Attenuated LTP in Hippocampal Dentate Gyrus Neurons of Mice Deficient in the PAF Receptor

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Chen, Chu, Jeffrey C. Magee, Victor Marcheselli, Mattie Hardy, and Nicolas G. Bazan. Attenuated LTP in hippocampal dentate gyrus neurons of mice deficient in the PAF receptor. J Neurophysiol 85: 384–390, 2001. Platelet-activating factor (PAF), a bioactive lipid (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) derived from phospholipase A₂ and other pathways, has been implicated in neural plasticity and memory formation. Long-term potentiation (LTP) can be induced by the application of PAF and blocked by a PAF receptor (PAF-R) inhibitor in the hippocampal CA1 and dentate gyrus. To further investigate the role of PAF in synaptic plasticity, LTP induction in the perforant path was induced by a high-frequency stimulation (HFS) and defined as >20% increase above baseline of the amplitude of excitatory postsynaptic potentials (EPSPs) from 26 to 30 min after HFS. HFS-induced enhancement of the EPSP amplitude was attenuated in cells from the PAF-R-deficient mice (163 ± 14%, mean ± SE; n = 32) compared with that in wild-type mice (219 ± 17%, n = 32). The incidence of LTP induction was also lower in the cells from the deficient mice (72%, 23 of 32 cells) than in the wild-type mice (91%, 29 of 32 cells). Using paired-pulse facilitation as a synaptic pathway discrimination, it appeared that there were differences in LTP magnitudes in the lateral perforant path but not in the medial perforant path between the two groups. BN52021 (5 μM), a PAF synaptosomal receptor antagonist, reduced LTP in the lateral path in the wild-type mice. However, neither BN52021, nor BN50730 (5 μM), a micosomal PAF-R antagonist, reduced LTP in the lateral perforant path in the receptor-deficient mice. These data provide evidence that PAF-R-deficient mice are a useful model to study LTP in the dentate gyrus and support the notion that PAF actively participates in hippocampal synaptic plasticity.

INTRODUCTION

Platelet-activating factor (PAF), a bioactive lipid (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) generated by phospholipase A₂ and other pathways (Bazan 1995), has been implicated in neural plasticity and memory formation. These functional implications of PAF are based on studies showing that tetanic stimulation-induced long-term potentiation (LTP) is blocked by a PAF receptor (PAF-R) antagonist in rat hippocampal CA1 (Arai and Lynch 1992; Kato et al. 1994), in dentate gyrus (Kato and Zorumski 1996), and in medial vestibular nuclei (Grassi et al. 1998). PAF also enhances excitatory postsynaptic responses (Clark et al. 1992; Kato and Zorumski 1996; Kato et al. 1994; Kornecki et al. 1996; Wieraszko et al. 1993) and increases the frequency of spontaneous miniature excitatory postsynaptic currents (Clark et al. 1992; Kato and Zorumski 1996; Kato et al. 1994) in hippocampal neurons. PAF inhibits ionotropic GABA receptor activity in primary cultured hippocampal neurons (Chen and Bazan 1999), further contributing to the notion that accumulation of PAF promotes enhanced excitatory neurotransmission by decreasing inhibitory synaptic input. There is evidence that PAF release from hippocampal slices is dramatically increased during the induction of LTP by a high-frequency stimulation (Kornecki et al. 1996). In addition, PAF has been proposed to be involved in memory formation (Izquierdo et al. 1995; Teather et al. 1998). Furthermore PAF content in brain is elevated during ischemia or seizures (Kumar et al. 1988; Nishida and Markey 1996), and PAF receptor antagonists reduce glutamate-induced neurotoxicity and cell death (Makherejee et al. 1999; Ogden et al. 1998). Therefore PAF is of significance both in cell function as well as in pathological conditions (Bazan and Allan 1998).

Recent progress in the introduction of targeted mutations into the mouse genome has made it possible to test specific gene products in synaptic function. A seven-transmembrane-spanning domain PAF-R has been cloned (Honda et al. 1991), and a mouse lacking this PAF-R has been generated (Ishii et al. 1998). To assess the usefulness of this mutant for studying synaptic plasticity and to further investigate the role of PAF in synaptic plasticity, LTP induction in the perforant path was evaluated in dentate granule cells from hippocampal slices of adult mice deficient in the PAF-R and their age-matched wild-type littermates using the whole cell patch-clamp technique. Our results indicate that LTP is attenuated in lateral perforant path-dentate granule cell synapses from mice deficient in the PAF receptor and support the notion that PAF is involved in hippocampal synaptic plasticity.

METHODS

Generation of deficient mice

PAF-R deficient mice (hybrids between C57BL/6J and 129/Ola strains) (Ishii et al. 1998) and their wild-type littermates were kindly provided by Takao Shimizu (Department of Biochemistry, Faculty of Medicine, University of Tokyo).

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Oxygenated (95% O2 -5% CO2) low-Ca2+ brain was rapidly removed after decapitation and placed in cold procedures as described previously (Magee et al. 1996). Briefly, the wild-type allele), water, and solution. Slices were cut at a thickness of 400 μm and transferred to a slicing solution (25 mM KCl, 7.0 MgCl2, 28.0 NaHCO3, 1.25 NaH2PO4, 0.5 CaCl2, 7.0 glucose, 3 pyruvic acid, 1 ascorbic acid, and 14 phosphocreatine (pH, 7.25 with KOH). Series resistance ranged from 15 to 30 MΩ as estimated directly from the amplifier and was monitored during recordings by injecting a 20-ms hyperpolarizing current (50 pA) before delivering a stimulus. The mean input resistance (estimated from a 50-pA hyperpolarizing current injection) was 138 ± 8 MΩ (n = 52) for cells from the wild-type mice and 135 ± 9 MΩ (n = 68) for cells from the deficient mice, and the mean resting membrane potential was −74 ± 1 mV (n = 52) for cells from the wild-type mice and 73 ± 1 mV (n = 68) for cells from the deficient mice. Excitatory postsynaptic potentials (EPSPs) were recorded in response to stimulation of the perforant path at a frequency of 0.05 Hz. Stimuli were elicited via a bipolar tungsten electrode. The synaptic input pathway was determined by stimulation electrode positioning and paired-pulse stimulating (inter-pulse interval, 80–100 ms). As reported (Colino and Malenka 1993; Min et al. 1998; Reid and Clements 1999; Zucker 1989), medial perforant path (MPP) stimulation elicited paired-pulse depression, whereas lateral perforant path (LPP) stimulation elicited facilitation. Paired-pulse ratio was calculated as P2/P1 (P1, the amplitude of the first EPSP; P2, the amplitude of the second EPSP). The amplitude range of the evoked EPSPs was always adjusted to 2–6 mV (<30% of threshold for generating an action potential). LTP in the perforant path was induced by a high-frequency stimulation (HFS) consisting of eight trains, each of eight pulses at 200 Hz with an intertrain interval of 2 s as described by Wang et al. (1996). Postsynaptic depolarization was induced by injecting a depolarizing current (0.5 nA) during HFS. LTP was operationally defined as >20% increase above baseline for the amplitude of EPSPs from 26 to 30 min after HFS.

**Drug solutions**

All chemicals and drugs were purchased from Sigma except for bicuculline, which was purchased from RBI (Natick, MA), and BN52021, which was purchased from Biomol (Plymouth Meeting, PA). BN52021 and BN50730 were dissolved in DMSO at 100 mM and diluted to a desired concentration with the standard ACSF. In experiments where the PAF-R antagonists were applied, slices were pretreated with either 5 μM BN52021 or BN50730 for ≥1.5 h and then were continuously perfused with the antagonists during recordings. All the bath perfused solutions, including drug solutions, contained 10 μM bicuculline to block ionotropic GABA receptors. All experiments were performed blind, i.e., the person who carried out the recordings did not know the genotypes of the animals. After finishing the recordings and basic data analyses, the codes were broken from the chips that were previously implanted in the animals for identification. The data were classified into two groups based on the recordings made from the deficient and wild-type mice. Data are presented as means ± SE. Unless stated otherwise, Student’s t-test and one-way ANOVA with Fishers PLSD post hoc were used for statistical comparison when appropriate. Differences were considered significant when P < 0.05. The care and use of the animals reported on this study were approved by the Animal Care and Use Committee of Louisiana State University Health Sciences Center.
RESULTS

Induction of LTP is attenuated in the lateral perforant path-dentate gyrus cell synapses of PAF-R-deficient mice

Synaptic transmission and plasticity were examined in dentate granule cells of PAF-R-deficient mice and their age-matched wild-type littermates. There were no abnormalities in basic membrane properties including resting membrane potential, input resistance (see METHODS), current required to evoke EPSPs (1–10 μA), and action potential generation (8–10 spikes per burst) in cells from the receptor deficient slices. As indicated in Fig. 2, however, the potentiation of EPSP amplitude by HFS was significantly reduced in cells from PAF-R-deficient mice (mean potentiation: 163 ± 14% of baseline 26–30 min after HFS, n = 32) when compared with that in the wild-type mice (219 ± 17%, n = 32). The incidence of LTP induction was also lower in the receptor deficient mice (72%, 23 of 32 cells) than in the wild-type mice (91%, 29 of 32 cells, Fig. 2D).

FIG. 2. Long-term potentiation (LTP) is attenuated in hippocampal perforant path-dentate gyrus neurons of mice deficient in the platelet-activating factor receptor. A: representative responses in cells recorded from platelet-activating factor receptor (PAF-R) wild-type (+/+ ) and deficient mice (−/−) before (1) and after high-frequency trains of stimulation (HFS). B: time course and extent of LTP induction following HFS (↑) in PAF-R wild-type and -deficient mice. Excitatory postsynaptic potential (EPSP) amplitude was normalized as percent of average baseline EPSP amplitude. Each point represents averages of 3 consecutive trials recorded at 0.05 Hz. C: mean potentiation of EPSP calculated by the average EPSP amplitude at 1–5, 16–20, and 26–30 min after HFS plotted as percent of baseline. D: incidence of LTP induction in the PAF-R wild-type and -deficient mice. LTP was operationally defined as ≥ 20% increase above baseline for the amplitude of EPSPs from 26 to 30 min after HFS. Data are represented as means ± SE. *P < 0.05, **P < 0.05, χ² test.

Using a standard PPF protocol, we attempted to determine if the differences in LTP induction were pathway specific. Input from the medial and lateral perforant pathways can be determined based on ratios of PPF (see METHODS). As shown in Fig. 3A, there were no significant differences in magnitudes of LTP in MPP between the PAF-R-deficient mice (179 ± 23% of baseline 26–30 min after HFS, n = 15) and wild-type mice (175 ± 21%, n = 7). However, significant differences in magnitudes of LTP in LPP were found between the deficient mice (161 ± 18% of baseline 26–30 min after HFS, n = 14) and wild-type mice (235 ± 23%, n = 20). In addition, there were significant differences in ratios of the paired-pulse facilitation in LPP before and after HFS in both PAF-R deficient mice (P2/P1: from 1.18 ± 0.04 to 1.02 ± 0.03, n = 14) and wild-type mice (P2/P1: from 1.18 ± 0.04 to 1.06 ± 0.03, n = 20), but there were no differences in changes in the ratios of the paired-pulse facilitation before and after HFS between the receptor-deficient and wild-type mice.
deficient (0.16 ± 0.02, n = 14) and wild-type mice (0.12 ± 0.02, n = 20).

**PAF-R antagonist reduces LTP in wild-type mice but not in PAF-R mutants**

Tetanic stimulation-induced LTP can be blocked by the synaptosomal PAF-R antagonist 52021 in rat hippocampus (Kato and Zorumski 1996; Kato et al. 1994). To examine whether HFS-induced LTP in LPP in PAF-R-deficient mice could be blocked by the PAF-R antagonist, slices were pretreated with 5 μM BN52021 for 1.5 h at 22–24°C, and then continuously perfused with the antagonist throughout the experiments at 34–35°C. It appears that LTP in LPP in wild-type mice was significantly reduced in 5 μM BN52021-treated slices (162 ± 24% of baseline 26–30 min after HFS, n = 20) when compared with that in nontreated slices (235 ± 23%, n = 20; Fig. 4). However, there was no difference in LTP in LPP in PAF-R-deficient mice in 5 μM BN52021-treated slices (164 ± 12% of baseline 26–30 min after HFS, n = 16) when compared with that in nontreated slices (161 ± 18%, n = 14; Fig. 5, B and D). To test whether BN50730, a microsomal PAF-R antagonist, has an effect on LTP in LPP in PAF-R-deficient mice, slices were pretreated with 5 μM BN50730 in an incubator for 1.5 h, then continuously perfused with it during recordings. As shown in Fig. 5, A and C, HFS-induced LTP in LPP was not reduced in BN50730-treated slices (172 ± 12% of baseline 26–30 min after HFS, n = 20) when compared with that in nontreated slices (161 ± 18%, n = 14). It has been demonstrated before that BN50730 has no effect on LTP induction in normal rat hippocampus (Kato and Zorumski 1996; Kato et al. 1994), therefore we did not test the effect of this microsomal PAF-R antagonist on LTP in wild-type animals.
These mice grow and reproduce normally (Ishii et al. 1998). These mice grow and reproduce normally targeted deletion of the PAF plasma membrane receptor gene cloned (Honda et al. 1991). To further investigate the role of 1990). But thus far only the synaptosomal PAF-R has been membrane) and microsomal, have been described (Marcheselli et al. 1994; Kornecki et al. 1996; Wieraszko et al. 1993). Two types of PAF binding sites in brain, synaptosomal (plasma mem-
brane) and microsomal, have been described (Marcheselli et al. 1990). But thus far only the synaptosomal PAF-R has been cloned (Honda et al. 1991). To further investigate the role of PAF in hippocampal synaptic plasticity, we used mice with a targeted deletion of the PAF plasma membrane receptor gene (Ishii et al. 1998). These mice grow and reproduce normally and do not present obvious behavioral and cellular abnormalities. However, HFS-induced LTP of the lateral perforant path input to dentate granule cells is attenuated in the receptor-deficient mice when compared with that in wild-type controls. This is further evidence that PAF participates in modulating hippocampal excitatory synaptic transmission.

In addition to reduced LTP induction, the synaptosomal PAF-R antagonist 52021 (5 μM) reduces the magnitude of LTP in wild-type mice but not in the PAF-R-deficient mice, indicating that the synaptosomal PAF-R has likely been physically and functionally deleted. This is consistent with previous reports (Kato and Zorumski 1996; Kato et al. 1994) showing that BNS0730 (5 μM), a microsomal PAF-R antagonist, has no effect on the LTP induction in PAF-R-deficient mice.

The medial and lateral perforant paths represent two separate input pathways to the dentate gyrus. The medial perforant path synapses on the central third and lateral perforant path onto the distal third of the dendritic tree of dentate granule cells (Witter 1993). It has been demonstrated that synaptic transmission in these pathways displays profound differences in terms of physiology and pharmacology (Abraham and McNaughton 1984; Kahle and Cotman 1989; McNaughton 1980; Min et al. 1998), and our data support this idea. While the mechanisms of LTP expression in these two pathways are still not clear, recent evidence suggests that LTP of the medial perforant path results from an increased postsynaptic response to neurotransmitter (Reid and Clements 1999). Our data indicate that LTP induction in lateral perforant path is reduced in the PAF-R-deficient mice when compared with that in wild-type controls, suggesting that the role of PAF in the expression of LTP in these two pathways may be different. It has been proposed that PAF may act as a retrograde messenger at a presynaptic receptor in hippocampal CA1 LTP because of increases in the frequency of miniature excitatory postsynaptic currents and synaptic evoked responses by postsynaptic application of PAF (Clark et al. 1992; Kato et al. 1994; but see Kobayashi et al. 1999). Similar phenomena have also been observed in rat hippocampal dentate region (Kato and Zorumski 1996). A PAF-mediated increase in the probability release may be a mechanism of LTP expression in the lateral perforant path. This may be the reason why there is a difference in LTP in the lateral perforant, but not in the medial perforant path between the PAF-R-deficient mice and wild-type controls. Interestingly, it has been reported recently that LTP in lateral perforant path, but not in medial perforant, is impaired in mice deficient in the protein phosphatase inhibitor-1 (Allen et al. 2000). This finding together with our results suggest that there may be heterogeneous molecular signaling regulation of LTP in the lateral perforant path—and medial perforant path—dentate granule cell synapses.

In summary, we have found that HFS-induced LTP is reduced in lateral perforant path-dentate granule cell synapses in PAF-R-deficient mice when compared with that of wild-type mice. BNS5201 (5 μM), a PAF synaptosomal receptor antagonist, reduced LTP in the lateral perforant path in wild-type mice. However, neither BNS5201, nor 50730 (5 μM), a microsomal PAF-R antagonist, reduced LTP in lateral perforant path in the receptor-deficient mice. These data provide evidence to support the notion that PAF participates in hippocampal synaptic plasticity and that the PAF-R-deficient mice are a useful model. In addition, hippocampal dentate gyrus is of significance in leaning and memory as well as for epileptogen-
thesis. Alterations in PAF synthesis or PAF-R function may contribute to synaptic dysfunction and pathogenesis. Thus it would be of interest and significance to employ these receptor deficient mice to perform behavioral studies that will further our understanding of relationships between LTP and learning and memory, and role of PAF in learning and memory as well as in epileptogenesis.

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REFERENCES


