PAF potentiation (3 cases).

The dose-effect curve of the MC-PAF also was analyzed under infusion of BN-52021 and CV-6209 at different concentrations. As shown in Fig. 4, BN-52021 showed a more potent blocking effect on MC-PAF potentiation than CV-6209, as 1 μM BN-52021 prevented the potentiation induced by 0.008 and 0.010 μM PAF, whereas CV-6209 was effective only at 2 μM. On the whole, the dose-effect curve showed a shift to the right greater under BN-52021 than under CV-6209. This result suggests the main dependence of MC-PAF potentiation on the activation of synaptosomal PAF receptors.

DISCUSSION

In this study, we have shown that PAF plays a role in the NMDA-dependent LTP taking place in the MVN after HFS of the primary vestibular afferents (Capocchi et al. 1992;
Grassi et al. 1996). In fact, BN-52021, a potent PAF competitive antagonist for synaptosomal binding sites (Marcheselli et al. 1990), reduced, at low concentrations, the magnitude of potentiation induced by HFS. The same effect could be obtained by using CV-6209, which is a more potent antagonist at microsomal receptors (Marcheselli et al. 1990), but with concentrations four times higher than those of BN-52021. Conversely, PAF receptor activation does not seem to be necessary for LTP maintenance, as both BN-52021 and CV-6209 did not affect potentiation, once induced. Moreover, further evidence to support the involvement of PAF in vestibular synaptic plasticity, has been attained by using MC-PAF. In fact, MC-PAF was capable of inducing LTP in the MVN independently of HFS. The full block of MC-PAF dependent LTP by NMDA antagonist, suggests that this potentiation is the result of a presynaptic action of MC-PAF leading to an increase of glutamate release, which activates NMDA receptors. The fact that HFS could not induce further potentiation indicates that HFS-LTP and PAF-LTP do share the same pathway.

Another contribution to understand the role of PAF in vestibular LTP is provided by the different blocking efficacy of BN-52021 and CV-6209. The lower doses able to reduce the HFS dependent LTP and the greater shift to the right of the dose-effect curve of exogenous PAF, when we used BN-52021, suggest that the PAF plays a role in the induction of LTP through an interaction with the synaptosomal receptors. It has been suggested that PAF acts as a retrograde messenger in LTP of hippocampus (Kato et al. 1994; Kornecki et al. 1993; Wiersasko et al. 1993). PAF may be synthesized postsynaptically after an elevation of Ca$^{2+}$ levels that might activate both phospholipase A$_2$ activity (Bonventre and Koroshetz 1993) and Lyso-PAF acetyltransferase (Goracci and Francescangeli 1991), thus producing this lipid mediator by the ‘‘remodelling pathway’’ (Goracci 1990). Then, PAF may act presynaptically by stimulating the increase of glutamate release (Bazan and Allan 1996; Bito et al. 1992; Clark et al. 1992; Kato et al. 1994; Wieraszko et al. 1993). Our results showing the reduction of HFS potentiation under PAF competitive antagonists and its full development after drug wash-out, together with the fact that the PAF interaction seems to occur at presynaptic level, appear to be consistent with a role of the PAF as a retrograde messenger. In fact, a block of the retrograde messenger receptors would reduce the amplitude of the potentiation, which still could be induced at the postsynaptic level. At the removal of receptor block, PAF, synthesised following the already activated postsynaptic events, would lead to the full LTP induction.

In conclusion, our results suggest that PAF could be involved in facilitating LTP induction in the MVN independently of HFS. The full block of MC-PAF dependent LTP by NMDA antagonist, suggests that this potentiation is the result of a presynaptic action of MC-PAF leading to an increase of glutamate release, which activates NMDA receptors. The fact that HFS could not induce further potentiation indicates that HFS-LTP and PAF-LTP do share the same pathway.

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