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Short communication

The synergism between ACPD and arachidonic acid on glutamate release in hippocampus is age-dependent

Bernadette McGahon, Marina A. Lynch *

Department of Physiology, Trinity College, Dublin 2, Ireland

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Abstract

Activation of the metabotropic glutamate receptor by the specific agonist *trans*-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) increases release of glutamate and activation of protein kinase C in the presence of a low concentration of arachidonic acid in hippocampal synaptosomes prepared from 4-month-old rats. The data presented indicate an age-related decrease in both [^{3}H]glutamate release and protein kinase C activation and an age-related decrease in the release response to arachidonic acid and ACPD, with no corresponding change in protein kinase C activation. The finding that the interaction between arachidonic acid and ACPD on release was absent in synaptosomes prepared in the presence of heparin, an antagonist at inositol trisphosphate receptors, suggests that mobilization of intracellular calcium stores plays a role in the synergism between arachidonic acid and ACPD on [^{3}H]glutamate release in hippocampal synaptosomes.

Keywords: Aging; ACPD (*trans*-1-amino-cyclopentyl-1,3-dicarboxylate); Arachidonic acid; Glutamate release; Protein kinase C; Inositol trisphosphate

1. Introduction

Activation of metabotropic glutamate receptors by the agonist, *trans*-1-amino-cyclopentyl-1,3-dicarboxylate (ACPD) in the presence of arachidonic acid increases release of glutamate in synaptosomes prepared from whole hippocampus (McGahon and Lynch, 1994), and dentate gyrus (McGahon and Lynch, 1996) in a protein kinase C-dependent manner similar to that described in cortex (Herrero et al., 1992). The recent observation that the synergism between arachidonic acid and ACPD on release of glutamate in dentate gyrus was occluded following induction of long-term potentiation (McGahon and Lynch, 1996) suggests a role for the interaction of these agents in the genesis of long-term potentiation. This thesis is supported by the observation that while arachidonic acid (Williams et al., 1989) and ACPD (Collins and Davies, 1993) induced a slowly developing form of potentiation, a rapidly developing form of potentiation was induced if tissue was exposed to arachidonic acid and ACPD coincidentally (Collins and Davies, 1993). Although the mechanism underlying the synergism between arachidonic acid and ACPD has not been elucidated, there is evidence of a parallel interaction resulting in enhancement of presynaptic inositol phospholipid metabolism and protein kinase C activation, both of which are attenuated by prior induction of long-term potentiation in dentate gyrus (McGahon and Lynch, 1996). One interpretation of this finding is that increased release may be modulated by the action of arachidonic acid and ACPD on phospholipase C activation.

Ability of aged animals to sustain long-term potentiation is impaired (Davis et al., 1993; Lynch and Voss, 1994) and this impairment is paralleled by decreased KCl-stimulated glutamate release (Lynch and Voss, 1994). In this study, we consider the possibility that these age-related deficits might be due to down-regulated sensitivity of the release process to the interaction between arachidonic acid and ACPD.

2. Materials and methods

Adult (4 months, 250–300 g) and aged (22 months, 400–500 g) male Wistar rats were killed by stunning and decapitation, the brain was rapidly removed, placed on ice and the hippocampus was dissected free. Tissue was homogenized in 0.32 M ice-cold sucrose and centrifuged at 5000 rpm for 5 min; the resulting supernatant was centrifuged at 15 000 rpm for 15 min to yield P_2. Glutamate release was assessed in the presence and absence of added

* Corresponding author. Tel.: 353 1 608 1770; fax: 353 1 679 3545.

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$CaCl_2$, and in the presence and absence of 40 mM KCl, as previously described (McGahon and Lynch, 1994). P_2 was resuspended in ice-cold Krebs solution (composition in mM: NaCl, 136; KCl 2.54; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.18; $NaHCO_3$, 16; glucose 10) containing 2 mM $CaCl_2$, incubated for 15 min at 37°C in the presence of [^{3}H]glutamate (Amersham, UK: specific activity 20–40 Ci/mmol; final concentration 5×10^{-7} mM), aliquoted onto Millipore filters (0.45 μm) and rinsed by addition of ice-cold, oxygenated, fresh Krebs. To examine unstimulated release, tissue was incubated for 10 s in the presence or absence of 2 mM $CaCl_2$ and filtrate was added to scintillant for counting. To examine stimulated release this step was repeated in the presence of 40 mM KCl. In some experiments arachidonic acid (final concentration 1 μM), ACPD (50 μM) or both, were added during this incubation period. In a separate series of experiments synaptosomes were prepared in the presence or absence of heparin (200 μg/ml), which has been shown to act as an antagonist at IP_3 receptors, and release was assessed as described above. Previous experiments indicated that under the conditions described here at least 80% of the radiolabel was recovered as [^{3}H]glutamate.

Protein kinase C activity was examined using an enzyme assay system (Amersham, UK) which assessed transfer of the radiolabelled phosphate group from adenosine-5-triphosphate ([^{32}P]ATP) to histone, a peptide specific for protein kinase C. P_2 was lysed by incubating at 4°C for 10 min in buffer (5 mM Tris-HCl (pH 7.5), containing 5 mM EDTA, 10 mM EGTA, 0.3% w/v β-mercaptoethanol, 10 mM benzamidine and 50 μl/ml phenylmethylsulphonyl fluoride). The membrane fraction was pelleted and resuspended in assay buffer (composition: 50 mM Tris-HCl, pH 7.5, containing 0.3% w/v β-mercaptoethanol, 10 mM benzamidine and 50 μl/ml phenylmethylsulphonyl fluoride). The reaction was started by addition of [^{32}P]ATP to the synaptosomal suspension; incubation continued at 25°C for 15 min. The effect of arachidonic acid (1 μM), ACPD (50 μM) or both was assessed; in this case the compounds were added prior to the 15 min incubation period. Phosphorylated peptide was separated by applying the reaction mixture to binding paper and washing with 5% v/v acetic acid. Binding papers were added to scintillant and [^{32}P] counted. Results were expressed as pmol phosphate transferred per minute.

When one-way analysis of variance (ANOVA) indicated significant differences between conditions, post-hoc Student-Newman-Keuls test analysis was used to determine statistical differences between conditions. When appropriate, Student's *t*-test for independent means was used to establish statistical significance.

3. Results

Unstimulated release of [^{3}H]glutamate was similar in the absence and presence of calcium, and no age-related difference was observed (0.06 ± 0.007 and 0.07 ± 0.006 in synaptosomes preapred from young rats, in the absence and presence of calcium, respectively; 0.052 ± 0.004 and 0.06 ± 0.008 in synaptosomes preapred from aged rats, in the absence and presence of calcium, respectively). Addition of 40 mM KCl increased release in the absence of calcium but the increase was significantly greater in its presence. In 4-month-old animals, KCl-stimulated release (i.e. release stimulated by the presence of 40 mM KCl minus unstimulated release) was significantly greater in the presence, compared to the absence, of calcium, while in aged animals the additional increase in the presence of calcium did not reach statistical significance (Fig. 1). In synaptosomes prepared from young animals, neither arachidonic acid nor ACPD alone had any significant effect on [^{3}H]glutamate release, but a significant increase was observed when both agents were present ($P < 0.001$; ANOVA). Neither arachidonic acid nor ACPD alone had any significant effect on release of glutamate in hippocampal synaptosomes prepared from aged animals. However, in contrast to the results observed in young animals, there was no evidence of synergism between arachidonic acid and ACPD in synaptosomes prepared from aged animals.

Fig. 2a shows that there was a decrease in protein kinase C activity in synaptosomes prepared from hippocampus of aged, compared to young, animals ($P < 0.01$; Student's *t*-test for unpaired means). Both arachidonic acid and ACPD significantly increased activity of protein ki-

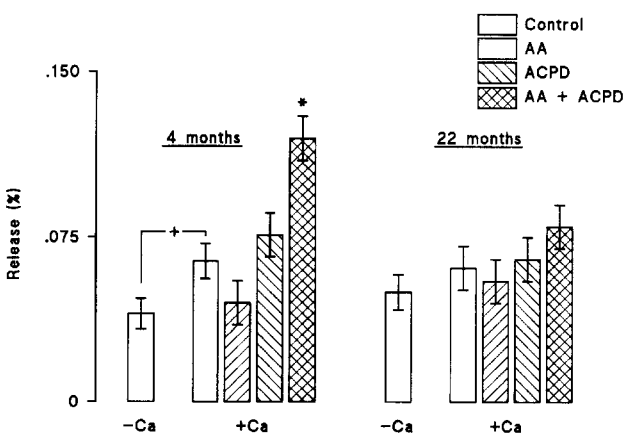

Fig. 1. KCl-stimulated release of [^{3}H]glutamate in hippocampal synaptosomes prepared from aged and young animals: effect of ACPD and arachidonic acid. Release of [^{3}H]glutamate was stimulated by addition of 40 mM KCl to Krebs solution containing 2 mM $CaCl_2$. Neither ACPD (50 μM) nor arachidonic acid (1 μM) had any significant effect on glutamate release in synaptosomes prepared from young or aged animals, but, in combination, arachidonic acid and ACPD significantly increased release ($P < 0.001$; ANOVA) in synaptosomes prepared from adults, but not aged animals. [^{3}H]Glutamate release was significantly greater in synaptosomes prepared from young, compared to aged animals ($P < 0.05$; Student's *t*-test for independent means). Results were calculated by subtracting basal release from KCl-stimulated release and individual values were used to calculate the mean ± S.E.M. for 9 observations. Fractional release is the ratio of the radiolabelled glutamate released during a given incubation period to the total radiolabel present at the start of that incubation period.

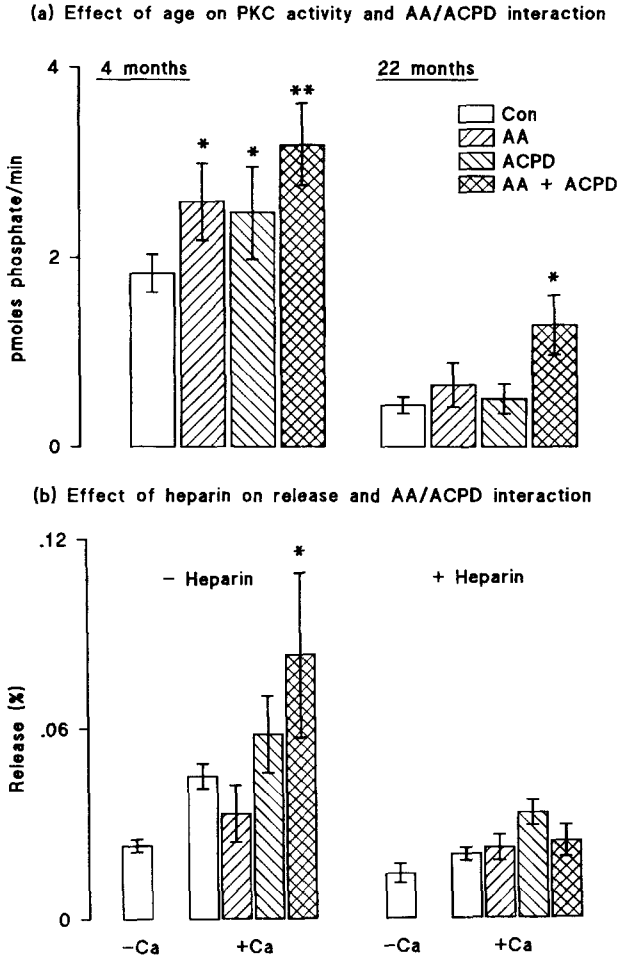

Fig. 2. (a) Protein kinase C activity in synaptosomes prepared from hippocampal synaptosomes prepared from young and aged animals: effect of ACPD and arachidonic acid. Protein kinase C activity was significantly decreased in synaptosomes prepared from aged animals compared to young animals ($P < 0.001$; Student's t-test for independent means). Arachidonic acid (1 μM) and ACPD (50 μM) significantly increased protein kinase C activity in synaptosomes prepared from young animals ($P < 0.05$; ANOVA); in combination, arachidonic acid and ACPD further increased protein kinase C activity ($P < 0.01$ compared to control; $P < 0.05$ compared to either arachidonic acid or ACPD alone). In contrast neither arachidonic acid nor ACPD had any significant effect on protein kinase C activity in synaptosomes prepared from aged animals, though in combination, a significant increase in protein kinase C activity was observed ($P < 0.01$; ANOVA). Values are means ± S.E.M. of 6 observations and are expressed as pmol phosphate incorporated/min. (b) Heparin blocks the synergism between arachidonic acid and ACPD on KCl-stimulated release of [^{3}H]glutamate in hippocampal synaptosomes prepared from young animals. Hippocampal synaptosomes were prepared in the absence or presence of heparin (200 μg/ml). Release of [^{3}H]glutamate was stimulated by 40 mM KCl. In control synaptosomes, neither ACPD (50 μM) nor arachidonic acid (1 μM) had any significant effect on glutamate release, but, in combination, arachidonic acid and ACPD significantly increased release ($P < 0.01$; ANOVA). Release of glutamate both in the presence and absence of calcium was decreased in heparin-treated synaptosomes and, in addition, the synergism between arachidonic acid and ACPD was blocked. Results are means ± S.E.M. of 7 observations.

nase C in hippocampal synaptosomes prepared from young animals ($P < 0.05$ in both cases, ANOVA) but in the presence of both agents a further enhancement of protein kinase C activity was observed ($P < 0.05$ compared to control; $P < 0.05$ compared to either agent alone). In aged animals, neither arachidonic acid nor ACPD alone had any significant effect on protein kinase C activity, but when both agents were included in the incubation medium, protein kinase C activity was significantly increased compared to control ($P < 0.01$; ANOVA).

To investigate the possibility that the interaction between arachidonic acid and ACPD on glutamate release involves calcium mobilization from intracellular stores, synaptosomes were prepared from young rats in the presence or absence of heparin, an antagonist at the inositol trisphosphate (IP_3) receptor. Fig. 2b shows that release in both the absence and presence of calcium was reduced in heparin-treated synaptosomes compared to control synaptosomes, and that KCl-stimulated release observed in the presence of calcium was attenuated in heparin-treated tissue. In control synaptosomes, the synergistic action of arachidonic acid and ACPD on glutamate release was observed, but the interaction between these agents was blocked in heparin-treated synaptosomes.

4. Discussion

Our findings indicate that while arachidonic acid and ACPD interact to increase glutamate release in hippocampal synaptosomes prepared from young animals, this effect was absent in hippocampal synaptosomes prepared from aged animals. The evidence presented here suggests that this compromised age-related response may be due to a change in IP_3 receptor interaction rather than a change in protein kinase C activation.

Arachidonic acid and ACPD act in synergy to increase glutamate release in hippocampal synaptosomes prepared from young rats which confirms earlier observations in hippocampus (McGahon and Lynch, 1994), dentate gyrus (McGahon and Lynch, 1996) and cortex (Herrero et al., 1992). In contrast to this effect in 4-month-old animals, there was no evidence of any synergism in synaptosomes prepared from 24-month-old animals. While 40 mM KCl significantly increased release of glutamate in synaptosomes prepared from both young and aged animals, a further contrast between young and aged animals was that the additional increase in release observed in the presence of calcium in young animals was markedly attenuated in aged animals. This observation, indicating an age-related decrease in transmitter release, supports previous observations in cortex (Aprikyan and Gekchyan, 1988) and dentate gyrus (Lynch and Voss, 1994).

The underlying cause of the age-related attenuation in transmitter release has not been elucidated, though calcium channel activity is decreased with increasing age (Reynolds and Carlen, 1989) and therefore calcium influx is

decreased (Martinez-Serrano et al., 1989; Blanco et al., 1994); these changes might be expected to impact upon KCl-stimulated release. There is evidence that calcium channel activity is modulated by phosphorylation by protein kinase C (Bartschat and Rhodes, 1995); for this reason, and the reason that the synergism between arachidonic acid and ACPD is dependent on (Herrero et al., 1992), and paralleled by (McGahon and Lynch, 1996), protein kinase C activation, we investigated the interaction between arachidonic acid and ACPD on protein kinase C activity in synaptosomes prepared from aged and young animals. Our data show that, while arachidonic acid and ACPD alone both increase protein kinase C activity in synaptosomes prepared from young animals, there was no effect in aged animals. Despite this attenuation, arachidonic acid and ACPD interacted to increase protein kinase C activity in hippocampal synaptosomes prepared from aged, as well as young animals. The present finding indicating a decreased protein kinase C activity with age supports the previous report of an age-related decrease in protein kinase C translocation in hippocampus and cortex (Battaini et al., 1995). The attenuated response to arachidonic acid and ACPD in aged, compared to young animals, is, we believe, the first indication of such an age-dependent change in hippocampal synaptosomes. These results also clearly indicate that the synergism between arachidonic acid and ACPD on glutamate release cannot be explained solely by a similar interaction on protein kinase C activity.

In addition to the interaction between arachidonic acid and ACPD on glutamate release and protein kinase C activity, we have reported that these agents interact to stimulate phospholipase C (McGahon and Lynch, 1994). The consequence of increased phospholipase C activity is increased diacylglycerol, leading to protein kinase C activation, and increased inositol trisphosphate (IP_3) which mobilizes calcium from intracellular stores. Either, or both, arms of this pathway may contribute to the synergistic action of arachidonic acid and ACPD on glutamate release. While a role for protein kinase C appears to be excluded on the basis of the present data, a role for IP_3 receptor activation is suggested by our finding that heparin, an IP_3 receptor antagonist, inhibits this synergism. In addition to the attenuated response of heparin-treated synaptosomes to arachidonic acid and ACPD, KCl-stimulated glutamate release was also decreased in treated synaptosomes. This suggests that IP_3 receptor activation plays a role in depolarization-induced glutamate release, therefore it might be predicted that any age-related change in IP_3 receptor number or function might impact on transmitter release in aged brains. A decrease in the number of IP_3 receptors (Martini et al., 1994) and a decrease in the efficacy of IP_3 to elicit calcium mobilization (Burnett et al., 1990) have been reported in age; these observations and the age-related decrease in phospholipase C activity (Lynch and Voss, 1994) support the idea that impaired glutamate release and also impaired responsiveness to arachidonic acid and ACPD, may be partly due to age-related changes in IP_3 receptor function.

Acknowledgements

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References

Aprikyan, G.V. and K.G. Gekchyan, 1988, Release of neurotransmitter amino acids from brain synaptosomes and its regulation in aging, Gerontology 34, 35.

Bartschat, D.K. and T.E. Rhodes, 1995, Protein kinase C modulates calcium channels in isolated presynaptic nerve terminals of rat hippocampus, J. Neurochem. 64, 2064.

Battaini, F., S. Elkabes, S. Bergamaschi, V. Ladisa, L. Lucchi, P.N.E. DeGrann, T. Schuurman, W.C. Wetsel, M. Trabucchi and S. Govoni, 1995, Protein kinase C activity, translocation, and conventional isoforms in aging rat brain, Neurobiol. Aging 16, 137.

Blanco, P., A. Huidobro, M. Villalba, P. Gomez-Puertas, E. Bogonez, R. Periera, A. Martinez-Serrano, J.J. Aparico and J. Satrustegui, 1994, The activity of synaptosomal calcium channels is inversely correlated with working memory performance in memory-impaired, aged rats, Neurosci. Lett. 165, 5.

Burnett, D.M., L.C. Daniell and N.R. Zahniser, 1990, Decreased efficacy of inositol-1,4,5-trisphosphate to elicit calcium mobilization from cerebrocortical microsomes of aged rats, Mol. Pharmacol. 37, 566.

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

Davis, S., A.L. Markowska, G.L. Wenk and C.A Barnes, 1993, Acetyl-L-carnitine: behavioural, electrophysiological and neurochemical effects, Neurobiol. Aging 14, 107.

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

Lynch, M.A. and K.L. Voss, 1994, Membrane arachidonic acid concentration correlates with age and induction of long-term potentiation in the dentate gyrus, Eur. J. Neurosci. 6, 1008.

Martinez-Serrano, A., E. Bogonez, J. Vitorica and J. Satrustegui, 1989, Reduction in K^+-stimulated $^{45}Ca^{2+}$ influx in synaptosomes with age involves inactivating and noninactivating calcium channels and is correlated with temporal modifications in protein dephosphorylation, J. Neurochem. 52, 576.

Martini, A., F. Battaini, S. Govoni and P. Volpe, 1994, Inositol 1,4,5-trisphosphate receptor and ryanodine receptor in the aging brain of Wistar rats, Neurobiol. Aging 15, 203.

McGahon, B. and M.A. Lynch, 1994, A study of the synergism between metabotropic glutamate receptor activation and arachidonic acid in the rat hippocampus, NeuroReport 5, 2353.

McGahon, B. and M.A. Lynch, 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.

Reynolds, J.N. and P.L. Carlen, 1989, Diminished calcium currents in aged hippocampal dentate gyrus granule neurons, Brain Res. 479, 384.

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