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Brief report

Impairment and reorganization of the phosphoinositide-specific phospholipase C enzymes in suicide brains

Vincenza Rita Lo Vasco [a,*], M. Leopizzi [b], C. Della Rocca [b], P. Fais [c], M. Montisci [d], G. Cecchetto [d]

[a] Sense Organs Department, Policlinico Umberto I, Faculty of Medicine and Dentistry, Sapienza University of Rome, viale del Policlinico 155, 00185 Rome, Italy
[b] Department of Medico-Surgical Sciences and Biotechnology, Sapienza University- Polo Pontino, Rome, Italy
[c] Unit of Forensic Medicine, Department of Public Health and Community, Policlinico G.B. Rossi, Verona University, Verona, Italy
[d] Department of Cardiological, Thoracic and Vascular Sciences, Institute of Legal Medicine, Padova University, Padova, Italy

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ABSTRACT

A number of studies suggested that suicide may be associated with specific neurobiological abnormalities. Neurobiology studies focused upon abnormalities of signalling mechanisms with special regard to the serotonin system and the related Phosphoinositide (PI) signalling system. Previous data suggested the involvement of the PI-specific phospholipase C (PLC) family in neuropsychiatric disorders. By using PCR and morphological microscopy observation we examined the whole panel of expression of PLC isoforms in the brains of 28 individuals who committed suicide and in normal controls in order to evaluate the involvement of specific PLC isoforms. The overall PLC expression was reduced and a complex reorganization of the isoforms was observed. The knowledge of the complex network of neurobiological molecules and interconnected signal transduction pathways in the brain of suicide victims might be helpful to understand the natural history and the pathogenesis of the suicidal behavior. That might lead to obtain prognostic suggestions in order to prevent suicide and to new therapeutic agents targeting specific sites in this signalling cascade.

1. Introduction

About one million people die by suicide worldwide each year, thus suicide is considered a major public health problem (Murphy et al., 2011). Evidences supported by a number of studies suggested that suicide may be associated with specific neurobiological abnormalities. Neurobiology studies focused upon abnormalities in the serotonergic pathway in post-mortem brain of suicide victims (Ressler and Nemeroff, 2000). Recently abnormalities in 5-hydroxytryptamine$_{2A}$ (HT2A) receptor for serotonin aroused great interest. The phosphoinositide (PI)-specific phospholipase C (PLC) signalling system is strictly related to serotonin (Ressler and Nemeroff, 2000; Pacheco and Jope, 1996; Pandey et al., 1999).

Evidences indicated that PLC enzymes are involved in the complex processes of neurite outgrowth and neuron positioning (Jang et al., 2013). PLC isoforms also participate in neurotrophin-mediated neuron functions and seem to be involved in brain development as well as in synaptic transmission (Jang et al., 2013). Abnormal expression and/or altered activation of PLC enzymes were/was suggested in a number of brain disorders, including epilepsy, mood disorders, Huntington's disease and Alzheimer's disease (Jang et al., 2013). Further studies revealed abnormalities in transcription factors and in their target genes (Numakawa et al., 2014). The most important gene seems to be the brain-derived neurotrophic factor (BDNF), which has been studied in suicide, as well as in schizophrenia. Interestingly, BDNF and its receptor TrkB induce glutamate release through activation of a PLC-dependent pathway in developing cultured cortical neurons (Numakawa et al., 2014).

The PLC family of enzymes is constituted from 13 isoforms divided into six subfamilies (β, γ, δ, ε, ζ and η) (Suh et al., 2008; Bunney and Katan, 2011; Katan, 2005; Nakamura and Fukami, 2009; Hisatsune et al., 2005). Activation of Gq-coupled 5-HT2A receptors results in PLC-catalyzed hydrolysis of membrane PI (Aghajanian and Sanders-Bush, 2002; Pandey et al., 1999), namely the phosphatidylinositol 4,5 bisphosphate (PIP2). That generates two crucial second messengers, inositol(1,4,5) triphosphate (IP3) and diacylglycerol (DAG) (Suh et al., 2008). IP3 plays an important role in a number of physiological processes by releasing calcium from intracellular stores. DAG activates protein kinase C (PKC) enzymes, which, in turn, phosphorylate target proteins in the cell.

* Tel.: +39 0649976814; fax: +39 0649976817.
E-mail address: ritalovasco@hotmail.it (V.R. Lo Vasco).

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The literature data described the involvement of PLC enzymes in suicide, with special regard to PLC β1 isoform (Pacheco et al., 1996a, Pandey et al., 1999; Pandey 2013a). Involvement of PLC enzymes in schizoaffective disorders was also suggested (Pandey 2013b; Lo Vasco et al., 2013a; Lo Vasco, 2012; Udawela et al., 2011).

Evidences demonstrated that the PLC enzymes are selectively expressed depending on the cell type and that each tissue owns a specific panel of expression, which may vary under different conditions, such as inflammation or tumor enhancement and progression (Lo Vasco et al., 2012a; Udawela et al., 2011; Nakamura et al., 2013; Lo Vasco et al., 2014a, b; Lo Vasco et al., 2013b, 2013c; Lo Vasco et al., 2012b; Lo Vasco, 2010 and Lo Vasco et al., 2010, 2007).

In the present experiments, we examined the post-mortem brains of 28 individuals who committed suicide and of 18 normal controls in order to compare the panel of expression PLC isoforms.

The knowledge of the complex network of molecules belonging to different signal transduction pathways in the brain of suicide victims might provide helpful insights in the suicidal behavior. That might contribute prognostic suggestions in order to prevent suicide, also paving the way to new therapeutic agents targeting specific sites in this signalling cascade.

2. Materials and methods

Brain cortex samples of 30 suicide victims and of 18 normal controls, collected following the ethical guidelines of the Ethics Committee of the Padua University Medicine School, were analyzed. Two out 30 suicide brain samples were not used as cDNA amplification was not possible, probably due to overfixation time. Twenty out 28 suicide victims were referred to present with major depression symptoms and 16 out 20 were suggested antidepressants assumption. Five out 28 subjects had attempted suicide before; for 2 out 28 substance abuse (1 cocaine and 1 alcohol) was referred. Brain samples were obtained from post-mortem autopsies performed in the Section of Legal Medicine of Padua University. The formalin fixed paraffin embedded specimens, stored at room temperature, were cut into 6–10-μm sections, RNA was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Ambion Inc., Austin, TX) as previously described (Lo Vasco et al. 2012a) following the manufacturer's indications. The concentration and quality of the RNA obtained was monitored using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Inc. USA).

RNA was reverse-transcribed into cDNA using High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Foster City, CA, USA) following the manufacturer's indications. Briefly, 2 μg RNA was incubated with 2 μl of 10× Reverse Transcription Buffer, 0.8 μl of 25× dNTPs (100 mM), 2 μl of 10× random primers, 1 μl of MultiScribeTM Reverse Transcriptase (50 U/μl) and 3.2 μl of DNase-free water. 10 μl of diluted sample RNA to a final volume of 20 μl and reverse transcribed 10 min at 25 °C, 120 min at 37 °C and 5 min at 85 °C in Gene Amp® PCR System 9700 thermocycler (Applied Biosystems). The primer pairs for each PLC isoform (Bio Basic Inc, Amherst, New York, USA) are listed in Table 1. The specificity of the primers was verified by searching in the NCBI database for possible homology to cDNAs of unrelated proteins. To amplify glyceraldehyde 3 phosphate dehydrogenase (*GAPDH*) (Bio Basic Inc, Amherst, New York, USA) the following primer pair was used: forward 5′-CGAGATCCCTCCAAAATCAA-3′ reverse 5′-GTCTTCTGGGTGGCAGTGAT-3′.

Standard analytical PCR reaction was performed with GoTaq Master Mix (Promega; Madison, WI, USA) as previously described (Lo Vasco et al., 2014b) following manufacturer's indications. Cycling conditions were conducted as follows: initial denaturation step at 95 °C for 1 min, 40 cycles consisting of denaturation (30 s) at 95 °C, annealing (30 s) at the appropriate temperature for each primer pair and extension (1 min) at 72 °C in Gene Amp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA).

Electrophoresis of the PCR products in 1.5% TAE ethidium bromide-stained agarose gel revealed single DNA bands with nucleotide lengths as expected for each primer pair. RNA samples were also amplified by PCR without RT. No band was observed, excluding DNA contamination during the procedure. Experiments were independently repeated at least 3 times for each line.

2.1. Immunohistochemistry

Immunofluorescence microscopy was performed in order to confirm the results of PCR experiments and to visualize the localization of selected PLC enzymes within the cell.

Table 1
List of PCR primers for *PLC* genes.

PI-PLC β1 (PLCB1; OMIM ⁎607120)	forward 5′-AGCTCTCAGAACAAGCCTCCAACA-3′ reverse 5′-ATCATCGTCGTCGTCACTTTCCGT-3′
PI-PLC β2 (PLCB2; OMIM ⁎604114)	forward 5′-AAGGTGAAGGCCTATCTGAGCCAA-3′ reverse 5′-CTTGGCAAACTTCCCAAAGCGAGT-3′
PI-PLC β3 (PLCB3; OMIM ⁎600230)	forward 5′-TATCTTCTTGGACCTGCTGACCGT-3′ reverse 5′-TGTGCCCTCATCTGTAGTTGGCTT-3′
PI-PLC β4 (PLCB4; OMIM ⁎600810)	forward 5′-GCACAGCACACAAAGGAATGGTCA-3′ reverse 5′-CGCATTTCCTTGCTTTCCCTGTCA-3′
PI-PLC γ1 (PLCG1; OMIM ⁎172420)	forward 5′-TCTACCTGGAGGACCCTGTGAA-3′ reverse 5′-CCAGAAAGAGAG CGTGTAGTCG-3′
PI-PLC γ2 (PLCG2; OMIM ⁎600220)	forward 5′-AGTACATGCAGATGAATCACGC-3′ reverse 5′-ACCTGAATCCTGATTTGACTGC-3′
PI-PLC δ1 (PLCD1; OMIM ⁎602142)	forward 5′-CTGAGCGTGTGGTTCCAGC-3′ reverse 5′-CAGGCCCTCGGACTGGT-3′
PI-PLC δ3 (PLCD3; OMIM ⁎608795)	forward 5′-CCAGAACCACTCTCAGCATCCA-3′ reverse 5′-GCCA TTGTTGAGCACGTAGTCAG-3′
PI-PLC δ4 (PLCD4; OMIM ⁎605939)	forward 5′-AGACACGTCCCAGTCTGGAACC- 3′ reverse 5′-CTGCTTCCTCTTCCTCATATTC- 3′
PI-PLC ε (PLCE; OMIM ⁎608414)	forward 5′-GGGGCCACGGTCATCCAC-3′ reverse 5′-GGGCCTTCATACCGTCCATCCTC-3′
PI-PLC η1 (PLCH1; OMIM ⁎612835	forward 5′-CTTTGGTTCGGTTCCTTGTGTGG-3′ reverse 5′-GGATGCTTCTGTCAGTCCTTCC-3′
PIPLC η2 (PLCH2; OMIM ⁎612836)	forward 5′-GAAACTGGCCTCCAAACACTGCCCGCCG-3′ reverse 5′-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3′
Glyceraldehyde 3 Phosphate Dehydrogenase (GAPDH)	forward 5′-CGAGATCCCTCCAAAATCAA-3′ reverse 5′-GTCTTCTGGGTGGCAGTGAT-3′