Protein kinases A and C in post-mortem prefrontal cortex from persons with major depression and normal controls

Richard C. Shelton¹, D. Hal Manier¹ and David A. Lewis²
¹ Department of Psychiatry, Vanderbilt University, Nashville, TN, USA
² Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Major depression (MDD) is a common and potentially life-threatening condition. Widespread neurobiological abnormalities suggest abnormalities in fundamental cellular mechanisms as possible physiological mediators. Cyclic AMP-dependent protein kinase [also known as protein kinase A (PKA)] and protein kinase C (PKC) are important components of intracellular signal transduction cascades that are linked to G-coupled receptors. Previous research using both human peripheral and post-mortem brain tissue specimens suggests that a subset of depressed patients exhibit reduced PKA and PKC activity, which has been associated with reduced levels of specific protein isoforms. Prior research also suggests that specific clinical phenotypes, particularly melancholia and suicide, may be particularly associated with low activity. This study examined PKA and PKC protein levels in human post-mortem brain tissue samples from persons with MDD (n = 20) and age- and sex-matched controls (n = 20). Specific PKA subunits and PKC isoforms were assessed using Western blot analysis in post-mortem samples from Brodmann area 10, which has been implicated in reward mechanisms. The MDD sample exhibited significantly lower protein expression of PKA regulatory Ia (RIa), PKA catalytic a (Ca) and Cb, PKCβ1, and PKCε relative to controls. The melancholic subgroup showed low PKA RIa and PKA Cb, while the portion of the MDD sample who died by suicide had low PKA RIa and PKA Ca. These data continue to support the significance of abnormalities of these two key kinases, and suggest linkages between molecular endophenotypes and specific clinical phenotypes.

Introduction

Converging evidence implicates signal transduction cascades, particularly protein kinases, as possible mediators of vulnerability to major depression (MDD). Protein kinases are key regulators of receptor-effector cascades, such as the relationship between receptor activation and gene expression (Edelman et al. 1987). Two of these, cyclic AMP-dependent protein kinase [also known as protein kinase A (PKA)] and protein kinase C (PKC), have been shown to be abnormal in both central and peripheral tissue in depression. PKA is widely distributed and serves as the principal effector mechanism for G-coupled receptors linked to adenylyl cyclase. PKA is a tetramer comprised of two regulatory and two catalytic subunits. Activation of receptors stimulates the formation of cyclic AMP, which binds to two binding sites on each regulatory subunit, resulting in conformation change and dissociation of the catalytic subunits. The free catalytic subunits are responsible for catalysing the transfer of phosphates from ATP to serine and threonine residues in proteins. There are a number of regulatory (RIα, RIβ, RIIα, RIIβ) and catalytic (Ca and Cb) subunit isoforms that make up the PKA holoenzyme. Similarly, activation of receptors linked to phospholipase C stimulates the conversion of inositol triphosphate to phosphatidylinositol and diacylglycerol (DAG). DAG binds to the regulatory portion of PKC, also producing...
serine–threonine phosphorylation. Like PKA, several PKC isoform proteins were analysed in this study (PKCa, β1, β2, γ, δ, and ε).

An important target of both PKA and PKC phosphorylation is the transcription-factor cyclic AMP response element-binding protein (CREB). Phosphorylation of CREB results in translocation to the nucleus, where it binds to cyclic AMP response elements (CREs) in a variety of genes, regulating their expression (Meinkoth et al. 1993; Zanassi et al. 2001). These include genes coding for factors that have been implicated in the pathophysiology of depression (Shelton 2007). The expression of these gene products by antidepressants is dependent on phosphorylation of CREB and CREB–CRE interactions (Duman et al. 1997, 1999, 2000; Hyman & Nestler 1996). Hence, abnormalities of either enzyme could result in altered expression of genes thought to be involved in the genesis of depression. Key expression products regulated by CREB include brain-derived neurotrophic factor (BDNF) (Finkbeiner, 2000; Karege et al. 2004; Shieh et al. 1998) and its receptor TrkB (Deogracias et al. 2004; Duman & Vaidya, 1998; Finkbeiner, 2000; Nibuya et al. 1996), and glucocorticoid receptors (Barrett & Vedeckis, 1996). Because of their extensive distribution in brain, abnormal signal transduction via either PKA or PKC could result in a wide range of physiological changes.

Work from our laboratory has demonstrated a variety of abnormalities of PKA and PKC using a peripheral fibroblast tissue model. These include: (1) decreased phosphorylation of target polypeptides by PKA (Shelton et al. 1996) and PKC (Akin et al. 2005); (2) reduced binding of cyclic AMP to the putative regulatory subunits of PKA (Manier et al. 1996); and (3) decreases in specific PKA isoforms (Akin et al. 2005). Findings from other laboratories implicate both PKA and PKC in depression as well. For example, prefrontal cortex post-mortem tissue samples from suicide victims with a history of a diagnosis of MDD have been shown to have decreased binding and activity of both PKA and PKC, as well as expression of specific kinase proteins (Dwivedi et al. 2004b; Pacheco et al. 1996; Pandey et al. 1997, 2005, 2007). This has also been shown to be associated with reduced expression of CREB and CREB-DNA binding (Dwivedi et al. 2002, 2004b; Pandey et al. 2005). Of note, however, Pandey et al. (2005) have shown that there are significant regional differences in kinase activity and protein levels. In post-mortem brain tissue samples, PKA regulatory subunits Rαa and Rββ were reduced in prefrontal cortex [Brodmann area (BA) 9] and nucleus accumbens, but not hippocampus. They also showed decreased mRNA expression in both subunits in prefrontal cortex, but only Rαa in nucleus accumbens. Notably, these were different than the PKA protein expression levels in the cultured fibroblasts in a study from our laboratory, which showed abnormalities in Rαa and both catalytic subunits, Ca and Cβ. Therefore, although reduced PKA and PKC activity and expression levels can been seen in a variety of human tissues in depression, these may vary considerably.

One purpose of this study was to evaluate the protein levels from a brain region that has received limited attention, BA10 (also known as the frontopolar area). This region appears to be involved in a variety of unique activities, including executive functioning and memory retrieval (Konishi et al. 2000; Leung et al. 2005; Okuda et al. 2007; Rogers et al. 1999). Of significance to depression, BA 10 has been shown to be involved in the mediation of reward (Rogers et al. 1999). For example, Rogers et al. (1999), using positron emission tomography scanning, showed that a task which involved choices between high probability–low reward vs. low probability–high reward options resulted in the activation of three adjacent regions in the orbitofrontal cortex: BA 10, BA 11 and BA 47. Moreover, BA 10 has been shown to be selectively activated with cocaine administration; Kufahl et al. (2005) evaluated fMRI brain oxygen level-dependent (BOLD) signal changes with cocaine administration in regular users of the drug. This resulted in decreased BOLD signal in ventral tegmental area (VTA), nucleus accumbens, subcallosal cortex, ventral pallidum, amygdala, parahippocampal gyrus, posterior orbital gyrus, inferior and superior temporal gyrus, but marked and sustained increases in BOLD signal in BA 10 and BA 11. These are similar to increased BA 8 and BA 10 activity with amphetamine administration (Devous et al. 2001). These findings are consistent with the observation that BA 10 receives significant dopamine innervation and has a relatively high density of dopamine receptors (Volkow et al. 2000). Depression is a condition which, at its core, involves abnormal responses to rewarding stimuli, making BA 10 a logical target for study. To our knowledge, only one prior study evaluated kinase activity specifically in BA 10, and found no abnormalities of specific PKC protein isoforms (α, β, γ, ε) (Hrdina et al. 1998).

A second purpose of the study was to assess the relationship between clinical phenotypes and kinase abnormalities. Human post-mortem brain tissue research has suggested that kinase abnormalities may be associated with the clinical phenotype of suicide, leading to the hypothesis that reduced PKA and PKC activity may be factors predisposing to suicide.
(Dwivedi et al. 2002, 2004b; Pandey et al. 1997, 2003, 2005, 2007). Our prior research in peripheral tissue suggested that the reduced PKA and PKC were associated with the melancholic subtype of MDD, but not necessarily limited to people with serious suicidal ideation or history of suicide attempts (Akin et al. 2005; Shelton et al. 1999). Melancholia is a type of depression characterized by insomnia (especially early morning awakening), persistent anhedonia, fatigue, marked agitation or motor retardation, excessive guilt, anorexia, and weight loss. The kinase abnormalities were not found in cells from persons with non-melancholic depression, including those with the atypical phenotype. It is clear that these are not mutually exclusive hypotheses, and it is possible that there is physiological convergence between both melancholia and suicide phenotypes at the level of protein kinases.

**Methods**

All procedures were approved by the University of Pittsburgh’s Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research and the Vanderbilt University Health Sciences Institutional Review Board. Freshly frozen brain specimens were obtained from the University of Pittsburgh’s Brain Tissue Donation Program during autopsies after consent was given by the next of kin. Samples were obtained from 20 persons with MDD and control post-mortem brains matched individually for gender, age, race, and post-mortem interval (PMI). All samples came from persons free from all known psychotropic agents and recent substance abuse.

Field evaluations were conducted by trained and experienced clinicians and consisted of structured interviews with family members of the deceased after written informed consent was obtained. Ancillary data was also collected from clinical records, toxicology and neuropathology examinations, and the Medical Examiner’s investigation. After field interviews were completed, a committee of experienced research clinicians held an independent diagnostic conference and assigned consensus DSM-IV diagnoses for each subject on the basis of medical records and the results of structured interviews conducted with family members of the deceased.

Twenty-millimetre cubed sections of coronal blocks containing BA 10 of the frontal cortex were taken. The sections transversed cortical grey matter and included a small amount of white matter. After cutting, the samples were stored in a Trizol reagent (Invitrogen, USA) and stored at −80 °C.

**Western blot analysis**

Tissues were homogenized in 10 vol ice-cold buffer containing 20 mM Tris–HCl (pH 7.4 at 25°C), 2 mM EDTA, 25 mM 2-mercaptoethanol, 0.5 mM AEBSF, plus 0.5% Triton X-100, 2 μg/ml leupeptin, 3 μg/ml aprotinin, and 0.2 mg/ml soybean trypsin inhibitor and sonicated. The homogenate was centrifuged at 12,000 g for 10 min at 4°C. Equal volumes of supernatant (20 μl containing 30 μg protein) and gel loading solution [50 mM Tris–HCl (pH 6.8), 4% β-mercaptoethanol, 1% sodium dodecyl sulphate (SDS), 40% glycerol, and a trace amount of Bromphenol Blue] were mixed, then boiled for 3 min and kept on ice for 10 min. Samples were loaded onto 10% (w/v) SDS-polyacrylamide gel using the Mini Protein II gel apparatus (Bio-Rad, USA). The gels were run using 25 mM Tris-base, 192 mM glycerine, and 0.1% (v/v) SDS at 200 V. The proteins were transferred electrophoretically to an enhanced chemiluminescence (ECL) nitrocellulose membrane (Amersham, USA) using the Mini TransBlot transfer unit (Bio-Rad) at 0.25 A constant current. Membranes were washed with PBS containing 0.05% Tween-20 for 10 min. The blots were blocked by incubation with 3% (w/v) powdered non-fat milk in PBS. They were incubated overnight at 4°C with primary antibody [anti-PKA Rα, Rβ, RILα, RILβ, Ca, Cβ; anti-PKCα, β1, β2, γ, δ, and ε; anti-β-actin [all antibodies were from Santa Cruz Biotechnologies, USA] at a dilution of 1:1000 to 1:3000 depending on the antibody used. The membranes were washed with PBS and incubated with horseradish peroxidase-linked secondary antibody (anti-rabbit IgG; 1:3000) for 1.5 h at room temperature. The membranes were washed with water followed by PBS containing 0.05% Tween-20 and exposed to ECL film, then standardized using 10–100 μg protein. The optical density of the bands varied linearly with a concentration of up to 100 μg of protein. The band optical density was quantified using Un-Scan-It gel digitizing software (Orem, USA). The gels were stripped and reprobed for β-actin; the optical density was corrected by β-actin. All samples were performed in triplicate, four matched pairs (depressed vs. matched controls) of triplicates run on the same gel (for representative blots, see Fig. 1). Triplicates with out-of-range values were repeated with their matched controls. If values remained out of range, the sample was excluded from the analysis.

**Statistical analysis**

Demographic and post-mortem (e.g. PMI) variables were compared via independent-samples t test or χ² analysis as appropriate. Western blot data were
Demographic, tissue sample, and other descriptors are shown in Table 1. There were no significant differences in age ($t = -317$, d.f. = 38, $p = 0.75$), PMI ($t = 1.47$, d.f. = 38, $p = 0.88$), pH of samples ($t = -1.184$, d.f. = 38, $p = 0.24$), or sex (both groups were 15% female). All samples were from Caucasians. All were psychotropic drug free at the time of death based on toxicology.

### Immunolabelling of PKA subunit and PKC isoform proteins

Western blot analyses were conducted on the following proteins: PKA RIIα, RIIβ, RIIγ, Ca, Cβ, PKCa, β1, β2, γ, δ, and ε. The primary contrast was between the total depressed sample ($n = 20$) and matched controls. The results are summarized in Table 2. There were significant reductions in PKA RIIα ($t = 3.21$, d.f. = 17, $p = 0.005$), PKA Ca ($t = 2.87$, d.f. = 19, $p = 0.01$), PKA Cβ ($t = 3.40$, d.f. = 17, $p = 0.004$), PKCβ1 ($t = 2.11$, d.f. = 19, $p = 0.048$), and PKCζ ($t = 2.09$, d.f. = 17, $p = 0.05$); there were statistical trends for reduction in PKA RIIβ ($t = 1.76$, d.f. = 17, $p = 0.096$) and PKCβ2 ($t = 1.85$, d.f. = 18, $p = 0.081$). Of these, PKA RIIα and PKA Cβ survived correction for multiple comparisons.

The next analyses evaluated samples from those with MDD, melancholic subtype ($n = 15$) and matched controls. There were statistically significant differences for PKA RIIα ($t = 2.55$, d.f. = 13, $p = 0.024$), PKA Cβ ($t = 2.78$, d.f. = 11, $p = 0.018$), and PKCβ2 ($t = 2.36$, d.f. = 13, $p = 0.035$). There were reductions at a trend level for PKA RIIβ ($t = 1.75$, d.f. = 13, $p = 0.10$), PKA Ca ($t = 1.98$, d.f. = 14, $p = 0.068$), PKCβ1 ($t = 1.95$, d.f. = 14, $p = 0.072$), PKCβ2 ($t = 2.02$, d.f. = 13, $p = 0.065$), and PKCζ ($t = 1.87$, d.f. = 13, $p = 0.085$). The non-melancholic subgroup was small ($n = 5$); hence the data are reported in Table 2 for comparison purposes only, although PKA Ca ($t = 2.65$, d.f. = 4, $p = 0.057$) and PKCγ ($t = 2.61$, d.f. = 3, $p = 0.80$) were reduced at a trend level.

Samples from persons who died by suicide ($n = 12$) were then compared to matched controls. Significantly lower protein levels were shown for PKA RIIα ($t = 3.91$, d.f. = 9, $p = 0.004$) and PKA Ca ($t = 2.66$, d.f. = 10, $p = 0.024$), with a trend for PKA Cβ ($t = 2.23$, d.f. = 8, $p = 0.056$) in the suicide group compared to controls. Exploratory analyses were also conducted for the group of depressed patients who did not die by suicide ($n = 8$). Although the group size was relatively small, there was a significant reduction in PKA Cβ relative to controls ($t = 2.70$, d.f. = 7, $p = 0.031$).

### The effects of potential confounding variables

The effects of age, PMI, and pH on PKA and PKC protein expression were analysed via Pearson product moment correlation. Significant correlations were
### Table 1. Sample characteristics

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Group</th>
<th>Pair</th>
<th>Sex (M/F)</th>
<th>Race</th>
<th>Age (yr)</th>
<th>PMI (h)</th>
<th>pH</th>
<th>Cause of death</th>
<th>Manner of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>511</td>
<td>MDD*</td>
<td>1</td>
<td>M</td>
<td>W</td>
<td>43</td>
<td>17.9</td>
<td>6.91</td>
<td>ASCVD</td>
<td>Natural</td>
</tr>
<tr>
<td>596</td>
<td>MDD*</td>
<td>2</td>
<td>M</td>
<td>W</td>
<td>68</td>
<td>20.5</td>
<td>6.88</td>
<td>Gunshot wound</td>
<td>Suicide</td>
</tr>
<tr>
<td>600</td>
<td>MDD*</td>
<td>3</td>
<td>M</td>
<td>W</td>
<td>63</td>
<td>9.9</td>
<td>6.72</td>
<td>Hanging</td>
<td>Suicide</td>
</tr>
<tr>
<td>613</td>
<td>MDD*</td>
<td>4</td>
<td>M</td>
<td>W</td>
<td>59</td>
<td>15.6</td>
<td>6.95</td>
<td>Gunshot wound</td>
<td>Suicide</td>
</tr>
<tr>
<td>614</td>
<td>MDD</td>
<td>5</td>
<td>M</td>
<td>W</td>
<td>39</td>
<td>19.5</td>
<td>6.67</td>
<td>CO poisoning</td>
<td>Suicide</td>
</tr>
<tr>
<td>628</td>
<td>MDD*</td>
<td>6</td>
<td>M</td>
<td>W</td>
<td>26</td>
<td>21.6</td>
<td>6.73</td>
<td>CO poisoning</td>
<td>Suicide</td>
</tr>
<tr>
<td>668</td>
<td>MDD*</td>
<td>7</td>
<td>M</td>
<td>W</td>
<td>34</td>
<td>24.3</td>
<td>7.00</td>
<td>Hanging</td>
<td>Suicide</td>
</tr>
<tr>
<td>699</td>
<td>MDD</td>
<td>8</td>
<td>M</td>
<td>W</td>
<td>65</td>
<td>5.5</td>
<td>6.71</td>
<td>Gunshot wound</td>
<td>Suicide</td>
</tr>
<tr>
<td>735</td>
<td>MDD*</td>
<td>9</td>
<td>F</td>
<td>W</td>
<td>40</td>
<td>14.0</td>
<td>6.84</td>
<td>Pulmonary embolism</td>
<td>Accidental</td>
</tr>
<tr>
<td>927</td>
<td>MDD*</td>
<td>10</td>
<td>M</td>
<td>W</td>
<td>58</td>
<td>24.9</td>
<td>6.11</td>
<td>ASCVD</td>
<td>Natural</td>
</tr>
<tr>
<td>949</td>
<td>MDD*</td>
<td>11</td>
<td>M</td>
<td>W</td>
<td>38</td>
<td>25.0</td>
<td>6.23</td>
<td>Cardiac arrhythmia</td>
<td>Natural</td>
</tr>
<tr>
<td>1013</td>
<td>MDD*</td>
<td>12</td>
<td>M</td>
<td>W</td>
<td>46</td>
<td>16.1</td>
<td>6.27</td>
<td>Nailgun wound</td>
<td>Suicide</td>
</tr>
<tr>
<td>1017</td>
<td>MDD</td>
<td>13</td>
<td>M</td>
<td>W</td>
<td>27</td>
<td>18.8</td>
<td>5.69</td>
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<td>Natural</td>
</tr>
<tr>
<td>1028</td>
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<td>M</td>
<td>W</td>
<td>39</td>
<td>14.5</td>
<td>6.18</td>
<td>Gunshot wound</td>
<td>Suicide</td>
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<td>MDD</td>
<td>15</td>
<td>M</td>
<td>W</td>
<td>47</td>
<td>24.0</td>
<td>6.57</td>
<td>ASCVD</td>
<td>Natural</td>
</tr>
<tr>
<td>1131</td>
<td>MDD*</td>
<td>16</td>
<td>M</td>
<td>W</td>
<td>29</td>
<td>26.6</td>
<td>6.92</td>
<td>Gunshot wound</td>
<td>Suicide</td>
</tr>
<tr>
<td>1186</td>
<td>MDD*</td>
<td>17</td>
<td>M</td>
<td>W</td>
<td>45</td>
<td>6.6</td>
<td>6.62</td>
<td>Traumatic asphyxiaton</td>
<td>Accidental</td>
</tr>
<tr>
<td>1215</td>
<td>MDD*</td>
<td>18</td>
<td>M</td>
<td>W</td>
<td>44</td>
<td>11.0</td>
<td>6.54</td>
<td>ASCVD</td>
<td>Natural</td>
</tr>
<tr>
<td>1221</td>
<td>MDD*</td>
<td>19</td>
<td>F</td>
<td>W</td>
<td>28</td>
<td>24.8</td>
<td>6.61</td>
<td>Pulmonary embolism</td>
<td>Natural</td>
</tr>
<tr>
<td>10028</td>
<td>MDD</td>
<td>20</td>
<td>F</td>
<td>W</td>
<td>72</td>
<td>23.1</td>
<td>6.66</td>
<td>Gunshot wound</td>
<td>Suicide</td>
</tr>
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</table>

Mean (s.d.) values

<table>
<thead>
<tr>
<th>MDD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.5 (14.2)</td>
<td>46.9 (13.4)</td>
</tr>
<tr>
<td>18.2 (6.4)</td>
<td>17.9 (5.6)</td>
</tr>
<tr>
<td>6.65 (0.23)</td>
<td>6.74 (0.27)</td>
</tr>
</tbody>
</table>

**MDD, Major depression; PMI, post-mortem interval; ASCVD, arteriosclerotic cardiovascular disease.**

*Melancholic subtype.
Table 2. PKA and PKC Western blot results (arbitrary density units)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Controls (n = 12)</th>
<th>Total sample (n = 20)</th>
<th>Not melancholic (n = 5)</th>
<th>Suicide (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKA RI</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>PKA RI</td>
<td>1.69 ± 1.25</td>
<td>1.14 ± 0.96</td>
<td>0.84 ± 0.62</td>
<td>0.71 ± 0.50</td>
</tr>
<tr>
<td>PKA RI</td>
<td>0.84 ± 0.86</td>
<td>0.88 ± 0.77</td>
<td>0.41 ± 0.31</td>
<td>0.32 ± 0.31</td>
</tr>
<tr>
<td>PKA RI</td>
<td>1.46 ± 1.70</td>
<td>1.28 ± 1.73</td>
<td>0.45 ± 0.40</td>
<td>0.45 ± 0.40</td>
</tr>
<tr>
<td>PKA C</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>PKA C</td>
<td>0.41 ± 0.24</td>
<td>0.42 ± 0.24</td>
<td>0.95 ± 0.62</td>
<td>0.89 ± 0.62</td>
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<tr>
<td>PKA C</td>
<td>1.46 ± 1.07</td>
<td>1.38 ± 1.07</td>
<td>1.05 ± 0.86</td>
<td>1.05 ± 0.86</td>
</tr>
<tr>
<td>PKA C</td>
<td>1.83 ± 0.31</td>
<td>1.62 ± 0.31</td>
<td>0.86 ± 0.86</td>
<td>0.86 ± 0.86</td>
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<tr>
<td>PKA C</td>
<td>0.86 ± 0.45</td>
<td>0.85 ± 0.45</td>
<td>1.04 ± 0.60</td>
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</tr>
</tbody>
</table>

- Expressed as percent matched control values; a p < 0.05 vs. matched control (corrected); b p < 0.05 vs. matched control (uncorrected).

Discussion

This set of MDD brain tissue samples from BA 10 demonstrated reduced levels of specific PKA and PKC isoform proteins relative to controls. These findings are similar to those previously reported (Dwivedi et al. 2002, 2004b; Pandey et al. 1997, 2005) with some differences. In the present study, protein levels of PKA RII, PKA Cα, PKA Cβ, PKCβ1, and PKCε were significantly reduced (with a trend for PKCβ2) in MDD samples. These results differ somewhat from those previously reported from other brain areas (Dwivedi et al. 2002, 2004b; Pandey et al. 1997, 2005) or peripheral tissues (Akin et al. 2005; Pandey et al. 2002; Perez et al. 2001; Tardito et al. 2000). For example, Dwivedi et al. (2004b) demonstrated reduced levels of PKA RIIβ and Cβ from BA 9 in two separate cohorts of depressed suicide victims from Budapest and USA (Maryland). Pandey et al. have shown reduced PKA RII and RIIβ (Pandey et al. 2005) and the α, β1, β2, and γ isoenzymes of PKC (Pandey et al. 2004) in BA 9 from samples from a cohort of teenage suicide victims (PKCε was not reported). This group also investigated PKA activity and subunit expression from other brain regions and showed low PKA RII and RIIβ in nucleus accumbens, but not hippocampus. These results, along with our previous work in peripheral fibroblast cultures, suggest that reduced kinase proteins may vary considerably from one tissue type to another. This, then, has significant implications regarding both the mechanisms by which these effects occur, and the impact on gene expression caused by the changes.

The one prior study that investigated PKC isoform proteins in this region found no differences between depressed suicide victims and control samples (Hrdina et al. 1998). That study also examined BA 9 and found no differences in PKC isofoms, which is at odds with findings from BA 9 by Pandey and colleagues who found decreases in PKCα, β1, β2, and γ (Pandey et al. 2004) as well as PKC-dependent phosphorylation of myristilated alanine-rich C kinase substrate (Pandey et al. 2003) in suicide victims compared to controls. Other than certain differences in methods, the reasons for these discrepancies are unclear.

Protein kinases serve as key translators of intracellular responses to G-coupled receptor activation,
serving a wide variety of functions, including gene expression, plasticity, memory, and cell differentiation. (Dwivedi et al. 2004a; Dwivedi & Pandey, 2008) Therefore, alterations of kinase protein availability as demonstrated in the present study would be expected to have broad effects. The significance of disruption of kinase signalling in BA 10 is unclear, but it is likely to have significant effects on the function of this important cortical region. As noted earlier, BA 10 is involved in the mediation of reward-related behaviours (Rogers et al. 1999) and it is also selectively activated by the administration of cocaine (Kufahl et al. 2005) or amphetamine (Devous et al. 2001) in humans. Notably, targeted disruption of PKA RI\(\beta\), which was found to be reduced in the present study, has been shown to decrease amphetamine-induced locomotor responses and both striatal fos induction and dynorphin expression in mice. (Brandon et al. 1998) For example, studies have shown activation of both PKA and PKC (Alvarez-Jaimes et al. 2005; Narita et al. 2007) as well as CREB phosphorylation (Alvarez-Jaimes et al. 2005) in reward-related reinforcement of behaviour. Inhibition of PKC activity in the nucleus accumbens reduced amphetamine-induced place preference learning and dopamine release in mice (Narita et al. 2004). A similar effect is achieved by inhibition of PKA in nucleus accumbens, which reverses a variety of reward-related behaviours, including conditioned approach behaviours and lever pressing for food, as well as conditioned place preference related to local injections of amphetamine or cocaine in rats (Beninger & Gerdjikov, 2004). Similar effects were observed with inhibition of PKA in the amygdala or medial prefrontal cortex (Beninger & Gerdjikov, 2004). Curiously, previously learned reward behaviours were not disrupted with inhibition of PKA, suggesting that it is important for the acquisition of incentive learning (Beninger & Gerdjikov, 2004). Hence, altered cell signalling via PKA or PKC, as shown in the present study, might be expected to diminish reward-related behavioural responses, which are key characteristics of depression.

A potentially significant finding of the present study was an apparent interaction between clinical phenotypes and molecular endophenotypes. Our previous work in peripheral tissues suggested that reduced PKA and PKC activity was specific to the melancholic subtype and was not found in non-melancholic patients (Akin et al. 2005). Melancholia appears to be a distinct clinical phenotype (Kendler, 1997), with evidence of hyperarousal of CNS (Gold & Chrousos, 2002). Symptoms of overactivation include insomnia, hypophagia, agitation, and inhibited sex drive (Parker et al. 1995). Melancholia also appears to be associated with elevated central and peripheral measures of noradrenaline activity (Wong et al. 2000) and HPA axis (Duval et al. 2006; Sahoo & Subho, 2007; Wong et al. 2000). However, in the present study the reductions in PKA and PKC isoforms did not appear to be specific to the melancholic subtype in this brain region (Table 2), although the sample of non-melancholics was too small to make definitive comparisons. Future research should investigate larger numbers of melancholic and non-melancholic patients.

Those who died by suicide in the present study \((n = 12)\) showed extremely low PKA RI\(\alpha\) at 53.5\% of matched controls, as well as a significant reduction in PKA CA, and a trend for PKC C\(\beta\). This stands in contrast to the samples from persons who did not die from suicide in which RI\(\alpha\) was 89\% of controls, a non-significant difference. In the non-suicide sample, PKA CA was not significantly reduced, although C\(\beta\) was significantly at 78.9\% of controls. As noted earlier, prior studies in other brain regions show reductions in different isoforms in suicide samples. One study (Dwivedi et al. 2004b) demonstrated low PKA RI\(\beta\) and C\(\beta\) in BA 9. Other studies have shown low PKA RI\(\alpha\) and RI\(\beta\) (Pandey et al. 2005) and PKCa, \(\beta\)1, \(\beta\)2, and \(\gamma\) (Pandey et al. 2004), also in BA 9, as well as reduced PKA RI\(\alpha\) and RI\(\beta\) in nucleus accumbens. This indicates considerable regional variability in differential expression of PKA and PKC isoforms. However, it also suggests that completed suicide may be a distinct clinical phenotype that may map on to certain molecular endophenotypes. The net effect of reduced PKA and PKC isoforms may be reduced signalling via CREB phosphorylation, which could have significant implications for the treatment of suicidal patients.

The apparent variability of individual PKA and PKC isoform proteins by brain region and clinical phenotype is intriguing and may have significant implications regarding causal models of depression. However, the actual mechanisms for this variability remain obscure. Prior research has indicated that mRNA expression may be reduced, which may account for the reduced protein concentrations shown in both this study and earlier post-mortem research. (Dwivedi et al. 2004b; Pandey et al. 2004, 2005) Notably, changes in isoform-specific protein levels have been shown in prior animal models of depression. For example, stress-induced learned helplessness in rats, a putative model of depression, resulted in decreased expression of PKA RI\(\beta\), CA, and C\(\beta\) in both prefrontal cortex and hippocampus, with a trend towards reduction of RI\(\alpha\) in prefrontal cortex in one study (Dwivedi et al. 2004a). Similarly, exogenously administered corticosterone in rats (a model of elevated
hypothalamic–pituitary–adrenal axis activity in human depression) was shown to be associated with altered PKA RIIα, Cα, and Cβ expression. However, further research must be performed to elucidate the mechanisms for tissue- and isoform-specific alterations in expression levels.

Caution needs to be exercised in interpreting these data. For example, the study only involved 20 depressed and control samples, all were from Caucasians and all but three in each group were from males. Therefore, the results may not be representative of all depressed patients. In addition, classification of both melancholic status and suicide were made retrospectively and, in the case of diagnosis, by second-hand report. However, the support for these findings from previous studies is encouraging.

In conclusion, the results of this study continue to show a significant association between reduced activity of two key serine–threonine kinases, PKA and PKC. Moreover, the results indicate that death by suicide represents a distinct clinical phenotype that appears to be associated with a particular set of molecular endophenotypes. Future research is needed to better understand the relationships between these clinical and molecular factors.

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Statement of Interest

None.

References


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