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Developmental Profile of Metabotropic Glutamate Receptor mRNA in Rat Brain

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SUMMARY

We have studied the expression of metabotropic glutamate receptor (mGluR) mRNA by Northern blot analysis with a specific cDNA probe (the pmGR1 probe). In 1-day-old rats, the steady state levels of mRNA were higher in the hypothalamus and olfactory bulb, with intermediate levels in the cerebellum and low levels in the hippocampus and cerebral cortex. In the olfactory bulb, hypothalamus, and cerebral cortex, the expression of mGluR mRNA remained constant at 8 and 30 days of postnatal life. In contrast, in the cerebellum and hippocampus, mRNA levels increased progressively with age. There was no correlation between levels of mGluR mRNA and stimulation of polyphosphoinositide hydrolysis by 1-aminocyclopentane-1*S*,3*R*-dicarboxylic acid (*trans*-ACPD), which was much greater in brain slices from 8-day-old rats and was nearly absent in the adult cerebellum and olfactory bulb, where we have found the highest levels of mRNA. In addition, mGluR mRNA was detectable in cultured cerebellar granule cells but not in cultured neurons from cerebral hemispheres or in cultured astrocytes, which responded to *trans*-ACPD with an increased formation of [^{3}H]inositol monophosphate. The discrepancies between levels of mGluR mRNA detected with the pmGR1 probe and *trans*-ACPD-stimulated polyphosphoinositide hydrolysis suggest either that different subtypes of mGluRs exist or that mRNA levels are not critical for the dynamic changes in the activity of mGluRs during development.

Specific glutamate receptors coupled to PPI (mGluR) have been described in cultured neurons and astrocytes (1–3), brain slices (4), and *Xenopus* oocytes injected with rat brain mRNA (5). In most of the systems, these receptors are activated by L-glutamate, quisqualate, ibotenate, and ACPD, but not by α-amino-3-hydroxy-5-methylisoxazolepropionate, kainate, or *N*-methyl-D-aspartate (for review, see Ref. 6). ACPD has been described as the most selective agonist of mGluRs, with no apparent activity on glutamate receptors associated with ion channels ("ionotropic receptors") (7). L-2-Amino-3-phosphonopropionic acid, L-2-amino-4-phosphonobutanoic acid, and L-serine-*O*-phosphate act as mGluR antagonists in brain slices, but they are less effective in cultured neurons (1, 4, 8, 9). In *Xenopus* oocytes and in cultured neurons (but not in cultured astrocytes), stimulation of PPI hydrolysis by mGluR agonists is attenuated by PTX (5, 10–12), suggesting that a PTX-sensitive G protein couples mGluRs with phospholipase C. The identity of this specific G protein is obscure at present.

Measurement of agonist-stimulated [^{3}H]inositol phosphate formation has revealed that mGluRs undergo plastic modification during development, as well as in adult life. In particular, stimulation of PPI hydrolysis by excitatory amino acids is high during the early stages of neuronal maturation and declines progressively with age (4, 13–15). In kitten striate cortex, stimulation of [^{3}H]inositol phosphate formation by ibotenic acid is greater between the 3rd and the 5th week of postnatal life, a time that corresponds to the "critical period" for synaptic modification (16). This suggests that functional expression of mGluRs is in relation to the activity-dependent modifications of synaptic efficacy. In adult rats, excitatory amino acid-stimulated PPI hydrolysis is enhanced in response to deafferentation (17), during electrical kindling of the hippocampus or amygdala (18, 19), after induction of long term potentiation (20), and in tissue prepared from animals trained in the radial maze (21). Attempts have been made to establish which component of the receptor complex (recognition site, G protein, or phospholipase C) is responsible for the functional changes of mGluRs during development or in adult life. In general, changes in [^{3}H]glutamate binding do not correlate with changes in agonist-stimulated [^{3}H]inositol phosphate formation (17, 20). However, binding sites are limited by the lack of specific radioactive ligands for mGluRs.

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ABBREVIATIONS: PPI, polyphosphoinositide; PTX, pertussis toxin; ACPD, 1-aminocyclopentane-1*S*,3*R*-dicarboxylic acid; mGluR, metabotropic glutamate receptor; G protein, GTP-binding protein; InsP, inositol monophoshate; SSC, standard saline citrate.

Recently, two independent groups have cloned and characterized the cDNA for a mGluR. Oocytes transfected with the derived mRNA respond to the application of mGluR agonists (such as quisqualate or *trans*-ACPD) with an increased formation of inositol-1,4,5-trisphosphate and activation of Ca^{2+}-dependent Cl^- current. Both effects are largely reduced after treatment of oocytes with PTX (22, 23). The cDNA encodes a protein of 1199 amino acids, containing a seven-transmembrane region motif flanked by large amino- and carboxyl-terminal domains. This protein is much larger than and has no apparent homology with any other member of the family of G protein-linked receptors for neurotransmitters (22, 23).

The availability of the cDNA allows determination of the levels of mGluR by hydribization techniques. We have studied the developmental profile of mGluR mRNA in relation to the functional activity of mGluRs in various brain regions.

Materials and Methods

Measurement of PPI hydrolysis in brain slices. PPI hydrolysis was determined by measuring the accumulation of [^{3}H]InsP in the presence of 10 mM Li^+, according to the method described by Berridge *et al.* (24), as slightly modified (1). In brief, slices from hippocampus, olfactory bulb, cerebellum, hypothalamus, or cerebral cortex were prelabeled with *myo*-[2-^{3}H]inositol and then stimulated with *trans*-ACPD in the presence of 10 mM Li^+. The reaction was terminated by addition of chloroform/methanol (1:2). After further addition of chloroform and water, phases were separated and the [^{3}H]InsP present in the aqueous phase was isolated by anion exchange chromatography (24).

Preparation of cell cultures. Primary cultures of cerebellar neurons were prepared from 8-day-old rats as described previously (1). In their final stage of maturation, these cultures contain >90% granule cells, with 4–6% γ-aminobutyric acid-ergic neurons and a small amount of glial and endothelial cells (1). Primary cultures of neurons were obtained from cerebral hemispheres of rat embryos (embryonic day 16–18), as described by Borg *et al.* (25). Primary cultures of astrocytes were obtained from cerebral hemispheres of newborn rats, as described by Avola *et al.* (26).

Measurement of PPI hydrolysis in cell cultures. PPI hydrolysis was measured in cultured neurons and astrocytes as described previously (1). In brief, cultures were prelabeled with 3 μCi of *myo*-[2-^{3}H]-inositol for 16–18 hr and then stimulated with *trans*-ACPD in the presence of 10 mM Li^+. [^{3}H]InsP formation was determined as described above.

RNA isolation, electrophoresis, and hybridization. Total RNA was extracted from brain tissue or cultured cells, as described by Chomczynski and Sacchi (27). Total cellular RNA (20 or 30 μg/sample) was electrophoresed through 1.1% agarose-2.2 M formaldehyde gels, stained with ethidium bromide, photographed under UV light, and then blotted onto nitrocellulose (Hybond-C Extra; Amersham). Prehybridization (4–6 hr) and hybridization (18–24 hr) were carried out at 42° in a mixture containing 50% formamide, 5× SSC (0.15 M NaCl, 0.015 M sodium citrate), 5× Denhardt's solution, 20 mM sodium phosphate buffer (pH 6.5), and 100 μg/ml denaturated, sheared, salmon sperm DNA. In the case of hybridization, the mixture was supplemented with 10% dextran sulfate and labeled denaturated probe. Full-length pmGR1 probe (23) was linearized with *Eco*RI; full-length β-actin cDNA probe (28) was linearized with *Bam*HI. Both plasmids were ^{32}P-labeled by the random-primed DNA-labeling method developed by Feinberg and Vogelstein (29). Nitrocellulose membranes were washed three times (5 min each) with a mixture containing 2× SSC and 0.1% sodium dodecyl sulfate, at room temperature, and then three times (30 min each wash) with a mixture of 0.1× SSC and 0.1% sodium dodecyl sulfate, at 52°; finally, they were exposed to X-ray film at −70°, using intensifying screens. The density of the hybridization signal was determined by scanning the autoradiogram with a LKB UltroScan XL laser densitometer.

Materials. *myo*-[2-^{3}H]Inositol (specific activity, 15.6 Ci/mmol) and [α-^{32}P]deoxycytidine 5′ triphosphate (specific activity, 3000 Ci/mmol) were purchased from NEN-DuPont (Florence, Italy). Quisqualate and *trans*-ACPD were purchased from Tocris Neuramin (Essex, UK). The pmGR1 cDNA probe, constructed by Dr. Masayuki Masu, was a generous gift of Prof. Shigetada Nakanishi (Institute for Immunology, Kyoto University, Japan).

Results and Discussion

RNA blot analysis in all the brain regions revealed two transcripts, with stronger hybridization at 7–7.4 kilobases and a weaker signal at about 4.4 kilobases, in agreement with previous results (22, 23) (see Fig. 1). The larger mRNA seems to encode the mGluR protein (22). In 1-day-old rats, the expression of mGluR mRNA was greater in the olfactory bulb, with intermediate levels in the hypothalamus and cerebellum and low levels in the cerebral cortex and hippocampus (Figs. 1–3). In the olfactory bulb, hypothalamus, and cerebral cortex, the steady state level of mGluR mRNA remained constant at 8 and

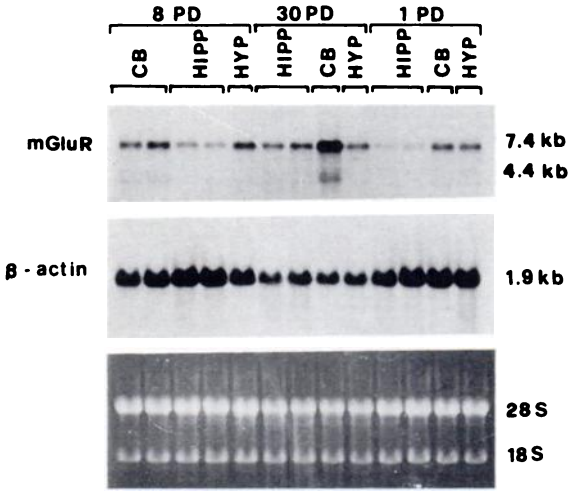

Fig. 1. Northern blot analysis of mGluR mRNA. Each lane was loaded with 20 μg of total RNA. *CB*, cerebellum; *HIPP*, hippocampus; *HYP*, hypothalamus; *PD*, postnatal days. For cerebellum and hippocampus at 8 postnatal days and hippocampus at 30 and 1 postnatal days, each of the two lanes was loaded with RNA samples from individual animals. The same blot was rehybridized with a β-actin cDNA probe. The ethidium bromide-stained gel shows that equal amounts of rRNA (28 S and 18 S) were loaded in each lane. RNA size was estimated by the position of rRNAs comigrating in the same lane, as well as by RNA markers loaded in a different lane. RNA markers (0.36–9.49 kilobases) were purchased from Promega (Madison, WI).

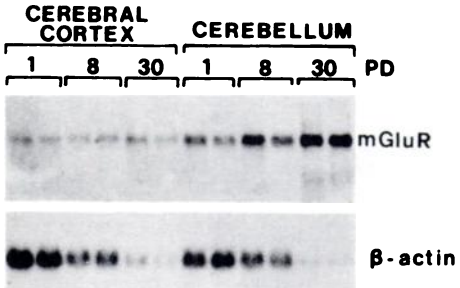

Fig. 2. Northern blot analysis of mGluR and β-actin mRNAs in cerebral cortex and cerebellum from rats at different days of postnatal life (*PD*). Each lane was loaded with 20 μg of total RNA. Each lane was loaded with RNA samples from individual animals.

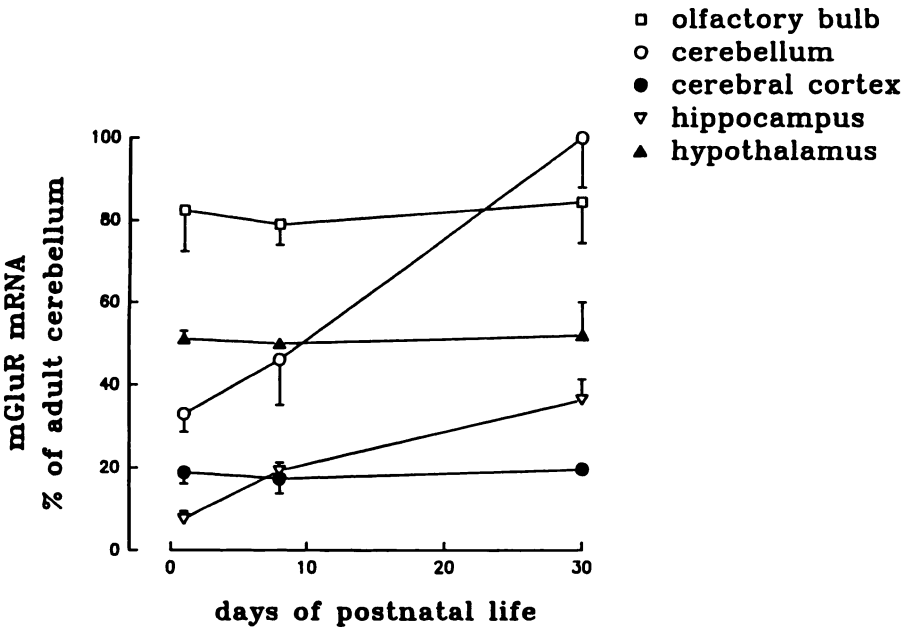

Fig. 3. Expression of mGluR mRNA at different days after birth. Results are expressed as percentage of values obtained in the adult cerebellum and represent the means ± standard errors of three determinations obtained from three different RNA preparations. Each preparation was derived from a single animal or a pool of brain regions to reach a sufficient amount of tissue.

30 days of postnatal life. In contrast, the expression of mGluR mRNA increased with age in the cerebellum and hippocampus. The rise was linear in the cerebellum, which expressed the highest levels of mGluR mRNA at 30 days after birth (Figs. 1–3). Levels of mRNA for the cytoskeletal protein β-actin exhibited a different developmental pattern, decreasing progressively with age in all the brain regions (Figs. 1 and 2). Hence, it is unlikely that the age-dependent increase in mGluR mRNA levels in cerebellum and hippocampus is due to regional or developmental differences in the relative amount of polyadenylated and nonpolyadenylated RNA.

As opposed to the levels of mGluR mRNA, the functional expression of mGluRs was much greater at 8 days of postnatal life, as reflected by a greater ability of maximal concentrations of *trans*-ACPD (100 μM) to stimulate [^{3}H]InsP formation in brain slices (Fig. 4). Stimulation of PPI hydrolysis by *trans*-ACPD was greater in the hippocampus and hypothalamus and substantially smaller in the cerebellum and olfactory bulb. At 30 days after birth, *trans*-ACPD stimulated PPI hydrolysis only in the hippocampus and cerebral cortex, whereas it was virtually inactive in the hypothalamus, olfactory bulb, and cerebellum (Fig. 4).

As expected, *trans*-ACPD and quisqualate stimulated [^{3}H]-InsP formation in cultured cerebellar granule cells, in cultured neurons from cerebral hemispheres, and in cultured astrocytes (Table 1). However, mGluR mRNA was expressed only by cultured cerebellar granule cells (Table 1), whereas no hybridization signal was found in RNA extracted from cultured neurons from cerebral hemispheres or from cultured astrocytes (Table 1; Fig. 5).

Taken collectively, these results indicate an absolute lack of correlation between the steady state levels of mGluR mRNA and the ability of *trans*-ACPD to stimulate PPI hydrolysis during postnatal development. The activity of mGluRs was homogeneously down-regulated in adult life, whereas the expression of mRNA was constant or even increased (as in the cerebellum and hippocampus). mGluR mRNA levels were substantially lower in the hippocampus, the only region that retained a robust response to *trans*-ACPD in adulthood. In contrast, mRNA levels were high in the adult olfactory bulb and cerebellum, which did not respond to *trans*-ACPD. We have used *trans*-ACPD because it is the most selective agonist of mGluRs. Although other agonists (such as ibotenate and quisqualate) may exhibit a different developmental profile in stimulating PPI hydrolysis, they are also more effective during the first 15 days of postnatal life than in adulthood (15). However, the use of different agonists could have changed the regional pattern of stimulation of [^{3}H]InsP formation. Accordingly, as

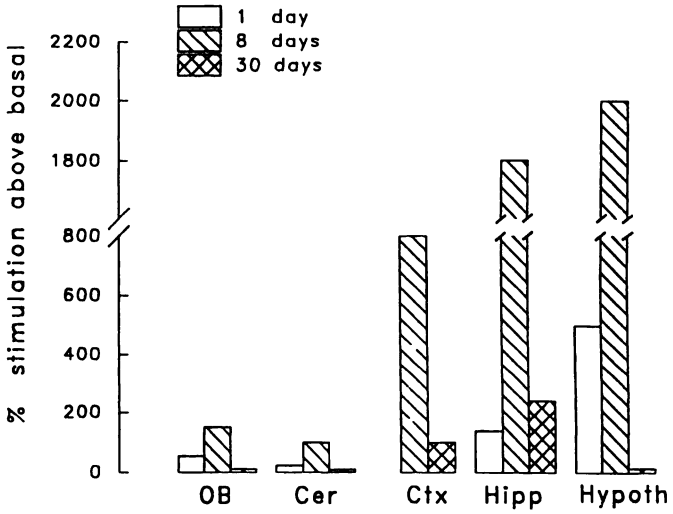

Fig. 4. Stimulation of [^{3}H]InsP formation elicited by *trans*-ACPD (100 μM) in slices prepared from olfactory bulb (*OB*), cerebellum (*Cer*), cerebral cortex (*Ctx*), hippocampus (*Hipp*), and hypothalamus (*Hypoth*) of rats at different days of postnatal life. In slices from 1- or 8-day-old rats, basal values of [^{3}H]InsP ranged from 2,400 to 3,600 dpm/mg of protein. Values remained constant (olfactory bulb, cerebellum, and hypothalamus) or decreased by 40–60% (cortex and hippocampus) in slices from 30-day-old rats. Results are expressed as percentage of stimulation above basal values and represent the means of six to nine determinations from two or three individual experiments performed in triplicate. Standard deviation was <10% of the mean value in all determinations.

TABLE 1

Steady state levels of mGluR mRNA and excitatory amino acid-stimulated [^{3}H]InsP formation in cultured neurons and astrocytes

All cultures were used at 7–9 days *in vitro*. Quisqualate and *trans*-ACPD were added at concentrations of 100 μM. Values are means ± standard errors of three determinations. mGluR mRNA levels are expressed as percentages of values detected in the cerebellum of 8-day-old rats (from which cultured cerebellar neurons were prepared).

	Cerebellar granule cells	Hemispheric neurons	Astrocytes
[^{3}H]InsP formation (dpm/mg of protein) × 10^{-3}			
Basal	12 ± 0.7	13 ± 0.6	5.8 ± 0.7
trans-ACPD	19 ± 0.3	18 ± 0.2	9.7 ± 0.5
Quisqualate	30 ± 0.8	24 ± 1.5	15 ± 0.3
mGluR mRNA (% of cerebellum)	35 ± 5	ND[a]	ND

[a] ND, not detectable.

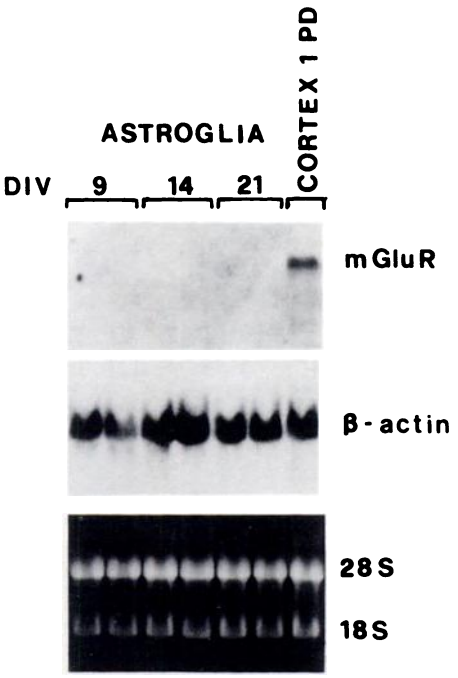

Fig. 5. *Upper*, Northern blot analysis of mGluR and β-actin mRNA in cultured astrocytes (*ASTROGLIA*) at different days *in vitro* (*DIV*). Each lane was loaded with 30 μg of total RNA. In the last lane, an equal amount of total RNA from 1-day-old rat cerebral cortex (*CORTEX 1 PD*) was also analyzed, as positive control. *Lower*, the ethidium bromide-stained gel shows that equal amounts of rRNA (28 S and 18 S) were loaded in each lane. In astroglia (9, 14, and 21 days *in vitro*), each of the two lanes was loaded with RNA samples from individual culture dishes.

opposed to our findings with *trans*-ACPD, quisqualate and glutamate activate mGluRs in adult rat cerebellum (30), whereas ibotenate is more effective in the olfactory bulb than in the hippocampus (31). For this reason, we restrict the discussion of the data to the specific subtype (or conformational state) of mGluR activated by *trans*-ACPD.

There are different explanations for the discrepancy between the levels of mGluR mRNA and the functional activity of mGluRs. Oocytes transfected with mGluR mRNA acquire the ability to respond to *trans*-ACPD and other receptor agonists with an increased formation of inositol-1,4,5-trisphosphate and activation of Ca^{2+}-dependent Cl^- current (22, 23), suggesting that this specific mGluR mRNA can be potentially translated into a functionally active receptor. The lack of activity of *trans*-ACPD in the adult olfactory bulb, cerebellum, and hypothalamus, despite the high levels of mGluR mRNA, may indicate that mature neurons (and/or astrocytes) do not possess the appropriate machinery that allows the expression of a functional active receptor.

It is possible that the translation of mGluR mRNA and/or the post-translational modifications of the precursor protein are developmentally regulated. Alternatively, different subtypes of mGluRs may be present in the central nervous system. If so, the protein encoded by the mRNA we have detected may not be the one responsible for the developmental changes in *trans*-ACPD-stimulated PPI hydrolysis. The existence of additional mGluR subtypes is consistent with the observation that no hybridization signal was obtained with the pmGR1 probe in cultured neurons from cerebral hemispheres and cultured astrocytes, which responded to *trans*-ACPD or quisqualate with increased [^{3}H]InsP formation.

A major difference between oocytes or developing neurons and adult neurons may reside in the levels of expression of the protein that couples the receptor to phospholipase C. A role for a G protein in the signal transduction pathway of mGluRs has been inferred by the use of PTX, which ADP-ribosylates the α subunit of a variety of G proteins, including G_i and G_o (32, 33). PTX attenuates the functional expression of mGluRs in oocytes (5), cultured cerebellar granule cells (12), and cultured striatal neurons (10) but not in cultured hippocampal neurons (34) or in cultured astrocytes (11). This suggests that the specific G protein associated with mGluRs may be differentially regulated in different tissues or cell types. The identification of the specific G protein is necessary for exploration of the nature of the changes in the activity of mGluRs during development, as well as in adult life.

If stimulation of PPI hydrolysis by *trans*-ACPD is entirely mediated by the receptor protein encoded by the pmGR1 cDNA, then the high levels of expression of mRNA in adult brain regions are apparently paradoxical. It is possible that the receptor protein translated in adult life serves as a reservoir and becomes functional under appropriate conditions, such as in response to lesions (17) or during learning and memory formation (20, 21). Alternatively, it cannot be excluded that the mGluR has functions other than the stimulation of PPI hydrolysis. Interestingly, the amino-terminal domain of mGluR protein exhibits a structural similarity with the membrane form of sea urchin guanylate cyclase (22). Coupling of mGluR to multiple signal transduction pathways would provide a novel mechanism by which the same receptor protein could serve different functions in relation to a specific stage of neuronal maturation.

In conclusion, we have found that the expression of mGluR mRNA does not correlate with the functional activity of the mGluR activated by *trans*-ACPD during postnatal development. However, these conclusions are limited by the use of brain slices or intact tissue in our study. We cannot exclude the possibility that a better correlation would exist if mGluR mRNA and agonist-stimulated PPI hydrolysis were examined at the single-cell level. Autoradiography can be applied to the study of mGluR for the detection of [^{3}H]cytidine diphosphate diacylglycerol, which is synthesized as a result of PPI hydrolysis (35). Combined autoradiographical and *in situ* hybridization studies will better clarify the relation between mGluR mRNA

and agonist-stimulated PPI hydrolysis during postnatal development.

Acknowledgments

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Note Added in Proof

After this report was submitted, Tanabe et al. (36) reported the isolation of three cDNA clones (mGluR2, 3 and 4) from a rat brain cDNA library by cross-hybridization with a cDNA for the metabotropic receptor used in this study (named mGluR1).

References

1. Nicoletti, F., J. T. Wroblewski, A. Novelli, H. Alho, A. Guidotti, and E. Costa. The activation of inositol phospholipid metabolism as a signal transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* **6**:1905–1911 (1986).
2. Pearce, B. B., J. Albrecht, C. Morrow, and S. Murphy. Astrocyte glutamate receptor activation promotes inositol phospholipid turnover and calcium influx. *Neurosci. Lett.* **72**:335-340 (1987).
3. Sladeczek, F., J. P. Pin, M. Récasens, J. Bockaert, and S. Weiss. Glutamate stimulates inositol phosphate formation in striatal neurons. *Nature* (*Lond.*) **317**:717–719 (1985).
4. Nicoletti, F., M. J. Iadarola, J. T. Wroblewski, and E. Costa. Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with α1-adrenoceptors. *Proc. Natl. Acad. Sci. USA* **83**:1931–1935 (1986).
5. Sugiyama, H., I. Ito, and C. Hirono. A new type of glutamate receptor linked to inositol phosphate metabolism. *Nature* (*Lond.*) **325**:531–533 (1987).
6. Sladeczek, F., F. Récasens, and J. Bockaert. A new mechanism for glutamate receptor action: phosphoinositide hydrolysis. *Trends Neurosci.* **11**:545–549 (1988).
7. Palmer, E., D. T. Monaghan, and C. W. Cotman. *Trans*-ACPD, a selective agonist of the phosphoinositide-coupled excitatory amino acid receptor. *Eur. J. Pharmacol.* **166**:585–587 (1989).
8. Schoepp, D. D., and B. G. Johnson. Excitatory amino acid agonist-antagonist interactions at 2-amino-4-phosphonobutyric acid-sensitive quisqualate receptors coupled to phosphoinositide hydrolysis in slices of rat hippocampus. *J. Neurochem.* **50**:1605–1613 (1988).
9. Schoepp, D. D., and B. G. Johnson. Inhibition of excitatory amino acid-stimulated phosphonositide hydrolysis in the neonatal rat hippocampus by 2-amino-3-phosphonoproprionate. *J. Neurochem.* **50**:1865–1870 (1989).
10. Ambrosini, A., and J. Meldolesi. Muscarinic and quisqualate-induced phosphoinositide hydrolysis in primary cultures of striatal and hippocampal neurons: evidence for differential mechanism of activation. *J. Neurochem.* **53**:825–833, (1988).
11. Glaum, S. R., J. A. Holzwarth, and R. J. Miller. Glutamate receptors activate Ca^{2+} mobilization and Ca^{2+} influx into astrocytes. *Proc. Natl. Acad. Sci. USA* **87**:3454–3458 (1990).
12. Nicoletti, F., J. T. Wroblewski, E. Fadda, and E. Costa. Pertussis toxin inhibits signal transduction at a specific metabotropic glutamate receptor in primary cultures of cerebellar granule cells. *Neuropharmacology* **27**:551–556 (1988).
13. Dudek, S. M., W. D. Bowen, and M. F. Bear. Postnatal changes in glutamate-stimulated phosphoinositide turnover in rat neocortical synaptoneurosomes. *Dev. Brain Res.* **47**:123–128 (1989).
14. Guiramand, J., I. Sassetti, and M. Recasens. Developmental changes in the chemosensitivity of rat brain synaptoneurosomes to excitatory amino acids, estimated by inositol phosphate formation. *Int. J. Dev. Neurosci.* **7**:257–266 (1989).
15. Schoepp, D. D., and C. C. Hillman. Developmental and pharmacological characterization of quisqualate, ibotenate and *trans*-1-amino-1,3-cyclopentanedicarboxylic acid stimulations of phosphoinositide hydrolysis in rat cortical brain slices. *Biogenic Amines* **7**:331–340 (1990).
16. Dudek, S. M., and M. F. Bear. A biochemical correlate of the critical period for synaptic modification in the visual cortex. *Science* (*Washington D. C.*) **246**:673–675 (1989).
17. Nicoletti, F., J. T. Wroblewski, H. Alho, C. Eva, E. Fadda, and E. Costa. Lesions of putative glutamatergic pathways potentiate the increase of inositol phospholipid hydrolysis elicited by excitatory amino acids. *Brain Res.* **436**:103-109 (1987).
18. Akiyama, K., Y. Norihito, and S. Mitsumoto. Increase in ibotenate-stimulated phosphatidylinositol hydrolysis in slices of the amygdala/pyriform cortex and hippocampus of rat by amygdala kindling. *Exp. Neurol.* **98**:499–508 (1987).
19. Iadarola, M. J., F. Nicoletti, J. R. Naranjo, and E. Costa. Kindling enhances the stimulation of inositol phospholipid hydrolysis elicited by ibotenic acid in rat hippocampus. *Brain Res.* **374**:174–178 (1986).
20. Aronica, E., U. Frey, M. Wagner, H. Schroeder, M. Krug, H. Ruthrich, M. V. Catania, F. Nicoletti, and K. G. Reymann. Enhanced sensitivity of "metabotropic" glutamate receptors after induction of long-term potentiation in rat hippocampus. *J. Neurochem.* **57**:376–383 (1991).
21. Nicoletti, F., C. Valerio, C. Pellegrino, F. Drago, U. Scapagnini, and P. L. Canonico. Spatial learning potentiates the stimulation of phosphoinositide hydrolysis by excitatory amino acids in rat hippocampal slices. *J. Neurochem.* **51**:725–729 (1988).
22. Houamed, K. M., J. L. Kuijper, T. L. Gilbert, B. A. Haldeman, P. J. O'Hara, E. R. Mulvihill, W. Almers, and F. S. Hager. Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science* (*Washington D. C.*) **252**:1318–1321 (1991).
23. Masu, M., Y. Tanabe, K. Tsuchida, R. Shigemoto, and S. Nakanishi. Sequence and expression of a metabotropic glutamate receptor. *Nature* (*Lond.*) **349**:760–765 (1991).
24. Berridge, M. J., C. P. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**:587–597 (1982).
25. Borg, J., B. Spitz, G. Hamel, and J. Mark. Selective culture of neurons from rat cerebral cortex: morphological characterization, glutamate uptake and related enzymes during maturation in various culture media. *Dev. Brain Res.* **18**:37–49 (1985).
26. Avola, R., D. F. Condorelli, S. Surrentino, L. Turpeenoja, A. Costa, and A. M. Giuffrida Stella. Effect of epidermal growth factor and insulin on DNA, RNA and cytoskeletal proteins labelling in primary rat astroglial cell cultures. *J. Neurosci. Res.* **19**:230–238 (1988).
27. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159 (1987).
28. Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes. Isolation and characterization of full-length cDNA clones for human *alpha-*, *beta-* and *gamma*-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. *Mol. Cell Biol.* **3**:787–795 (1983).
29. Feinberg, A. P., and B. Vogelstein. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**:266–267 (1984).
30. Blackstone, C. D., S. Supattapone, and S. H. Snyder. Inositol phospholipid-linked glutamate receptors mediate parallel-fiber-Purkinje-cell synaptic transmission. *Proc. Natl. Acad. Sci. USA* **86**:4316–4320 (1989).
31. Desai, M. A., and P. J. Conn. Selective activation of phosphoinositide hydrolysis by a rigid analogue of glutamate. *Neurosci. Lett.* **109**:157–162 (1990).
32. Gilman, A. G. G protein and dual control of adenylate cyclase. *Cell* **36**:577–579 (1984).
33. Ui, M. Islet activation protein, pertussis toxin: a probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends Pharmacol. Sci.* **5**:277–279 (1984).
34. Murphy, S. N., J. A. Holzwarth, and R. J. Miller. Quisqualic acid-mediated intracellular calcium mobilization is inhibited by phorbol ester but not by pertussis toxin. *Soc. Neurosci. Abstr.* **15**:277–279 (1989).
35. Hwang, P. M., D. S. Bredt, and S. H. Snyder. Autoradiographic imaging of phosphoinositide turnover in the brain. *Science* (*Washington D. C.*) **249**:802–804 (1990).
36. Tanabe, W., M. Matsu, T. Ishii, R. Shigemoto, and S. Nakanishi, A family of metabotropic glutamate receptors. *Neuron* **8**:1–20 (1992).

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