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Interactions between arachidonic acid and metabotropic glutamate receptors in the induction of synaptic potentiation in the rat hippocampal slice

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Abstract

Perfusion of neither the metabotropic glutamate receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), nor arachidonic acid caused any long-term enhancement of synaptic transmission in the CA1 region of the rat hippocampal slice. However, co-perfusion of ACPD (50 μM) and arachidonic acid (10 μM) for 5 min induced a rapidly evoked and long-lasting enhancement of synaptic transmission. This enhancement persisted in the presence of D(−)-2-amino-5-phosphonopentanoic acid (40 μM) and is therefore independent of NMDA receptor activation. The potentiation was mimicked by perfusion of the phospholipase A_2 activator melittin (10 μg/ml) for 5 or 10 min, or exogenous phospholipase A_2 (1 μg/ml) for 5 min, immediately before ACPD application. We propose a role for arachidonic acid in the induction of synaptic potentiation, possibly as a retrograde transmitter substance.

Keywords: Arachidonic acid; Long-term potentiation; NMDA (*N*-methyl-D-aspartate); ACPD ((1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid); Melittin; Phospholipase A_2

1. Introduction

Long-term potentiation describes a form of synaptic enhancement, widely regarded as a model for the neural mechanisms underlying learning and memory (Bliss and Collingridge, 1993). Transient activation of the NMDA subtype of glutamate receptor has been shown to be a prerequisite for the induction of long-term potentiation (Collingridge et al., 1983). However, application of NMDA alone is insufficient to induce long-term potentiation, evoking only a short-term enhancement of synaptic transmission (Collingridge et al., 1983; Kauer et al., 1988; McGuinness et al., 1991a; Collins and Davies, 1994; but see Thibault et al., 1989; Collingridge et al., 1991). Antagonists of the metabotropic subtypes of glutamate receptor can also block the formation of long-term potentiation (Bashir et al., 1993; Behnisch and Reymann, 1993). Application of an agonist for the metabotropic receptors (1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid: ACPD) has also been reported to induce potentiation, but usually under conditions where there would be concurrent NMDA receptor activation (McGuinness et al., 1991b; Otani and Ben-Ari, 1991; Musgrave et al., 1993; Collins, 1993; Behnisch and Reymann, 1993; Collins and Davies, 1994; O'Connor et al., 1994). Hence, it is likely that the induction of long-term potentiation requires activation of both NMDA and metabotropic receptors.

The present study was initiated by the report that application of ACPD in the presence, but *not* in the absence, of arachidonic acid enhanced the release of glutamate from synaptosomes (Herrero et al., 1992). Activation of postsynaptic NMDA receptors raises postsynaptic intracellular Ca^{2+} levels and activates a range of enzymes including phospholipase A_2 (PLA_2) which generates arachidonic acid (Dumuis et al., 1988). Arachidonic acid has previously been implicated as a retrograde messenger which could be released from

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the postsynaptic cell and trigger increased transmitter release from the presynaptic terminal (Williams et al., 1989, Williams and Bliss, 1989). Induction of long-term potentiation might therefore require activation of presynaptic metabotropic glutamate receptors, and concurrent activation of postsynaptic NMDA receptors to trigger release of arachidonic acid.

Here we have tested some predictions of this hypothesis by comparing the effects on synaptic transmission of activation of metabotropic receptors alone, with those under conditions where arachidonic acid levels would be raised. Preliminary results have previously appeared in rapid form (Collins and Davies, 1993).

2. Materials and methods

Transverse hippocampal slices (400 μm thick) were prepared from halothane anaesthetized rats (weight 140–150 g) and maintained in a constantly perfused interface chamber at 29–31°C. The artificial cerebrospinal fluid (aCSF) for perfusion contained (in mM): NaCl 124, KCl 3, $NaHCO_3$ 26, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 1, D-glucose 10. In the initial experiments involving arachidonic acid the perfusion medium also included 1 μM picrotoxin, since this was found to increase the likelihood of obtaining potentiation.

Bipolar electrodes were used to stimulate the Schaffer collateral-commissural fibre pathway at a frequency of 0.033 Hz. Field excitatory postsynaptic potentials (field e.p.s.p.s) were recorded from the dendritic region of the CA1 with a 3 M NaCl filled glass microelectrode (resistance 2–10 MΩ). For all experiments the CA3 region was removed.

On stabilisation of the response a 15 min control period was recorded prior to drug application. ACPD, arachidonic acid, PLA_2 and melittin were applied for 5 min, either singly or in combination, within the aCSF. In experiments including the selective NMDA antagonist D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), the D-AP5 was perfused for 15 min prior to drug application and until 5 min post application. ACPD and D-AP5 were obtained from Tocris Cookson; arachidonic acid (shipped in dry ice), melittin, PLA_2 (prepared from bovine pancreas) and picrotoxin were obtained from Sigma.

Statistical comparisons of pre- and post-treatment field e.p.s.p. amplitudes were made using the Student's *t*-test.

3. Results

In picrotoxin containing medium, perfusion of 10 μM arachidonic acid for 5 min evoked a gradually developing and persistent depression of the response

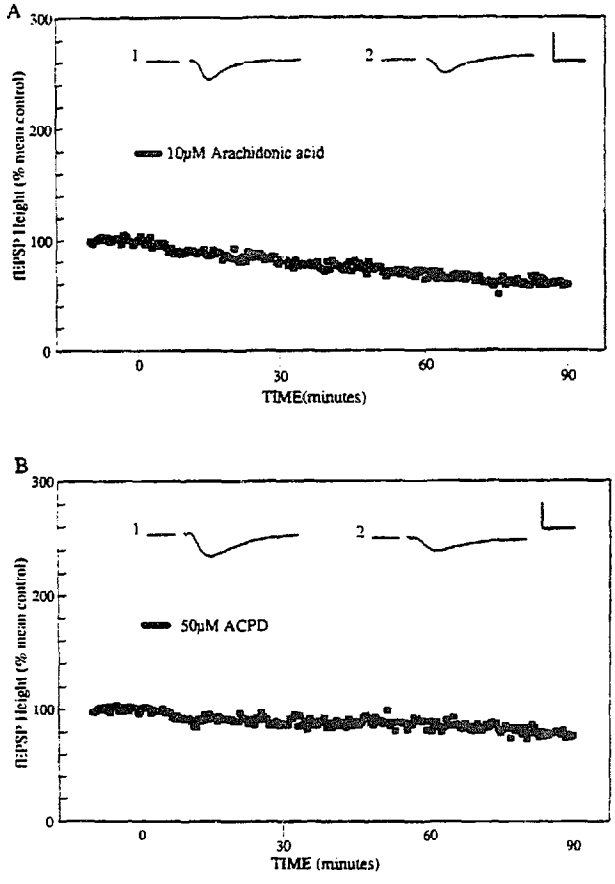

Fig. 1. Neither arachidonic acid, nor ACPD, perfused alone potentiate synaptic transmission. A: Effect of 10 μM arachidonic acid perfused for 5 min ($n = 5$). B: Effect of 50 μM ACPD perfused for 5 min ($n = 5$). In this and all other figures points on the graphs represent pooled and normalised data for the amplitude of the field e.p.s.p. for the stated number of slices. The inset traces show the average of 3 consecutive synaptic responses from a single slice during the control period (trace 1) and at the end of the time period shown (trace 2). The bar indicates the period of drug application; scale bar represents 2 mV and 10 ms.

in all 5 slices tested (mean field e.p.s.p. amplitude was 88% of control at 10 min, 58% of control at 90 min post application, $n = 5/5$, $P < 0.05$, Fig. 1A). Perfusion of 50 μM ACPD for 5 min elicited no long-term effects. Immediately following perfusion there was a small and short-lasting reduction in the field e.p.s.p. amplitude, which quickly recovered, and in 3 out of 8 slices was followed by a return to control levels. In the other 5 slices the initial reduction was followed by a slight gradual attenuation of the response (mean field e.p.s.p. amplitude was 86% at 10 min, 76% at 90 min post application, $n = 5/8$, not significant, Fig. 1B).

In picrotoxin containing medium, co-application of 10 μM arachidonic acid and 50 μM ACPD for 5 min elicited in 7 out of 11 slices a rapidly induced enhancement of the synaptic response during perfusion, followed by a transient decay towards control levels within 10 min, but this then gave rise to a slowly developing

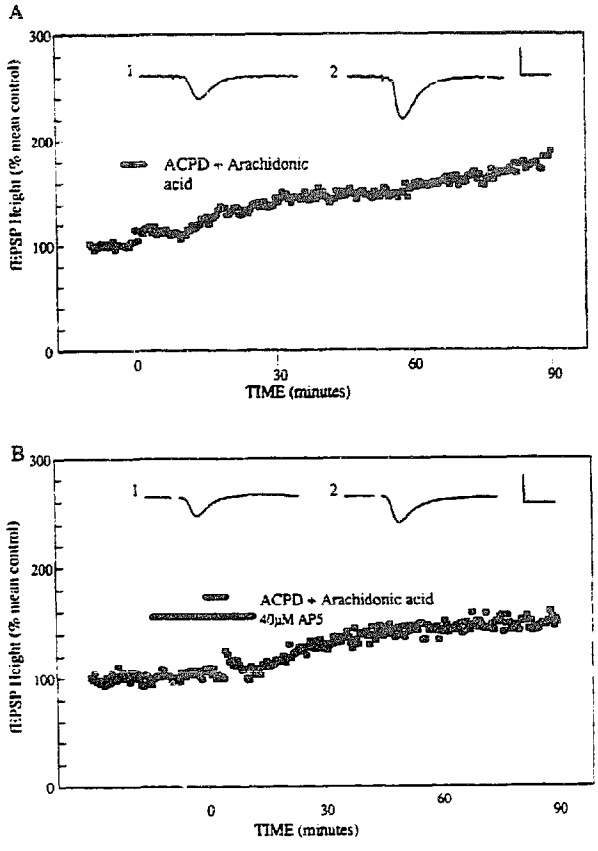

Fig. 2. Co-perfusion of arachidonic acid and ACPD induces potentiation which does not rely on NMDA receptor activation. A: Arachidonic acid (10 μM) and ACPD (50 μM) were co-perfused for 5 min ($n = 7$). B: D-AP5 (40 μM) was perfused from 15 min prior to, until 5 min after, co-perfusion of arachidonic acid and ACPD ($n = 5$). Scale bar represents 2 mV and 10 ms.

and long-lasting potentiation of the response (mean field e.p.s.p. amplitude was 110% at 10 min, 181% at 90 min post application, $n = 7/11$, $P < 0.05$, Fig. 2A). The remaining 4 slices showed either no long-term effect, or a marginal depression.

To determine whether this potentiation was independent of NMDA receptor activation, we repeated co-application of arachidonic acid and ACPD, in the presence of the selective NMDA receptor antagonist D-AP5. Inclusion of D-AP5 had no effect on the induction of potentiation by arachidonic acid and ACPD. In 5 out of 7 slices the responses consistently exhibited the same rapid induction, brief decay and slowly developing potentiation (mean field e.p.s.p. amplitude was 103% at 10 min, 150% at 90 min post application, $n = 5/7$, $P < 0.01$, Fig. 2B). There was no apparent short- or long-term effect in the other 2 slices.

We also perfused arachidonic acid and ACPD in the absence of picrotoxin. Under these conditions arachidonic acid or ACPD perfused alone produced comparable long-term effects to that seen in the presence of picrotoxin (mean field e.p.s.p. amplitude was 100% at 10 min, 98% at 90 min post application of arachidonic acid, $n = 6/8$, not significant, data not illustrated, and 86% at 10 min and 66% at 90 min post application of ACPD, $n = 5/6$, not significant, data not illustrated). Co-perfusion of arachidonic acid and ACPD in the absence of picrotoxin still induced potentiation, so long as fresh arachidonic acid was prepared from sealed vials each day. With this restriction, in 7 out of 10 slices, co-perfusion of 10 μM arachidonic acid and 50 μM ACPD for 5 min induced the same two-phase potentiation with a rapid short-lived potentiation during perfusion, followed by a slower rising and persistent potentiation of the synaptic response. The time course of this effect was identical to that seen previously, although the maximum potentiation was somewhat smaller (mean field e.p.s.p. amplitude was 108% at 10 min, 141% at 90 min post application, $n = 7/10$, $P < 0.01$, data not illustrated). The remaining 3 slices showed the same effect as that caused by perfusion of ACPD alone.

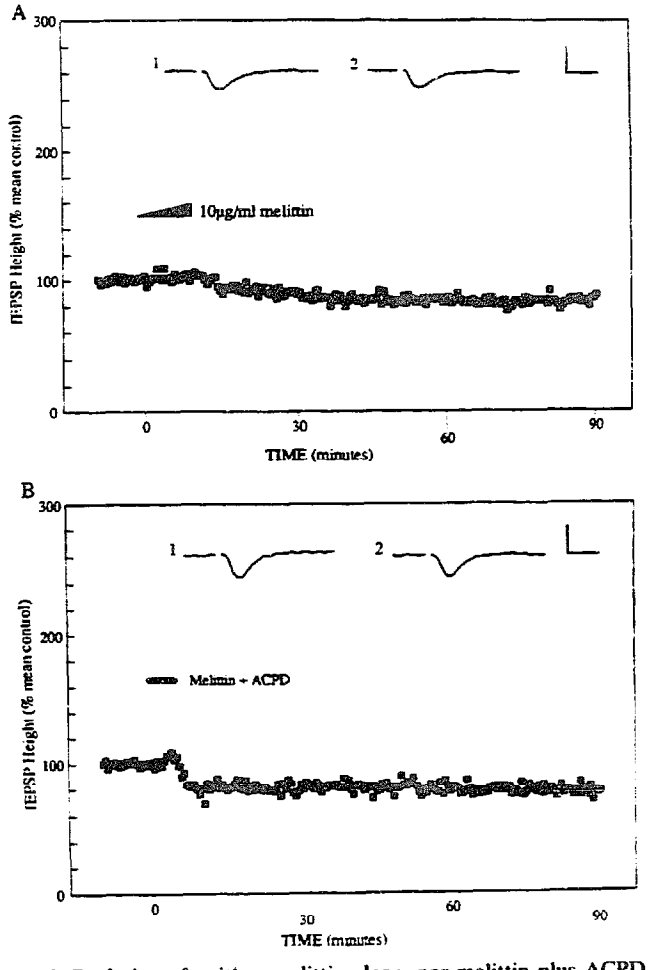

Fig. 3. Perfusion of neither melittin alone, nor melittin plus ACPD, potentiates synaptic transmission. A: Melittin (10 μg/ml) was perfused for either 5 or 10 min ($n = 5$). B: Melittin (10 μg/ml) and ACPD were co-perfused for 5 min ($n = 8$). Scale bar represents 2 mV and 10 ms.

We then used a series of alternative strategies designed to increase endogenous arachidonic acid levels within the slice using the PLA_2 activator melittin, or using exogenous PLA_2.

First, perfusion of high concentrations of melittin (50 μg/ml or over) onto naive slices for 5 min caused a marked hyperexcitability of the slice with a transient increase in the height of the field e.p.s.p. and/or an increase in the number of population spikes evoked ($n = 3$). This excitatory effect was short lived and was followed by persistent depression, or even total loss, of the synaptic responses (data not shown). Perfusion of lower concentrations of melittin (10 μg/ml) for 5 or 10 min had no significant effect on synaptic transmission (mean field e.p.s.p. amplitude was 88% at 30 min, 84% at 90 min post application, $n = 5/6$, not significant, Fig. 3A). Co-perfusion of 10 μg/ml melittin and 50 μM ACPD for 5 min evoked only a brief period of marginal enhancement during perfusion which, in 8 out of 11 slices, was followed by a long-lasting depression of the response (mean field e.p.s.p. amplitude was 80% at 10 min, 76% at 90 min post application, $n = 8/11$, $P < 0.05$, Fig. 3B).

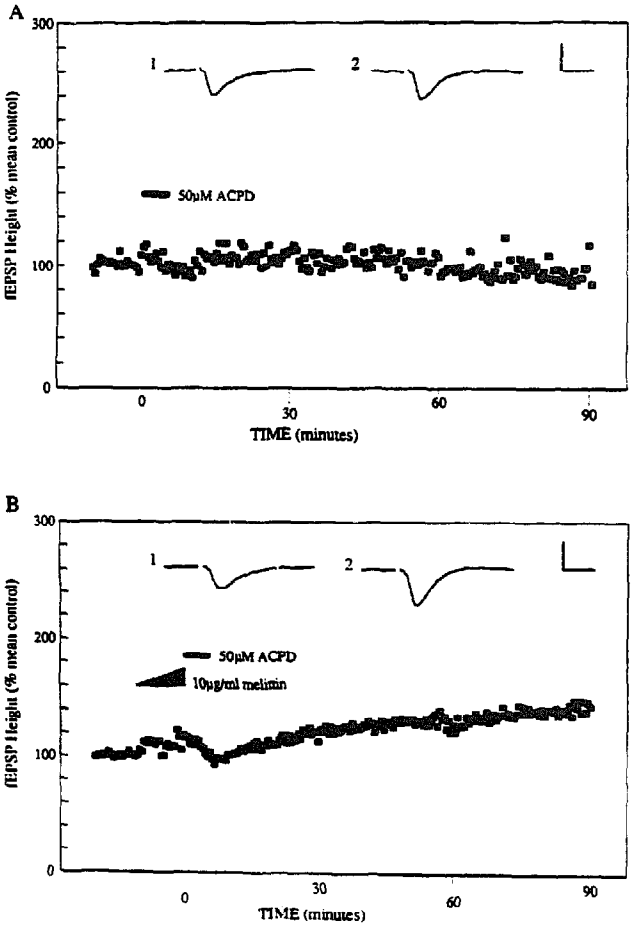

Fig. 4. Administration of ACPD to slices preincubated with melittin does not induce potentiation, but administration of ACPD immediately after perfusion of melittin does. A: Slices were preincubated for 30–60 min with 10 μg/ml melittin and ACPD (50 μM) was perfused for 5 min ($n = 3$). B: Slices were perfused with melittin (10 μg/ml) for 5 or 10 min, and then with ACPD (50 μM) for 5 min ($n = 5$). Scale bar represents 2 mV and 10 ms.

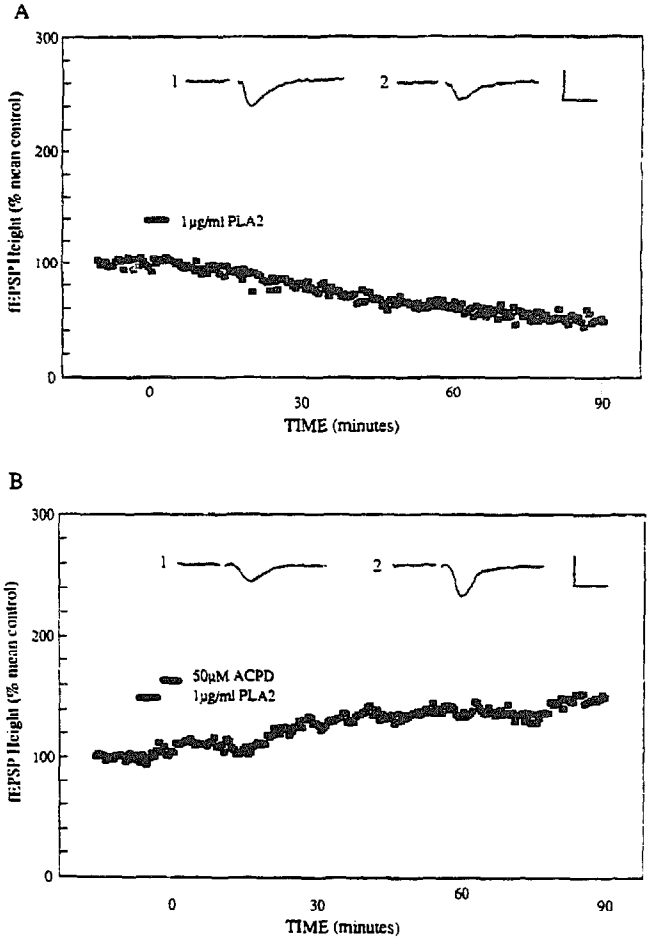

Fig. 5. Administration of PLA_2 alone does not induce potentiation, but perfusion of PLA_2 followed by ACPD does. A: Slices were perfused with PLA_2 (1 μg/ml) for 5 min ($n = 4$). B: Slices were perfused with PLA_2 (1 μg/ml) for 5 min, immediately followed by ACPD (50 μM) for 5 min ($n = 5$). Scale bar represents 2 mV and 10 ms.

Secondly, we attempted to elicit potentiation by perfusing 50 μM ACPD for 5 min onto slices that had been incubated in melittin. Synaptic responses from these slices were typically small and sometimes comprised multiple population spikes. Perfusion of ACPD in slices that had been incubated with 10 μg/ml melittin for 30–60 min beforehand, had little or no effect on the synaptic response (mean field e.p.s.p. amplitude was 108% at 30 min, 95% at 90 min post ACPD application, $n = 3/3$, not significant, Fig. 4A).

We suspected that co-application of the drugs did not allow time for any delay in the activation of PLA_2 and production of arachidonic acid, and that preincubation with melittin caused depletion of arachidonic acid. Therefore, we ran trials in which 10 μg/ml melittin was perfused for 5 or 10 min immediately prior

to perfusion of 50 μM ACPD for 5 min. In 5 out of 7 slices pre-perfusion of melittin followed by ACPD application gave rise to a rapidly initiated and long-lasting enhancement of the response, comparable in time course to that evoked by co-application of arachidonic acid and ACPD (mean field e.p.s.p. amplitude was 98% at 10 min, 144% at 90 min post ACPD application, $n = 5/7$, $P < 0.05$, Fig. 4B). The remaining 2 slices showed no long-term effects.

Finally, we examined the effects of perfusion with PLA_2. Perfusion of 10 μg/ml PLA_2 for 5 min caused an immediate increase in the size of the field e.p.s.p. and appearance of multiple population spikes during perfusion, but this was short lived and was followed by a marked depression of the response ($n = 3$). Perfusion of 1 μg/ml PLA_2 for 5 min most commonly caused no visible effects during perfusion, which was followed by a very gradual depression of the synaptic response (mean field e.p.s.p. amplitude was 95% at 10 min, 50% at 90 min post ACPD application, $n = 4/5$, $P < 0.05$, Fig. 5A). The remaining slice showed a slight potentiation. In 6 slices, co-perfusion of 50 μM ACPD and 1 μg/ml PLA_2 for 5 min caused variable results with equal numbers of slices showing either no long-term effect, or potentiation (mean field e.p.s.p. amplitude was 119% at 10 min, 142% at 90 min post ACPD application, $n = 3/6$, $P < 0.05$, data not illustrated). However, perfusion of 1 μg/ml PLA_2 for 5 min immediately prior to application of ACPD more reliably evoked potentiation in a total of 5 out of 7 slices (mean field e.p.s.p. amplitude was 111% at 10 min, 149% at 90 min post ACPD application, $n = 5/7$, $P < 0.05$, Fig. 5B). In the remaining 2 slices the drug application caused no long-term effects.

4. Discussion

4.1. Effect of ACPD

The lack of any persistent potentiation caused by perfusion of ACPD alone is in agreement with our previous experiments (Collins and Davies, 1994), and with those from other laboratories (Bortolotto and Collingridge, 1993) performed on slices from which the CA3 region had been removed. Some laboratories report that ACPD causes a short-term depression of synaptic transmission (e.g. Baskys and Malenka, 1991) though this is by no means evident in the records from all laboratories (e.g. Bortolotto and Collingridge, 1993). Although such a depression is not clearly visible in the records illustrated in Fig. 1 or 4, it should be noted that both of these are recorded in modified conditions (picrotoxin-containing medium or from slices preincubated in melittin respectively). We have previously noted such a depression in normal medium (Collins and Davies, 1994) and the lack of depression in the current records probably reflects the multiplicity of effects of metabotropic glutmate receptors on the synaptic responses. For instance, ACPD also causes a short-term enhancement of the NMDA receptor mediated component of synaptic transmission which might be unmasked in picrotoxin containing medium.

4.2. Effect of arachidonic acid

Previous experiments have found that perfusion of arachidonic acid facilitates the induction of long-term potentiation by a subthreshold tetanus (Williams et al., 1989; O'Dell et al., 1991), or by low frequency stimulation in low Mg^{2+} medium (Kato et al., 1991). Effective concentrations used in these experiments are very difficult to compare since they will depend on the methods used to apply the drug. For instance, Williams et al. (1989) prepared the arachidonic acid in ethanol (to aid dispersion) and ascorbic acid (to limit oxidative breakdown). Since ascorbic acid has been shown to have various actions on excitable tissue (Grünemald, 1993), it was not included in our drug preparation and instead arachidonic acid was prepared freshly from a sealed vial and either used immediately, or stored under nitrogen at −20°C for a maximum of 4 days. Also, our slices show a high sensitivity to ethanol; 5 min perfusion of ethanol concentrations as low as 0.01% resulted in a gradual reduction in the field e.p.s.p. amplitude (data not shown) and as a result our stock arachidonic acid was prepared in water (according to the method of Barbour et al. (1989)) or stock aCSF solution. Although the method might not guarantee uniform dispersion of arachidonic acid in the aCSF, the consistency of the results would suggest that distribution was even throughout the solution.

4.3. Effect of ACPD and arachidonic acid

The potentiation produced by co-perfusion of arachidonic acid and ACPD appeared to consist of two phases. There was a rapidly induced potentiation occurring during perfusion of the drugs, followed by a more slowly developing but persistent potentiation developing over the 90 min thereafter. From the relative time courses it is hard to determine whether this apparent biphasic effect results from a reversal of the normal inhibitory effect of ACPD applied alone followed by a separate slowly developing potentiation, or whether it represents a continuous gradual potentiation superimposed on which is the transient depression caused by ACPD alone. The fact that potentiation was observed in approximately two thirds of the slices tested parallels the consistency of results obtained by Kovalchuk et al. (1994) who investigated the effects of higher concentrations of bath applied arachidonic acid

on the synaptically evoked AMPA receptor mediated currents in hippocampal slices. They attributed the failure of arachidonic acid to inhibit these currents to the presumed breakdown of arachidonic acid within the slice.

4.4. Picrotoxin

Although 1 μM picrotoxin was present during the first set of experiments involving arachidonic acid, and was found to improve the potentiation, it is important to note that this is not a consequence of unmasking any NMDA receptor mediated component of synaptic transmission. This cannot be the case because the potentiation persisted in the presence of D-AP5 (see below). Instead we suspect that breakdown products of arachidonic acid potentiate inhibitory GABAergic transmission and limit the potentiation seen. This would explain why potentiation was reliably observed in the absence of picrotoxin, so long as the arachidonic acid was prepared freshly from sealed vials, whereas freezing and storage rapidly reduced its activity.

4.5. Persistence of potentiation in D-AP5

A previous report by O'Dell et al. (1991) showed that 40 μM DL-AP5 blocks the potentiation caused by a subthreshold tetanus in the presence of arachidonic acid, and they therefore concluded that the effect of arachidonic acid must be 'up-stream' of NMDA receptor activation. In our experiments the potentiation evoked by coperfusion of arachidonic acid and ACPD persisted in the presence of 40 μM D-AP5 (a concentration sufficient to block tetanus induced long-term potentiation in our hands, data not shown) suggesting that we were effectively bypassing the requirement for NMDA receptor activation. Hence we suggest that we are observing effects 'down-stream' of NMDA receptor activation.

4.6. Melittin

In our hands high concentrations of melittin (50 μM or above) had clear acute excitatory effects which were followed by subsequent depression of the synaptic response. The failure of ACPD to induce potentiation in slices which were either preincubated in, or co-perfused with, lower concentrations (10 μM) of melittin may be due to depletion of, or inappropriate timing of the release of, arachidonic acid respectively. In contrast, 5 or 10 min perfusion of melittin, followed by 5 min perfusion of 50 μM ACPD replicated the results obtained by co-perfusion of arachidonic acid and ACPD. This would suggest that activation of the phospholipid cascades which liberate endogenous arachidonic acid can substitute for perfusion of exogenous arachidonic acid in inducing potentiation.

In this study melittin was used as an alternative method to activate PLA_2 and release endogenous arachidonic acid. Melittin activates some, but not all, types of PLA_2, e.g. 10 μg/ml increases activity of a low molecular weight (14 kDa) PLA_2 isolated from murine T-cells approximately 5-fold (Steiner et al., 1993). However, it also has other actions; most notably it inhibits some kinases with an EC_{50} of approximately 8 μM (Mazzei et al., 1982), and has been used previously as a kinase inhibitor to prevent the induction of long-term potentiation the dentate gyrus (Lovinger et al., 1987). Since these experiments were performed in vitro using local microapplication of the drug it is hard to estimate the effective concentration used. However, these issues complicate the results and it would clearly be useful to use more specific activators of PLA_2, and to measure the rate of arachidonic acid release associated with these different protocols.

4.7. PLA_2

It has been reported that prolonged incubation with PLA_2 increases binding of α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) in rat brain slices (Tocco et al., 1992) and in our experiments higher concentrations of PLA_2 (10 μg/ml) did cause an increase in the field e.p.s.p. amplitude and the introduction of multiple population spikes, but this effect did not outlast the perfusion period and was usually followed by a gradual reduction in field e.p.s.p. amplitude. At the concentrations used in these experiments (1 μg/ml) there was little or no effect of PLA_2 during perfusion, but there was consistently a gradual reduction in the amplitude of the response over the next 90 min. Whether this represents a physiological or pathological response is unclear, nevertheless, it does make the potentiation induced by ACPD and PLA_2 all the more striking. As with melittin, it was found that application of ACPD immediately after perfusion of PLA_2 was more effective in inducing potentiation than was co-perfusion of the two drugs. This difference may reflect the accessability of PLA_2 to the recording site within the slice, and/or the time required to generate arachidonic acid by this means.

4.8. Scheme for involvement of arachidonic acid as retrograde transmitter

We speculate that during the special circumstances of long-term potentiation induction, NMDA receptor gated channels allow a highly localised and transient influx of Ca^{2+} into the postsynaptic spine head (Smith, 1987). This is sufficient to activate PLA_2 which gener-

ates arachidonic acid from membrane phospholipids (Dumuis et al., 1988; Sanfeliu et al., 1990). Arachidonic acid may then diffuse across the synaptic cleft to the presynaptic terminal where it acts in concert with the metabotropic receptor activation to increase transmitter release (Herrero et al., 1992). Arachidonic acid has two short-term actions, namely inhibition of L-glutamate uptake (Barbour et al., 1989) and potentiation of NMDA receptor mediated responses (Miller et al., 1993), which would effectively make this an all-or-none event. Furthermore, diffusion of L-glutamate to neighbouring synapses which have not undergone long-term potentiation induction may activate presynaptic metabotropic receptors alone, hence inhibiting those synapses (Baskys and Malenka, 1991) and effectively enhancing the signal-to-noise ratio of the potentiated pathway.

The mechanism governing the interaction between ACPD and arachidonic acid in the control of synaptic plasticity is open to speculation. Indeed, since arachidonic acid is a relatively short-lived compound which is rapidly broken down to biologically active metabolites (Piomelli and Greengard, 1990) the observed effects may not be a direct effect of arachidonic acid at all. However, one possibility concerns the regulation of PKC. Both metabotropic glutamate receptors (Tanabe et al., 1992), and arachidonic acid (Sekiguchi et al., 1987, 1993; Schaechter and Benowitz, 1993) have been implicated in the regulation of PKC. Metabotropic glutamate receptors, arachidonic acid and PKC have in turn all been implicated in the control of voltage dependent K^+ and Ca^{2+} channels (Charpak et al., 1990; Keyser and Alger, 1990; Meves, 1994; Swartz and Bean, 1992; Yang and Tsien, 1992; Zona et al., 1993) which might play an important role in regulating transmitter release. It will therefore be interesting to establish whether ACPD and arachidonic acid exert a comparable interaction in the control of these channels.

Acknowledgements

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