ELSEVIER

European Journal of Pharmacology 342 (1998) 213–216

ejp

Short communication

Arachidonic acid metabolites and the synaptic potentiation evoked by activation of metabotropic glutamate receptors

Dawn R. Collins [1], Stephen N. Davies [*]

Department of Biomedical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

Received 31 July 1997; revised 8 December 1997; accepted 12 December 1997

Abstract

We have previously shown that coapplication of arachidonic acid (10 μM) and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 50 μM) evokes an enhancement of synaptic transmission in the CA1 region of the rat hippocampal slice. Here we have investigated whether the metabolites of arachidonic acid are implicated in this potentiation. Inclusion of the cyclo-oxygenase inhibitor indomethacin (10 μM) did not block the potentiation induced by coapplication of arachidonic acid and ACPD. However, the presence of either the cyclo-, lipo- and epoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA, 20 μM), or the lipoxygenase inhibitor nordihydroguaiaretic acid (10 μM), prevented the long-lasting enhancement. The results suggest that the lipoxygenase and epoxygenase metabolites of arachidonic acid may be involved in the induction of this form of synaptic potentiation.

Keywords: Long-term potentiation; Arachidonic acid; Metabotropic glutamate receptor; Lipoxygenase; Epoxygenase; Cyclooxygenase

1. Introduction

There is evidence for the involvement of both NMDA and metabotropic subtypes of glutamate receptor (mGlu receptors) in the induction of synaptic long-term potentiation in the CA1 region of the rat hippocampal slice (Collingridge et al., 1983; Bashir et al., 1993; Collins and Davies, 1994; Izumi and Zorumski, 1994), although the nature of their interaction is unclear. One hypothesis suggests that activation of postsynaptic NMDA receptors is required for upregulation of phospholipase A_2 and release of the putative retrograde transmitter, arachidonic acid (Williams et al., 1989). A refinement of the hypothesis is that arachidonic acid may then interact with presynaptic mGlu receptors to increase transmitter release (Herrero et al., 1992; Sanchez-Prieto et al., 1996).

In support of a role for NMDA receptor-mediated release of arachidonic acid interacting with mGlu receptors, we have previously shown that co-application of exogenous arachidonic acid with the mGlu receptor agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) evokes synaptic potentiation and that this potentiation is not blocked by NMDA receptor antagonists, suggesting that the effect is downstream of NMDA receptor activation (Collins and Davies, 1993; Collins et al., 1995).

Arachidonic acid is a relatively unstable and short-lived substance, being rapidly broken down by three principal enzymes (Piomelli and Greengard, 1990). Metabolism by lipoxygenase enzymes produces various hydroxyeicosatetranoic acids and leukotrienes, epoxygenase enzymes give rise to epoxides and cyclooxygenase enzymes produce prostaglandins and thromboxanes. The aim of the present study was to establish whether arachidonic acid itself, or one of its biologically active metabolites, was responsible for the interaction with mGlu receptors. We have therefore investigated the effects of the cyclo-, lipo- and epoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) (Capdevila et al., 1988), the lipoxygenase inhibitor nordihydroguaiaretic acid (Egan and Gale, 1984) and the cyclooxygenase inhibitor indomethacin (Salari et al., 1984), on the potentiation induced by co-application of arachidonic acid and ACPD.

[*] Corresponding author. Tel.: +44-1224-273086; fax: +44-1224-273019; e-mail: s.n.davies@abdn.ac.uk

[1] Current address: Département de Physiologie, Université Laval, Quebec, Que., Canada G1K 7P4.

0014-2999/98/$19.00

2. Materials and methods

400 μm thick transverse hippocampal slices were prepared from halothane anaesthetised rats (weight 140–160 g) and maintained in a constantly perfused and humidified interface chamber at 29–31°C. Artificial cerebrospinal fluid 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, and was constantly bubbled with 95% O_2/5% CO_2. For all experiments the CA3 region was removed. Bipolar electrodes were used to stimulate the Schaffer collateral commissural fibre pathway at a frequency of 0.033 Hz. Field excitatory post synaptic potentials (field e.p.s.p.'s) were recorded from the dendritic region of the CA1 with a 3 M NaCl filled glass electrode (resistance 2–10 MΩ). All drugs were applied by addition to the perfusion medium.

Arachidonic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), nordihydroguaiaretic acid and indomethacin were obtained from Sigma and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) was obtained from Tocris Cookson. Statistical comparison of pre- and post-treatment field e.p.s.p. amplitudes were made using the Student's *t*-test.

3. Results

Perfusion of either arachidonic acid (10 μM) or ACPD (50 μM) for 5 min had no significant long-term effects on synaptic transmission (for arachidonic acid: the mean field e.p.s.p. amplitude was 99 $\pm$ 9% of the control at 90 min post application, not significant, $n = 6$, Fig. 1a; for ACPD, the mean field e.p.s.p. amplitude was 91 $\pm$ 8% of the control at 90 min post application, not significant, $n = 5$, Fig. 1b). However, we found that coperfusion of arachidonic acid and ACPD evoked a long-lasting enhancement of synaptic transmission. As we have previously observed, the potentiation showed a clear biphasic time course with an early transient phase which peaked during perfusion of the drugs (maximum potentiation was 113 $\pm$ 7% of control during perfusion) and a slower developing but persistent phase which followed (mean field e.p.s.p. amplitude was 144 $\pm$ 5% at 90 min post application, $P < 0.05$, $n = 8$, Fig. 1c).

5 min perfusion of the cyclo-, lipo- and epoxygenase inhibitor ETYA (20 μM) had no effect on synaptic transmission (mean field e.p.s.p. height was 90 $\pm$ 9% at 90 min post application, not significant, $n = 5$, data not shown). Co-perfusion of arachidonic acid and ACPD in the presence of ETYA evoked a transient potentiation of the field e.p.s.p. during perfusion comparable to that in control conditions (maximum potentiation was 112 $\pm$ 5% of control during perfusion), but no long-term potentiation was observed (mean field e.p.s.p. amplitude was 86 $\pm$ 7% at 90 min post application, not significant, $n = 7$, Fig. 2a).

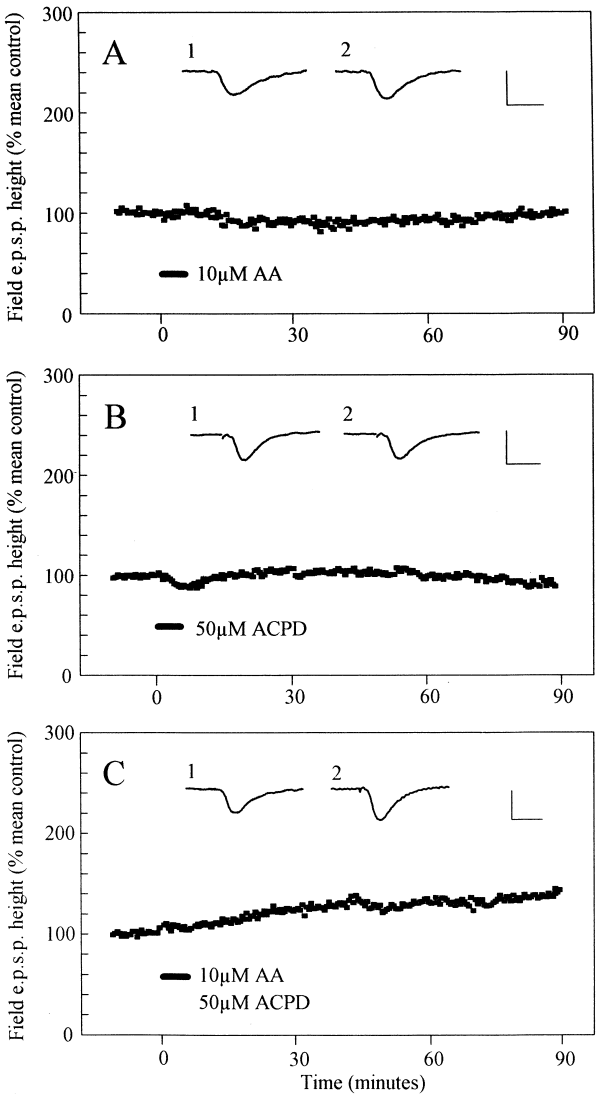

Fig. 1. Perfusion of arachidonic acid and ACPD together, but not alone, potentiates synaptic transmission. (A) Effect of 10 μM arachidonic acid perfused for 5 min ($n = 6$). (B) Effect of 50 μM ACPD perfused for 5 min ($n = 5$). (C) Effect of co-perfusion of 10 μM arachidonic acid and 50 μM ACPD for 5 min ($n = 8$). In this and Fig. 2, points on the graph 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 three consecutive synaptic responses from a single slice taken during the control period (trace 1), or at the end of the time period shown (trace 2). The thick bar indicates the period of drug perfusion and the scale bar represents 2 mV and 10 ms.

5 min perfusion of the lipoxygenase inhibitor nordihydroguaiaretic acid (10 μM) had no significant effect on synaptic transmission (mean field e.p.s.p. amplitude was 81 $\pm$ 11% at 90 min post application, not significant, $n = 6$, data not shown). Co-perfusion of arachidonic acid and ACPD in the presence of nordihydroguaiaretic acid evoked a transient depression of the field e.p.s.p., which returned to pre-treatment levels within 20 min, but no long-term effects were observed (mean field e.p.s.p. ampli-

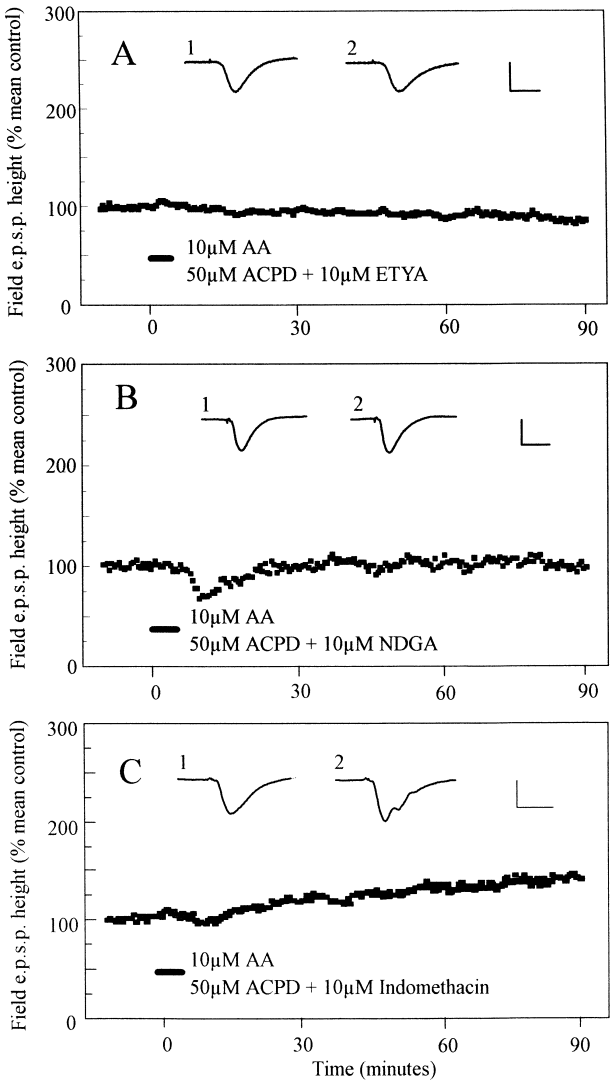

Fig. 2. ETYA and nordihydroguaiaretic acid, but not indomethacin, block the slowly developing persistent potentiation induced by co-perfusion of arachidonic acid and ACPD. (A) Effect of co-perfusion of 10 μM arachidonic acid (AA) and 50 μM ACPD in the presence of 10 μM ETYA ($n = 7$). (B) Effect of co-perfusion of 10 μM arachidonic acid (AA) and 50 μM ACPD in the presence of 10 μM nordihydroguaiaretic acid (NDGA) ($n = 6$). (C) Effect of co-perfusion of 10 μM arachidonic acid (AA) and 50 μM ACPD in the presence of 10 μM indomethacin ($n = 4$).

tude was 99 ± 18% at 90 min post application, not significant, $n = 6$, Fig. 2b).

Application of the cyclo-oxygenase inhibitor indomethacin (10 μM) also had no effect on synaptic transmission (mean field e.p.s.p. amplitude was 92 ± 11% at 90 min post application, not significant, $n = 7$, data not shown).

Interestingly, in the presence of indomethacin, perfusion of arachidonic acid and ACPD still evoked a biphasic potentiation almost identical to that seen in control slices with a transient potentiation observed during perfusion (maximum potentiation observed during perfusion of the drugs was 111 ± 5%) and a slower persistent phase which followed (mean field e.p.s.p. amplitude was 140 ± 16% at 90 min post application, $P < 0.05$, $n = 4$, Fig. 2c).

4. Discussion

The results indicate that the metabolites of arachidonic acid are involved in the induction of the slowly developing synaptic potentiation induced by arachidonic acid and ACPD. Since indomethacin did not block the potentiation, the cyclo-oxygenase metabolites of arachidonic acid are probably not involved, but since ETYA and nordihydroguaiaretic acid both did, the lipoxygenase and epoxygenase metabolites probably are.

These conclusions are comparable to those of Williams and Bliss (1989) who found that nordihydroguaiaretic acid but not indomethacin inhibited tetanus induced long term potentiation in the CA1 region of the rat hippocampal slice. They also reported that although NGDA prevented the development of long-term potentiation, the short-term potentiation observed following tetanic stimulation was unaffected and they suggested that this may reflect the involvement of arachidonic acid in the induction of short term potentiation but the involvement of the metabolites in the later phases.

This also has parallels in our results where ETYA blocked the slowly developing potentiation, but did not affect the early transient potentiation observed during perfusion of the drugs. This is the first time that we have been able to pharmacologically separate these two temporal phases of potentiation and suggests that they may have different underlying mechanisms, with the early transient phase depending on arachidonic acid and the slowly developing transient phase depending on the metabolites.

The rapid transient phase of potentiation was not observed in our experiments with nordihydroguaiaretic acid. We suspect this may be due to the non-specific actions of nordihydroguaiaretic acid which, apart from inhibiting the formation of lipoxygenase metabolites, has also been shown to inhibit phospholipase A_2 (Billah et al., 1986). So far as we are aware its action on other phospholipases has not been established, but since it has been suggested that arachidonic acid and the metabotropic glutamate receptors may potentiate synaptic transmission via phospholipase activation (McGahon and Lynch, 1996), this inhibition of phospholipase activity may block the initial brief enhancement of transmission. Furthermore, such an action may explain the pronounced depression observed in the presence of nordihydroguaiaretic acid, since blocking the actions of phospholipase linked group I mGlu receptors leaves the effect of the cAMP linked group II and III mGlu receptors in isolation and it is these which are thought to be responsible for the transient depression of synaptic transmission (Sanchez-Prieto et al., 1996).

In summary, we suggest that the lipoxygenase and epoxygenase, but not the cyclooxygenase metabolites of arachidonic acid may be involved in the slowly developing persistent potentiation observed after coperfusion of arachidonic acid and ACPD. In contrast, the early transient potentiation observed during perfusion of the drugs may be mediated by arachidonic acid itself.

Acknowledgements

This work was supported by the Wellcome trust.

References

Billah, M.M., Bryant, R.W., Seigel, M.I., 1986. Lipoxygenase products of arachidonic acid modulate biosynthesis of platelet activating factor (1-O-alkyl-2-acetyl-sn-glycerol-3-phosphocholine) by human neutrophils via phospholipase A_2. J. Biol. Chem. 260, 6899–6906.

Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berretta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C., Collingridge, G.L., 1993. Induction of LTP in the hippocampus needs synaptic activation of metabotropic glutamate receptors. Nature 363, 347–350.

Capdevila, J., Gil, L., Orellana, M., Marnett, L.J., Yadagari, P., Falk, J.R., 1988. Inhibitors of cytochrome P-450-dependent arachidonic acid metabolism. Arch. Biochem. Biophys. 261, 257–263.

Collingridge, G.L., Kehl, S.J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus. J. Physiol. 334, 33–46.

Collins, D.R., Davies, S.N., 1993. Co-administration of (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid and arachidonic acid potentiates synaptic transmission in rat hippocampal slices. Eur. J. Pharmacol. 240, 325–326.

Collins, D.R., Davies, S.N., 1994. Potentiation of synaptic transmission in the rat hippocampal slice by exogenous L-glutamate and selective L-glutamate receptor sub-type agonists. Neuropharmacology 33, 1055–1063.

Collins, D.R., Smith, R.C., Davies, S.N., 1995. Interactions between arachidonic acid and metabotropic glutamate receptors in the induction of synaptic potentiation in the rat hippocampal slice. Eur. J. Pharmacol. 294, 147–154.

Egan, R., Gale, P.H., 1984. In: Bailey, J.M. (Eds.), Prostaglandins, Leukotrienes and Lipoxins. Plenum, New York, pp. 593–607.

Herrero, I., Miras-Portugal, M.T., Sanchez-Prieto, J., 1992. Positive feedback of glutamate exocytosis by metabotropic presynaptic receptor stimulation. Nature 360, 163–166.

Izumi, Y., Zorumski, C.F., 1994. Developmental changes in the effects of metabotropic glutamate receptor antagonists on CA1 long-term potentiation. Neurosci. Lett. 176, 89–92.

McGahon, B., Lynch, M.A., 1996. The synergism between metabotropic glutamate receptor activation and arachidonic acid on glutamate release is occluded by induction of long-term potentiation in the dentate gyrus. Neuroscience 72, 847–855.

Piomelli, D., Greengard, P., 1990. Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signalling. Trends Pharmacol. Sci. 11, 367–373.

Salari, H., Braquet, P., Borgeat, P., 1984. Comparative effects of indomethacin, acetylenic acids, 15-HETE, nordihydroguaretic acid and BW 755C on the metabolism of arachidonic acid in human leucocytes and platelets. Prostaglandins Leukotrienes Med. 13, 53–60.

Sanchez-Prieto, J., Budd, D.C., Herrero, I., Vasquez, E., Nicholls, D.G., 1996. Presynaptic glutamate receptors and the control of glutamate exocytosis. Trends Neurosci. 19, 235–239.

Williams, J.H., Bliss, T.V.P., 1989. An in vitro study of the effect of lipoxygenase and cyclo-oxygenase inhibitors of arachidonic acid on the induction and maintenance of long-term potentiation in the hippocampus. Neurosci. Lett. 107, 301–306.

Williams, J.H., Errington, M.L., Lynch, M.A., Bliss, T.V.P., 1989. Arachidonic acid induces a long-term activity-dependent enhancement of synaptic transmission in the hippocampus. Nature 341, 739–742.