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Protein and mRNA expression of protein kinase C (PKC) in the postmortem brain of bipolar and schizophrenic subjects

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Abstract

Abnormalities of protein kinase C (PKC) have been implicated in the pathophysiology of bipolar (BP) illness. This is primarily based on studies of PKC in platelets of BP patients. Whether such abnormalities of PKC activity and isoforms exist in the brain is unclear. We have therefore determined PKC activity, protein and mRNA expression of PKC isoforms in the prefrontal cortex (PFC), cingulate cortex (CING) and temporal cortex (TEMP) from BP (n = 19), schizophrenic (SZ) (n = 20) and normal control (NC) (n = 25) subjects. The brain samples were obtained from the Harvard Brain Bank, and the subjects were diagnosed according to DSM-IV criteria. Protein levels were determined using Western blot technique and mRNA levels were determined using real-time PCR (qPCR) method. We found that there was a significant decrease in the PKC activity in the cytosol and membrane fractions of PFC and TEMP obtained from BP subjects but not from SZ subjects. When we compared the expression of PKC isozymes, we found that the protein and mRNA expression of several isozymes was significantly decreased in the PFC (i.e., PKCα, PKCβI, PKCβII and PKCε) and TEMP (i.e., PKCα, PKCβI, PKCβII, PKCε and PKCγ) of BP subjects, but not in the CING. Overall, there was no difference in the mRNA or protein expression of PKC isozymes between SZ and NC subjects in any of the three brain areas we studied. Our results show that there is a region-specific decrease of certain PKC isozymes in the membrane and cytosol fractions of BP but not SZ subjects.

Keywords

Protein Kinase C isozymes; Bipolar Disorders; Postmortem Brain; Protein Kinase C activity

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Contributors

This manuscript is an original article. All co-authors qualify for authorship as all have made a substantial contribution to conception and design (GNP), data generation (HSR and XR). The article was drafted by me and revised critically by other co-authors. All authors approve manuscript submission.

Conflict of interest

All authors declare that they have no financial interests or potential conflicts of interest directly or indirectly related to this work.

1. Introduction

Bipolar (BP) illness has been identified as one of the leading causes of disability and is generally characterized by recurrent fluctuations between periods of both positive (mania) and negative (depression) extremes of mood states. It is a common, severe and life-threatening illness (Goodwin and Jamison, 2007; Muller-Oerlinghausen et al., 2002).

Several studies suggest abnormalities of signal transduction systems in the pathophysiology of BP illness (Niciu et al., 2013; Zarate et al., 2003). One of these abnormal signal transduction systems is the phosphoinositide (PI) signaling cascade (Jope, 1999a, b; Jope et al., 1996; Li et al., 2002; Pandey, 2013a, b) where stimulation of receptors, such as 5-hydroxytryptamine $(5HT)_{2A}$ by agonists increases intracellular calcium (Ca^{2+}) and diacylglycerol (DAG). PKC is an important component of the PI signaling system and increased DAG formation activates PKC which in turn increases the transcription factor cAMP response element-binding protein (CREB) (Abrial et al., 2011; Berridge, 1984; Pandey, 2013a, b). PKC also phosphorylates many substrates, such as myristoylated alanine-rich C-kinase substrate (MARCKS), growth associated protein 43 (Gap-43), neurogranin, which are involved in pre- and post-synaptic regulation of neurotransmitter release and synaptic plasticity [see Saxena et al. (2017)].

The PKC is divided into three classes depending on their structure and requirements for activation: (*i*) the conventional PKC isozymes (PKCα, PKCβI, PKCβII, and PKCγ), which require both Ca^{2+} and DAG for their activation; (*ii*) the novel PKC isozymes (PKCδ, PKCε, PKCη, and PKCθ) which are also activated by DAG but not by Ca^{2+}; and (*iii*) the atypical PKC isozymes (PKCζ; PKCι, and PKCλ) consisting of PKC isoforms that do not require either Ca^{2+} or DAG for their activation (Ohno and Nishizuka, 2002; Tanaka and Nishizuka, 1994).

There is both direct and indirect evidence suggesting that PKC abnormalities may be associated with the pathophysiology of BP illness. The indirect evidence is derived mainly from the effect of lithium on PKC. Lithium is one of the most important mood stabilizing drugs and its mode of therapeutic action in BP illness is not very well understood (Malhi et al., 2013). Lithium treatment affects many systems, including the serotonergic and the PI signaling systems (Malhi et al., 2013). Some studies suggest that both acute and chronic lithium treatment causes PKC changes (Manji et al., 1993). For example, acute lithium treatment increases while chronic treatment decreases PKC activity (Manji et al., 1993). It has also been shown that lithium downregulate PKC expression and activity in the rat brain (Jope and Williams, 1994). These common effects of lithium and other mood stabilizing drugs suggest that PKC may be involved not only in the therapeutic effects of lithium but that PKC abnormalities may also be involved in the pathophysiology of BP illness.

The possible involvement of PKC in the pathophysiology of BP disorder is also based on clinical studies. For example, some studies show both increased as well as decreased PKC activity in platelets of BP patients (Hahn and Friedman, 1999; Pandey et al., 2002). Pandey et al. (2002) found that some of the specific PKC isozymes, such as PKCα and PKCβI are decreased in platelets of BP patients compared with NC subjects. Although these studies

suggested PKC abnormalities in peripheral tissues of BP patients, it is not clear if similar abnormalities may also exist in the brain of these subjects.

BP illness and schizophrenia share many common symptoms as well as structural and biological abnormalities (Berrettini, 2000; Jablensky, 1999; Potash and Bienvenu, 2009). Both disorders include psychosis and depression, and both diseases are responsive to treatment with antipsychotic drugs (Berrettini, 2000).

In this study, therefore, we determined PKC activity and mRNA expression of PKC isozymes, including the conventional, novel and atypical isozymes and the protein expression of the conventional and novel isozymes in three different brain areas [prefrontal cortex (PFC), cingulate cortex (CING), and temporal cortex (TEMP)] obtained from subjects with BP illness and schizophrenia. In order to examine if changes in mRNA and protein expression of these isozymes are also related to their biological function, we determined PKC activity in the PFC, CING and TEMP of BP and schizophrenic (SZ) subjects.

2. Materials and methods

2.1 Acquisition of Human Postmortem Brain Samples and Clinical Assessment

The frozen postmortem brain samples from the PFC (Brodmann area 9 [BA9]), CING (BA24), and TEMP (BA38) were obtained from the Harvard Brain Tissue Resource Center (HBTRC) at McLean Hospital, Belmont, MA, consisting of BP, SZ, and normal control (NC) subjects. The psychiatric diagnoses were established by two senior psychiatrists based on clinical and family histories and according to Feighner et al. (1972) for SZ and the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for BP subjects. Individuals with a documented history of substance dependence or neurological illnesses were excluded from the collection. The BP and SZ groups also included subjects who died by suicide. This study was approved by the Institutional Review Boards of McLean Hospital and the University of Illinois at Chicago.

The demographics associated with these subjects are listed in Table 1. The sample included 19 BP subjects, 20 SZ subjects, and 25 normal control (NC) subjects. Mean age, postmortem interval (PMI), and pH of the frozen brain samples did not differ significantly between the three groups (Table 1). The BD and SZ subjects had been exposed to various psychotropic medications (Table 1).

2.2 Determination of mRNA levels of PKC isozymes in the PFC, TEMP and CING of BP, SZ and NC subjects

2.2.1 RNA extraction—Total RNA was extracted using 100 mg of tissue with TRIZOL (Invitrogen) followed by DNase treatment, as per the manufacturer's instructions. RNA concentration was measured on the NanoDrop®ND-1000 (NanoDrop Technologies) and quality was assessed using Agilent Bioanalyzer 2100 (Agilent). Only samples with RNA integrity number (RIN) greater that 5.5 were used for further analysis.

2.2.2 Relative real-time PCR—

cDNA was synthesized using 1ug of RNA from each sample as previously described (Ren et al., 2014), see the Supplemental Methods for details. The list of PKC isoform probes is shown in Table 2.

2.3 Quantitation of PKC isozymes in membrane and cytosol fractions by Western blot

Immunolabeling of PKC α, βI, βII, γ, δ and ε isozymes was determined in the postmortem brain samples as described previously (Pandey et al., 2004), see the Supplemental Methods for details.

2.4 Determination of PKC Activity in Membrane and Cytosol Fractions

PKC activity in membrane and cytosol fractions was measured by the procedure previously described (Pandey et al., 2004), see the Supplemental Methods for details.

2.5 Statistical analysis and effect of confounding variables

We analyzed this data using IBM SPSS Statistics version 24. To jointly compare control subjects, BP subjects and SZ subjects we used a MANCOVA analysis adjusting the effects of confounding variables age, postmortem interval (PMI), and brain pH as covariates. This analysis was individually done in the PFC, TEMP and CING regions of the brain for both mRNA and protein to determine the difference of PKC isoform levels between the subjects. For multiple comparisons, we used Bonferroni correction to adjust type I error rates. We also used Post-Hoc t test for paired comparisons. To examine if the observed changes in PKC isozymes expression are related to the covariates, we examined the effect of age, gender, PMI, brain pH on the protein and mRNA expression levels of PKC isozymes and PKC activity.

3. Results

3.1 mRNA expression of PKC isozymes in PFC, CING, TEMP of BP, SZ and NC subjects

In order to examine if the mRNA expression of various PKC isozymes was altered in the PFC of BP and SZ subjects, we determined these isozymes in BP, SZ and NC subjects. The overall group effect using a MANCOVA generalized linear model (GLM) shows that the mRNA expression of PKCα, PKCβI, PKCβII, PKCγ and PKCε differed significantly between the three groups (Fig. 1 A; Supplemental Figure 1A). However, these three groups did not differ significantly in the mRNA expression of PKCδ, PKCθ, PKCη, PKCζ and PKCι. Post hoc comparisons using t-test showed significant differences in the mRNA expression of PKCα [$F(5,58) = 1.103$, $p = 0.05$], PKCβI [$F(5,58) = 3.789$, $p = 0.0024$], PKCβII [$F(5,58) = 6.700$, $p = 0.000016$], PKCγ [$F(5,58) = 3.269$, $p = 0.031$] and PKCε [$F(5,58) = 2.638$, $p = 0.0044$]. We next performed multiple group comparisons using the Bonferroni test [$p \leq 0.005$] which revealed that there was a significant difference in mRNA expression of PKCβI, PKCβII and PKCε in BP compared with NC subjects, but there was no significant difference in the mRNA expression of PKCα, PKCγ, PKCδ, PKCθ, PKCη, PKCζ and PKCι between BP and NC subjects, as shown in Figure 1A. When we compared the mRNA expression of these PKC isozymes in the PFC between NC and SZ subjects we found that there were no significant differences in any of the PKC isozymes studied (Fig. 1A; Supplemental Figure 1A).

We then compared the mRNA expression of these PKC isozymes in the CING obtained from NC, BP, and SZ subjects. We found that the overall group effect using a MANCOVA GLM shows that the mRNA expression of PKCα [F (5,58) =1.571, p = 0.05], PKCβI [F (5,58) = 3.134, p = 0.034] and PKCβII [F (5,58) = 5.606, p = 0.046] was significantly decreased in the CING of BP compared with NC subjects, with no significant change in PKCδ, PKCε, PKCγ, PKCη, PKCι, PKCθ, PKCζ isozymes (Fig. 1B; Supplemental Figure 1B). However, when we performed multiple group comparisons using the Bonferroni correction [p ≤ 0.005], there was no significant difference in the mRNA expression of any of the PKC isozymes between BP and NC subjects. There were no significant differences in the mRNA expression of any of the PKC isozymes in the CING between SZ and NC subjects (Fig. 1B; Supplemental Figure 1B).

When we performed a MANCOVA using GLM, it showed that the mRNA expression of PKCα, PKCβI, PKCβII, PKCγ, and PKCε was significantly decreased between the three groups in the TEMP (Fig. 1C; Supplemental Figure 1C). Post hoc t-test showed significant decreases in mRNA of PKCα [F (5,56) = 2.269, p = 0.006], PKCβI [F (5,56) = 1.436, p = 0.021], PKCβII [F (5,56) = 3.324, p = 0.00032] and PKCε [F (5,56) = 1.927, p = 0.004].

Further comparison of individual groups by Bonferroni correction [p ≤ 0.005] showed that there was a significant decrease in the mRNA expression of only PKCβII and PKCγ in the TEMP obtained from BP subjects compared with NC subjects, whereas there was no significant difference in the other PKC isozymes (i.e., PKCα, PKCβI, PKCδ, PKCε, PKCη, PKCι, PKCθ, PKCζ) between BP and NC subjects (Fig. 1C; Supplemental Figure 1C). When we compared the mRNA expression of these PKC isozymes between SZ and NC subjects, we did not find any significant differences in the TEMP.

3.2 Protein expression of PKC isozymes in the PFC, CING and TEMP of BP, SZ and NC subjects

Since we found a significant decrease in the mRNA expression of some PKC isozymes in the BP but not in the SZ subjects, we then examined if changes in mRNA expression of these isozymes were also associated with their altered protein expression. Using the Western blot method, we determined protein expression in both cytosol and membrane fractions of PKCα, PKCβI, PKCβII, PKCγ, PKCδ and PKCε in the PFC, CING and TEMP of BP, SZ and NC subjects. The immunoblots showing protein expression of PKC isozymes in the PFC (membrane and cytosol fraction) of two BP, two SZ and two NC subjects are shows in Figure 2. As can be seen, the protein expression of PKCα, PKCβI, PKCβII and PKCε appears to be decreased in BP compared with NC subjects (Fig. 2). We found that overall group effect using a MANCOVA showed significant differences in the expression of PKC isozymes between the groups. Post hoc analysis using t-test showed significant decreases in the protein expression of PKCα [Cyto: F (5,59) = 2.911, p = 0.0017, Memb: (5,59) = 6.321, p ≤ 0.00069], PKCβI [Cyto: F (5,59) = 9.198, p = 0.00005, Memb: (5,59) = 8.824, p = 0.0000054], PKCβII [Cyto: F (5,59) = 9.140, p = 0.000089, Memb: (5,59) = 7.394, p = 0.000078] and PKCε [Cyto: F (5,59) = 4.552, p = 0.0028, Memb: (5,59) = 4.832, p = 0.0019] in the PFC of BP compared with NC subjects in both membrane and cytosol fractions (Fig. 3A; Supplemental Figure 3A).

When we analyzed the results using the Bonferroni correction [$p \leq 0.0042$] we found that, similar to t-test, the protein expression levels of PKCα, PKCβI, PKCβII, and PKCε were significantly decreased in both membrane and cytosol fraction of PFC obtained from BP compared with NC subjects (Fig. 3A; Supplemental Figure 3A). However, there was no significant difference in PKCγ and PKCδ between NC and BP subjects. We observed a significant decrease in protein levels of PKCγ in the PFC of SZ subjects, only in the cytosol but not in the membrane fraction (Fig. 3A; Supplemental Figure 3A).

We also determined the protein expression of these PKC isozymes in the membrane and cytosol fraction of CING obtained from BP, SZ and NC subjects. To our surprise, in the CING we did not find any significant changes in any of the isozymes, except that PKCβI was significantly ($p = 0.025$) increased in the membrane fraction of SZ subjects compared with NC (Supplemental Figure 3B).

We also examined the protein expression of these isozymes in membrane and cytosol fractions obtained from BP and SZ subjects in the TEMP. We found a significant decrease in the expression of PKCα [Cyto: $F(5,46) = 2.801$, $p = 0.011$, Memb: $(5,46) = 2.901$, $p = 0.0061$], PKCβI [Cyto: $F(5,46) = 2.790$, $p = 0.023$, Memb: $(5,46) = 3.815$, $p = 0.0029$], and PKCγ [Cyto: $F(5,46) = 4.976$, $p = 0.0027$, Memb: $(5,46) = 4.128$, $p = 0.0047$] in both cytosol and membrane fractions in the TEMP of BP compared with NC subjects (Fig. 3C; Supplemental Figure 3C). However, we did not find a significant difference in the expression of PKCβII [Cyto: $F(5,46) = 4.932$, $p = 0.52$, Memb: $(5,46) = 1.203$, $p = 0.34$] in the TEMP of BP subjects although it tended to be lower compared with NC subjects. After Bonferroni correction [$p \leq 0.0042$] only PKC α (in membrane), PKCβI (in membrane) and PKCγ (in cytosol) were significantly decreased in the TEMP of BP subjects (Fig. 3C; Supplemental Figure 3C). There were no significant differences in any of the PKC isozymes between SZ and NC subjects in the TEMP cortex (Fig. 3C; Supplemental Figure 3C).

3.3 PKC activity in the PFC, CING and TEMP of BP, SZ and NC postmortem brain samples

In order to examine if there is a functional change in the PKC due to decreased mRNA and protein expression of some of its isozymes, we determined the PKC activity in the cytosol and membrane fractions of PFC, CING and TEMP from NC, BP and SZ subjects. We found that PKC activity in the cytosol [$F(5,59) = 3.680$, $p = 0.0018$] and membrane fractions [$F(5,59) = 5.700$, $p = 0.000025$] of PFC was significantly decreased in BP compared with NC subjects after Bonferroni correction [$p \leq 0.025$] (Fig. 4). However, there was no change in the PKC activity either in the cytosol or membrane fractions of the PFC of SZ subjects (Fig. 4; Supplemental Figure 4). We found no significant difference in the cytosol or membrane PKC activity between BP and NC subjects, or SZ and NC subjects in the CING. When we compared the PKC activity in the cytosol [$F(5,46) = 5.407$, $p = 0.00004$] and membrane fractions [$F(5,46) = 3.169$, $p = 0.021$] obtained from the TEMP, we found that there was a significant decrease in the PKC activity in the cytosol and membrane fractions obtained from the TEMP in BP but not in SZ compared with NC subjects after Bonferroni correction [$p \leq 0.025$] (Fig. 4; Supplemental Figure 4).

4. Discussion

In this study we determined mRNA and protein expression of PKC isozymes and PKC activity in the cytosol and membrane fractions in the PFC, TEMP and CING obtained from BP, SZ and NC subjects. The main finding of this study was that the mRNA and protein expression of PKC isozymes PKCα, PKCβI, PKCβII, and PKCε, and PKC activity was significantly decreased in the PFC of BP compared with NC subjects after Bonferroni correction.

In the TEMP, the mRNA and protein expression of PKCα, PKCβI, PKCε, PKCγ and PKC activity was significantly decreased in BP compared with NC subjects. The PKCβII mRNA but not protein expression was significantly decreased in BP compared with NC subjects, although protein expression of PKCβII was lower, but not significantly.

In the CING there was no significant difference in the mRNA and protein expression of any of the PKC isozymes or PKC activity in BP or SZ subjects compared with NC after Bonferroni Correction.

In SZ subjects there were generally no significant differences in the protein or mRNA expression of PKC isozymes or PKC activity between SZ and NC subjects in the PFC, CING or TEMP.

In summary, our studies indicate region-specific (PFC and TEMP) abnormalities (decreases) in PKC function in BP illness as evidenced by decreased PKC activity and that these abnormalities of PKC function are related to decreased levels of PKC isoforms, specifically PKCα, PKCβI, PKCβII and PKCε. Also, there is no abnormality of PKC function or PKC isozymes expression in CING of BP subjects.

We studied PKC isozymes in three brain areas (i.e., PFC, CING and TEMP cortex) of BP, SZ and NC subjects. The major rationale for the study of these areas is based on previous structural studies and the postmortem brain studies, showing the involvement of these areas in BP disorders and SZ [see review by Giridharan et al. (2020)]. For example, Rajkowska et al. (2001) found a reduction in neuronal and glial distribution in the PFC of BP disorder subjects. Benes et al. (2001) found altered density of pyramidal neurons in the anterior CING cortex of SZ and BP disorder subjects. Brauch et al. (2006) found reduced glial area in TEMP cortex of BP disorder subjects. The structural and imaging studies also show abnormalities in these areas of BP disorder [see Drevets et al. (1997)]. They found abnormalities in the PFC of BP patients. This was also observed by Lyoo et al. (2004).

The mechanism and reasons for decreased PKC isozymes are not clear, but a possible reason may be Ca^{2+} signaling changes observed in BP disorders. Recent resurgence of interest in Ca^{2+} and BP disorder has been fostered by the genomic data showing that voltage-gated calcium channels (VGCCs) are a part of genetic risk factors, suggesting a role for Ca^{2+} signaling in BP disorders. As described in the Introduction section, the binding of agonists, such as serotonin, causes hydrolysis of phosphatidylinositide-4,5 bisphosphate (PIP_2) by phospholipase C (PLC) resulting in the formation of two second messengers – inositol 1,4,5 triphosphate (IP_3) and DAG. IP_3 mobilizes Ca^{2+} and DAG activates an enzyme protein

kinase C (PKC), which phosphorylates proteins such as MARCKS and causes the release of serotonin, cell differentiation, nerve growth and gene expression (Berridge, 1984; Pandey, 2013a). DAG stimulates PKC, and this is the second arm of the PI signaling system. We have focused on the study of PKC (i.e., the second arm of the PI signaling system).

The first arm of the PI signaling system (i.e., the generation of Ca^{2+}) has been extensively studied in BP disorders. Many investigators have studied Ca^{2+} levels in platelets and lymphocytes of BP disorder patients [see review by Warsh et al. (2004), Berridge (2014), Harrison (2016)]. A meta-analysis of Ca^{2+} studies in BP disorder by Harrison et al. (2019) found that free intra-cellular Ca^{2+} is increased in platelets and lymphocytes of medication-free BP patients. The Ca^{2+} levels are also increased in platelets of BP patients after serotonin or thrombin stimulation. These studies indicated increased Ca^{2+} levels in BP illness.

Genome-wide association studies (GWAS) have identified risk genes associated with BP disorder. The two most prominent susceptibility genes associated with BP disorders are ANK3 and CACNA1C (Fiorentino et al., 2014). Of these, CACNA1C is an L-type voltage gated Ca^{2+} gene which encodes L-type calcium channel Cav1.2 subunit and is the best BP susceptibility gene. These L-type calcium channels have been identified as important BP disorder susceptibility genes [see the Psychiatric GWAS Consortium Bipolar Disorder (PGC-BD) Working Group (2011)]. Among Ca^{2+} channel genes CACNA1C is most strongly implicated with the C allele conferring risk for BP illness. Besides CACNA1C another Ca^{2+} channel genes associated with BP disorders is CACNB2 gene (Berridge, 2014) which encodes the β2 subunit of Cav1.2 channel (Ferreira et al., 2008; Sklar et al., 2008). Mutations of CACNA1C and CACNB2 genes have been linked to BP disorders and schizophrenia (Ferreira et al., 2008; Giegling et al., 2010; Sklar et al., 2008).

Thus, studies of Ca^{2+} levels and Ca^{2+} signaling have been linked to BP disorders. Activation of PI-PLC signaling causes formation of Ca^{2+}. On the other hand, it also causes the formation of DAG and the activation of PKC. Therefore, it was of interest to examine if along with the Ca^{2+} pathway abnormalities, PKC abnormalities are also associated with BP disorder. We observed a decrease in the expression of PKCα, PKCβI and PKCβII in the PFC of BP subjects. These are Ca^{2+}-dependent isozymes and require Ca^{2+} and DAG for their activation. One of the reasons for their dysregulation may be due to altered Ca^{2+} levels which have been observed and reported in BP disorders.

Most of the PKC isozymes are highly expressed in the CNS (Naik et al., 2000; Wetsel et al., 1992), particularly the ε and γ forms. PKCγ is exclusively localized in the brain. Most of those PKC isozymes highly expressed in the brain regions such as PFC and hippocampus are located both within the neurons and gray matter (Amadio et al., 2006; Tanaka and Nishizuka, 1994).

Since PKC is also expressed in some peripheral tissue such as platelets (Naik et al., 2000) PKC has also been studied in platelets obtained from BP subjects. Wang et al. (1999) determined the PKC activity in the platelets from BP patients and found that it was increased in BP patients and that lithium treatment decreased PKC activity in membrane and cytosole fractions of BP patients. On the other hand, Soares et al. (2000) found that platelets from

lithium-treated BP patients had lower cytosole PKCα isozyme and lower membrane PIP_2 levels. Pandey et al. (2002) studied the PKC activity, PKC isozymes and expression of MARKS (the molecular target of PKC) in platelets obtained from BP patients, depressed patients, and NC subjects. They found that the PKC activity was significantly decreased in the platelets of BP patients compared with NC subjects, but not in unipolar patients. They studied the protein expression of some of the PKC isoforms and found that the protein expression of PKCα, PKCβI, and PKCβII was significantly decreased in the membrane and cytosole fractions of the platelets of BP subjects but not in the unipolar subjects. They also found that MARCKS, a target of PKC, was significantly elevated in membrane and cytosol factions in patients with BP disorder (Pandey et al., 2002). Pandey et al. (2008) studied the PKC in the platelets obtained from pediatric patients with BP disorder and very similar to that found in adult BP patients they observed the PKC activity and the expression of PKCβI, and PKCβII, but PKCα or PKCδ was decreased in the platelets of pediatric BP patients. However, they also found that 8 weeks of treatment with mood stabilizers normalized, i.e., increased the levels of PKC activity and these isozymes.

Young et al. (1999) determined the protein expression of PKCα in platelets of BP patients and found no difference in protein expression in cytosol or membrane fractions of PKCα in either drug-free or lithium-treated patients compared with controls. They also did not find any difference in the membrane to cytosol ratio of PKCα between BP subject and controls.

Wang and Friedman (1996) determined PKC activity and its redistribution in the frontal cortex of postmortem brain samples from BP subjects and found higher membrane-associated PKC activity in BP subjects compared with controls.

There are very few studies of PKC either in platelets or postmortem brain of SZ subjects. Opeskin et al. (1996) measured PKC in the striatum from SZ subjects and controls and found no difference in [^{3}H]PDBu binding (density of PKC) in SZ subjects compared with non-SZ subjects.

The reasons for decreased PKC activity and some of the PKC isozymes in the postmortem brain of BP subjects are not clear. Since lithium inhibits PKC activity it is generally believed that PKC activity and isozymes may be higher in BP illness and the inhibition of PKC may be one of the mechanisms of the therapeutic action of lithium. However, our results showing decreased PKC contradicts this hypothesis. One of the reasons for this may be that most of our BP patients were either on lithium treatment or on treatment with mood stabilizing drugs at the time of death and that this may have affected PKC activity and the expression of its isozymes. On the other hand, we not only found decreased PKC activity and decreased expression of PKC isozymes in the platelets of drug-free adult and pediatric BP patients (Pandey et al., 2008) but also found that treatment with mood stabilizing drugs might even increase the expression of PKC isozymes and its activity (Pandey et al., 2008). Koenigsberg et al. (2012) found that platelet protein expression of PKCα and PKCβII was lower in borderline personality disorder patients compared with controls. Also, we (Pandey et al., 1997; Pandey et al., 2003; Pandey et al., 2004) and Shelton et al. (2009) found decreased levels of PKC isozymes and PKC activity in the postmortem brain of suicide subjects. Therefore, it is quite possible but unlikely that our present results of decrease PKC activity

and specific isozymes are related to previous exposure to mood stabilizing drugs including lithium.

Another question is what is the significance of decreased PKC activity and some of the PKC isozymes in BP postmortem brain? PKC produces its biological effects, such as synaptic plasticity, and this may be related to the phosphorylation of several downstream targets of PKC. Some of these targets include neurogranin, neurotrophic factors, Gap-43 and neuromodulin, MARCKS, synaptosome associated protein 25 (SNAP-25) [see Saxena et al. (2017) and Deloulme et al. (1990)].

MARCKS is one of the most important substrates for PKC. MARCKS contains a large middle region, termed the effector domain which binds acidic phospholipids, actin and calcium/calmodulin (Yamauchi et al., 1998). It also contains PKC phosphorylation sites in close proximity to the effector domain. MARCKS has been implicated in many cellular genetics (Myat et al., 1997). Some studies indicate that levels of MARCKS and its phosphorylation are decreased in depression and suicide (Pandey et al., 2003). Our findings of decreased levels of PKC in BP subjects suggest that this may cause decreased phosphorylation of MARCKS and its decreased function.

5. Conclusion

Our studies indicate region-specific decreases in PKC activity in the postmortem brain of BP subjects, such that this decrease in PKC activity was observed in the PFC and TEMP but not in CING. Our studies also indicate that this decrease in PKC activity in the PFC and TEMP may be related to decreased mRNA and protein expression of specific PKC isozymes, primarily PKCα, PKCβI, PKCβII and PKCε in PFC and TEMP of BP subjects. PKC has been targeted for the treatment of BP disorders. Our studies suggest that targeting specific isozymes, such as PKCα, PKCβI, PKCβII and PKCε may be more appropriate. Our study also strongly suggests region-specific alterations of PKC in the pathophysiology of BP disorders.

6. Limitation

The main limitation of our study is that most of the subjects in this study were on treatment with mood stabilizing drugs, particularly lithium at the time of death. Hence, it is difficult to conclude if the changes in PKC are related to lithium treatment or to BP illness. The number of subjects in each group is relatively small, which is an inherent limitation of postmortem studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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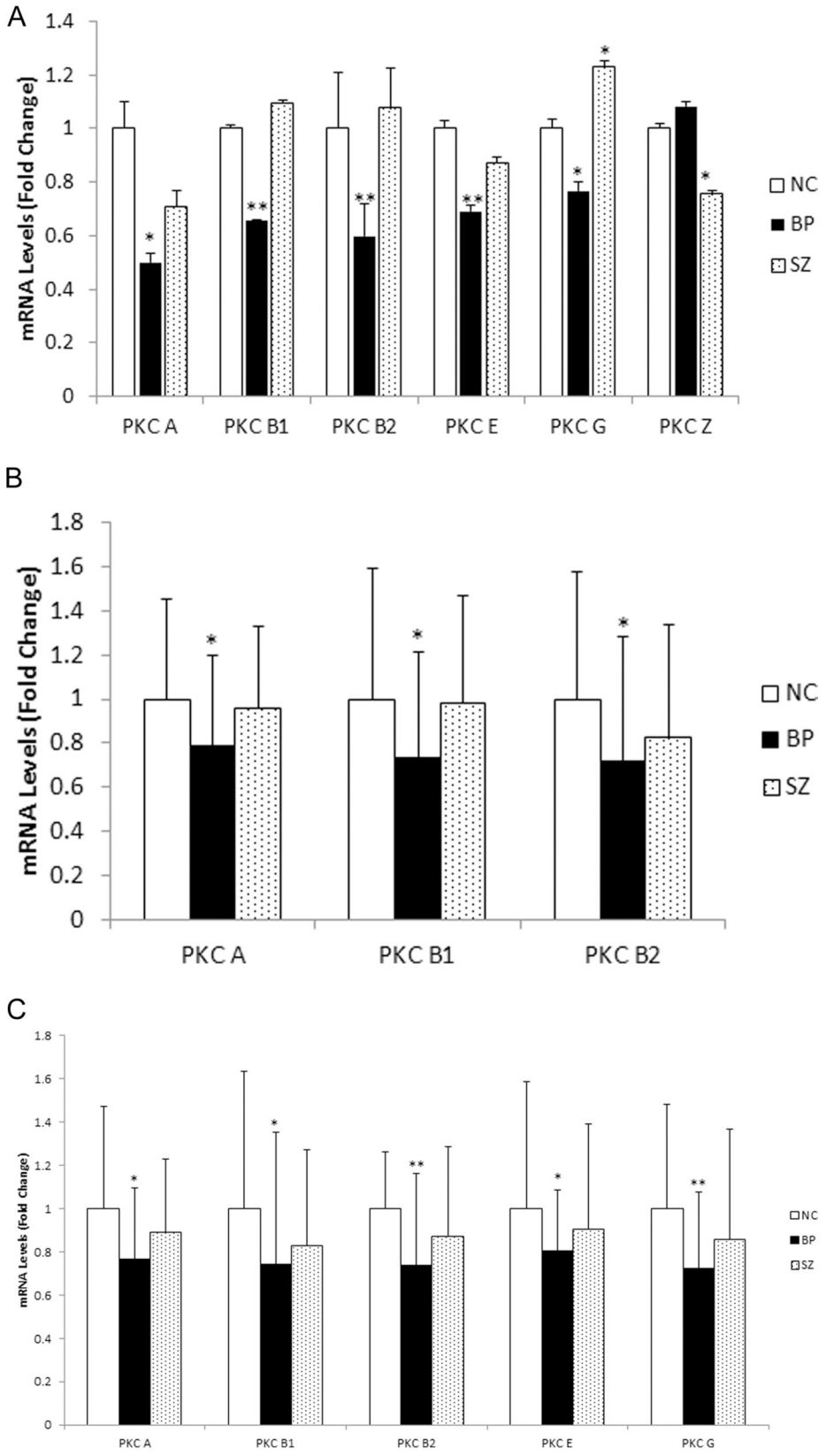

Fig. 1.

A. Mean mRNA levels of PKC isozymes in the prefrontal cortex (PFC) obtained from 25 normal control (NC), 16 bipolar (BP) and 18 schizophrenic (SZ) subjects. Error bars represent SD. *P ≤ 0.05; **P ≤ 0.005 (significance after Bonferroni correction).

B. Mean mRNA levels of PKC isozymes in the cingulate cortex (CING) obtained from 25 NC, 16 BP and 18 SZ subjects. Error bars represent SD. *P ≤ 0.05; **P ≤ 0.005 (significance after Bonferroni correction).

C. Mean mRNA levels of PKC isozymes in the temporal cortex (TEMP) obtained from 24 NC, 18 BP and 15 SZ subjects. Error bars represent SD. *P ≤ 0.05; **P ≤ 0.005 (significance after Bonferroni correction).

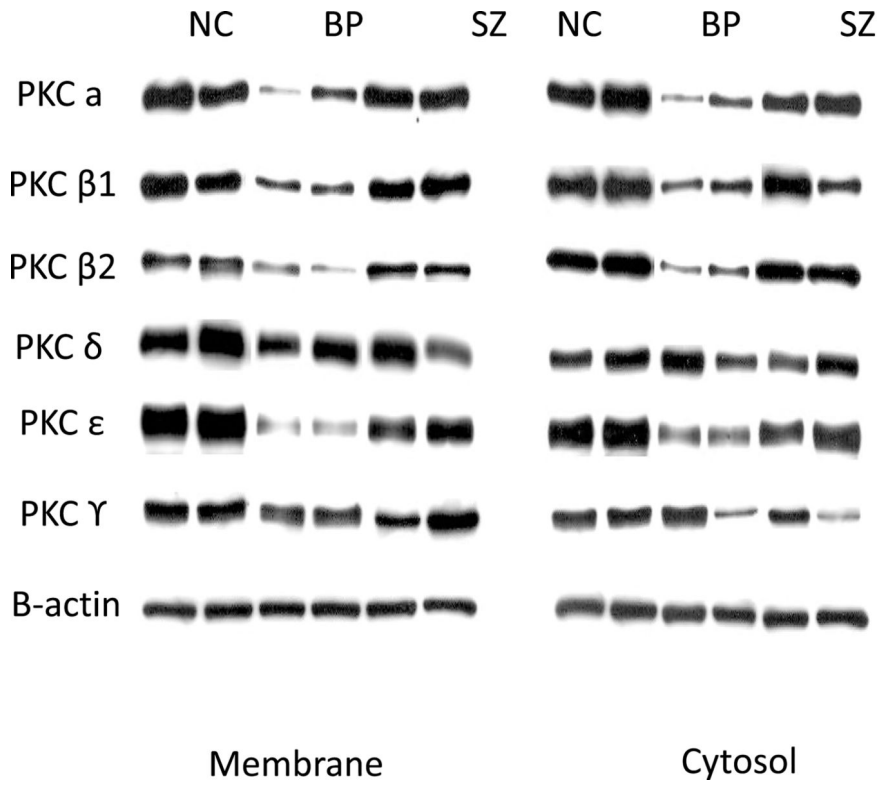

Fig. 2.

Representative Western blots showing immunolabeling of PKCα, PKCβI, PKCβII, PKCδ, PKCε, PKCγ and β-actin in the PFC (membrane and cytosol fractions) of two BP, two SZ and two NC subjects.

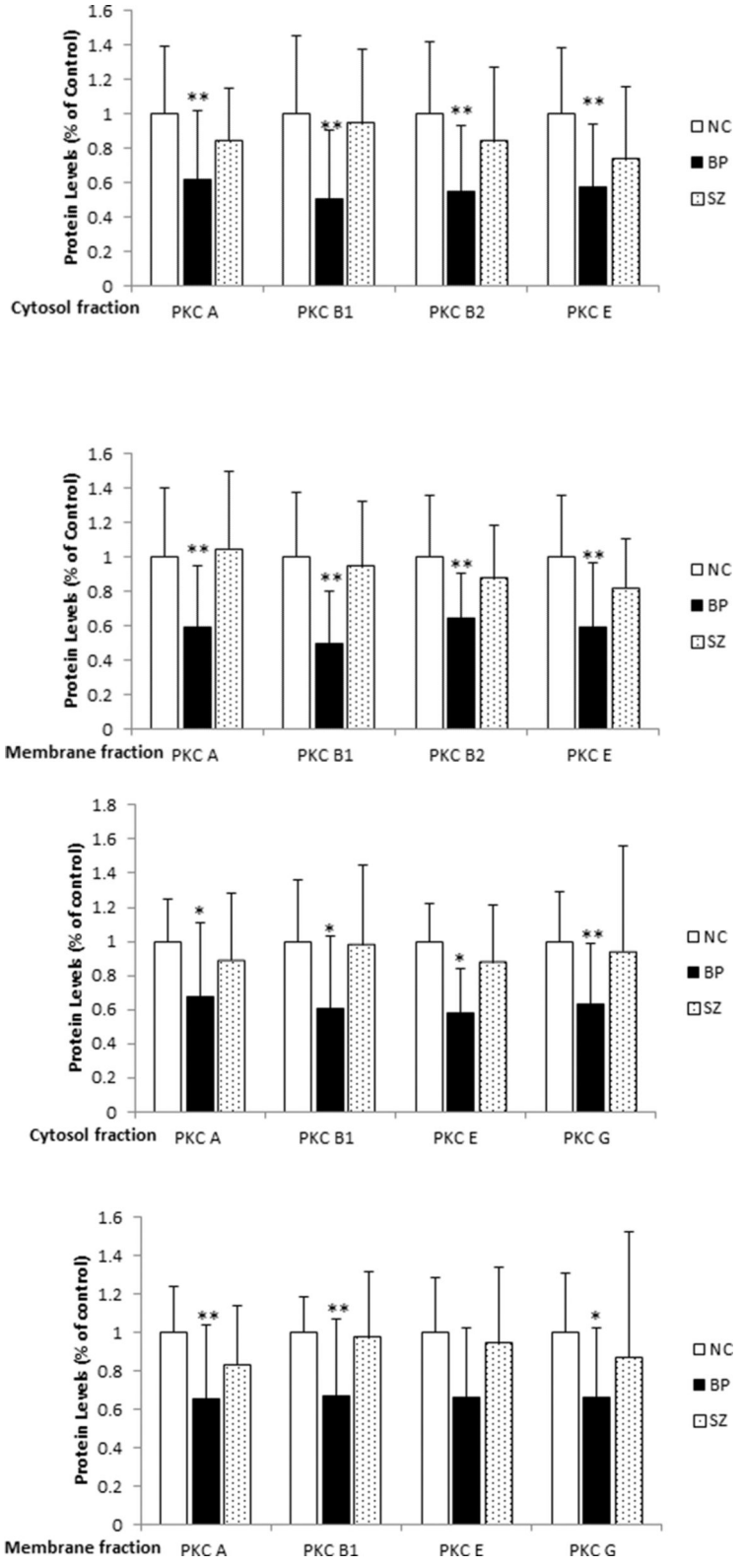

Fig. 3.

A. Mean protein levels of PKC isozymes in membrane and cytosol fractions of PFC obtained from 21 NC, 19 BP and 20 SZ subjects. Error bars represent SD. *P ≤ 0.05 **P ≤ 0.0042 (significance after Bonferroni correction).

B. Mean protein levels of PKC isozymes in membrane and cytosol fractions of TEMP obtained from 16 NC, 16 BP and 15 SZ subjects. Error bars represent SD. *P ≤ 0.05 **P ≤ 0.0042 (significance after Bonferroni correction).

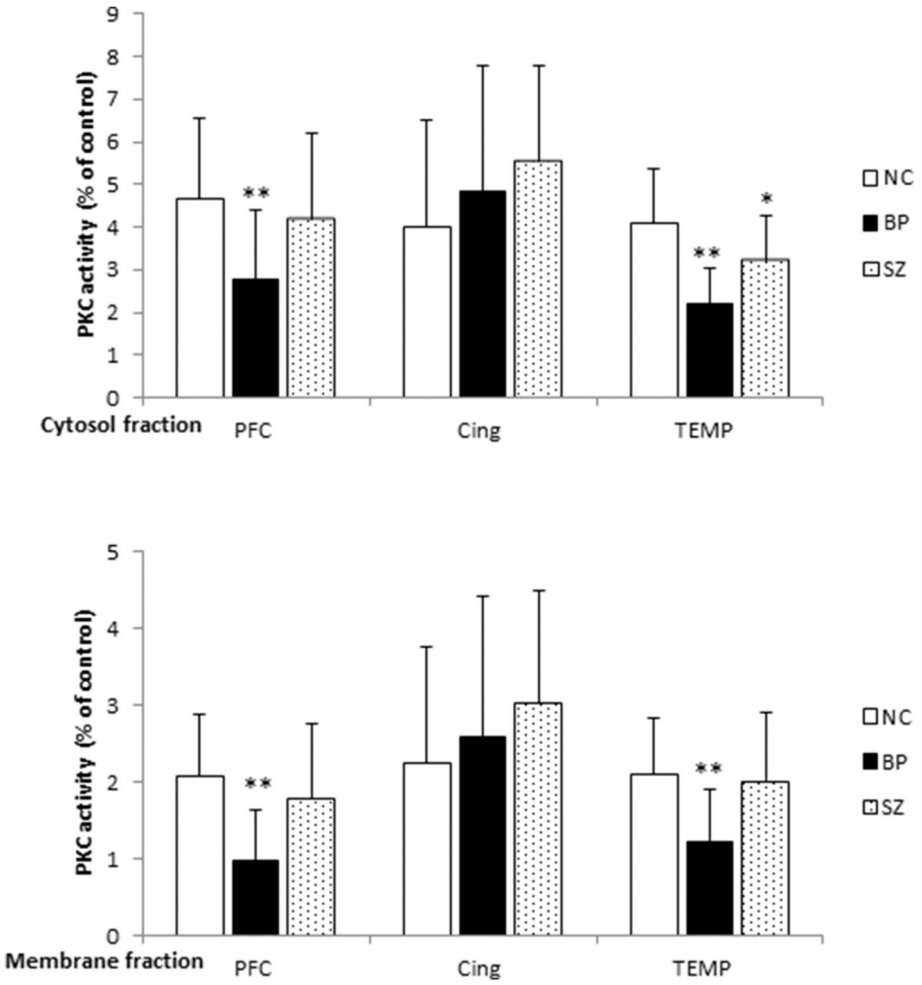

Fig. 4.

Mean PKC catalytic activity in PFC, CING and TEMP (membrane and cytosol fractions) from BP, SZ and NC subjects. Error bars represent SD. *P ≤ 0.05 **P ≤ 0.025 (significance after Bonferroni correction).

Table 1.

Summary of Demographic and Clinical Data Available on Subjects and Tissue Samples Used in the Present Study

Group and Subject	Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychiatric Diagnosis	Medication at the time of death
CONTROLS								
1.	49	White	M	24.60	6.76	Myocardial Infarction	Control	
2.	53	Unknown	F	24.00	5.8	Cancer	Control	
3.	74	White	F	12.50	6.33	Unknown	Control	
4.	54	White	M	24.20	6.53	Congestive Heart Failure	Control	
5.	70	White	F	22.50	6.26	Cancer	Control	
6.	67	White	M	22.33	6.42	Heart Attack/Disease	Control	
7.	37	White	M	18.75	6.68	Accidental	Control	
8.	73	Unknown	M	20.53	6.05	Unknown	Control	
9.	35	Unknown	M	25.67	6.33	Unknown	Control	
10.	89	White	M	7.42	6.39	Cancer	Control	
11.	79	White	M	20.92	6.28	Cancer	Control	
12.	78	White	F	23.91	6.67	Cancer	Control	
13.	38	White	M	28.83	6.53	Myocardial Infarction	Control	
14.	65	White	F	24.25	6.4	Unknown	Control	
15.	66	White	F	7.42	6.03	Cancer	Control	
16.	50	Unknown	M	24.13	6.01	Unknown	Control	
17.	84	White	M	28.58	6.42	Pneumonia	Control	
18.	40	White	M	16.60	6.24	Myocardial Infarction	Control	
19.	66	White	M	18.70	6.76	Heart Attack/Disease	Control	
20.	40	White	M	28.00	6.5	Ski Accident	Control	
21.	36	White	M	20.00	5.97	Myocardial Infarction	Control	
22.	42	White	M	18.33	6.78	Myocardial Infarction	Control	
23.	69	White	M	15.3	6.88	Respiratory Failure	Control	
24.	70	White	F	15.00	6.59	Heart Attack/Disease	Control	
25.	78	White	F	23.91	6.67	Cancer	Control	
Mean:	**60.08**			**20.66**	**6.41**			
S.D.:	**16.91**			**5.80**	**0.29**			

Group and Subject	Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychiatric Diagnosis	Medication at the time of death
BIPOLAR								
1.	80	Unknown	F	11.60	6.38	Unknown	BP	Unknown
2.	74	White	M	7.18	6.7	Pneumonia	BP	Gabapentin, zolpidem, olanzapine, lorazepam
3.	73	White	F	20.83	6.3	Sepsis	BP	Carbamazepine, risperidone, diltiazem
4.	74	White	M	14.25	6.27	Pneumonia	BP	Lithium,olanzapine, divalproex, zolpidem, lorazepam
5.	73	White	F	17.00	6.4	Renal Failure	BP	Divalproex, risperidone, sertraline, donepezil
6.	40	White	M	30.75	6.03	Suicide - hanging	BP	Risperidone, gabapentin, nefazodone, topiramate, ziprasidone
7.	38	White	M	22.00	6.24	Suicide - CO poisoning	BP	Divalproex, Paroxetine, clonazepam, olanzapine, metoclopramide
8.	83	Unknown	M	17.50	6.6	Cardiopulmonary arrest	BP	Divalproex, Paroxetine
9.	72	White	M	27.66	6.24	Sepsis	BP	Lithium, Trazadone,
10.	82	White	M	5.02	6.37	Cardiopulmonary arrest	BP	Unknown
11.	78	White	M	30.20	6.3	Cardio-respiratory arrest	BP	Thorazine, clonazepam, divalproex, lithium, levodopa-carbidopa
12.	42	White	F	15.80	6.26	Medication overdose	BP	Divalproex, lithium, perphenazine, zolpidem
13.	29	White	F	10.70	6.7	Suicide - hanging	BP	Valproic acid, lithium, clonozepam, phenelzine, olanzapine, propranolol
14.	64	White	F	11.00	6.69	Emphysema	BP	Divalproex, carbamazepine, trifluoperazine, Doxepin, trihexyphenidil, clonazepam
15.	38	White	M	41.50	6.52	Suicide - Gunshot Wound	BP	Unknown (? Not taking meds at time of death)
16.	51	White	M	31.00	7.02	Unknown	BP	Unknown
17.	76	White	F	22.80	6.6	Heart Attack/Disease	BP	Lithium, lorazepam
18.	50	White	M	30.50	6.35	Heart Attack/Disease	BP	Lithium
19.	74	White	M	24.80	6.53	Pneumonia	BP	Divalproex, quetiapine
Mean:	**62.68**			**20.64**	**6.45**			
S.D.:	**17.91**			**9.70**	**0.23**			
SCHIZO.								
1	55	Unknown	F	18.00	6.48	Unknown	SA	Unknown
2.	66	White	M	22.1	6.43	Pneumonia	SZ	Haloperidol
3.	61	White	M	19.9	6.68	Renal failure	SZ	Haloperidol, lorazepm
4.	73	White	F	24.00	6.08	Cancer	SZ	Risperidone,fluoxetine, clorazepate, fentanyl

Group and Subject	Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychiatric Diagnosis	Medication at the time of death
5.	63	White	M	22.35	6.55	Cardiac arrest	SZ	Clozapine, haloperidol, trazadone, lorazepam
6.	44	White	M	19.0	6.05	Pneumonia	SZ	Clozapine
7.	35	White	M	28.00	6.25	Heart Attack/Disease	SZ	None
8.	42	White	M	18.1	6.26	Suicide -CO poisoning	SZ	Trazadone
9.	78	White	F	13.40	6.81	Sick sinus syndrome	SA	Unknown
10.	46	White	M	18.5	6.31	Cancer - sepsis	SZ	Olanzapine,divalproex
11.	26	White	M	16.00	6.75	Suicide - hanging	SZ	Prolixin decanoate
12.	42	White	M	27.1	6.64	Cancer	SZ	None
13.	47	White	M	19.25	6.57	Cancer	SZ	Clonazepam, hydroxyzine
14.	83	White	F	23.25	5.91	GI bleed	SZ	Haloperidol decanoate
15.	84	White	F	25.75	6.14	Congestive Heart Failure	SA	Risperidone, divalproex, temazepam
16.	31	White	M	15.0	6.46	Unknown	SZ	Risperidone, olanzapine, bupropion
17.	72	White	F	21.75	6.65	Cancer	SZ	Risperidone, paroxetine, clonidine
18.	48	White	F	33.78	6.63	Heart Attack/Disease	SA	Risperidone, divalproex
19.	80	White	M	10.97	6.44	Heart Attack/Disease	SZ	Thioridazine, mirtazapine
20.	49	White	M	19.08	6.6	Suicide - hanging	SZ	Haloperidol decanoate, lorazepam
Mean:	**56.25**			**20.76**	**6.43**			
S.D.:	**17.90**			**5.35**	**0.25**			

Table 2:

List of PKC isoform probes

PKC Isoforms		Taqman accession	Probe Location (exon boundary)	Assay Function
	ACTB	Hs99999903_m1	1–1	House Keeping (HK)
	GAPDH	Hs99999905_m1	3–3	HK
PKCα	PRKCA	Hs00176973_m1	11–12	target gene
PKCβI	PRKCB1	Hs01030676_m1	16–17	target gene
PKCβII	PRKCB2	Hs01034075_m1	16–17	target gene
PKCγ	PRKCG	Hs00177010_m1	3–4	target gene
PKCδ	PRKCD	Hs00178914_m1	18–19	target gene
PKCε	PRKCE	Hs00178455_m1	5–6	target gene
PKC θ	PRKCQ	Hs00234697_m1	2–3	target gene
PKC η	PRKCH	Hs00178933_m1	7–8	target gene
PKCζ	PRKCZ	Hs00177051_m1	8–9	target gene
PKC ι	PRKCN	Hs00702263_g1	3–4	target gene