

Metabotropic Glutamate Receptor Agonists Increase Release of Soluble Amyloid Precursor Protein Derivatives from Rat Brain Cortical and Hippocampal Slices¹

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Accepted for publication December 23, 1996

ABSTRACT

The proteolytic processing of the β -amyloid precursor protein (APP) is regulated by neurotransmitters. Stimulation of metabotropic glutamate receptors (mGluRs) has been shown to increase the release of soluble amyloid precursor protein derivatives (APPs) from cultured cells. We examined the effects of mGluR agonists on APP processing in cortical and hippocampal slices from rat brain. Incubation of the slices in the presence of L-glutamic acid (500 μ M), *trans*-(1*S*,3*R*)-1-amino-1,3-cyclopentane dicarboxylic acid (1–100 μ M) or quisqualic acid (1–100 μ M) increased APP release into the medium, relative to the amount of APPs released during incubation in normal Krebs-Ringer buffer under basal conditions. N-Methyl-D-aspartate (1–320 μ M), (\pm)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (1–100 μ M) or kainic acid (5–500 μ M) did not alter APP release. The increases in APP release induced by L-glutamic acid (500 μ M), *trans*-(1*S*,3*R*)-1-amino-1,3-cyclopentane dicarboxylic acid (10 μ M) or quisqualic acid (10 μ M) were blocked by 100 μ M (\pm)- α -methyl-4-carboxyphenylglycine, a selective antagonist of mGluRs. Incubation of the slices in the presence of 1 μ M phorbol-12-myristate-13-acetate, an activator of protein kinase C (PKC), also increased APP release, and an inhibitor of PKC, GF-109203X (1 μ M), blocked this response as well as the release evoked by mGluR agonists. These data show that activation of mGluR increases APP release from brain slices *via* PKC-dependent mechanisms.

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The β -amyloid deposits found in brains of patients with Alzheimer's disease are composed of 39 to 42 amino acid peptides (A β) derived by proteolytic cleavage of the APP (for reviews, see Checler, 1995; Maury, 1995; Selkoe, 1994). APP is a membrane-spanning secretory glycoprotein constitutively that is expressed in many types of mammalian cells and present in high levels in brain cells (Kang *et al.*, 1987; Selkoe *et al.*, 1988; Weidemann *et al.*, 1989).

Two major pathways of APP processing have been described. In the constitutive secretory pathway, APP is cleaved within its A β domain at residue 16 by an uncharacterized protease known as α -secretase (Esch *et al.*, 1990; Sisodia, 1990; Sisodia *et al.*, 1990). This process releases large soluble fragments, APPs, into the extracellular me-

dium, and the smaller membrane-associated intracellular fragment is retained for subsequent cleavage and endocytotic processing (Haass *et al.*, 1993). The secreted APPs exhibit neuroprotective and neurotrophic activities in some experimental systems, protecting primary neuronal cultures from excitotoxic damage (Mattson *et al.*, 1993; Barger *et al.*, 1995) and promoting neurite outgrowth in PC-12 cells (Milward *et al.*, 1992) and cell-to-cell adhesion (Koo *et al.*, 1993). The alternative APP secretory processing involves cleavage at both the amino and the carboxyl termini of A β , followed by its rapid secretion (Haass *et al.*, 1992; Schubert *et al.*, 1989). The secreted A β peptides, when present in high concentrations or exposed to amyloidotrophic factors, can form insoluble amyloid aggregates that may be toxic to neurons (Bush *et al.*, 1994; Jarrett and Lansbury, 1993). The rate of A β production appears to be inversely coupled to that of APP secretion. In several cell culture systems, enhanced APP secretion is associated with diminished A β production (Buxbaum *et al.*, 1993; Gabuzda *et al.*, 1993; Hung *et al.*, 1993; Wolf *et al.*, 1995), suggesting that the secretory processing of APP to

Received for publication June 24, 1996

¹ These studies were supported in part by grants from the National Institute of Mental Health (M. H.-28783) and the Center for Brain Sciences and Metabolism Charitable Trust.

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ABBREVIATIONS: APP, β -amyloid precursor protein; mGluR, metabotropic glutamate receptor; APP, soluble amyloid precursor protein derivative; *trans*-(1*S*,3*R*)-ACPD, *trans*-(1*S*,3*R*)-1-amino-1,3-cyclopentane dicarboxylic acid; NMDA, N-methyl-D-aspartic acid; AMPA, (\pm) α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid; MCPG, (\pm)- α -methyl-4-carboxyphenylglycine; PMA, phorbol-12-myristate-13-acetate; PKC, protein kinase C; LDH, lactic dehydrogenase; DAG, diacylglycerol; PtdInsP₂, phosphatidylinositol-4,5-bisphosphate; TBST, Tris-buffered saline/0.05% Tween.

secreted APPs reduces the formation of potentially amyloidogenic derivatives.

Studies performed in cell culture show that a variety of extracellular and intracellular signals can modulate APP processing to favor the secretion of APPs. Such intracellular signals include PKC (Buxbaum *et al.*, 1990; Caporaso *et al.*, 1992; Slack *et al.*, 1993), tyrosine kinases (Slack *et al.*, 1995) and calcium levels (Buxbaum *et al.*, 1994). Activation of cell-surface receptors enhances the formation of DAG and inositol-1,4,5-triphosphate from PtdInsP₂ and also increases APP release. Such receptors have been shown to include muscarinic M₁ and M₃ (Buxbaum, 1992; Nitsch *et al.*, 1992; Wolf *et al.*, 1995), metabotropic glutamate (Lee *et al.*, 1995), serotonin 5-HT_{2a} and 5-HT_{2c} (Nitsch *et al.*, 1996), vasopressin (V1a) and bradykinin (B2) types (Nitsch *et al.*, 1995).

Our laboratory previously showed that electrical stimulation of rat brain cortical and hippocampal slices caused enhanced APP secretion in a frequency-dependent, tetrodotoxin-sensitive fashion (Nitsch *et al.*, 1993); exposure of the slices to muscarinic receptor agonists had similar effects (Farber *et al.*, 1995). These findings demonstrated that the proteolysis of APP to form APPs also occurs in the central nervous system and that this process can be regulated in brain by neuronal activation and neurotransmitter receptors. Thus, we hypothesize that impairments in neurotransmission could exacerbate amyloid formation in Alzheimer's disease, particularly in the cortex and hippocampus. The role of glutamate in cortical or hippocampal APP processing might be expected to be particularly important inasmuch as glutamatergic corticocortical connections and hippocampal projections are highly vulnerable to damage in early stages of Alzheimer's disease (Francis *et al.*, 1993).

Using cell cultures, we recently observed that the release of APPs was rapidly enhanced by stimulation of mGluRs but not of glutamate receptors coupled to ligand-gated channels (Lee *et al.*, 1995). In the present study, we examine the effects of the activation of various glutamate receptors on APP processing in rat cortex and hippocampus. We find that stimulation of mGluRs in cortical and hippocampal slices rapidly increases APP release into the medium.

Methods

Preparation and perfusion of slices. Male Sprague-Dawley rats (280–350 g) were decapitated, and the brains were rapidly removed and placed in chilled (4°C) oxygenated Krebs-Ringer buffer (see below). After removal of remaining meninges and choroid plexuses, hippocampal and cortical tissues were dissected, and slices 0.3 mm thick were prepared using a McIlwain tissue chopper (Brinkman Instruments, Westbury, NY). Slices were washed four times to remove most of the membrane debris and then transferred into eight superfusion chambers (four for hippocampal slices and four for cortical slices). The chambers were kept at 37°C in a water bath, and the slices were perfused for 60 min (for equilibration and for washing away excess APP released during slice preparation) with Krebs-Ringer buffer (120 mM NaCl, 3.5 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 25 mM NaHCO₃ and 10 mM glucose) at a constant flow rate (0.8 ml/min) using an eight-channel peristaltic pump (Rainin Instrument Co., Woburn, MA). This solution was bubbled continuously with a mixture of 95% O₂ and 5% CO₂. After this period, five or six hippocampal and seven or eight cortical slices were transferred into each of eight incubation wells (made from a 24-well tissue culture dish, with the bottom covered with nylon mesh). Slices were incubated in 1 ml of Krebs-Ringer buffer for 20

min; incubation media were replaced by 1 ml of buffer (37°C, oxygenated) every 5 min.

After the 20-min incubation period, slices were incubated for 10 additional min in 1 ml of buffer. The incubation medium was carefully removed, and slices were rinsed with 0.5 ml of the buffer (heated and oxygenated). The incubation and rinsing media were combined for measurement of basal APP release. Slices were then incubated again for one or more 10-min periods in 1 ml of buffer solutions containing the desired concentrations of drugs to be tested. In one set of experiments, KCl concentration was elevated from 3.5 to 50 mM and NaCl concentration was reduced to 73.5 from 120 mM in the buffer during second, third and fourth 10-min incubation periods. At the end of each incubation period, media were removed, and slices were rinsed with 0.5 ml of buffer. The incubation and rinsing media were again combined for measurements of APP release. Such release during the second incubation period was expressed as the percentage of APP release during first 10-min incubation period, and each well was used as its own control.

Measurements of secreted APPs. Incubation and rinsing media were collected into 1.5-ml Eppendorf tubes on ice and centrifuged for 15 min at 2500 × *g* at 4°C to remove debris. Two aliquots (0.4 and 0.8 ml) of the media were blotted directly onto polyvinylidene difluoride membranes (Immobilon-P, Waters, Milford, MA) by vacuum filtration, using a slot-blot microfiltration apparatus (Bio-Dot SF, BioRad, Hercules, CA). The remaining binding sites were blocked for 30 min with 4% nonfat dry milk (Carnation, Glendale, CA) in TBST. Membranes were then rinsed five times in TBST and immersed in TBST solution containing the monoclonal antibody 22C11 (Boehringer-Mannheim Biochemicals, Indianapolis, IN). After overnight incubation, membranes were rinsed in TBST and then treated for 1 hr with a peroxidase-linked sheep anti-mouse secondary antibody (Amersham Corp., Arlington Heights, IL). Bands were visualized by chemiluminescence using linear Kodak X-ray films. Immunoreactive bands were compared densitometrically using a laser scanner (UltraScan XL, Pharmacia LKB, Bromma, Sweden) set at 40-μm vertical intervals and a 3-mm horizontal slit width. Areas under the absorbance curves were expressed as arbitrary units. Areas generated by immunoreactive proteins secreted during the second 10-min incubation under test conditions were normalized as percentages of those generated by immunoreactive proteins secreted from the same slices during the first 10-min period under basal conditions. Measurements were always performed in the linear range. Typically, two aliquots (0.4 and 0.8 ml) of incubation media obtained under basal and test conditions were handled in parallel, processed identically and run in parallel on the same blot. During test incubation periods, control samples (containing no drug and usually from duplicate chambers) were always run in parallel, and the changes in APP release under test conditions were compared with its release from control slices during a second incubation period.

In previous studies from our laboratory (Farber *et al.*, 1995; Nitsch *et al.*, 1993), it was shown that the most of the immunoreactive protein found in the medium is APP. Intact APP is present in high concentrations in the slices but is not released into the media (Farber *et al.*, 1995). In pilot experiments, this relationship was verified using a polyclonal antiserum directed against the carboxyl terminus of APP; the antibody reacted strongly with protein extracts from brain slices, but no signal was detected with the incubation medium. Thus, the amounts of intact APP released into the media were extremely low and below the detection limit of our assay system.

Glutamate and LDH assays. Glutamate in incubation media was determined by high performance liquid chromatography-electrochemical detection as described previously (Bogdanov and Wurtman, 1994). LDH activity in 0.5 ml of incubation medium was assayed by using a commercial assay kit (Sigma Chemical, St. Louis, MO).

Drugs. L-Glutamic acid hydrochloride, NMDA, *trans*-(1S,3R)-ACPD, AMPA, kainic acid, (±)-quisqualic acid and MCPG were purchased from Research Biochemicals (Natick, MA). PMA and GF-109203X were purchased from LC Laboratories (Woburn, MA).

TABLE 1

APP release from incubated cortical and hippocampal slices, basally and with exposure to depolarizing concentrations of potassium

Cortical and hippocampal slices were perfused for 60 min (0.8 ml/min) and then incubated for 20 min with Krebs' medium. After an 80-min equilibration period, slices were incubated in 1 ml of Krebs' medium for four consecutive 10-min periods (second through fourth periods). During the second through fourth 10-min incubation periods, some slices were depolarized by incubation in K⁺-containing Krebs' buffer (50 mM KC1 plus 73.5 mM NaCl). Incubation media were removed at the end of each 10-min incubation period, and the slices were rinsed with 0.5 ml of Krebs' medium. Incubation and rinsing media were collected in iced plastic tubes and assayed for APPs. APP release during the second through fourth incubation periods was normalized (as percent) to the APP release observed during the first 10-min period. Data are mean \pm S.E.M. ($n = 8-12$).

Incubation period	APP release			
	Cortical slices		Hippocampal slices	
	Basal	High K ⁺	Basal	High K ⁺
	% of first incubation period			
Second 10 min	95 \pm 7	158 \pm 11 ^a	95 \pm 7	162 \pm 14 ^a
Third 10 min	85 \pm 8	99 \pm 14	85 \pm 9	101 \pm 13
Fourth 10 min	79 \pm 5	90 \pm 12	89 \pm 9	N.D.

^a Significantly higher than corresponding basal value.
N.D., no data.

Data analysis. Data are expressed as mean \pm S.E.M. Analysis of variance and Student's *t* test (two-tailed) were used to evaluate differences between groups. Differences were taken to be statistically significant at $P < .05$.

Results

Basal APP release from cortical and hippocampal slices. The cortical and hippocampal slices incubated in normal Krebs-Ringer media released APPs into the medium at more or less constant rates during four consecutive 10-min incubation periods (table 1).

When the slices were incubated in high (50 mM) potassium media for three consecutive 10-min incubation periods, APP release increased significantly, by 1.6- or 1.5-fold from hippocampal and cortical slices, respectively (table 1). APP release subsequently returned to basal levels even in the continued presence of high potassium concentrations (table 1).

Effects of glutamate receptor agonists on APP release. Effects of L-glutamic acid, *trans* (1S,3R)-ACPD, quisqualic acid, NMDA, AMPA and kainic acid on APP release from cortical and hippocampal slices are summarized in table 2.

The endogenous and nonspecific glutamate receptor agonist L-glutamic acid (500 μ M) increased APP secretion by \sim 1.5- and \sim 1.3-fold from cortical and hippocampal slices, respectively (table 2). At lower concentrations (5 or 50 μ M), L-glutamic acid failed to modify APP release from either brain region (table 2).

A selective mGluR agonist, *trans*-(1S,3R)-ACPD (Brabet *et al.*, 1995; Kingston *et al.*, 1995; Palmer *et al.*, 1989; Pin and Duvoisin, 1995), at concentrations of 1, 10 or 100 μ M, increased APP release by \sim 1.7-, \sim 2.1- and \sim 1.9-fold, respectively, from cortical slices and by \sim 1.4-, \sim 1.5- and \sim 1.5-fold from hippocampal slices, respectively (table 2).

The nonselective glutamate receptor agonist quisqualic acid, which activates both mGluRs and quisqualate/kainate receptors, increased APP release by \sim 1.3- to \sim 1.4-fold from both cortical and hippocampal slices (table 2).

The kainate receptor agonist kainic acid (5–500 μ M), the

TABLE 2

Effects of glutamate agonists on APP release from cortical and hippocampal slices

Cortical and hippocampal slices were perfused for 60 min (0.8 ml/min) and incubated for 20 min with Krebs' medium. After an 80-min equilibration period, slices were incubated in 1 ml of Krebs' medium for two consecutive 10-min periods. During the second 10-min incubation period, the incubation medium contained the concentrations of glutamate agonist indicated. Incubation media were removed at the end of each 10-min incubation period, and the slices were rinsed with 0.5 ml of Krebs' medium and assayed as described in table 1. Data are mean \pm S.E.M. The number of measurements is given in parentheses.

Glutamate agonist	APP release	
	Cortical slices	Hippocampal slices
	% of first 10-min period	
L-Glutamic acid (μ M)		
0	93 \pm 4 (12)	93 \pm 3 (11)
5	100 \pm 6 (4)	116 \pm 10 (6)
50	102 \pm 8 (7)	114 \pm 6 (6)
500	139 \pm 9 (13) ^a	124 \pm 7 (11) ^a
<i>trans</i> -(1S,3R)-ACPD (μ M)		
0	88 \pm 6 (9)	93 \pm 6 (9)
1	152 \pm 17 (6) ^a	128 \pm 20 (8)
10	188 \pm 13 (8) ^a	137 \pm 18 (9) ^a
100	163 \pm 16 (7) ^a	159 \pm 17 (9) ^a
Quisqualic acid (μ M)		
0	83 \pm 4 (11)	91 \pm 5 (9)
1	108 \pm 5 (11) ^a	127 \pm 7 (9) ^a
10	114 \pm 5 (11) ^a	125 \pm 6 (9) ^a
100	118 \pm 6 (11) ^a	114 \pm 9 (9)
Kainic acid (μ M)		
0	92 \pm 6 (9)	89 \pm 4 (8)
5	97 \pm 7 (9)	114 \pm 6 (8)
50	95 \pm 8 (9)	97 \pm 8 (8)
500	109 \pm 8 (9)	100 \pm 8 (8)
AMPA (μ M)		
0	86 \pm 9 (9)	92 \pm 4 (8)
1	96 \pm 7 (9)	109 \pm 7 (8)
10	89 \pm 6 (9)	100 \pm 6 (8)
100	92 \pm 7 (9)	107 \pm 7 (8)
NMDA (μ M)		
0	99 \pm 6 (8)	97 \pm 7 (8)
10	115 \pm 15 (9)	94 \pm 7 (8)
100	100 \pm 12 (9)	98 \pm 9 (8)
320	111 \pm 14 (8)	102 \pm 6 (8)

^a Significantly higher than the corresponding control (0) value.

AMPA receptor agonist AMPA (1–100 μ M) and the NMDA receptor agonist NMDA (10–320 μ M) all failed to alter APP release from cortical or hippocampal slices (table 2).

Effects of mGluR antagonist on APP release. As seen in table 3, the increases in APP release from cortical or hippocampal slices induced by *trans*-(1S,3R)-ACPD (10 μ M), quisqualic acid (10 μ M) or glutamic acid (500 μ M) were blocked by MCPG (100 μ M). At this dose, MCPG did not alter APP release from either cortical or hippocampal slices (table 3).

Effects of PKC activators and inhibitors on APP release. The PKC activator PMA (1 μ M) increased APP release by 1.9- or 1.6-fold from cortical or hippocampal slices, respectively (table 4).

The PKC inhibitor GF-109203X (1 μ M) did not alter APP release from cortical or hippocampal slices (table 5); however, it prevented the increases in APP release induced by PMA (table 5).

Effects of PKC inhibitors on APP release induced by glutamate receptor agonists. As seen in table 6, GF-109203X (1 μ M) prevented the increases in APP release

TABLE 3

Effects of MCPG on APP release from cortical and hippocampal slices induced by L-glutamic acid, *trans*-(1S,3R)-ACPD or quisqualic acid

Cortical and hippocampal slices were perfused and incubated and the media were assayed as in table 1. During the second 10-min incubation period, the incubation medium contained L-glutamic acid (500 μ M), *trans*-(1S,3R)-ACPD (10 μ M) or quisqualic acid (10 μ M) with or without MCPG (100 μ M) or its vehicle (10 μ l of 0.1 N NaOH), as indicated. Data are mean \pm S.E.M. ($n = 7$).

Treatment	APP release	
	Cortical slices	Hippocampal slices
	% of first 10-min period	
Vehicle	93 \pm 2	94 \pm 4
Vehicle + L-glutamic acid	129 \pm 9 ^a	128 \pm 13 ^a
Vehicle + <i>trans</i> -(1S,3R)-ACPD	133 \pm 10 ^a	148 \pm 12 ^a
Vehicle + quisqualic acid	128 \pm 12 ^a	120 \pm 9 ^a
MCPG	92 \pm 6	88 \pm 5
MCPG + L-glutamic acid	85 \pm 4	84 \pm 5
MCPG + <i>trans</i> -(1S,3R)-ACPD	86 \pm 6	75 \pm 5
MCPG + quisqualic acid	89 \pm 6	90 \pm 5

^a Significantly higher than vehicle and corresponding value with MCPG.

TABLE 4

Increases in APP release from cortical and hippocampal slices induced by PMA

Cortical and hippocampal slices were perfused and incubated and the media were assayed as in table 1. During the second 10-min incubation period, the incubation medium contained PMA (1 μ M) or its vehicle (1 μ l of ethanol plus 9 μ l of Krebs' buffer). Data are mean \pm S.E.M. ($n = 7$).

Treatment	APP release	
	Cortical slices	Hippocampal slices
	% of first 10-min period	
Vehicle	89 \pm 3	90 \pm 4
PMA	179 \pm 5 ^a	160 \pm 11 ^a

^a Significantly higher than the vehicle value.

TABLE 5

Inhibition by GF-109203X of APP release from cortical and hippocampal slices induced by PMA

Cortical and hippocampal slices were perfused and incubated and media were assayed as in table 1. During the second 10-min incubation period, the incubation medium contained vehicle (1 μ l of ethanol plus 9 μ l of Krebs' buffer) or PMA (1 μ M), GF-109203X (1 μ M) or both. Data are mean \pm S.E.M. ($n = 7$).

Treatment	APP release	
	Cortical slices	Hippocampal slices
	% of first 10-min period	
Vehicle + vehicle	92 \pm 7	94 \pm 4
Vehicle + PMA	176 \pm 8 ^a	153 \pm 15 ^a
GF-109203X + vehicle	77 \pm 7	89 \pm 7
GF-109203X + PMA	80 \pm 6	90 \pm 6

^a Significantly higher than the vehicle value.

from cortical and hippocampal slices induced by glutamic acid (500 μ M), *trans*-(1S,3R)-ACPD (10 μ M) or quisqualic acid (10 μ M).

Effects of glutamate agonists on LDH activity and glutamate levels in the medium. LDH activities in media incubated with cortical or hippocampal slices were 104 \pm 15 μ units/ml ($n = 16$) or 55 \pm 7 μ units/ml, respectively ($n = 16$), after a 10-min incubation period. Incubation with L-glutamic acid (500 μ M), *trans*-(1S,3R)-ACPD (10 μ M) or quisqualic acid (10 μ M) failed to affect the activity of the enzyme (table 7).

TABLE 6

Blockade by GF-109203X of the increases in APP release induced by glutamate agonists

Cortical and hippocampal slices were perfused and incubated and media were assayed as in table 1. During the second 10-min incubation period, the incubation medium contained L-glutamic acid (500 μ M), *trans*-(1S,3R)-ACPD (10 μ M) or quisqualic acid (10 μ M), as well as GF-109203X (1 μ M) or its vehicle (1 μ l of ethanol plus 9 μ l of Krebs' buffer). Data are mean \pm S.E.M. ($n = 6$).

Treatment	APP release	
	Cortical slices	Hippocampal slices
	% of first 10-min period	
Vehicle	86 \pm 6	89 \pm 3
Vehicle + L-glutamic acid	134 \pm 13 ^a	129 \pm 9 ^a
Vehicle + <i>trans</i> -(1S,3R)-ACPD	141 \pm 11 ^a	146 \pm 9 ^a
Vehicle + quisqualic acid	124 \pm 5 ^a	129 \pm 8 ^a
GF-109203X	77 \pm 7	79 \pm 4
GF-109203X + L-glutamic acid	71 \pm 7	69 \pm 8
GF-109203X + <i>trans</i> -(1S,3R)-ACPD	100 \pm 7	73 \pm 9
GF-109203X + quisqualic acid	96 \pm 7	71 \pm 5

^a Significantly higher than corresponding GF-109203X value.

Endogenous glutamate was released into the media during 10-min incubations of cortical or hippocampal slices; concentrations were 66 \pm 9 nM ($n = 16$) and 48 \pm 8 nM ($n = 16$), respectively. Glutamate concentrations in the media did not change (table 7) during incubation of slices in the presence of *trans*-(1S,3R)-ACPD (10 μ M) or quisqualic acid (10 μ M).

Discussion

These data show that the selective mGluR agonist *trans*-(1S,3R)-ACPD and the nonselective glutamate receptor agonists L-glutamic acid and quisqualic acid, but not the ionotropic glutamate receptor agonists NMDA, AMPA and kainic acid, increase APP release from rat brain cortical and hippocampal slices. Furthermore, a selective mGluR antagonist, MCPG, blocks the increases in APP release from cortical and hippocampal slices induced by *trans*-(1S,3R)-ACPD, L-glutamic acid or quisqualic acid without affecting basal APP release. Incubation of the slices in physiological media containing a depolarizing concentration of potassium (50 mM) or PMA, an activator of PKC, also increases APP release. A PKC inhibitor, GF-109203X, blocks the increases in APP release induced by PMA as well as by L-glutamic acid, *trans*-(1S,3R)-ACPD or quisqualic acid. Therefore, activation of mGluRs in rat brain enhances APP processing to APPs, as we previously showed occurs in cultured cells (Lee *et al.*, 1995).

These results confirm and extend other reports from our laboratory (Farber *et al.*, 1995; Nitsch *et al.*, 1993) showing that brain slices release APPs into the medium and that release is enhanced by depolarizing the slices electrically or by exposing them to M₁ cholinergic receptor agonists. The increase in APP release after potassium depolarization was smaller in magnitude and shorter lasting than the increases observed in APP release during electrical stimulation (Farber *et al.*, 1995). Indeed, in the present experiments, incubation of cortical and hippocampal slices in the presence of high potassium concentrations for 30 min (*i.e.*, three consecutive 10-min periods) caused 1.6- or 1.5-fold increases in APP release from hippocampal and cortical slices during first 10-min incubation period (table 1), but APP release returned to basal levels during the second and third 10-min periods (ta-

TABLE 7

Effects of glutamate agonists on LDH activities and glutamate concentrations in incubation media

Cortical and hippocampal slices were perfused for 60 min (0.8 ml/min) and incubated for 20 min with Krebs' medium. After an 80-min equilibration period, slices were incubated in 1 ml of Krebs' medium for two consecutive 10-min periods. During the second 10-min incubation period, incubation media contained the concentrations of glutamate agonists indicated. Incubation media were removed at the end of each 10-min incubation period, and slices were rinsed with 0.5 ml of Krebs' medium. Incubation and rinsing media were collected in iced plastic tubes and assayed for glutamate and LDH. For each set of slices, glutamate levels and LDH activities in the second incubation period were normalized (as percent) for those observed in the first 10-min incubation period. Data are mean \pm S.E.M. ($n = 4-6$).

Treatment	Cortical slices		Hippocampal slices	
	LDH	Glutamate	LDH	Glutamate
	% of first 10-min period			
Control	88 \pm 13	94 \pm 7	98 \pm 10	95 \pm 12
L-Glutamic acid (500 μ M)	95 \pm 8	N.D.	93 \pm 16	N.D.
<i>trans</i> -(1 <i>S</i> ,3 <i>R</i>)-ACPD (10 μ M)	92 \pm 14	85 \pm 6	88 \pm 9	105 \pm 15
Quisqualic acid (10 μ M)	90 \pm 11	124 \pm 19	93 \pm 7	94 \pm 16

N.D., no data.

ble 1). In contrast, the increases in APP release from electrically depolarized cortical or hippocampal slices were 2-fold and were maintained for \sim 30 min (Farber *et al.*, 1995).

The increases in APP release from hippocampal and cortical brain slices induced by *trans*-(1*S*,3*R*)-ACPD (table 3) are in good accordance with our laboratory's recent finding (Lee *et al.*, 1995) that mGluR stimulation increases APP release from primary hippocampal primary neuronal cultures. In the present study, we also observed that L-glutamic acid and quisqualic acid increase APP release from cortical and hippocampal slices. It is well known that L-glutamic acid and quisqualic acid stimulate ionotropic glutamate receptor in addition to mGluR. This raised the possibility that L-glutamic acid and quisqualic acid might also enhance APP secretion by stimulating Ca^{++} influx *via* ligand-gated channels activated by ionotropic glutamate receptor. This seems unlikely for two reasons. First, selective stimulation of ionotropic NMDA receptors by NMDA or of AMPA/kainate receptors by AMPA or kainate failed to affect APP release (table 2) across a wide range of drug concentrations (1–500 μ M). Second, the increases in APP release induced by the selective mGluR agonist *trans*-(1*S*,3*R*)-ACPD, as well as by L-glutamic acid or quisqualic acid, could be prevented by MCPG (table 3), which effectively and competitively blocks mGluR-mediated effects without inhibiting ionotropic glutamate receptor-mediated effects (Eaton *et al.*, 1993; Thomsen *et al.*, 1994).

The mGluR family is heterogeneous and comprises eight different subtypes classified into three major groups on the basis of receptor sequence homologies, agonist and antagonist pharmacology and the signal transduction pathway with which they couple (for reviews, see Nakanishi, 1992; Schoepp and Conn, 1993; Pin and Duvoisin, 1995). Group I mGluRs, comprising mGluR1 α and mGluR5, are coupled to PtdInsP₂ hydrolysis/calcium mobilization (Abe *et al.*, 1992; Aramori and Nakanishi, 1992). Group II (mGluR2 and mGluR3) and group III (mGluR4–8) metabotropic receptors are linked to inhibition of adenylate cyclase and reduced cAMP formation (Nakanishi, 1992; Pin and Duvoisin, 1995; Schoepp and Conn, 1993). mGluR1/mGluR5 and mGluR2/mGluR3 receptors are sensitive to *trans*-(1*S*,3*R*)-ACPD, as well as to quisqualate and glutamate, as an agonist (Nakanishi, 1992; Pin and Duvoisin, 1995; Schoepp and Conn, 1993) and to MCPG as a competitive antagonist (Hayashi *et al.*, 1994; Thomsen *et al.*, 1994). It is well known that the activation of mGluR1/mGluR5 by glutamate, quisqualate or *trans*-(1*S*,3*R*)-ACPD results in PtdInsP₂ hydrolysis/calcium mobilization (Hayashi

et al., 1994; Kingston *et al.*, 1995; Tanabe *et al.*, 1993), which is effectively blocked by MCPG (Hayashi *et al.*, 1994; Kingston *et al.*, 1995; Roberts, 1995) and, consequently, PKC activation (Nishizuka, 1992). In the present study, direct activation of PKC by PMA increased APP release from both cortical and hippocampal slices. This effect of PMA was blocked by the specific PKC inhibitor GF-109203X. Moreover, the increase in APP release induced by glutamatergic agonists was also blocked effectively by GF-109203X. These observations agree with our laboratory's previous report on cultured cells (Lee *et al.*, 1995) and show that activation of PKC plays a major role in the stimulation by mGluR of APP release from cortical and hippocampal slices. In other studies using established cell cultures, it has been shown that PMA (Caporaso *et al.*, 1992; Lee *et al.*, 1995; Slack *et al.*, 1993) or activation of cell-surface receptors coupled to PtdInsP₂ hydrolysis (Buxbaum *et al.*, 1992; Lee *et al.*, 1995; Nitsch *et al.*, 1992) can increase APP secretion and decrease A β formation.

In summary, the present study demonstrates that mGluRs are coupled to APP proteolysis and APP release in cortical and hippocampal slices. The increase in APP induced by mGluR agonists is mimicked by phorbol esters and blocked by PKC inhibitors, suggesting that PKC activation mediates APP secretion.

In Alzheimer's disease brains, glutamatergic transmission is severely impaired by the early degeneration of corticocortical connections and hippocampal projections (Francis *et al.*, 1993). Because both of these brain regions accumulate amyloid and are components of neural systems involved in memory and learning, the decrease in glutamatergic transmission may contribute to the accumulation of amyloid plaques and, secondarily, to memory dysfunction and progressive dementia. The present observations suggest that metabotropic glutamate agonists might be clinically useful for the treatment of Alzheimer's disease, all the more so when it is borne in mind that glutamate agonists can also increase cortical acetylcholine release (Ulus *et al.*, 1992).

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