Is arachidonic acid a retrograde messenger in long-term potentiation?

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Introduction

Long-term potentiation (LTP) is the term used to describe a long-lasting increase in synaptic efficacy which occurs following a brief train of high-frequency stimulation to an afferent input to the hippocampus [1]. The persistence of the response resulting from a relatively modest tetanus has led to the suggestion that LTP may represent a neural correlate of learning and/or memory.

Induction and maintenance of LTP

Since its description, the mechanisms underlying LTP have been sought and in the past few years the events leading to its induction have been elucidated. Experiments have shown that conjunction of presynaptic and postsynaptic activity is required for induction [2, 3]. The high-frequency train stimulates release of glutamate, which binds to postsynaptic receptors, including the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, causing strong depolarization. The voltage-dependent block by Mg\(^{2+}\) of the NMDA-receptor-associated Ca\(^{2+}\) channel is relieved by strong depolarization allowing Ca\(^{2+}\) to enter the postsynaptic neurone [4, 5]. The resultant increase in Ca\(^{2+}\) is considered to be the trigger for induction of LTP [6].

Despite considerable efforts, the mechanisms underlying the maintenance of LTP are ill-understood and the debate concerning presynaptic [7, 8] and postsynaptic [9, 10] involvement remains unresolved. Recently, data obtained from quantal analysis studies strongly suggest that there is an increase in transmitter release in LTP [11, 12], as previously proposed by this laboratory on the basis of push–pull perfusion studies in the anaesthetized animals. LTP in the perforant path-granule cell synapses is associated with a sustained increase in the perfusate of both newly synthesized [7] and endogenous [8, 14, 15] glutamate. An increase in glutamate release has also been found after induction of LTP in the hippocampal slice preparation [16]. In addition, in the ex vivo preparation, K\(^{+}\)-induced Ca\(^{2+}\)-dependent release of \(^{3}H\)glutamate is increased in potentiated tissue compared with control, and is blocked when induction of LTP is blocked [17]. These data have led us to propose that maintenance of LTP is dependent, at least in part, on presynaptic mechanisms. The proposal is strengthened by the finding that blocking induction of LTP in dentate gyrus-granule cell synapses also blocks the LTP-associated increase in glutamate release [8, 14, 15]. Because induction of LTP is triggered postsynaptically and maintenance of LTP requires an increase in transmitter release, we have proposed that a retrograde messenger, derived from a postsynaptic site is released into the synaptic cleft to stimulate an increase in glutamate release [13]. One candidate for this role is arachidonic acid, a small lipid-soluble molecule capable of crossing membranes, which has been shown to act as an intracellular messenger in sensory neurones of *Aplysia* [18].

Evidence that arachidonic acid plays a role in LTP

The first evidence that arachidonic acid played a role in LTP was suggested by the observation that nordihydroguaiaretic acid (NDGA), a lipoxygenase and phospholipase A\(_2\) inhibitor, blocked induction of LTP [15]. It was subsequently reported that arachidonic acid [19, 20], but not other fatty acids [20] (oleic, linolenic, linoleic, docosahexanoic or palmitic acid), induced a delayed activity-dependent potentiation, which occluded with tetanus-induced potentiation [19, 20]. The observation that other fatty acids failed to induce the effect, coupled with the fact that arachidonic acid, in the absence of increased activity in afferent pathways, failed to induce potentiation, argues strongly against the idea that the observed potentiation was due to a non-specific action on membrane fluidity rather than to a specific effect of arachidonic acid. These two findings pointed to a role for arachidonic acid in LTP though neither advanced the specific hypothesis that arachidonic acid may function as a retrograde messenger.

A role for protein kinase C (PKC) in induction of LTP has been established for some time [22,
Evidence that arachidonic acid is a retrograde messenger in LTP  

Three basic criteria are required of a retrograde messenger. First, it must be shown to appear in the synaptic cleft; secondly, it must act presynaptically to increase release of glutamate and finally, it must be released from a postsynaptic site.

(1) Is arachidonic acid released into the synaptic cleft?

We investigated this first question by collecting perfusate, using push-pull perfusion, from urethane-anaesthetized rats before and after induction of LTP. Perfusate was analysed for arachidonic acid as well as oleic, linoleic, linolenic, stearic and palmitic acids. Two 12-lipoxygenase metabolites of arachidonic acid, hydroperoxyeicosatetraenoic acid (HPETE) and hydroxyeicosatetraenoic acid (HETE) were also analysed. Fatty acids were converted to their 2-nitrophenylhydrazine derivatives, separated by reverse-phase h.p.l.c. on a Microsorb C5 column and detected by u.v. spectrometry at 230 nm [25], while HPETE and HETE were detected without derivatization at 230 nm. Fig. 1 shows the results of these experiments. Induction of LTP was associated with an immediate increase in the slope of the excitatory postsynaptic potential (EPSP) and population spike height (spike). A second high-frequency train increased both parameters, but the additional increase was not significant. LTP was associated with an increase in the concentration of arachidonic acid (Fig. 1c), but not of other fatty acids (Fig. 1c), and a significant increase in both HPETE and HETE (Fig. 1b). In a group of related experiments (results not shown) d-(-)-aminophosphonovaleric acid (APV), an antagonist at the NMDA receptor, was perfused for 30 min before, until 5 min after the tetanus. APV blocked induction of LTP and the accompanying changes in the release of arachidonic acid, HPETE and HETE, providing evidence that these changes were specifically associated with induction and maintenance of LTP.

(2) Does arachidonic acid increase glutamate release?

In an attempt to investigate the second criterion, we used established in vitro methods. In the first instance, it was important to assess the effect of arachidonic acid on high-affinity uptake of glutamate in the hippocampus, since it has been shown to inhibit uptake into glial cells prepared from the salamander retina [26], and synaptosomes and slices prepared from cortex [27]. In the dentate gyrus, arachidonic acid had no significant effect on glutamate uptake at concentrations up to 10 µM. At 50 µM, a concentration which significantly affected lactic dehydrogenase activity, arachidonic acid inhibited uptake [28]. The effect of arachidonic acid was then investigated on release of glutamate. Our data indicate that it increases both K+-induced Ca2+-dependent and Ca2+-independent release of glutamate from slices and synaptosomes (Fig. 2a). Ca2+-dependent release of glutamate (release in the presence of Ca2+ minus release in its absence) was significantly increased by arachidonic acid, 5 µM. It is generally believed that Ca2+-dependent release represents vesicular release, while Ca2+-independent release is either cytoplasmic or non-neuronal in origin [29]. Arachidonic acid failed to stimulate release of glutamate from a preparation of glia and so it appears that in dentate gyrus, arachidonic acid-induced Ca2+-independent release is cytoplasmic, and not non-neuronal, in origin. We must conclude, therefore, that arachidonic acid significantly affects both vesicular and cytoplasmic release of glutamate.

The mechanism underlying the increase in release of glutamate is unknown, but one possibility is that it is stimulated by increased phospholipase C (PLC) activity and the subsequent production of inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] and diacylglycerol. Both second messengers have been shown to enhance transmitter release, by Ins(1,4,5)P3-induced increase in intracellular Ca2+ or diacylglycerol-induced increase in PKC activity, respectively [30]. We have examined the possibility that the increase in glutamate release stimulated by arachidonic acid may be secondary to increased activity of PLC. Fig. 2(b) shows that arachidonic acid stimulates PLC activity, with maximum effect at 5 µM. Since PLC activation is generally stimulated by receptor activation, and since receptors for arachidonic acid metabolites have been identified in neuronal tissue [31], we examined the possibility that there are receptors for arachidonic acid on synaptosomal membranes. Although there was a concentration-dependent increase in arachidonic acid binding, saturation was not achieved even with arachidonic acid concentrations as high as 30 µM, which suggests that classical receptors probably do not exist. Therefore, the mechanism by which...
LTP is accompanied by an increased release into perfusate of arachidonic acid, HPETE and HETE

(a) Induction of LTP in dentate gyrus-granule cell synapses is described in detail in [8]. Delivery of a high-frequency train of stimuli to the medial perforant path was accompanied by an immediate increase in excitatory postsynaptic potential (EPSP) slope and population spike height. A second high-frequency train, delivered 45 min later induced a further small change in both parameters. (b) Changes in perfusate concentration of HPETE and HETE after induction of LTP. Samples of perfusate collected during the experiments were analysed by h.p.l.c. for concentration of HPETE and HETE (see text). Results are expressed as µg/ml and are the means (±SEM) of six observations. The concentration of HPETE was significantly increased for 1 h after induction of LTP and then returned to baseline, while the concentration of HETE remained significantly higher than baseline for the duration of the experiment (P<0.05; Student's t-test). (c) Changes in perfusate concentration of fatty acids after induction of LTP. Samples of perfusate were analysed for fatty acid concentration according to the method of Miwa et al. [25]. Results are expressed in nM and are means (±SEM) of six determinations. After induction of LTP there was a significant increase in perfusate concentration of arachidonic acid compared with baseline (P<0.05; Student's t-test for independent means). There was no significant change in any of the other fatty acids studied.

_arachidonic acid stimulates PLC activity is unclear. To rule out the possibility that PLC activity was stimulated by a non-specific action on membrane fluidity, oleic, palmitic and stearic acids were investigated and shown to be inactive [28].

Arachidonic acid increases Ca$^{2+}$ concentrations in pancreatic islet cells [32]. Increased intrasynaptosomal Ca$^{2+}$ is one requirement for an increase in transmitter release and therefore arachidonic-acid-induced change in intrasynaptosomal concentration of Ca$^{2+}$ was examined in the dentate gyrus. Fig. 2(c) shows that arachidonic acid increases intrasynaptosomal Ca$^{2+}$ in a concentration-dependent manner. Ins(1,4,5)P$_3$ also increased

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Fig. 1

(b) Changes in perfusate concentration of fatty acids after induction of LTP. Samples of perfusate were analysed for fatty acid concentration according to the method of Miwa et al. [25]. Results are expressed in nM and are means (±SEM) of six determinations. After induction of LTP there was a significant increase in perfusate concentration of arachidonic acid compared with baseline (P<0.05; Student’s t-test for independent means). There was no significant change in any of the other fatty acids studied.
to examine this question, we first analysed the concentration of arachidonic acid in membranes prepared from slices obtained from control and potentiated tissue at three time intervals after induction of LTP. Table 1A shows that 2.5 min, 45 min and 3 h after induction, there was a significant increase in arachidonic acid concentration in potentiated tissue compared with control. This was not found to be the case for membrane concentrations of oleic, palmitic and stearic acids, which were similar in control and potentiated preparations (not shown). Results from preliminary experiments indicated that there was no similar increase in arachidonic acid concentration in membranes prepared from synaptosomes obtained from control tissue and tissue in which LTP had been sustained for 45 min. Since slices contain both presynaptic and postsynaptic elements, and synaptosomes mainly postsynaptic elements, it was tentatively concluded that the increase in arachidonic acid concentration was associated with a postsynaptic area.

To characterize further the locus of the LTP-associated increase, Percoll-purified synaptosomes, or tissue preparations enriched in glia or postsynaptic densities, were prepared from control and potentiated dentate gyrus. Details of the methodology are given in [8].

Table 1
Membrane arachidonic acid concentrations measured in control and potentiated dentate gyrus

<table>
<thead>
<tr>
<th>Arachidonic acid concn.</th>
<th>Control</th>
<th>Potentiated</th>
</tr>
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<tbody>
<tr>
<td>A Time after LTP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 min</td>
<td>3.6 (0.6)</td>
<td>5.3 (1.3)</td>
</tr>
<tr>
<td>45 min</td>
<td>1.8 (0.5)</td>
<td>5.0 (1.0)</td>
</tr>
<tr>
<td>3 h</td>
<td>4.0 (0.8)</td>
<td>8.6 (0.9)</td>
</tr>
<tr>
<td>B Preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synaptosomes</td>
<td>37.5 (5)</td>
<td>40.0 (5)</td>
</tr>
<tr>
<td>PSDs</td>
<td>8.5 (1)</td>
<td>15.5 (2)</td>
</tr>
<tr>
<td>Glia</td>
<td>10.5 (3)</td>
<td>9.5 (2)</td>
</tr>
</tbody>
</table>
tiated dentate gyr and membrane arachidonic acid concentrations were measured. As shown in Table 1B, there was a significant increase in the concentration of arachidonic acid in postsynaptic densities prepared from potentiated tissue compared with control, while the concentrations in glia and synaptosomes were similar in both conditions. This result is consistent with the hypothesis that arachidonic acid is released from a postsynaptic site, although the possibility remains that arachidonic acid is released from another site and preferentially taken up into the membrane of the postsynaptic density. This possibility was addressed in separate experiments, where 8,11,14-eicosatrienoic acid, an inhibitor of arachidonic acid uptake, was found to have no effect on arachidonic acid concentration in postsynaptic densities prepared from control tissue or potentiated tissue in which LTP was sustained for 45 min. This experiment did not examine the possibility that uptake of arachidonic acid into postsynaptic density membrane occurred only at the time of induction of LTP. However, this seems unlikely since the increase in perfusate concentration of arachidonic acid persists for 1.5 h after induction. Thus, although the question of the site of arachidonic acid release in LTP remains an open one, our evidence to date suggests that release is postsynaptic in origin.

**Liberation of arachidonic acid from phospholipids**

An increase in membrane concentration of free arachidonic acid requires an increase in the activity of one or more of the enzymes responsible for its liberation from phospholipids, i.e. phospholipase \( A_1 \) (PLA\(_1\)), phospholipase \( A_2 \) (PLA\(_2\)) or PLC. We have shown that there is an increase in activity of PLC in slices of dentate gyrus 45 min and 3 h after induction of LTP, but that activity in control and potentiated tissue was similar 2.5 min after induction. Thus an increase in PLC activity may be responsible for the increase in arachidonic acid concentration in the later phases of LTP, but is clearly not associated with earlier changes. Activity of PLA\(_1\) was similar in control and potentiated tissue at the three time intervals studied, but 2.5 min after induction there was a small but significant increase in PLA\(_2\) activity. Further examination of this change in PLA\(_2\) activity led to the finding that phosphatidylcholine and phosphatidylinositol, but not phosphatidylethanolamine, were used as substrates for PLA\(_2\). On the basis of these findings, we have formulated a working hypothesis relating to release of arachidonic acid. At the time of induction of LTP, there is a local influx of \( Ca^{2+} \) into the postsynaptic region through NMDA-receptor-associated channels, which stimulates a number of changes, including activity of PLA\(_1\). Although we have not tested this in the dentate gyrus, it has been shown in cultures of cerebellar granule cells [34] and hippocampal pyramidal cells [35] that NMDA activation results in a PLA\(_1\)-dependent increase in arachidonic acid liberation. Thus the early increase in arachidonic acid concentration in perfusate may be the result of increased PLA\(_2\) activity. At later time points, the LTP-associated increase in glutamate release stimulates the quisqualate metabotropic receptor; this in turn stimulates PLC activity, and with a concomitant increase in diacylglycerol lipase, results in liberation of arachidonic acid which accounts for the increase in release observed 45 min and 3 h after induction of LTP.

The evidence presented is consistent with the hypothesis that arachidonic acid is a retrograde messenger in LTP. Three basic predictions of the hypothesis have been examined and two have been confirmed, i.e. (i) there is an increase in arachidonic acid concentration in the perfusate after induction of LTP and (ii) arachidonic acid exerts significant effects on the presynaptic terminal which stimulates glutamate release. The third prediction, that the locus of arachidonic acid release is postsynaptic, remains to be firmly established, but the data presented suggest that liberation of the fatty acid, as a result of increased activities of PLA\(_2\) and PLC, is from the postsynaptic density.

A number of questions relating to the role of arachidonic acid as a retrograde messenger in LTP need to be addressed. First, the delayed effect of arachidonic acid in inducing potentiation implies the existence of an additional, immediate retrograde factor. One possibility is nitric oxide, which has been shown to be released in cerebellar granule cells following NMDA receptor stimulation [36]. Secondly, is the relatively early appearance of increased arachidonic acid concentration in the perfusate (within the first 15 min) inconsistent with the delayed onset of arachidonic-acid-induced potentiation? Thirdly, why is there a persistent increase in arachidonic acid release after induction of LTP? One would have predicted that the messenger would be released, trigger a change and undergo immediate inactivation by metabolism or reuptake. As long as these intriguing questions remain unanswered, the hypothesis that arachidonic acid plays a role of retrograde messenger in LTP remains an issue for debate.

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