Protein Kinase C Expression and Activity After Global Incomplete Cerebral Ischemia in Dogs

Frederick E. Sieber, MD; Richard J. Traystman, PhD; Phillip R. Brown, DVM; Lee J. Martin, PhD

Background and Purpose—Studies suggest that protein kinase C (PKC) activation during ischemia plays an important role in glutamate neurotoxicity and that PKC inhibition may be neuroprotective. We tested the hypothesis that elevations in the biochemical activity and protein expression of Ca\(^{2+}\)-dependent PKC isoforms occur in hippocampus and cerebellum during the period of delayed neurodegeneration after mild brain ischemia.

Methods—We used a dog model of 20 minutes of global incomplete ischemia followed by either 6 hours, 1 day, or 7 days of recovery. Changes in PKC expression (Western blotting and immunocytochemistry) and biochemical activity were compared with neuropathology (percent ischemically damaged neurons) by means of hematoxylin and eosin staining.

Results—The percentage of ischemically damaged neurons increased from 13±4% to 52±10% in CA1 and 24±11% to 69±6% in cerebellar Purkinje cells from 1 to 7 days, respectively. The occurrence of neuronal injury was accompanied by sustained increases in PKC activity (240% and 211% of control in hippocampus and cerebellum, respectively) and increased protein phosphorylation as detected by proteins containing phosphoserine residues. By Western blotting, the membrane-enriched fraction showed postischemic changes in protein expression with increases of 146±64% of control in hippocampal PKC\(\alpha\) and increases of 138±38% of control in cerebellar PKC\(\alpha\), but no changes in PKC\(\beta\) and PKC\(\gamma\) were observed. By immunocytochemistry, the neurell of CA1 and CA4 in hippocampus and the radial glia in the molecular layer of cerebellum showed increased PKC\(\alpha\) expression after ischemia.

Conclusions—This study shows that during the period of progressive ischemic neurodegeneration there are regionally specific increases in PKC activity, isoform-specific increases in membrane-associated PKC, and elevated protein phosphorylation at serine sites. (Stroke. 1998;29:1445-1453.)

Key Words: cerebral ischemia, global ▪ cerebral ischemia, transient ▪ neuronal damage ▪ neuronal death ▪ protein kinase C ▪ dogs

The PKC family consists of at least 12 different isozymes that catalyze serine/threonine phosphorylation of target proteins. Depending on their mode of activation, these isoforms are divided into Ca\(^{2+}\)-dependent isoforms \((\alpha, \beta, \beta, \gamma)\) and Ca\(^{2+}\)-independent isoforms \((\delta, \epsilon, \theta)\). PKC isoforms are differentially distributed within different tissue types. In the central nervous system, PKC activation leads to phosphorylation of many intracellular proteins that initiate and regulate various signal transduction processes that function in neuronal excitability, release of neurotransmitters, synaptic plasticity, and cell proliferation. PKC is activated by interaction/binding with specific activators such as DAG and Ca\(^{2+}\) at the regulatory binding site. For example, PKC is activated by increased intracellular Ca\(^{2+}\) occurring as a result of glutamate receptor activation. In addition, the activation of phospholipases A\(_2\) and C leads to accumulation of DAG. These alterations in the neuronal intracellular milieu may lead to pathological activation of PKC during cerebral ischemia and reperfusion.
Selected Abbreviations and Acronyms

DAG = diacylglycerol
H&E = hematoxylin and eosin
OD = optical density
PKC = protein kinase C

Temporary Global Incomplete Ischemia Model
Twenty minutes of global incomplete ischemia was induced in dogs (n = 20) with the use of halothane (1% to 2% inspired) anesthesia as previously described.2,9 Epidural temperature was maintained at 37°C to 38°C. End-tidal CO₂ was monitored, and ventilation was adjusted to maintain PaCO₂ at 35 to 40 mm Hg.

In this compression model of global incomplete ischemia, cerebral perfusion pressure is precisely regulated, and cerebral blood flow, cerebral oxygen consumption, ATP, and intracellular pH are reproducibly altered. The percent decrease in cerebral blood flow is uniform in cortex and subcortical regions. Therefore, the level of ischemic insult is similar in all brain regions. The heart and other organs are not ischemic. Recovery of somatosensory evoked potentials is similar to that in cardiac arrest models of similar duration.10

Neuropathology
This model allows the evaluation of selective neuronal damage in several brain regions and evaluation of mechanisms of injury in all selectively vulnerable regions in response to the same insult independent of damage to peripheral organs. The neuropathology is similar bilaterally.59 There is a reproducible level of selective neuronal damage that occurs continuously over 7 days without infarct. In addition, cerebellar damage occurs in this model, unlike most rodent models of global ischemia.

Neuropathologic analysis of hippocampal and cerebellar damage in sham control and ischemic animals was performed as previously described.39 Sections of two brain regions were stained for H&E and evaluated quantitatively for neuronal injury: the hippocampus (CA1) and the anterior cerebellar lobule at the midline. In sections stained for H&E, the number of neurons was counted, and the percentage of neurons with damage was determined as previously described.2,3 The percent neuronal damage in each region was averaged for each animal, and a group mean was calculated. The number of neurons per square millimeter was calculated for each region in all animals, and the percentage of neurons remaining was calculated for the ischemic animals.

Immunocytochemistry
We examined changes in the Ca²⁺-dependent PKC isofoms α, β, and γ. The right cerebrum and cerebellum were systematically cut into 1-cm-thick coronal slabs, cryoprotected in 20% buffered glycerol, frozen in isopentane, and stored at −80°C for immunocytochemical analyses. Immunocytochemistry was performed in hippocampus and cerebellum sections with the use of an immunoperoxidase method11–13 to localize PKC isoforms

PKC Enzyme Activity
To evaluate whether changes in PKC expression are associated with a gain or loss of function, biochemical assays of PKC activity were performed. PKC determinations in S2 and P2 fractions were done with a commercially available PKC enzyme assay system (Amer sham) that measures the transfer of 32P from ATP to a specific PKC substrate peptide in fresh-frozen brain homogenates of membrane and soluble fractions. Linearity of this assay was verified by using different amounts of protein in a constant reaction volume.

Experimental Design
In ischemic and sham animals, we quantitatively and qualitatively evaluated protein expression of PKC isoforms α, β, and γ by immunocytochemistry and Western blotting in cerebellar cortex and hippocampus. To demonstrate that increased PKC expression was specific for selectively vulnerable regions, in ischemic and sham animals we quantitatively evaluated protein expression of PKC isoforms α and γ by Western blotting in thalamus and medulla (regions with minimal neuronal damage).7 To demonstrate that increased PKC expression correlates with activity, in ischemic and sham animals we quantitatively evaluated total PKC activity in cerebellar cortex and hippocampus. To demonstrate that increases in PKC activity were most likely a result of Ca²⁺-dependent isoforms, we quantitatively evaluated protein expression of PKCα by Western blotting in cerebellum and hippocampus. To demonstrate that postischemic changes in PKC expression and activity resulted in altered protein phosphorylation, we quantitatively evaluated expression of phosphoserine-containing proteins by Western blotting in cerebellar cortex and hippocampus.

We determined protein expression at four time points: control sham-operated dogs, 6 hours after insult, 1 day after insult, and 7 days after insult. The sham-operated animals were anesthetized, underwent surgical procedures similar to those in their experimental counterparts, and were then killed (n = 7 total; n = 3 for immunocy-
Delayed Neuronal Damage After Incomplete Global Ischemia

In cerebellum the sham-operated animals had no Purkinje cell damage and a baseline density of 34 ± 6 Purkinje cells per square millimeter. The percentage of damaged Purkinje cells increased from 24 ± 11% to 69 ± 6% at 1 and 7 days after ischemia, respectively. At 1 and 7 days after ischemia, the numbers of remaining Purkinje cells were 82 ± 5% and 78 ± 16% of control, respectively.

In hippocampus the sham-operated animals had no CA1 pyramidal cell damage and a baseline density of 1012 ± 85 CA1 pyramidal neurons per square millimeter. CA1 pyramidal cell damage increased from 13 ± 4% to 52 ± 10% of remaining neurons damaged at 1 and 7 days after ischemia, respectively. At 1 and 7 days after ischemia the numbers of remaining CA1 pyramidal cells were 97 ± 10% and 43 ± 10% of control, respectively.

Global Ischemia Causes Increased PKC Activity

Table 1 shows the total PKC activity in membrane and soluble fractions. Total PKC activity in the membrane fraction was ≤ 10% of the soluble fraction. In hippocampus, total PKC activity in the membrane fraction was 281 ± 47% and 228 ± 28% of control at 1 and 7 days after ischemia, respectively. In cerebellum, total PKC activity in the membrane fraction was 280 ± 23% and 261 ± 36% of control at 1 and 7 days after ischemia, respectively. Postischemic total PKC activity in the soluble fraction increased to 152 ± 62% and 225 ± 100% of control in cerebellum and hippocampus, respectively.

Isoform-Specific Augmentation of PKC Protein Expression After Global Cerebral Ischemia

These results will focus on the postischemic changes in the membrane fraction. Postischemic increases in protein expression in the P2 fraction represent activation and translocation of PKC.1

In the hippocampus a postischemic increase in PKCα expression in the P2 fraction occurred starting 1 day after ischemia (Table 2, Figure 1). At 6 hours after ischemia, there was no increase in PKCα expression in the P2 fraction (data not shown). This indicates that postischemic translocation and activation of PKCα does not occur until sometime between 6 and 24 hours. PKCβ expression in P2 was similar to control in the P2 fraction after ischemia. PKCγ expression in the P2 fraction decreased by 7 days after ischemia. The increases in total PKC activity measured in the hippocampal

### Table 1. Total PKC Biochemical Activity

<table>
<thead>
<tr>
<th>Region</th>
<th>Fraction</th>
<th>Sample</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>S2</td>
<td>Control</td>
<td>3.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day after ischemia</td>
<td>4.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 day after ischemia</td>
<td>4.5 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>Control</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day after ischemia</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 day after ischemia</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>S2</td>
<td>Control</td>
<td>5.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day after ischemia</td>
<td>12.6 ± 2.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 day after ischemia</td>
<td>10.6 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>Control</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 day after ischemia</td>
<td>1.3 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 day after ischemia</td>
<td>1.0 ± 0.1*</td>
</tr>
</tbody>
</table>

*Activity values are mean ± SD, measured as picomoles phosphate transferred per minute per microgram protein.

### Table 2. PKC Isoform Expression by Western Blotting

<table>
<thead>
<tr>
<th>Region</th>
<th>Fraction</th>
<th>Isoform</th>
<th>Day After Ischemia</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>S2</td>
<td>α</td>
<td>1</td>
<td>81 ± 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>7</td>
<td>123 ± 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>7</td>
<td>135 ± 23*</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>α</td>
<td>1</td>
<td>159 ± 27*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>7</td>
<td>55 ± 16*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>7</td>
<td>60 ± 18*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>S2</td>
<td>α</td>
<td>1</td>
<td>154 ± 19*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>7</td>
<td>149 ± 30*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>7</td>
<td>67 ± 27</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>α</td>
<td>1</td>
<td>129 ± 12*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>7</td>
<td>63 ± 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>7</td>
<td>85 ± 29</td>
</tr>
</tbody>
</table>

*Percent control values are mean ± SD. No 50-kD fragments were observed in any Western blotting preparation.

*P < 0.05 compared with nonischemic control.
P2 fraction most closely parallel the increased expression of PKCα. In addition, the increases in total PKC activity measured in the hippocampal S2 fraction (Table 1) are consistent with the increased expression of PKC isoforms α and γ.

In membrane fractions of cerebellum both PKCγ and PKCβ expression were decreased after ischemia (Table 2, Figure 2). However, increases in total PKC activity measured in the cerebellar P2 fraction are consistent with an increase in PKCα expression (Figure 2). In addition, the increases in total PKC activity measured in the cerebellar S2 fraction are consistent with the postischemic increases in expression of PKCβ and PKCγ.

No changes in postischemic expression of PKCδ occurred in the P2 fractions of either hippocampus or cerebellum, suggesting that the Ca2+–dependent isoforms are most likely responsible for the increased total PKC activity of the P2 fractions.

To evaluate whether P2 fraction changes in PKC expression are regionally selective, Western blotting of P2 fractions for PKCα and PKCγ was performed in thalamus and medulla of both ischemic and sham animals. These two regions undergo minimal postischemic damage by H&E microscopy.7 In thalamus, PKCγ was 48% and 37% of control at 1 and 7 days after ischemia, respectively, while PKCα was 50% and 42% of control at 1 and 7 days after ischemia, respectively. In medulla, PKCγ was 39% and 30% of control at 1 and 7 days after ischemia, respectively, while PKCα was 63% and 42% of control at 1 and 7 days after ischemia, respectively. These results demonstrate that PKC protein levels in membrane fractions are decreased in ischemia-resistant regions. These results suggest that postischemic translocation of Ca2+–dependent PKC isoforms is specific for selectively vulnerable regions.

Immunocytochemistry Shows Selective Regional and Cellular Changes in PKC Localization After Ischemia

Hippocampus

In control dogs PKCα was expressed in the pyramidal cell bodies of CA1 (Figure 3A). Pyramidal cell bodies of CA2 through CA4 and granule cells of dentate gyrus had moderate expression (Figure 4D). On day 1 after ischemia there was increased labeling of both CA2 and CA4 cell bodies, which continued through day 7 after ischemia. In addition, at day 1 after ischemia the neuropil of CA4 exhibited increased labeling of PKCα (Figure 4E). Densitometric analysis showed that CA4 neuropil immunoreactivity increased from 0.41±0.04 in sham animals to 0.47±0.01 and 0.87±0.08 OD units at 1 and 7 days after ischemia, respectively. Furthermore, immunoreactivity of CA1 neuropil increased by 7 days after ischemia (0.56±0.05 and 0.71±0.04 OD units in sham animals and 7 days after ischemia, respectively). This pattern of increased PKCα expression at day 7 after ischemia is illustrated in Figure 4F. Thus, there is a neuronal component to increased PKCα expression. The contribution of glial elements to the increased PKCα immunoreactivity in the neuropil could not be determined by light microscopy.

In control dogs, PKCβ was uniformly expressed in the cell bodies of CA1 through CA4 (data not shown). The labeling
was darkest in CA3. In addition, there was expression of PKCβ in the granule cell layer of the dentate gyrus. The neuropil was poorly labeled throughout. On day 1 after ischemia there were increases in PKCβ expression in the neuropil (0.24±0.02 and 0.40±0.02 OD units in sham animals and 1 day after ischemia, respectively) as well as the inferior blade of the dentate in the molecular layer. The cell bodies of CA1 through CA4, which had previously expressed PKCβ, were now poorly labeled. The pattern of increased neuropil labeling but decreased cell body labeling persisted 7 days after the insult.

In control dogs, PKCγ immunoreactivity was present in both the perikaryal and proximal dendritic compartments of CA1 pyramidal cells (Figure 3D). A similar pattern of expression occurred in CA3 (not shown). Granule cells of the dentate gyrus were heavily labeled, with the inferior region staining darker than the superior region of the dentate gyrus (Figure 4A). At 1 day after ischemia, PKCγ labeling in CA1 pyramidal cells was similar to that in control (Figure 3E). However, the neuropil, especially the stratum radiatum, was more heavily labeled (0.45±0.05 and 0.50±0.03 OD units in sham animals and 1 day after ischemia, respectively). In the dentate gyrus, PKCγ labeling was increased in the inferior region of the granule cell layer (1.03±0.13 and 1.31±0.39 OD units in sham animals and 1 day after ischemia, respectively) and the molecular layer (0.55±0.03 and 0.58±0.02 OD units in sham animals and 1 day after ischemia, respectively). This pattern persisted at 7 days after insult, with the granule cells maintaining their increased PKCγ expression (Figure 4C). Discrete labeling of astrocyte cell bodies was not prominent.

**Cerebellum**

In control animals, light staining of PKCα occurred in the Purkinje cell bodies (Figure 5C). Their proximal dendrites were poorly labeled. There was some labeling of small cell bodies in the molecular layer. At 1 day after ischemia there was decreased labeling of both Purkinje cells and small cell bodies in the molecular layer. However, glial labeling occurred in white matter (Figure 5B). At 7 days after insult neither Purkinje cell bodies nor their dendrites as well as interneuron-like cells in the molecular layer were labeled, whereas the radial glia in the molecular layer were PKCα immunoreactive (Figure 5D).

There was more PKCβ immunoreactivity in cerebellum than in hippocampus. There was light labeling of Purkinje cells and the molecular layer. At 1 day after ischemia, the
PKC Expression Is Increased After Ischemia

Figure 6. Western blotting analysis of phosphoserine-containing proteins in hippocampus. Molecular weight standards (in kilodaltons) are indicated (right). Bracketed lanes labeled 7 are dogs 7 days after ischemia. Bracketed lanes labeled 1 are dogs 1 day after ischemia. Bracketed lanes labeled C are control nons ischemic dogs.

Purkinje cells and proximal dendrites expressed more PKCβ than did controls (0.32±0.01 and 0.39±0.03 OD units in sham animals and 1 day after ischemia, respectively). This pattern persisted by 7 days after insult, with increased Purkinje cell labeling and increased labeling of the neuropil.

In controls neither granule nor molecular layers stained for PKCγ. The cell bodies and proximal dendrites of the Purkinje cells expressed PKCγ, and faint labeling of the axons in subcortical white matter was observed. At 1 day after insult there was increased staining in Purkinje cells (0.37±0.02 and 0.49±0.03 OD units in sham animals and 1 day after ischemia, respectively) and their dendrites as well as in the molecular layer (0.33±0.01 and 0.50±0.06 OD units in sham animals and 1 day after ischemia, respectively) and subcortical white matter (0.12±0.01 and 0.21±0.01 OD units in sham animals and 1 day after ischemia, respectively). A similar pattern of increased PKCγ expression was observed at 1 and 7 days after ischemia.

Protein Phosphorylation of Serine Sites Is Increased After Ischemia

In hippocampus, increases in phosphoserine-containing proteins occurred at 1 and 7 days after ischemia (Figure 6). Phosphorylation of proteins with a molecular weight of approximately 79 kD was 135% and 108% of control at 1 and 7 days after ischemia, respectively (Figure 6). In addition, phosphorylation of proteins with a molecular weight of approximately 50 kD was 120% and 157% of control at 1 and 7 days after ischemia, respectively. In cerebellum, there were no obvious postischemic increases in phosphoserine-containing proteins (data not shown). These results show that sustained postischemic increases in protein phosphorylation at serine residues occur in selectively vulnerable regions in conjunction with increased PKC expression/activity.

Discussion

This study shows that during the progression of neurodegeneration after ischemia there is a sustained increase in total PKC activity and isoform-specific translocation and activation in hippocampus and cerebellum. These changes are accompanied by increased protein phosphorylation, as determined by regionally specific increases in phosphoserine-containing proteins. In hippocampus, an increase in Ca2+-dependent PKC protein expression may account for a significant portion of the enzyme activity increases. However, the cellular specificity for this change is uncertain because it was not possible to rule out the contribution of glial elements to the increased PKCα immunoreactivity in the neuropil. For example, in cerebellum, Ca2+-dependent PKC activation may represent a glial response.

During and after ischemia, increases in neuronal intracellular Ca2+ occur as a result of glutamate excitotoxicity. In addition, the activation of phospholipases A2 and C leads to accumulation of DAG. These alterations in the neuronal intracellular milieu lead to activation of PKC during ischemia and reperfusion. However, changes in brain PKC biochemical activity appear to depend on the severity of ischemia. In gerbils, PKC activation occurs at a cerebral blood flow of 35 to 40 mL/100 g per minute compared with the level at which energy failure occurs (20 mL/100 g per minute). This suggests that mild levels of ischemia, not associated with energy failure, cause PKC activation. This is consistent with the requirement of ATP for PKC function. When the model of bilateral carotid occlusion in gerbils is used, the following occur: 2 minutes of ischemia causes no PKC activation; 5 minutes of ischemia causes increased PKC activity in the membranous fraction of CA1 and CA3 hippocampus by 3 days of reperfusion; 6 minutes of ischemia causes increased PKC activation in CA1 by 1 hour of reperfusion and increased PKC activity in the membranous fraction of hippocampus by 24 hours of reperfusion; and 10 minutes of ischemia causes a decrease in total PKC activity by 2 hours of reperfusion. When the model of cardiac arrest in rats is used, the following occur: in neocortex and hippocampus there is a continuous decrease in PKC activity to approximately 60% of control by 30 minutes of ischemia, and after 11 to 13 minutes of arrest there is no recovery of PKC activity with reperfusion. When the four-vessel occlusion model in rats is used, the following occur: 5 minutes of ischemia causes no change in PKC activity; 10 minutes of ischemia causes a decrease in hippocampal PKC activity to 65% of control by 2 hours of reperfusion; and 20 minutes of ischemia causes a decrease in hippocampal PKC activity to 40% to 50% of control during ischemia and reperfusion. When the two-vessel occlusion plus hypotension model in rats is used, the following occur: 15 minutes of ischemia causes a 52% decrease in PKC activity in the membranous fraction of striatum and neocortex during ischemia, and 20 minutes of ischemia causes decreases in total PKC activity in hippocampus, cortex, and striatum. Taken together, these results in different species and ischemic models suggest that PKC activity is dependent on the severity of ischemia, with milder insults associated with increases in activity and more severe insults causing decreases in activity. Enzyme activation and translocation do occur even with severe ischemia and reperfusion and are isoform specific. The decreases in PKC activity, despite documented PKC translocation to the membrane compartment during severe ischemia, suggest that an endogenous PKC inhibitor is produced or that translocation and denaturation occur concurrently under these conditions. It is important to emphasize that the model of ischemia in the present study produces a mild to moderate insult. This conclusion is based on two pieces of evidence. First, previous 31P MR spectroscopic studies have shown that the 20-minute global incomplete ischemia dog model causes end-ischemic
phosphocreatine and ATP concentrations of 14±6% and 32±13% of baseline, respectively. On the other hand, the 15-minute two-vessel occlusion plus hypotension model in rats causes end-ischemic phosphocreatine and ATP concentrations of 4% and 6% of baseline, respectively. Second, the present model of transient ischemia does not produce frank infarct or pancellular necrosis. Instead, regionally selective neuronal death is the primary neuropathology. Thus, previous studies showing decreases in PKC activity used more severe ischemia. In the present model, regionally specific increases in total PKC activity occurred at 1 and 7 days after ischemia. However, it must be emphasized that the absolute increase in biochemical activity after ischemia was threefold to eightfold greater in S2 than in P2. Thus, smaller changes in isoform expression in P2 are consistent with increased biochemical activity.

Changes in PKC expression occur after both focal and global ischemia. Initial mRNA increases occur even with severe ischemia and are isoform specific. After 30 or 90 minutes of transient focal ischemia in rats, PKCα mRNA is upregulated in cerebral cortex for as long as 7 days. When the model of bilateral carotid occlusion in gerbils is used, 10 minutes of ischemia causes global increases in the major mRNA transcripts for most of the Ca²⁺-independent isoforms by 1 hour of recirculation, followed by a return to control by 3 days of recirculation. However, it is uncertain whether these transcripts are efficiently translated into increased PKC protein expression in the presence of a persistent suppression of protein synthesis. It appears that postischemic PKC protein levels depend on the severity of ischemia. When the two-vessel occlusion plus hypotension model in rats is used, 15 minutes of ischemia causes downregulation of PKC expression. When the model of bilateral carotid occlusion in gerbils is used, 7.5 minutes of ischemia causes enhanced PKCγ immunoreactivity in CA1 at 6 and 24 hours of reperfusion, and 10 minutes of ischemia causes increased PKCγ and PKCα expression at 4 hours of reperfusion in CA1, which returns to normal by 24 hours. Thus, in keeping with the biochemical activity, subsequent PKC protein expression depends on the severity of the ischemia. Milder insults increase PKC protein expression, whereas severe insults decrease PKC protein expression.

The present study focused on expression of Ca²⁺-dependent PKC isoforms and found isoform-specific changes in postischemic PKC expression. Regional increases in PKCα expression occurred in the P2 fraction, and this change appears to account for the activity increases in both hippocampus and cerebellum. In the S2 fraction, hippocampal increases occurred in PKCα and PKCγ expression, with these two isoforms possibly accounting for the activity increases of these respective regions. At a cellular level, we found a redistribution of Ca²⁺-dependent PKC isoforms from CA1 pyramidal cell bodies to their dendritic processes after global incomplete ischemia. In addition, the granule cell bodies of dentate gyrus show increased expression of PKCγ and PKCα after ischemia. These results agree with our Western blotting data and suggest that in some hippocampal neuronal populations, PKC isoform specific expression is increased postischemically. However, the glial contribution to the increased protein levels determined by immunoblotting is uncertain. Our observations suggest that the PKCα upregulation in cerebellum is a result of a glial response. In contrast, increases in PKCβ and PKCγ expression are due to Purkinje cells.

The significance of the postischemic change in PKC is unclear. PKC increases may be damaging or may represent reparative events. Evidence concerning the protective effects of PKC blockers both in vivo and in vitro strongly suggests that PKC is harmful.

In summary, this study shows that postischemic PKC activation is both regionally and isoform specific. Increased PKC activity occurs within 1 day after insult and remains elevated for at least 7 days in both hippocampus and cerebellum. This observation in hippocampus is underscored by postinsult increases in proteins containing phosphoserine residues, but we have not shown directly that PKC is the kinase responsible for this phosphorylation. Regional localization suggests that in hippocampus an increase in Ca²⁺-dependent PKC isoforms occurs in the neuropil of CA1 and CA4, whereas in cerebellum Ca²⁺-dependent PKC isoform increases occur in glial cells. In both hippocampus and cerebellum, Ca²⁺-dependent PKC isoform translocation and activation occur postischemically. Increased total PKC activity after ischemia is consistent with increased expression of Ca²⁺-dependent isoforms in the P2 subfraction. This finding is substantiated by the lack of change in Ca²⁺-independent isoform expression in the P2 fraction.

Acknowledgments

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References

PKC Expression Is Increased After Ischemia


17. Domanska-Janik K, Zalewska T. Effect of brain ischemia on protein transduction mechanisms. The role of protein kinase C in cerebral ischemia is potentially complex and poorly defined. The present study suggests that increased expression of protein kinase C occurs in a model of global cerebral ischemia. This finding is consistent with recent findings in humans in which increased expression of protein kinase C isoforms was detected after ischemic stroke.1

What is the functional importance of increased expression of protein kinase C? As discussed in the accompanying article, activation of protein kinase C may contribute to brain...
injury after ischemia, including glutamate-induced cytotoxicity. In addition, activation of protein kinase C in cerebral blood vessels produces vasoconstriction, reduced expression of the endothelial isoform of nitric oxide synthase, and reductions in activity of potassium channels (including the frequency of calcium sparks that activate some potassium channels), resulting in depolarization and contraction of vascular muscle. All of these vascular effects may contribute to brain injury after ischemia.

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References
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