Memory enhancement by intrahippocampal, intraamygdala, or intraentorhinal infusion of platelet-activating factor measured in an inhibitory avoidance task

(platelet-activating factor antagonist/long-term potentiation/CA1 neurons/retrograde messengers/learning)


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ABSTRACT Platelet-activating factor (PAF; 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine), which is thought to be a retrograde messenger in long-term potentiation (LTP), enhances glutamate release and LTP through an action on presynaptic nerve endings. The PAF antagonist BN 52021 blocks CA1 LTP in hippocampal slices, and, when infused into rat dorsal hippocampus pre- or posttraining, blocks retention of inhibitory avoidance. Here we report that memory is affected by pre- or posttraining infusion of the PAF analog 1-O-hexadecyl-2-N-methylcarbamoyl-sn-glycero-3-phosphocholine (mc-PAF) into either rat dorsal hippocampus, amygdala, or entorhinal cortex. Male Wistar rats were implanted bilaterally with cannulae in these brain regions. After recovery from surgery, the animals were trained in step-down inhibitory avoidance or in a spatial habituation task and tested for retention 24 h later. Mc-PAF (1.0 μg per side) enhanced retention test performance of the two tasks when infused into the hippocampus before training without altering training session performance. In addition, mc-PAF enhanced retention test performance of the avoidance task when infused into (i) the hippocampus 0 but not 60 min after training; (ii) the amygdala immediately after training; and (iii) the entorhinal cortex 100 but not 0 or 300 min after training. In confirmation of previous findings, BN 52021 (0.5 μg per side) was found to be amnestic for the avoidance task when infused into the hippocampus or the amygdala immediately but not 30 or more minutes after training or into the entorhinal cortex 100 but not 0 or 300 min after training. These findings support the hypothesis that memory involves PAF-regulated events, possibly LTP, generated at the time of training in hippocampus and amygdala and 100 min later in the entorhinal cortex.

Learning and memory probably involve activity-dependent modifications of synaptic efficiency (1, 2). Although this concept of synaptic plasticity in information storage originated from the century-old work of Santiago Ramon y Cajal, the cellular and molecular events underlying memory and learning are not clearly understood.

LTP (long-term potentiation) is a model of activity-dependent synaptic plasticity (1) and is a candidate mechanism for certain forms of memory in the mammalian brain (3). LTP is a persistent enhancement of excitatory neurotransmission that results from high-frequency activation of hippocampal synapses as well as other brain regions (1, 4). Activity-dependent potentiation involves the N-methyl-d-aspartate (NMDA) receptor and Ca2+ influx as well as several postsynaptic events. The enhanced release of excitatory neurotransmitters is thought to involve a presynaptic component sensitive to a retrograde messenger generated by the postsynaptic neuron (5, 6). Arachidonic acid, nitric oxide, carbon monoxide, and, most recently, a membrane-derived lipid second messenger platelet-activating factor (PAF; 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine) have been suggested as retrograde messengers in LTP. A PAF antagonist (BN 52021) that elicits neuroprotection (7) actually displaces [3H]PAF binding from presynaptic membranes (8), blocks PAF-induced glutamate release (9), and inhibits LTP (10). Arachidonic acid is released from membrane phospholipids by a phospholipase A2 and enhances synaptic transmission when coupled with presynaptic stimulation (6). A finding that limits the role of arachidonic acid as a retrograde messenger is the observation that the action of the fatty acid requires NMDA receptor activation (11).

Retrograde messengers generated in the postsynaptic neuron promote glutamate release from the presynaptic nerve endings and are believed to be critical for the synapse specificity of LTP (1). This postsynaptic to presynaptic communication is in accordance with the Hebbian postulate of synaptic potentiation (12). PAF is produced in brain during stimulation (13), is released from neurons in culture, enhances hippocampal excitatory synapses by an influence on glutamate release (9), and enhances hippocampal CA1 LTP (10). Free polyunsaturated fatty acids are also produced under these conditions (14, 15) and may be related to PAF formation since a deacylation reaction, possible through a phospholipase A2 (16), is required for both events. PAF has been proposed to be a retrograde messenger or a part of a network in the induction of LTP (10, 17–20). Moreover, rapid changes in lipid second messengers upon stimulation may also be involved in brain damage (21).

The development of LTP in the hippocampus and amygdala at the time of training, and 90–100 min after training in entorhinal cortex, has been suggested to play a central role in memory formation (22, 23). A large number of drugs known to affect hippocampal LTP on memory have been studied by infusing them into these brain structures and were found in all cases to have effects similar in nature and in time course to those reported on LTP (2, 22, 24). It must be pointed out that those findings suggest, but do not prove, a correlation between LTP and memory (1, 2). Other plastic synaptic phenomena may participate in memory in addition to or instead of LTP in memory processes (2). The effects of PAF are mediated by synaptic membrane receptors sensitive to BN 52021 (8–10), a

Abbreviations: LTP, long-term potentiation; PAF, platelet-activating factor; mc-PAF, PAF analog.

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PAF antagonist. Alone, BN 52021 has effects opposite those of PAF, which suggests that PAF is a physiological factor (9, 10). The effects of 1-O-hexadecyl-2-N-methylcarbamoyl-n-glycero-3-phosphocholine (mc-PAF) are similar to those of the parent compound but have a longer duration because mc-PAF is metabolized more slowly (25). When infused in the hippocampus before or immediately after training, BN 52021 hinders memory of inhibitory avoidance in the rat; memory is not hindered when BN 52021 is infused later, 30 or 60 min after training (26). Recently, effects similar to those of BN 52021 on memory were reported with an inhibitor of heme oxygenase, the enzyme that produces carbon monoxide, and with an inhibitor of NO synthase, the enzyme the produces nitric oxide (24). Carbon monoxide and nitric oxide are other putative retrograde messengers involved in LTP. In the present study, we report the effects of mc-PAF on retention test performance of step-down inhibitory avoidance habituation and learning in rats and confirm the effect of BN 52021 on this test. mc-PAF and BN 52021 were bilaterally infused into the hippocampus, amygdala, and entorhinal cortex before or at various time points after training, and the animals were tested for memory retention 24 h later.

MATERIALS AND METHODS

Surgery. Under thiobarbiturate (thiopental sodium) anesthesia (30 mg/kg, i.p.), 439 male Wistar rats (3–4 months old; body weight, 240–320 g) were bilaterally implanted with a 27-g guide cannula aimed 1.0 mm above area CA1 of the dorsal hippocampus (n = 224), 1.0 mm above the junction between the central and the lateral nuclei of the amygdala (n = 88), and 1.0 mm above the entorhinal cortex (n = 127). The coordinates used were as follows: hippocampus, A 4.3, L 4.0, V 2.0; amygdala, A 2.3, L 4.5, V 7.4; and entorhinal cortex, A 7.0, L 5.0, V 2.0, according to the method of Paxinos and Watson (27). Postmortem histological controls of the cannula placements (23, 28) showed that the injection tips were within 1 mm3 of the intended sites in 206 of the 224 animals implanted in the hippocampus, in 81 of the 88 implanted in the amygdala, and in 126 of 127 implanted in the entorhinal cortex. Behavioral data from only the animals with the cannula located in the intended sites were used.

Step-Down Inhibitory Avoidance Task. After recovery from surgery, animals were trained in a step-down inhibitory avoidance task and tested for retention 24 h later (29). Rats were placed on a 7 × 25 cm platform (2.5 cm high). The platform faced a 42 × 25 cm grid of parallel 0.1-cm-caliber stainless steel bars spaced 1 cm apart. The latency of the rats to step down placing all four paws on the grid was then measured. In training sessions, the animals received a 0.3-mA, 2-sec scramble footshock immediately upon stepping down. No footshock was given in test sessions. A 180-sec ceiling was imposed on test session latency measurements; latencies ≥180 sec were counted as 180 sec. Differences in training session latency among groups were not significant (Table 1). In control groups, training-test latency differences were significant at P < 0.002 in a nonparametric Mann–Whitney U test (two tailed). Differences in test latency among groups were evaluated by a Kruskal–Wallis ANOVA and were found to be significant at P < 0.001 in all experiments (n > 20). Individual differences between groups were evaluated by Mann–Whitney U tests.

Spatial Habituation Task. Twenty-four animals were submitted, on the 2 days before step-down avoidance training, to a spatial habituation task (23, 24, 29). In this task, the same apparatus was used without the platform. The grid floor was divided into four equal rectangles (12.5 × 25 cm) by imaginary lines. The animals were gently placed on the leftmost rectangle facing the rear left corner of the box. The number of line crossings and the number of rearings were measured over 3 min in a training session and again in a test session 24 h later. The decrease in crossings and rearings between the training and the test session in the saline group was taken as a measure of retention of habituation (24).

Infusion of PAF Antagonist, mc-PAF or Vehicle. At the time of infusion, 30-g cannulae were fitted into the guide cannula at the following times: (i) 10 min before training or immediately after (0 min), 30 or 60 min after training, or 10 min before testing in the animals implanted in the hippocampus; (ii) immediately after training in the animals implanted in the amygdala; and (iii) immediately, 100 min, or 300 min after training in the animals implanted in the entorhinal cortex. The infusion cannula was connected to a microsyringe by a polyethylene tube. The tip of the infusion cannula protruded 1 mm beyond that of the guide cannula and was aimed at CA1 in the dorsal hippocampus, at the junction between the central and the basolateral amygdaloid nuclei, or the entorhinal cortex. Two experiments were carried out: one using mc-PAF and the other using BN 52021. The analog of PAF (mc-PAF) from Calbiochem was used. In the mc-PAF experiments, each animal received a bilateral infusion (0.5 μl) of either the

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Time, min</th>
<th>Training step-down latency, sec</th>
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<tr>
<td></td>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>Dorsal hippocampus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretraining</td>
<td>10</td>
<td>9 ± 2 (12)</td>
</tr>
<tr>
<td>Posttraining</td>
<td>0</td>
<td>11 ± 2 (11)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>11 ± 2 (12)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>12 ± 2 (11)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Amygdala</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretraining</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Posttraining</td>
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<td>6 ± 2 (10)</td>
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<td></td>
<td>30</td>
<td>—</td>
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<tr>
<td>Entorhinal cortex</td>
<td></td>
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<tr>
<td>Pretraining</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Posttraining</td>
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<td>10 ± 2 (11)</td>
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<tr>
<td></td>
<td>100</td>
<td>11 ± 2 (10)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>11 ± 3 (11)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals used is in parentheses. Rats received bilateral infusions of mc-PAF (1 μg per side), BN 52021, or vehicle alone. The interval between training footshock (0.3 mA) and training test was 24 h. Differences among groups in training session latencies were not significant in one-way ANOVA (P = 0.1).
vehicle alone (0.5% ethanol in 0.9% saline) or mc-PAF (1.0 μg per side) dissolved in the vehicle. In the BN 52021 experiment, 0.5 μg per side of BN 52021 dissolved in the vehicle (50% dimethyl sulfoxide in saline) or vehicle alone was used.

RESULTS AND DISCUSSION
Test session step-down latency in the inhibitory avoidance task, when either mc-PAF or BN 52021 was infused into brain areas, is depicted in Figs. 1 and 2, respectively.

The intrahippocampal infusion of mc-PAF 10 min before training or immediately posttraining significantly enhanced retention test scores in this task. However, 30 min posttraining, intrahippocampal infusion of mc-PAF caused only a marginal increase in test scores, and infusion at 60 min posttraining had no effect on retention. The intraamygdala infusion of mc-PAF immediately posttraining, as well as mc-PAF infusion into the entorhinal cortex 100 min posttraining significantly increased retention test performance. Nevertheless, mc-PAF infused into the entorhinal cortex 0 or 300 min posttraining had no effect on retention (Fig. 1).

The effect of mc-PAF infused into the hippocampus 10 min before training was studied, as was its effect on the habituation task. The drug had no effect on crossings and rearings in the training session but markedly reduced performance of both responses in the retention test carried out 24 h later. This suggests that the drug had no effect on general exploratory activity or on acquisition parameters but instead specifically enhanced retention of the task (see refs. 23, 24, and 29).

The present data using BN 52021, a PAF antagonist that decreases release of glutamate through a presynaptic action (9), confirms and extends a recent report using the inhibitory avoidance task (26). The present results confirm that pre- or immediate posttraining intrahippocampal or intraamygdala infusion of BN 52021 produces amnesia for this task (Fig. 2; ref. 28). Administration of BN 52021 in either brain area 30 min after training or into the hippocampus 100 min after training had no effect. BN 52021 infused into the entorhinal cortex was amnestic only at 100 min posttraining (Fig. 2).

Intrahippocampal administration of either mc-PAF or BN 52021 prior to the test session had no influence on retention test performance of the inhibitory avoidance task (Table 2). Since 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) given prior to testing into the hippocampus does have an effect on retention test performance (see refs. 2 and 24), it seems likely that the effect of pre- or posttraining PAF on retention is not due to an influence on glutamatergic CNQX-sensitive transmission.

Table 2. Step-down latency in rats having received mc-PAF, BN 52021, or vehicle 10 min before testing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Training</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle 1</td>
<td>9</td>
<td>9.0 ± 1.2</td>
<td>45 (38/55)</td>
</tr>
<tr>
<td>mc-PAF</td>
<td>8</td>
<td>12.6 ± 2.6</td>
<td>46 (28/67)</td>
</tr>
<tr>
<td>Vehicle 2</td>
<td>9</td>
<td>12.4 ± 2.1</td>
<td>51 (21/75)</td>
</tr>
<tr>
<td>BN 52021</td>
<td>8</td>
<td>10.9 ± 2.5</td>
<td>53 (25/145)</td>
</tr>
</tbody>
</table>

Mean ± SE training step-down latency and median (interquartile range) test session step-down latency in seconds of rats that received a bilateral infusion of vehicle or mc-PAF (1.0 μg per side) into brain regions before or after training (0.3-mA training footshock; training-to-test interval, 24 h). All training test differences are significant at P < 0.02 or 0.002 by Mann-Whitney U tests (two tailed). a, Significant difference from vehicle group at P < 0.002 by Mann-Whitney U tests (two tailed). Number of animals used is given in Table 1.
The results suggest memory facilitation by PAF and confirm earlier findings that showed that presynaptic nerve ending PAF receptors (8), sensitive to BN 52021 in hippocampus, amygdala, and entorhinal cortex, are engaged in the early stages of memory formation of inhibitory avoidance in rats (26). The presynaptic sites may be the PAF receptor that mobilizes intracellular Ca2+ in hippocampal neurons (30). PAF enhances, and BN 52021 blocks, CA1 LTP (10), which led to the proposal that endogenous PAF may play a role as a retrograde messenger in LTP by stimulating glutamate release (10, 17–20). PAF may play a role in LTP through the induction of structural changes, as it does in platelets (17). The present findings support the idea that LTP, initially in the hippocampus and the amygdala and later in the entorhinal cortex, plays a role in memory formation (see Introduction).

The behavioral effects of mc-PAF or BN 52021 described here cannot be attributed to direct or delayed nonspecific influences on performance for three reasons. First, neither drug had any effect on training or test session performance of the avoidance task when administered into the hippocampus 10 min before training (Table 1) or testing (Table 2) in spite of the fact that pretraining infusions had a marked effect on retention of this task (Figs. 1 and 2). Second, delayed posttraining infusion of any of the two drugs in any of the three structures had no influence on retention test performance of the avoidance task measured on the next day. Third, in the habituation task, which involves the measurement of general exploratory and ambulatory behavior during 3 min, the pretraining administration of mc-PAF did not influence these variables in the training session but markedly reduced their subsequent performance in the retention test carried out 24 h later (Table 3).

The effects of PAF and BN 52021 on memory appear to be specific for certain brain areas and there is a time window during which the infusion of either the agonist or the antagonist elicits these effects relative to the training. The posttraining infusion of mc-PAF (n = 6) or BN 52021 (n = 8) into the thalamus or BN 52021 into the striatum (n = 8) had no effect on retention test scores (data not shown). Furthermore, the diffusion of intrahippocampal, intraamygdala, or intraentorhinal infusions of drugs in a volume such as was used here has been shown not to exceed a radius of 1 mm, using both radioactive substances (31) and India ink or methylene blue (23, 32).

The time course of the effect of both mc-PAF and BN 52021 given into the hippocampus or amygdala, on the one hand, or into entorhinal cortex on the other, were different and agree with earlier observations suggesting a temporarily later role of entorhinal cortex in memory processing (22, 23, 28). A possible relationship between LTP in these structures and memory mechanisms has been described (2, 22).

Finally, two cautionary comments appear necessary. The first concerns the hypothesis that LTP may underlie memory processes (1, 2, 22). The present findings support that hypothesis if, and only if, both mc-PAF and BN 52021 are assumed to act mainly or specifically at the synapses that had just been stimulated in the hippocampus, amygdala, and entorhinal cortex. This is easier to assume in the case of the blocker than in that of the agonist; unless, of course, the recently stimulated synapses had been primed by other retrograde messengers released simultaneously, like carbon monoxide (24), nitric oxide, or arachidonic acid (1, 2). If this were not the case, and the actions of mc-PAF and BN 52021 on memory result from some general, widespread effect in those structures, then the possibility that they may influence memory modulation rather than presumably synapse-specific memory formation must be entertained.

Second, PAF is one of the most potent biologically active lipids known (16). It is possible that, when PAF is infused into a brain region, a wide variety of actions may be elicited such as an effect on the microcirculation. PAF has been reported to elicit vascular constriction (33) that, in turn, could produce transient ischemia. These effects could, through an indirect route, affect the behavioral parameters. However, the effects of mc-PAF on specific neuroanatomical regions as well as the time window during which the effect is elicited argue in favor of a selective action. It is also important to stress the fact that the mc-PAF memory-enhancing action is assessed by a test performed 24 h after the infusion of the lipid. Moreover, the data on the effect of PAF antagonists further support the involvement of a presynaptic PAF receptor since BN 52021 has an amnesic action but BN 520730 is devoid of effects (26).

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<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Session training</th>
<th>Session test</th>
<th>Session training</th>
<th>Session test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>12</td>
<td>36.7 ± 2.9</td>
<td>25.5 ± 2.3</td>
<td>23.5 ± 1.4</td>
<td>14.5 ± 4.3</td>
</tr>
<tr>
<td>mc-PAF</td>
<td>12</td>
<td>35.8 ± 1.7</td>
<td>16.6 ± 2.1*</td>
<td>24.7 ± 1.4</td>
<td>5.2 ± 0.7†</td>
</tr>
</tbody>
</table>

In two 3-min exposures to the training apparatus, 24 h apart (training and test sessions), rats that received a bilateral infusion of vehicle or mc-PAF (1.0 μg) into the dorsal hippocampus 10 min before training were scored for crossings and rearings. Data are expressed as means ± SEM. Training-test differences in both crossings and rearings are significant at P < 0.01 in both groups.

*Significant difference from saline at P < 0.02.

†Significant difference from saline at P < 0.001 (Student’s t test).
