ELSEVIER

European Journal of Pharmacology 338 (1997) R1–R2

ejp

Rapid communication

Metabotropic glutamate receptor agonists stimulate polyphosphoinositide hydrolysis in primary cultures of rat hepatocytes

Francesc Sureda [a], Agata Copani [b], Valeria Bruno [a], Thomas Knöpfel [c], Gainer Meltzger [d], Ferdinando Nicoletti [a,b,*]

[a] *I.M.N., Pozzilli, Italy*
[b] *Institute of Pharmacology, School of Pharmacy, University of Catania, Viale A. Doria 6, 95125 Catania, Italy*
[c] *IRCCS S. Lucia, Rome, Italy*
[d] *Cell Control, Martinsried, Germany*

Received 21 August 1997; accepted 26 August 1997

Abstract

The metabotropic glutamate (mGlu) receptor agonists, quisqualate and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), but not (*RS*)-3,5-dihydroxyphenylglycine or (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine, stimulated [^{3}H]inositolmonophosphate ([^{3}H]InsP) formation in primary cultures of rat hepatocytes. 1*S*,3*R*-ACPD-stimulated [^{3}H]InsP formation was inhibited by α-methyl-4-carboxyphenylglycine, indicating that cultured hepatocytes express functional mGlu receptors coupled to polyphosphoinositide hydrolysis. The identity of these receptors is not similar to that of any of the known mGlu receptor subtypes characterized in heterologous expression systems. © 1997 Elsevier Science B.V.

Keywords: Metabotropic glutamate receptor; Hepatocyte, cultured; Polyphosphoinositide hydrolysis

Three groups of G-protein-coupled metabotropic glutamate (mGlu) receptors have been identified in the nervous system by molecular cloning and pharmacological studies. Group I includes $mGlu_1$ and $-_5$ receptors, which are coupled to polyphosphoinositide hydrolysis, whereas group II ($mGlu_2$ and $-_3$) and group III ($mGlu_4$, $-_6$, $-_7$ and $-_8$) mGlu receptors are negatively linked to adenylate cyclase (reviewed in Pin and Duvoisin, 1995). While ionotropic glutamate receptors have been found in the guinea pig myenteric plexus (Moroni et al., 1986) and in rat pancreatic islet cells (Inagaki et al., 1995), there is no evidence for the presence of mGlu receptors in peripheral organs, with the exception of $mGlu_4$ receptors, which are present in taste buds (Chaudhari et al., 1996). We now report that activation of mGlu receptors stimulates polyphosphoinositide hydrolysis in primary cultures of rat hepatocytes.

Adult rat hepatocytes were isolated by collagenase perfusion, plated onto Falcon Primaria 24-multiwell dishes (3×10^6 cells/well) and grown up to 5 days under conditions supporting survival and a liver-specific phenotype (Ham's F12 medium containing 10% foetal calf serum, 0.2% albumin, 100 nM insulin and 100 nM dexamethasone) (according to the methods of Guguen-Guillouzo et al., 1986 and Kimball et al., 1995, slightly modified). Culture medium was changed every day. Cultures were incubated overnight with 1 μCi/ml of myo-2-[^{3}H]inositol (NEN-DuPont, sp. act. 16.5 Ci/mmol) to label inositol phospholipids. Cultures were then washed in Krebs–Henseleit buffer (equilibrated with O_2/CO_2 to pH 7.4) and incubated in the same buffer containing 10 mM Li^+. After 30 min of incubation in the presence of transmitter receptor agonists, the reaction was terminated by the addition of methanol:water (1:1 v/v). [^{3}H]inositolmonophosphate ([^{3}H]InsP) was extracted, separated and detected as described previously (Nicoletti et al., 1986).

In cultures at 2 days in vitro [1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 100 μM) and quisqualate (100 μM) increased [^{3}H]InsP formation by about 80%. The action of 1*S*,3*R*-ACPD was concentration-dependent with an apparent EC_{50} value of 25 μM and a maximal stimulation at 100 μM. At 5 days in vitro, stimulation of polyphosphoinositide hydrolysis by both mGlu receptor agonists was maintained, albeit reduced by half; (*RS*)-3,5-dihydroxyphenylglycine (DHPG) and (2*S*,3*S*,4*S*)-α-(carboxycyclopropyl)glycine (L-CCG-I)

* Corresponding author at address b. Tel./Fax: (39-95) 337-167.

Table 1
Stimulation of polyphosphoinositide hydrolysis by mGlu receptor agonists in cultured hepatocytes at 2 or 5 days in vitro

	[^{3}H]InsP formation (dpm/mg protein)
2 days in vitro	
Basal	6075 ± 202
1*S*,3*R*-ACPD (100 μM)	11325 ± 150 [a]
Quisqualate (100 μM)	10800 ± 675 [a]
5 days in vitro	
Basal	4162 ± 225
1*S*,3*R*-ACPD (100 μM)	5887 ± 350 [a]
Quisqualate (100 μM)	5650 + 120 [a]
DHPG (100 μM)	3973 ± 360
L-CCG-I (100 μM)	3862 ± 187
MCPG (500 μM)	4312 ± 185
1*S*,3*R*-ACPD + MCPG	3900 ± 150 [b]

Values are means ± S.E.M. of 4–8 determinations. $P < 0.01$, if compared to the respective basal (a) or 1*S*,3*R*-ACPD (b) values (one-way analysis of variance + Fisher's test to isolate the differences). MCPG was added to the cultures 1 min before 1*S*,3*R*-ACPD. MCPG was inactive at 100 μM (not shown).

were inactive. 1*S*,3*R*-ACPD-stimulated polyphosphoinositide hydrolysis was inhibited by the mixed mGlu receptor antagonist, α-methyl-4-carboxyphenylglycine (MCPG, 500 μM) (Table 1).

These results indicate that cultured hepatocytes express functionally active mGlu receptors coupled to polyphosphoinositide hydrolysis. In recombinant cells, DHPG behaves as a pure agonist of $mGlu_1$ and $-_5$ receptors whereas quisqualate activates $mGlu_1$ and $-_5$ receptors with high potency and $mGlu_3$ receptors with lower potency and 1*S*,3*R*-ACPD and L-CCG-I activate both group I and II mGlu receptors with low and high potency, respectively (Pin and Duvoisin, 1995). The lack of effect of DHPG and L-CCG-I suggests that the activation of polyphosphoinositide hydrolysis in cultured hepatocytes is not mediated by conventional $mGlu_1$ or $-_5$ receptors and does not result from functional synergism between group I and II mGlu receptor subtypes. We speculate that cultured hepatocytes express a novel mGlu receptor coupled to polyphosphoinositide hydrolysis, the identity of which remains to be determined.

References

Chaudhari, N., Yang, H., Lamp, C., Delay, E., Cartford, C., Than, T., 1996. The taste of monosodium glutamate: Membrane receptors in taste buds. J. Neurosci. 16, 3817–3828.

Guguen-Guillouzo, C., Bourel, M., Guillouzo, A., 1986. Human hepatocyte cultures. Prog. Liver Dis. 8, 33–50.

Inagaki, N., Kuromi, H., Gonoi, T., Okamoto, Y., Ishida, H., Seino, Y., Kaneko, T., Iwanaga, T., Seino, S., 1995. Expression and role of ionotropic glutamate receptors in pancreatic islet cells. FASEB J. 9, 686–691.

Kimball, S.R., Horetsky, R.L., Jefferson, L.S., 1995. Hormonal regulation of albumin gene expression in primary cultures of rat hepatocytes. Am. J. Physiol. 268, 6–14.

Moroni, F., Luzzi, S., Franchi-Micheli, S., Zilletti, L., 1986. The presence of *N*-methyl-D-aspartate-type receptors for glutamic acid in the guinea pig myenteric plexus. Neurosci. Lett. 68, 57–62.

Nicoletti, F., Wroblewski, J.T., Novelli, A., Alho, H., Guidotti, A., Costa, E., 1986. The activation of inositol phospholipid metabolism as a signal transduction system for excitatory amino acids in primary cultures of cerebellar granule cells. J. Neurosci. 6, 1905–1911.

Pin, J.-P., Duvoisin, R., 1995. The metabotropic glutamate receptors: Structure and functions. Neuropharmacology 34, 1–26.