IMPORTANCE OF PROTEIN KINASE C EPSILON FOR THE ALLOSTERIC MODULATION OF GABA EVOKED CURRENTS IN CORTICAL CULTURE AT DIFFERENT DIV

Garzon Giorgio, Baraldi Mario, Puja Giulia

Dept. of BioMedical Sciences, University of Modena and Reggio Emilia, Via G. Campi 287, 41100 Modena, ITALY. Author for correspondence: Garzon Giorgio, tel +39-059-2055384; fax +39-059-2055376; e-mail: garzon.giorgio@unimore.it

The subunit composition of $GABA_A$ receptor determines the physiological and pharmacological features of the receptor, i.e. agonist potency, desensitization kinetics, allosteric properties and receptor trafficking. Phosphorilation is another important mechanism to regulate channel response not only to agonist but also to allosteric modulators such as drugs (i.e. benzodiazepines, BZs) or endogenous substances (i.e. neurosteroids, NSs). $GABA_A$ receptor can be phosphorylated by several types of kinase among those PKC, PKA, CamKII or TK. The protein kinase C family comprises of a group of at least 10 different isoenzymes (α, βI, βII, γ, δ,ε, η, θ, ι, and ζ). Behavioural and biochemical experiments showed that mice knock out for a specific PKC, the PKCε, express an increased sensitivity to $GABA_A$ allosteric modulators (ethanol, NSs, barbiturates and BZs) (1).
The aim of our study was to understand the role played by PKCε in the modulation of $GABA_A$ receptor by BZs and NSs in primary culture of cortical cells at different days in vitro (DIV).
For this purpose we either blocked PKCε activity with a selective inhibitor (εV1-2) or we potentiated PKCε function by incubating the cells with (+)-α-methyl-4-carboxyphenylglycine [(+)-MCPG], an antagonist of metabotropic glutamate receptors group I and II, which induces membrane translocation and consequent activation of kinase. Using the patch clamp technique in the whole-cell configuration we analyzed the effect of neurosteroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, ALLO) and of the benzodiazepine Flunitrazepam (FNZ) when the activity of PKCε was decreased or potentiated. In all experiments GABA affinity wasn't changed by the presence of εV1-2 peptide in the recording pipette. The effect of ALLO is more pronounced in cells dyalized with PKCε inhibitor compared to control at 7 DIV but reverse in older cultures (14DIV). Conversely FNZ modulation was decreased in the presence of the inhibitor at 7DIV but didn't change at 14 DIV.
Stimulation of PKCε with (+)-MCPG decreased ALLO potentation, compared to control cultures, at 7DIV but was without effect a 14 DIV. (+)-MCPG treatment increased the activity of FNZ at 7DIV and this effect was reverted by PKCε inhibitor. Our results could be explained by a different expression of $GABA_A$ receptor subunit subtypes or by variation in PKCε levels at 7 vs 14DIV.
In conclusion the allosteric modulation of $GABA_A$ receptors by ALLO and FNZ is dependent on the state of PKCε activation. Physiological events that affect PKCε activity coud alter the modulation of GABAergic neurotrasmission by exogenous or endogenous substances.
In conclusion the allosteric modulation of $GABA_A$ receptors by ALLO and FNZ is dependent on the state of PKCε activation. Physiological and phatological events that affect PKCε activity could alter the modulation of GABAergic neurotrasmission by exogenous or endogenous substances.

1. Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF, Wang D, Sanchez-Perez AM, Messing RO.(1999) *Nature Neurosci*. 2:997-1002