Neuroscience Letters, 109 (1990) 146–151
Elsevier Scientific Publishers Ireland Ltd.

NSL 06602

The glutamate receptor of the Q_p-type activates protein kinase C and is regulated by protein kinase C

Olivier J.J. Manzoni[1], Françoise Finiels-Marlier[2], Isabelle Sassetti[1], Joël Blockaert[1], Christian le Peuch[2] and Fritz A.J. Sladeczek[1]

[1]*Centre CNRS–INSERM de Pharmacologie–Endocrinologie, Montpellier (France) and* [2]*Centre de Recherches de Biochimie macromoléculaire, CNRS LP8402 and INSERM U249, Montpellier (France)*

(Received 28 June 1989; Revised version received 28 August 1989; Accepted 4 September 1989)

Key words: Glutamate receptor; Metabotropic quisqualate receptor; Protein kinase C; Activation; Phosphoinositide metabolism; Primary culture of striatal neurons

In striatal neurons in primary culture quisqualate potently stimulated the formation of inositol phosphates via a metabotropic receptor we recently termed Q_p in order to distinguish it from the classical ionotropic quisqualate receptor termed Q_i. Here we show that 10 μM of quisqualate activated in a rapid and transient manner protein kinase C as assessed by its translocation from the cytosolic to the membrane fraction. As 10 μM α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), the Q_i specific agonist, was without effect, this translocation was most probably mediated by the Q_p receptor. Phorbol 12,13-dibutyrate blocked in a dose-dependent manner the Q_p receptor-induced inositol phosphate formation ($IC_{50} = 2 \pm 0.4$ nM). The inactive ester 4α-phorbol-12,13-didecanoate was without effect. Very low concentrations of staurosporine completely reversed the phorbol 12,13-dibutyrate-induced blockade ($IC_{50} = 2.2 \pm 1.3$ nM). It can therefore be concluded that the Q_p receptor is able to activate protein kinase C and that the activity of this metabotropic receptor is regulated by protein kinase C.

The classical action of excitatory amino acids is the activation of different types of ion channels via different receptors. These receptors had been termed following their specific agonists *N*-methyl-D-aspartate, kainate and quisqualate (Q) receptors [18]. A certain amount of recent evidence suggests that the kainate and Q receptor might in fact be the same molecular entity [10]. We have previously demonstrated that in addition to these ionotropic receptors, excitatory amino acid receptors are able to stimulate the formation of inositol phosphates via a Q receptor [12, 14]. In a recent review we termed this new metabotropic receptor Q_p and the classical ionotropic receptor Q_i [15]. Similar findings have now been reported in other systems (see review in ref. 15). A second metabotropic excitatory amino acid receptor discovered

Correspondence: F.A.J. Sladeczek, Centre CNRS–INSERM de Pharmacologie–Endocrinologie, Rue de la Cardonille, F-34094 Montpellier Cedex 2, France.

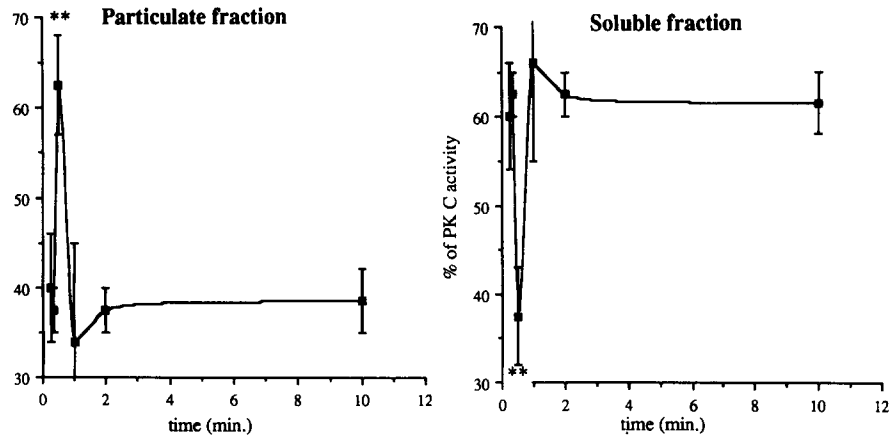

Fig. 1. Time course of the quisqualate-induced redistribution of protein kinase C activity. 14 DIV striatal neurons were incubated for designated times with 10 μM quisqualate. Protein kinase C activity was assayed in the particulate (left figure) and in the soluble fraction (right figure). Values represent mean $\pm$ S.E.M. of 3 independent determinations. Statistical significance was determined using Student's t-test. $^{**}P < 0.01$ as compared to control.

in the hippocampus [8] has a slightly different pharmacology from the Q_p receptor. This receptor was most potently stimulated by ibotenate and could be blocked by 2-amino-4-phosphonobutyrate (APB). A link between these metabotropic receptors and protein kinase C has not been reported yet.

Cultured striatal neurons were prepared and inositol phosphate formation was measured as previously described [14]. For protein kinase C assay, cultures were treated with phorbol esters or drugs in the same buffer used in inositol phosphate experiments but in absence of LiCl. Incubations were stopped by removing the medium and putting the cultures on ice. The cells were scraped in 1.0 ml per well of cold buffer A containing 20 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid], pH 7.4, 2 mM ethylene glycol-bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid, 2 mM phenylmethylsulfonyl fluoride, 2 mM D,L-dithiothreitol, 10 μg/ml soybean trypsin inhibitor, 10 μg/ml pepstatin, 10 μg/ml leupeptin, 25 μg/ml aprotinin. The cells were disrupted mechanically with a teflon-glass homogenizer and centrifuged 25 min at 50,000 *g*. The soluble fraction was saved and the pellet resuspended in buffer A containing 0.1% Triton X-100. The suspension was kept during 45 min at 0°C, and then centrifuged for 25 min at 50,000 *g*. The resulting supernatant contained solubilized membrane components and is called the particulate fraction. For protein kinase C assay, both fractions were adjusted to contain 0.05% Triton. Their kinase activities were tested in the presence and in the absence of phorbol ester and phosphatidylserine. Assays (final volume 100 μl) contained 20 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid], pH 7.5, 1 mg/ml Histones III S as substrates, 80 μg/ml phosphatidylserine, 1 μM phorbol 12-myristate 13-acetate (PMA), 10 mM $MgCl_2$, 1.83 mM $CaCl_2$, 2 mM ethylene glycol-bis-(β-aminoethyl ether)-

N,N,N',N'-tetraacetic acid, 1 mM D,L-dithiothreitol, 0.01 % Triton X-100, 0.1 mM [γ-^{32}P]ATP (spec. act. 300 cpm/pmol) and 20 μl of soluble or particulate fractions. The reaction was initiated by addition of the [γ-^{32}P]ATP. After 5 min of incubation, a 50 μl aliquot was spotted on Whatman P81 cation exchange paper. Papers were washed 3 times in ice-cold water, dried and counted by liquid scintillation spectrometry. Protein kinase C activity was defined as the difference between the activities measured in the presence and absence of PMA and phosphatidylserine.

Activation of protein kinase C was determined by measuring its translocation. In striatal neurons under basal conditions 37.2 ± 2.9 % ($n=4$) of the total cellular protein kinase C activity was found in the particulate fraction and 62.8 ± 2.9 % ($n=4$) in the soluble fraction. Treatment of the cultures during 10 min with 100 nM of phorbol 12,13-dibutyrate increased the membrane bound activity to 64.8 ± 4.5% ($n=4$) and decreased the soluble activity to 35.2 ± 5.5% ($n=4$). Total kinase activities were 3.86 ± 0.38 nmol PO_4/min/mg protein ($n=4$) and 5.82 ± 1.013 nmol PO_4/min/mg protein ($n=4$) in control and phorbol 12,13-dibutyrate-treated cells, respectively.

The Q-induced translocation of protein kinase C was very rapid and transient. At 30 s there was a significant increase in the membrane fraction and a significant decrease of the cytosolic fraction (Fig. 1). 10 μM of the Q_i selective agonist AMPA had no effect on the distribution of protein kinase C activity at 30 s (data not shown). The time course of the Q-induced protein kinase C translocation was very different to the one reported for phorbol 12,13-dibutyrate [19]. The phorbol 12,13-dibutyrate-

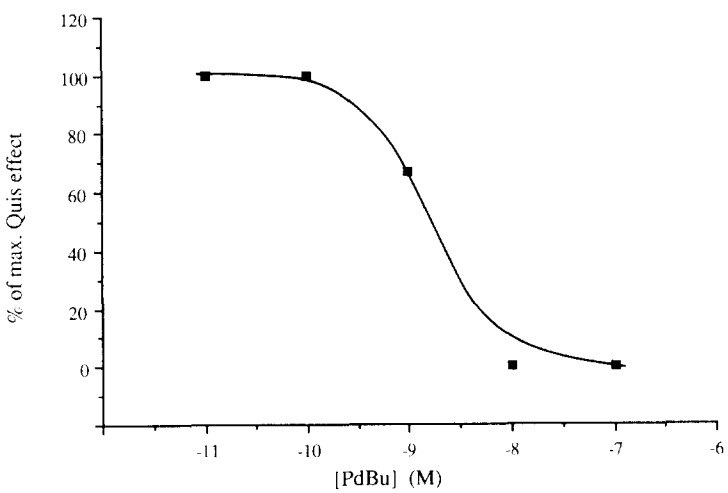

Fig. 2. Dose-response relationship for phorbol 12,13-dibutyrate action on quisqualate induced inositol phosphate formation. Inositol phosphate formation was examined as described in the text. Phorbol 12,13-dibutyrate was added to obtain the indicated final concentrations, 10 min prior to the 30 min stimulation period with 10 μM quisqualate. Phorbol 12,13-dibutyrate did not alter basal formation of inositol phosphates, at the maximal concentration tested (0.1 μM). The action of 0.1 μM phorbol 12,13-dibutyrate was not mimicked by 0.1 μM 4α-phorbol 12,13-didecanoate, a non-tumor-promoting phorbol ester. Results are means of duplicate determinations and the experiment shown was typical for 2 others.

induced translocation reached its maximal value 5 min after phorbol 12,13-dibutyrate addition and remained constant thereafter [19]. A rapid and transient effect similar to the one shown in Fig. 1 has also been observed after activation of other transmembrane receptors as for example the muscarinic cholinoceptor [17] or the receptor for thyrotropin releasing hormone [2]. An explanation for these different types of kinetics could be that very rapidly after stimulation of phosphoinositidase by a transmembrane receptor there is an activation of a second enzyme able to inhibit protein kinase C. Sphingomyelinase is a good candidate for such a role. This enzyme cuts sphingomyelin into ceramide and phosphocholine. Ceramide is then transformed into sphingosine, thought to be the endogenous inhibitor of protein kinase C (for a review see ref. 4). Phorbol esters do not stimulate this shut off pathway and induce therefore a long lasting translocation of protein kinase C.

Activation of protein kinase C by phorbol esters leads in general to the inhibition of hormone or neurotransmitter receptor-induced inositol phosphate formation in a great number of tissues (see for example ref. 13). In Fig. 2 we show that phorbol 12,13-dibutyrate inhibited in a dose-dependent manner the Q_p receptor-evoked inositol phosphate-formation ($IC_{50} = 2 \pm 0.4$ nM). One hundred nM of the inactive phorbol ester 4α-phorbol-12,13-didecanoate did not decrease significantly the Q_p receptor stimulated inositol phosphate formation (data not shown). It has recently been shown that phorbol esters and other diacylglycerol analogs are able to depress Ca^{2+} currents in chick sensory neurons [3]. The protein kinase C inhibitors staurosporine, sphingosine and H-7 did not reverse this Ca^{2+} current depression. This effect

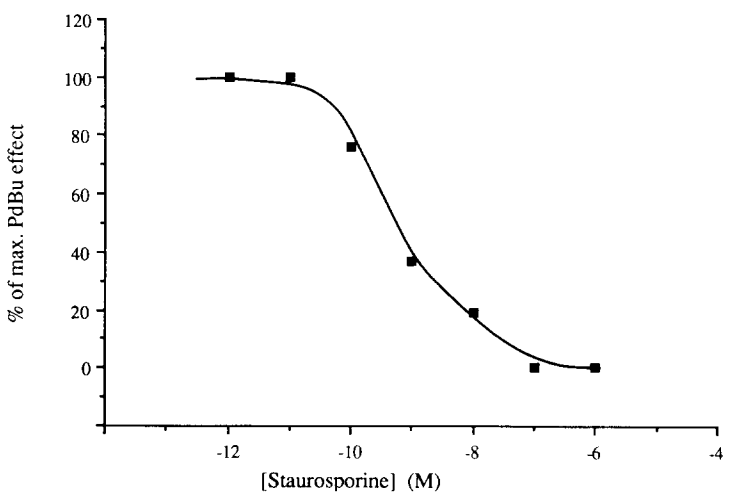

Fig. 3. Dose–response relationship for staurosporine inhibition of phorbol 12,13-dibutyrate blockade. Staurosporine was added to obtain the indicated final concentrations, 4 min prior to and 10 nM phorbol 12,13-dibutyrate 2 min prior to the 30 min stimulation period with 10 μM quisqualate. Staurosporine did not alter both basal and quisqualate induced inositol phosphate formation. The results are means of duplicate determinations and the experiment shown was typical for 2 others.

of phorbol esters was therefore not due to an activation of protein kinase C. In view of this report it was essential to confirm our finding by testing the effect of staurosporine on the phorbol 12,13-dibutyrate-induced blockade of the Q_p response. Already very low concentrations of staurosporine reversed the phorbol 12,13-dibutyrate blockade ($IC_{50} = 2.2 \pm 1.3$ nM; Fig. 3) indicating that the phorbol 12,13-dibutyrate-induced inhibition of the Q_p receptor mediated inositol phosphate formation was due to an activation of protein kinase C and not to a side effect of the phorbol ester.

What could be the physiological role of metabotropic excitatory amino acid receptor activation leading to an increase of the inositol phosphate formation and, as demonstrated for the first time in the present study, to an activation of protein kinase C? It has been shown that the inositol phosphate effects mediated by the Ibo preferring receptor in the hippocampus and by the Q_p receptor in rat brain synaptoneurosomes (see review in ref. 15) were highest during the major period of synaptogenesis and remained detectable in the adult. The great activity of metabotropic excitatory amino acid receptors in the developing brain as well as the observation that there were high concentrations of protein kinase C and its substrates (as for example the brain-specific protein kinase C substrate termed F1 having a molecular weight of 47 kDa [7] in growth cone-rich regions of the central nervous system [6] may suggest a role for these receptors in synaptogenesis.

The metabotropic excitatory amino acid receptors could also be implicated in the long-term potentiation (LTP) phenomenon. LTP is a long-lasting increase in synaptic efficacy induced by high-frequency stimulation of specific pathways in hippocampus. It has been shown that excitatory amino acids mediate this effect (for reviews see refs. 1, 16). There is a lot of evidence that activation of protein kinase C is necessary for the maintenance of LTP [5, 11]. Our finding that metabotropic excitatory amino acid receptors of the Q_p type are able to activate protein kinase C indicates that this type of receptor might be implicated in this phenomenon.

This work was supported by grants from INSERM, CNRS, Bayer (France) and DRET 88/163.

1 Collingridge, G.L. and Bliss, T.V.P., NMDA receptors — their role in long-term potentiation, Trends Neurosci., 10 (1987) 288–293.

2 Drust, D.S. and Martin, T.F.J., Protein kinase C translocates from cytosol to membrane upon hormone activation: effects of thyrotropin-releasing hormone in GH_3 cells, Biochem. Biophys. Res. Commun., 128 (1985) 531–537.

3 Hockberger, P., Toselli, M., Swandulla, D. and Lux, H.D., A diacylglycerol analogue reduces neuronal calcium currents independently of protein kinase C activation, Nature (Lond.), 338 (1989) 340–342.

4 Hunnun, Y.A. and Bell, R.M., Functions of sphingolipids and sphingolipid breakdown products in cellular regulation, Science, 243 (1989) 500–507.

5 Lovinger, D.M. and Routtenberg, A., Synapse-specific protein kinase C activation enhances maintenance of long-term potentiation in rat hippocampus, J. Physiol. (Lond.), 400 (1988) 321–333.

6 Nagle, D.S., Jake, S., Castagna, M. and Blumberg, P.M., Variation with embryonic development and regional localization of specific [^{3}H]phorbol 12,13-dibutyrate binding to brain, Cancer Res., 41 (1981) 89–93.

7 Nelson, R.B., Linden, D.J., Hyman, C., Pfenninger, K.H. and Routtenberg, A., The two major phosphoproteins in growth cones are probably identical to two protein kinase C substrates correlated with persistence of long-term potentiation, J. Neurosci., 9 (1989) 381–389.
8 Nicoletti, F., Meek, J.L., Iadarola, M.J., Chuang, D.M., Roth, B.L. and Costa, E., Coupling of inositol phospholipid metabolism with excitatory amino acid recognition sites in rat hippocampus, J. Neurochem., 46 (1986) 40–46.
9 Nicoll, R.A., Kauer, J.A. and Malenka, R.C., The current excitement in long-term potentiation, Neuron, 1 (1988) 97–103.
10 Pin, J.P., Van Vliet, B.J. and Bockaert, J., Complex interaction between quisqualate and kainate receptors as revealed by measurement of GABA release from striatal neurons in primary culture, Eur. J. Pharmacol., 172 (1989) 81–91.
11 Reymann, K.G., Brödemann, R., Kase, H. and Matthies, H., Inhibitors of calmodulin and protein kinase C block different phases of hippocampal long-term potentiation, Brain Res., 461 (1988) 388–392.
12 Sladeczek, F., Putative role of inositol phospholipid metabolism in neurons, Biochimie, 69 (1987) 287–296.
13 Sladeczek, F., Kirk, C.J., Bockaert, J. and Schmidt, B.H., Non-classical multiple-site interaction of (^{3}H)-prazosin with the α_1-adrenoceptor of intact BC_3H_1 cells, Br. J. Pharmacol., 97 (1989) 1101–1110.
14 Sladeczek, F., Pin, J.P., Récasens, M., Bockaert, J. and Weiss, S., Glutamate stimulates inositol phosphate formation in striatal neurones, Nature (Lond.), 317 (1985) 717–719.
15 Sladeczek, F., Récasens, M. and Bockaert, J., A new mechanism for glutamate receptor action: phosphoinositide hydrolysis, Trends Neurosci., 11 (1988) 545–549.
16 Smith, S.J., Progress on LTP at hippocampal synapses: a post synaptic Ca^{2+} trigger for memory storage?, Trends Neurosci., 10 (1987) 142–144.
17 TerBush, D.R., Bittner, M.A. and Holz, R.W., Ca^{++} influx causes rapid translocation of protein kinase C to membranes, J. Biol. Chem., 263 (1988) 18873–18879.
18 Watkins, J.C. and Evans, R.H., Excitatory amino acid transmitters, Annu. Rev. Pharmacol. Toxicol., 21 (1981) 165–204.
19 Weiss, S., Ellis, J., Hendley, D.D. and Lenox, R.H., Translocation and activation of protein kinase C in striatal neurons in primary culture: relationship to phorbol dibutyrate actions on the inositol phosphate generating system and neurotransmitter release, J. Neurochem., 52 (1989) 530–536.