

# Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: Developmental changes and interaction with $\alpha_1$ -adrenoceptors

(glutamate/norepinephrine/hippocampus/phosphatidylinositol/rat brain)

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**ABSTRACT** Glutamate, aspartate, ibotenate, and quisqualate activate inositol phospholipid hydrolysis in hippocampal slices prepared from brains of 6- to 8-day-old rats. The stimulation by glutamate and aspartate progressively declines during postnatal development and is negligible after the 24th day of life. In contrast, the stimulation of inositol phospholipid hydrolysis by norepinephrine is low in hippocampal slices from newborn animals and increases during development, reaching mature values after the 35th day of life. In adult hippocampal slices, the stimulation of inositol phospholipid hydrolysis elicited by norepinephrine is inhibited by glutamate in a concentration-dependent fashion. This inhibition can also be brought about by aspartate, 2-amino-4-phosphonobutanoate, and L-phosphoserine, a product of endogenous phosphatidylserine hydrolysis.

Glutamate and aspartate activate central nervous system (CNS) neurons by interacting with specific membrane recognition sites (for review, see ref. 1) coupled with biochemical processes acting as signal transducers (2–7). We have recently reported (8) that a specific excitatory amino acid recognition site, sensitive to the inhibitory action of 2-amino-4-phosphonobutanoate (APB), is coupled with phosphatidylinositol (PtdIns) metabolism in the rat hippocampus. In adult rats, this site is highly responsive to ibotenate, a rigid heterocyclic analogue of glutamate, but is not substantially activated by glutamate or aspartate (8). In contrast, glutamate and aspartate greatly enhance PtdIns hydrolysis in hippocampal slices prepared from brains of newborn rats. The intensity of this response progressively declines during postnatal development and is nearly absent after the 24th day of life. In the adult hippocampus, the occupancy of excitatory amino acid recognition sites by glutamate and aspartate fails to activate PtdIns metabolism but it inhibits the stimulation of PtdIns hydrolysis elicited by norepinephrine.

## MATERIALS AND METHODS

**Estimation of Inositol Phospholipid Hydrolysis.** The stimulation of inositol phospholipid hydrolysis elicited by transmitter receptor agonists was evaluated by measuring the accumulation of [ $^3$ H]inositol 1-phosphate ([ $^3$ H]Ins-1-P) in brain slices treated with Li $^+$ . Li $^+$  has been found to block the conversion of [ $^3$ H]Ins-1-P into inositol by inhibiting the enzyme inositol-1-phosphatase (9, 10).

Sprague-Dawley rats of various ages (6, 8, 12, 15, 19, 24, and 35 days) were decapitated. The brains were rapidly removed from the skulls and dissected on ice. Hippocampus,

corpus striatum, cerebral cortex, and hypothalamus were sliced (350  $\times$  350  $\mu$ m) with a McIlwain tissue chopper, and the slices immediately were suspended in Krebs-Hensleit buffer (118 mM NaCl/4.7 mM KCl/1.3 mM CaCl $_2$ /1.2 mM K $_2$ HPO $_4$ /1.2 mM MgSO $_4$ /25 mM NaHCO $_3$ /11.7 mM glucose, equilibrated with 95% O $_2$ /5% CO $_2$  to raise the pH to 7.4) and incubated at 37°C for 30–45 min with three intermediate changes of the buffer. Forty microliters of gravity-packed slices were then transferred to 3-ml vials containing 0.3  $\mu$ M *myo*-[2- $^3$ H]inositol (New England Nuclear, specific activity 16.5 Ci/mmol; 1 Ci = 37 GBq) in a final volume of 275  $\mu$ l. After 60 min of incubation, LiCl (7 mM) was added, followed, 20 min later, by transmitter receptor agonists. Antagonists were sometimes added 5 min prior to the agonists. After 60 min, the slices were washed three times with buffer and the reaction (cleavage of inositol phosphates from membrane phospholipids) was stopped by addition of 0.9 ml of chloroform/methanol (1:2). Identical results were obtained when slices were washed free of [ $^3$ H]inositol before the addition of transmitter receptor agonists. The content of [ $^3$ H]Ins-1-P was determined according to Berridge *et al.* (10). Proteins were measured as described by Lowry *et al.* (11).

**$\alpha_1$ -Adrenergic Receptor Binding Assay.** The characteristics of  $\alpha_1$ -adrenergic recognition sites were studied in crude synaptic membranes prepared from rat hippocampus, using [ $^3$ H]prazosin as a selective ligand, as described (12).

## RESULTS

**Age-Dependent Stimulation of PtdIns Hydrolysis by Excitatory Amino Acids.** Addition of glutamate, aspartate, ibotenate, or quisqualate to hippocampal slices prepared from brains of “newborn” (8-day-old) rats markedly enhances [ $^3$ H]Ins-1-P formation (Table 1). This effect results from an enhanced hydrolysis of inositol phospholipids rather than from an increased incorporation of [ $^3$ H]inositol into the phospholipids; in fact, a marked increase in [ $^3$ H]Ins-1-P formation is also observed in slices that have been washed free of [ $^3$ H]inositol before the addition of glutamate, aspartate, or ibotenate. The accumulation of [ $^3$ H]Ins-1-P induced by glutamate is linear for at least 90 min after a latency period of 10 min (data not shown). This pattern is similar to that observed with other transmitter receptor agonists in slices prepared from adult rats. The stimulation of [ $^3$ H]Ins-1-P formation elicited by glutamate in newborn hippocampal slices is concentration-dependent with an apparent EC $_{50}$  value of 300  $\mu$ M and a maximal stimulation of 14-fold. Ibotenate is more potent (EC $_{50}$  = 15  $\mu$ M) and has a

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Abbreviations: CNS, central nervous system; PtdIns, phosphatidylinositol; Ins-1-P, inositol 1-phosphate; APB, 2-amino-4-phosphonobutanoate.

Table 1. Stimulation of [<sup>3</sup>H]Ins-1-*P* formation elicited by various transmitter receptor agonists in hippocampal slices from 8-day-old rats

Agonist(s)	[ <sup>3</sup> H]Ins-1- <i>P</i> formation	
	Bq/mg of protein	% basal
None (basal)	160 ± 18	100
L-Glutamate	1300 ± 45*	800
L-Aspartate	1700 ± 300*	1040
Ibotenate <sup>†</sup>	2300 ± 120*	1460
Quisqualate	1900 ± 170*	1200
Kainate	150 ± 20	92
<i>N</i> -methyl- <i>D</i> -aspartate	160 ± 17	100
Quinolate	150 ± 24	92
Carbamoylcholine	690 ± 27*	430
Norepinephrine <sup>‡</sup>	310 ± 23*	190
L-Glutamate + norepinephrine <sup>‡</sup>	1500 ± 130*	930

All compounds were used at 1 mM unless otherwise indicated. Values are means ± SEM of at least 4 determinations.

\**P* < 0.01, when compared with basal values.

<sup>†</sup>500 μM.

<sup>‡</sup>100 μM.

greater efficacy (maximal stimulation 20-fold) than glutamate in enhancing [<sup>3</sup>H]Ins-1-*P* formation (Fig. 1). In newborn hippocampal slices, maximal responses to excitatory amino acids are greater than those to carbamoylcholine or norepinephrine (Table 1 and Fig. 1). This evidence suggests that the action of excitatory amino acids is not mediated through an enhanced release of acetylcholine or norepinephrine. Accordingly, atropine and phentolamine, which block the actions of carbamoylcholine and norepinephrine, respectively, fail to antagonize the stimulation of PtdIns hydrolysis elicited by excitatory amino acids (data not shown). In addition, the increase in PtdIns hydrolysis elicited by glutamate, aspartate, ibotenate, and quisqualate is not an epiphenomenon of a nonspecific excitatory action of these amino acids, since kainate, quinolate, and *N*-methyl-*D*-aspartate, which also excite CNS neurons (13, 14), fail to activate PtdIns hydrolysis even at high concentrations (Table 1).

A stimulation of PtdIns hydrolysis by glutamate or ibotenate also occurs in slices of corpus striatum, frontal cortex, or hypothalamus from newborn rats. However, in these brain regions, the extent of such stimulation is lower than in the hippocampus (Table 2).

The efficacy of glutamate and ibotenate in stimulating PtdIns hydrolysis progressively declines during postnatal development in the hippocampus (Table 3) as well as other brain regions (data not shown). The stimulation of PtdIns hydrolysis elicited by glutamate decreases >70% (from 14-fold to 4-fold) between the 6th and the 15th day after birth. Mature values are reached between the 19th and the 24th day (Table 3). A similar time course in the reduction of the stimulation is observed with aspartate (data not shown). After the 24th day, glutamate and aspartate are substantially

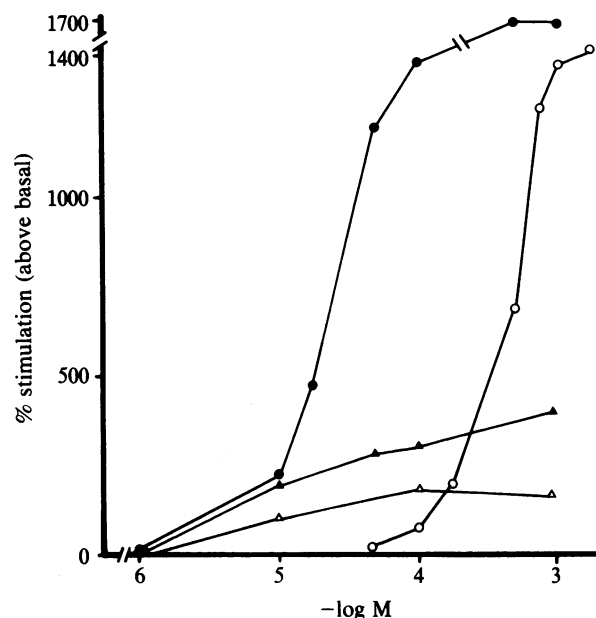


FIG. 1. Concentration-dependent stimulation of [<sup>3</sup>H]Ins-1-*P* formation elicited by L-glutamate (O), ibotenate (●), carbamoylcholine (▲), and norepinephrine (Δ) in hippocampal slices from 6-day-old rats. Each point is the mean of at least 4 determinations; SEM is less than 10% of the mean for each point.

devoid of intrinsic activity, but they inhibit the stimulation of PtdIns hydrolysis elicited by ibotenate (8), the only excitatory amino acid receptor agonist that stimulates PtdIns hydrolysis in hippocampal slices prepared from brains of adult rats (Table 3). APB and *O*<sup>3</sup>-phosphono-L-serine, which enhance PtdIns hydrolysis in hippocampal slices prepared from 6-day-old rats, are devoid of intrinsic activity in hippocampal slices prepared from brains of 15-day-old rats, where they antagonize the action of high concentrations of glutamate and ibotenate (Table 4, Fig. 2). In hippocampal slices from 6-day-old rats, APB and phosphoserine fail to antagonize the action of glutamate and ibotenate, even though relatively low concentrations of these amino acids are used (Table 4). Other excitatory amino acid receptor antagonists, including 2-amino-5-phosphonopentanoate; glutamic acid diethylester, and *cis*-2,3-piperidinedicarboxylate, fail to antagonize the stimulation of PtdIns hydrolysis elicited by glutamate or ibotenate during development (Table 4) as well as in adult rats (8).

The maturational changes in the stimulation of PtdIns hydrolysis elicited by norepinephrine differ from those relative to glutamate (Table 3). The efficacy of norepinephrine, which is relatively low in hippocampal slices from newborn animals, increases during development and reaches adult values after the 35th day of life. A similar time course in the "PtdIns response" to norepinephrine has been reported in slices from cerebral cortex (15).

Table 2. Stimulation of [<sup>3</sup>H]Ins-1-*P* formation elicited by different transmitter receptor agonists in slices of brain regions from 6-day-old rats

Agonist	[ <sup>3</sup> H]Ins-1- <i>P</i> , Bq/mg of protein			
	Hippocampus	Cortex	Corpus striatum	Hypothalamus
None	190 ± 23	120 ± 13	190 ± 21	290 ± 18
L-Glutamate (1 mM)	2700 ± 170*	550 ± 54*	730 ± 16*	931 ± 110*
Ibotenate (500 μM)	3300 ± 400*	460 ± 64*	820 ± 120*	800 ± 96*
Carbamoylcholine (1 mM)	720 ± 64*	610 ± 55*	960 ± 120*	ND
Norepinephrine (100 μM)	330 ± 44*	270 ± 19*	ND	ND

Values are means ± SEM of at least 4 determinations. ND, not done.

\**P* < 0.01, when compared with basal values.

Table 3. Stimulation of [<sup>3</sup>H]Ins-1-*P* formation elicited by L-glutamate, ibotenate, carbamoylcholine, and norepinephrine in hippocampal slices from rats of different ages

Agonist	Net increase in [ <sup>3</sup> H]Ins-1- <i>P</i> formation, Bq/mg of protein (% basal)						
	6 days	8 days	12 days	15 days	19 days	24 days	35 days
L-Glutamate (1 mM)	2500 ± 140 (1470%)	1100 ± 91 (800%)	400 ± 80 (550%)	350 ± 4 (520%)	150 ± 4 (300%)	21 ± 8 (135%)	17 ± 2 (145%)
Ibotenate (500 μM)	3100 ± 380 (1900%)	2200 ± 97 (1450%)	1600 ± 150 (1900%)	1300 ± 170 (1600%)	440 ± 4 (680%)	390 ± 19 (630%)	230 ± 38 (660%)
Carbamoylcholine (1 mM)	530 ± 48 (390%)	530 ± 9 (370%)	ND	280 ± 22 (440%)	150 ± 13 (300%)	170 ± 26 (305%)	125 ± 18 (400%)
Norepinephrine (100 μM)	140 ± 22 (175%)	150 ± 14 (190%)	ND	180 ± 8 (320%)	230 ± 13 (400%)	250 ± 30 (540%)	370 ± 21 (980%)

Values are means ± SEM of at least 4 determinations. The basal formation of [<sup>3</sup>H]Ins-1-*P* decreases from the 6th to the 35th day of postnatal life as follows: 6th day, 190 ± 23 Bq/mg of protein; 8th day, 160 ± 18; 12th day, 90 ± 22; 15th day, 83 ± 8; 19th day, 77 ± 8; 24th day, 56 ± 3; 35th day, 42 ± 2. ND, not done.

No substantial changes in carbamoylcholine-induced stimulation of PtdIns hydrolysis are observed during the postnatal development if the stimulation is expressed as percentage of the basal values (Table 3).

**Interactions Between Norepinephrine and Excitatory Amino Acids in Hippocampal Slices from Adult Rats.** In hippocampal slices from adult rats, norepinephrine stimulates [<sup>3</sup>H]Ins-1-*P* formation in a concentration-dependent fashion, with an apparent EC<sub>50</sub> of 5–10 μM and a maximal stimulation of 9- to 10-fold achieved with 100 μM. We have found that the same concentrations of glutamate that enhance PtdIns hydrolysis in immature hippocampal slices (Tables 1 and 3, Fig. 1) inhibit the increase in [<sup>3</sup>H]Ins-1-*P* formation induced by norepinephrine in adult slices (Fig. 3). This action is mimicked by aspartate, quisqualate, APB, and phosphoserine, but not by kainate or *N*-methyl-D-aspartate (Table 5); ibotenate and norepinephrine stimulate PtdIns hydrolysis in an additive fashion (Table 5). Glutamate also inhibits the action of norepinephrine in slices from corpus striatum and frontal cortex of adult rats. The inhibitory actions of glutamate, aspartate, APB, and phosphoserine are specific for norepinephrine, since these amino acids fail to affect the stimulation of PtdIns hydrolysis elicited by carbamoylcholine (Table 5). In hippocampal slices from newborn rats, where norepinephrine is not a potent activator of PtdIns hydrolysis, glutamate and norepinephrine stimulate [<sup>3</sup>H]Ins-1-*P* formation in an additive fashion (see Table 1).

To test whether the glutamate inhibition of the stimulation of PtdIns hydrolysis elicited by norepinephrine in the adult hippocampus results from alterations in the characteristics of the α<sub>1</sub>-adrenergic recognition site (which mediates the action of norepinephrine on PtdIns hydrolysis) (16, 17), we measured [<sup>3</sup>H]prazosin binding in synaptic membranes prepared

from adult hippocampus. Glutamate, aspartate, APB, or phosphoserine at 1 μM–1 mM fail to inhibit [<sup>3</sup>H]prazosin binding. In addition, high concentrations (1 mM) of glutamate fail to affect the displacement of [<sup>3</sup>H]prazosin by norepinephrine (data not shown), excluding any allosteric modulation of the α<sub>1</sub>-adrenergic recognition sites by excitatory amino acids.

## DISCUSSION

Increases in PtdIns hydrolysis are believed to be involved in receptor-mediated transmembrane signaling in the CNS (18, 19). This receptor-mediated signal transduction involves an activation of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate to yield inositol phosphates and diacylglycerol. Both compounds act as second messengers, triggering a chain of intracellular reactions leading to mobilization of Ca<sup>2+</sup> from intracellular stores, translocation and activation of protein kinase C, and release of arachidonic acid (18–20).

The excitatory transmitters glutamate and aspartate activate inositol phospholipid metabolism in the CNS. The need for relatively high concentrations of glutamate and aspartate to activate PtdIns hydrolysis may reflect the rapid removal of these amino acids from the extracellular space by several mechanisms, including specific carrier-mediated uptake systems located in neurons and glial cells (21, 22). Ibotenate, which is not taken up by excitatory amino acid uptake systems, activates PtdIns hydrolysis even at low concentrations.

The actions of glutamate and ibotenate on PtdIns hydrolysis seem to be mediated by specific recognition sites that cannot be identified with any of the three major subtypes

Table 4. Stimulation of [<sup>3</sup>H]Ins-1-*P* formation by L-glutamate and ibotenate in hippocampal slices from 6- or 15-day-old rats in the presence of excitatory amino acid receptor antagonists

Antagonist	[ <sup>3</sup> H]Ins-1- <i>P</i> , Bq/mg of protein							
	6 days old			15 days old				
	No agonist	Glutamate (150 μM)	Ibotenate (20 μM)	No agonist	Glutamate		Ibotenate	
				150 μM	500 μM	20 μM	100 μM	
None	190 ± 8	310 ± 42	770 ± 110	96 ± 9	150 ± 12	240 ± 8	480 ± 40	770 ± 16
APB	240 ± 19*	350 ± 16	900 ± 30	90 ± 20	88 ± 7*	150 ± 4*	120 ± 14*	210 ± 95*
Ser( <i>P</i> )	244 ± 14*	370 ± 19	1000 ± 180	100 ± 20	95 ± 8*	110 ± 10*	ND	170 ± 58*
APP	180 ± 13	280 ± 45	670 ± 36	110 ± 16	160 ± 32	280 ± 41	510 ± 28	750 ± 83
Glu(OEt)-OEt	180 ± 29	330 ± 46	830 ± 25	110 ± 27	ND	240 ± 13	ND	ND
Pip(COOH) <sub>2</sub>	210 ± 33	310 ± 58	ND	110 ± 13	ND	260 ± 23	ND	ND

Values are means ± SEM of at least 3 determinations. Antagonist concentration was 1 mM. Ser(*P*), phosphoserine; APP, 2-amino-5-phosphonopentanoate; Glu(OEt)-OEt, glutamic acid diethyl ester; Pip(COOH)<sub>2</sub>, *cis*-2,3-piperidinedicarboxylate. ND, not done.

\**P* < 0.01 when compared with values obtained in the absence of antagonist.

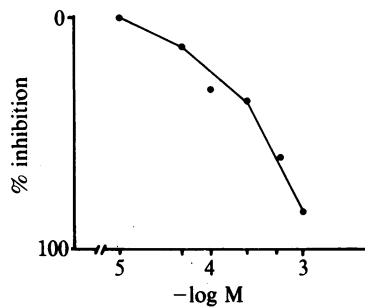


FIG. 2. Concentration-dependent inhibition of ibotenate-stimulated PtdIns hydrolysis by APB in hippocampal slices from 15-day-old rats. Each point represents the mean of at least 4 determinations; SEM is less than 10% of the mean for each point. Ibotenate concentration, 150  $\mu$ M.

("N-methyl-D-aspartate-," "quisqualate-," and "kainate-") preferring receptors) defined by electrophysiological and ligand-binding studies (23–25). The structure–activity relationship elucidated by the stimulation of [ $^3$ H]Ins-1-P formation suggests that, in the CNS, the APB-sensitive,  $Ca^{2+}/Cl^{-}$ -dependent recognition site for glutamate (24, 26) is coupled with PtdIns metabolism. Glutamate, aspartate, ibotenate, and quisqualate enhance PtdIns hydrolysis and displace specifically bound [ $^3$ H]APB and [ $^3$ H]glutamate in the presence of  $Ca^{2+}$  and  $Cl^{-}$  (24, 26); kainate and N-methyl-D-aspartate, which fail to activate PtdIns metabolism, do not displace [ $^3$ H]APB or inhibit  $Ca^{2+}/Cl^{-}$ -dependent [ $^3$ H]glutamate specific binding even at high concentrations (24, 26). In addition, phosphoserine, a selective ligand of the  $Ca^{2+}/Cl^{-}$ -dependent [ $^3$ H]glutamate binding site (27), antagonizes the stimulation of PtdIns hydrolysis elicited by glutamate or ibotenate.

During postnatal development, the density of  $Ca^{2+}/Cl^{-}$ -dependent [ $^3$ H]glutamate binding sites increases (28), but the efficacy of the coupling of these sites with PtdIns metabolism progressively declines. In hippocampal slices, the stimulation of PtdIns hydrolysis is very high 6 and 8 days after birth, but it decreases during the next 10 days and reaches adult values between 19 and 24 days after birth. This temporal pattern corresponds with an intense period of hippocampal synaptogenesis that occurs during the first postnatal week and slows down after the 10th day of life, when the major contacts of hippocampal pyramidal and granule cells (which use glutamate and/or aspartate as neurotransmitter, refs. 24 and 29) are formed (30). In the rat hippocampus, an enhanced

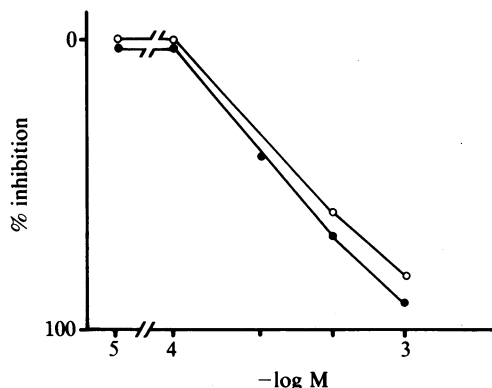


FIG. 3. Concentration-dependent inhibition of norepinephrine-stimulated PtdIns hydrolysis by L-glutamate (○) and L-phosphoserine (●) in hippocampal slices from adult rats. Each point is the mean of at least 4 determinations; SEM is less than 10% of the mean for each point.

Table 5. Stimulation of [ $^3$ H]Ins-1-P formation elicited by norepinephrine and carbamoylcholine in the presence of various excitatory amino acid receptor ligands

Ligand	[ $^3$ H]Ins-1-P, Bq/mg of protein		
	Control	Norepinephrine (100 $\mu$ M)	Carbamoylcholine (100 $\mu$ M)
None	48 $\pm$ 4	460 $\pm$ 25	120 $\pm$ 8
Glutamate	65 $\pm$ 6	150 $\pm$ 18*	116 $\pm$ 12
Aspartate	68 $\pm$ 2	125 $\pm$ 13*	126 $\pm$ 5
Quisqualate	80 $\pm$ 7	136 $\pm$ 25*	ND
APB	46 $\pm$ 3	120 $\pm$ 8*	122 $\pm$ 10
Phosphoserine	44 $\pm$ 2	105 $\pm$ 6*	122 $\pm$ 12
Ibotenate	280 $\pm$ 15	770 $\pm$ 56*	ND
Kainate	44 $\pm$ 3	450 $\pm$ 25	ND
N-Me-D-Asp	47 $\pm$ 5	500 $\pm$ 42	ND

Values are means  $\pm$  SEM of at least 4 determinations. Ligand concentration was 1 mM, except for ibotenate, which was used at 500  $\mu$ M. ND, not done.

\* $P < 0.01$  when compared with value obtained with norepinephrine in the absence of ligand.

hydrolysis of membrane inositol phospholipids by glutamate and aspartate and the consequent chain of intracellular reactions might have an important role in the regulation of neurocytomorphogenesis and synaptogenesis.

The progressive reduction of the coupling of the glutamate recognition site with PtdIns metabolism during development might be due to conformational changes in the recognition sites for glutamate, to changes in the coupling mechanisms, or to the appearance of a negative coupler that avoids the activation of PtdIns hydrolysis when glutamate binds to the recognition site. We do not have data that preferentially support one of these three hypotheses. Among the excitatory amino acids that we have tested, only ibotenate substantially activates PtdIns hydrolysis in mature hippocampal slices. Evidently, the rigid conformation of ibotenate allows the activation of PtdIns hydrolysis in spite of the reduced coupling of the glutamate recognition site with phospholipase C in the adult hippocampus. Whether there is an endogenous ligand of excitatory amino acid receptors that mimics the action of ibotenate remains to be elucidated.

In slices from mature rat hippocampus, where glutamate and aspartate lose their capacity to stimulate PtdIns hydrolysis, a new function for these amino acids emerges: they inhibit the activation of PtdIns hydrolysis elicited by norepinephrine. This inhibitory action is shared by other ligands of the  $Ca^{2+}/Cl^{-}$ -dependent glutamate recognition sites which, in slices of adult rat brain, have low or no activity in stimulating PtdIns hydrolysis. These ligands are quisqualate, APB, and phosphoserine. These amino acids specifically inhibit norepinephrine response; they fail to affect the stimulation of PtdIns hydrolysis elicited by the cholinergic agonist carbamoylcholine. The reduction of norepinephrine-induced stimulation of PtdIns hydrolysis is not due to a reduction in the number of the  $\alpha_1$ -adrenergic recognition sites, which appear to be coupled with PtdIns metabolism (16, 17). In fact, the curve for displacement of [ $^3$ H]prazosin by norepinephrine is not affected by glutamate even in the presence of  $Ca^{2+}$  at 37°C, when the  $Ca^{2+}/Cl^{-}$ -dependent glutamate recognition sites become exposed to the ligand (24, 26). It is possible that, if the  $\alpha_1$ -adrenergic recognition sites are located in the membrane of the same neurons, they share a common pool of phospholipase C. The interaction of the glutamate recognition site with "inactive" endogenous molecules, such as glutamate, aspartate, or phosphoserine, might lead to a sequestration of the enzyme, which would then become insensitive to activation by norepinephrine. However, from our data, we cannot rule out that the inhibition of norepi-

nephine response by excitatory amino acids is mediated by a transsynaptic mechanism rather than by interactions of two adjacent recognition sites in the membrane of the same neuron.

Our findings may imply that, in the adult brain, the functional expression of the  $\alpha_1$ -adrenoceptor might be regulated by glutamate and aspartate released in the synaptic cleft as well as by phosphoserine that may be produced by phosphatidylserine hydrolysis. The inhibition of norepinephrine response may represent a "fail-safe" mechanism to prevent an excessive stimulation of PtdIns hydrolysis by norepinephrine.

From a practical standpoint, our results indicate a type of mechanism that can be followed to pharmacologically inhibit  $\alpha_1$ -adrenoceptors in the CNS. Conventionally, these receptors are competitively inhibited by drugs endowed with high affinity for the  $\alpha_1$ -adrenoceptor recognition site and low intrinsic activity in stimulating receptor transduction. Our results suggest that analogues or precursors of glutamate, aspartate, or phosphoserine may be capable of inhibiting  $\alpha_1$ -adrenoceptor-stimulated PtdIns hydrolysis through a "receptor-receptor" interaction. The molecular mechanism by which such interaction occurs remains unclear.

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