

# Perspectives on Reperfusion-induced Damage in Rodent Models of Experimental Focal Ischemia and Role of $\gamma$ -Protein Kinase C

Jaroslawn Aronowski and Lise A. Labiche

## Abstract

Ischemic stroke represents the leading cause of death and disability among elderly people. Most stroke survivors are left with lifelong disability. With the exception of tissue-type plasminogen activator (t-PA), no effective therapy exists for the management of acute stroke. Understanding the role of various extrinsic and intrinsic pathogenic factors of ischemic damage represents a prime objective of ongoing stroke research. An important variable affecting stroke outcome is the presence or absence of reperfusion (recanalization of the occluded vessel) following an ischemic event. It appears that early reperfusion after a stroke is beneficial and capable of reversing the majority of ischemic dysfunctions. However, in some instances, late reperfusion may contrarily trigger deleterious processes and lead to more ischemic damage. Examples of ischemia/reperfusion damage using an experimental model of focal ischemia in rodents are provided, along with evidence that the brain-enriched  $\gamma$ -isoform of protein kinase C may represent an important mediator of reperfusion-induced brain injury in mutant mice.

**Key Words:** focal ischemia; mouse strains; protein kinase C; reperfusion injury

## Reperfusion and Brain Damage After Focal Ischemia in Rats

When evaluating factors contributing to ischemic neuronal damage, one cannot disregard the potential implication of reperfusion-induced damage. During ischemia, oxygen supply to the brain areas distal to the occlusion site is significantly limited. The most underperfused brain region, the ischemic core, displays cerebral blood flow values that are often less than 10% of the normal blood flow and most often undergoes irreversible damage. It is very important to recognize the ischemic penumbra, the tissue surrounding the ischemic core that represents a region of ischemia with intermediate cerebral blood flow reduction, with chances for survival and a primary

target for antistroke therapies. Within the ischemic core, early death of selected neurons can be induced by even very short durations of ischemia (Aronowski et al. 1999).

In general, durations of ischemia less than 30 min are well tolerated, as documented in many experimental models of focal ischemia (Aronowski et al. 1994, 1999; Kaplan et al. 1991; Memezawa et al. 1992). However, such tolerance to ischemic damage is dramatically lost once the ischemic period reaches more than 30 to 60 min (Aronowski et al. 1994, 1997, 1999). When ischemia lasts more than 30 min, damage to the brain cells (primarily neurons in the ischemic core) is so advanced that even re-establishment of blood flow to the ischemic brain cannot reverse the damage already done. In general, the longer the duration of ischemia, the more ischemic brain tissue will be irreversibly damaged (Aronowski et al. 1997, 1999). Many investigators have demonstrated a positive correlation between increased duration of ischemia and infarct volume (Aronowski et al. 1994, 1999; Buchan et al. 1992; Kaplan et al. 1991; Memezawa et al. 1992). However, in some instances (e.g., ischemia of intermediate intensity), reperfusion may trigger multiple adverse processes that can increase brain damage beyond that produced by the permanent ischemia (no reperfusion) (Aronowski et al. 1997).

During ischemia, cessation of oxidative phosphorylation leads to energy failures throughout the affected regions of brain as a result of severe cerebral blood flow reduction. Consequently, inability to sustain energy-dependent functions leads to neuronal death and irreversible brain damage following ischemia (Farber 1973). However, recent studies indicate that ischemia-associated energy failure is not the only factor contributing to the damage produced by focal ischemia. It was originally recognized in small intestine and later confirmed that in many other organs, including stomach, pancreas, liver, heart, and kidney, reperfusion following ischemia can contribute to additional damage (so-called "reperfusion-injury") (Bulkley 1987; Kloner et al. 1989). Alteration in production of various cytotoxic substances have been postulated to act as deleterious factors that augment brain damage during the reperfusion phase. These substances include free radicals (Dugan et al. 1995), excitatory amino acids (Matsumoto et al. 1996), free fatty acids, pro-inflammatory cytokines, and adhesion molecules (del Zoppo et al. 2000; Yoshimoto et al. 1997), as well as secondary  $\text{Ca}^{2+}$  influx (Uematsu et al. 1989) and changes in activation of protein kinases (Aronowski et al. 2000; Saluja et al. 1997, 1999; Waxham et al. 1996). However,

Jaroslawn Aronowski, Ph.D., is Associate Professor of Neurology and Director of Stroke Research in the Department of Neurology, University of Texas-Houston Medical School, Houston, Texas. Lise A. Labiche, M.D., is Cerebrovascular Fellow in the Stroke Program, Department of Neurology, University of Texas-Houston.

until recently, no direct evidence of the damaging effect of reperfusion on ischemic brain has been provided.

Using Long-Evans rats, we compared the effect of transient ischemia (3 hr of ischemia followed by 21 hr of reperfusion) versus permanent ischemia (24 hr of permanent focal ischemia) on infarct size using a tandem middle cerebral artery (MCA<sup>1</sup>) and common carotid artery (CCA<sup>1</sup>) occlusion. We demonstrated that infarct volume following reversible MCA/CCA occlusion was at least three-fold larger than the infarct volume after permanent occlusion (Aronowski et al. 1997). This experiment produced explicit evidence that reperfusion after reversible focal ischemia may trigger biological responses leading to the augmentation of cerebral damage established during ischemia (occlusion).

The nature of this reperfusion-induced damage is not entirely clear. In our study, reperfusion-augmented infarct volume was amenable to treatment with a free radical scavenger (*N*-tert-butyl-alpha-phenylnitron) and protein synthesis inhibitor (cycloheximide) (Aronowski et al. 1997), suggesting that oxidative stress and reperfusion-induced synthesis of potentially deleterious proteins may play an important role in executing reperfusion damage. Susceptibility to reperfusion injury in our study appeared to be closely related to the intraischemic reduction of cerebral perfusion and was evident only in animals with low to intermediate cerebral blood flow reduction (Aronowski et al. 1997). Animals with more profound reductions in cerebral perfusion, due either to vascular pathology (as in the case of spontaneously hypertensive rats) or to occlusion pattern (as in the case of unilateral MCA plus bilateral CCA occlusion), displayed large indistinguishable infarcts following both reversible and permanent ischemia, which indicated an absence of reperfusion damage (Aronowski et al. 1997). Corroborating our model of reperfusion damage, Watson et al. (2002) elegantly demonstrated that 120 min of reversible MCA occlusion in rats (using photothrombotic model) followed by 3 days of reperfusion resulted in a 2- to 2.5-fold larger infarct volume than 3 days of permanent ischemia with no reperfusion. Interestingly, in this model, the ischemia produced an intermediate reduction of cerebral perfusion in the ischemic cortex, reinforcing the notion that intermediate, but not severe, cerebral blood flow reduction is necessary to uncover reperfusion damage.

## Focal Ischemia in Mice: Strain Differences and Reperfusion Damage

Although evidence exists for the injurious role of reperfusion in ischemia/reperfusion stroke rat models, no information characterizing this phenomenon exists for mice. A

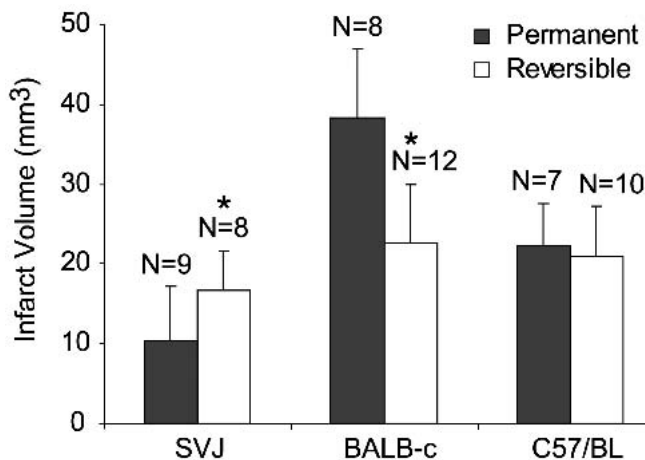
better understanding of the intrinsic mechanisms participating in brain damage after focal ischemia in mice is very important. Use of mice to model ischemic stroke has increased exponentially since the mid-1990s. Genetically engineered mutant mice represent a very useful, and often the only, tool to establish the causal relationship between various gene products and susceptibility/resistance to ischemic damage. Hundreds of experiments to date have utilized mice with mutations of various enzymes, receptors, adhesion molecules, structural proteins, and other molecules involved in cell to cell signaling in the context of stroke research (Crumrine et al. 1994; Ferriero et al. 1996; Guegan et al. 1998; Iadecola et al. 1997; Kamii et al. 1994; Kinouchi et al. 1991; Schneider et al. 1999; Soriano et al. 1996, 1999; Wang et al. 1998; Waxham et al. 1996; Yang et al. 1997).

Despite the criticism that these animals exhibit developmental adaptations following embryonic mutation, which may confound the real phenotypic picture, overall past experience has been positive. In general, it appears that phenotypic changes in response to stroke in mutant animals are in agreement with the phenotypic changes seen after use of specific pharmacological agents and mimic the effect of genetic mutation. Apparently, the use of genetically altered mice to investigate mechanisms of stroke-induced damage represents a reliable experimental tool. For these reasons, it is extremely important to better characterize and understand the potential intrinsic and extrinsic factors affecting ischemic damage in mice models to study human stroke.

Of the many variables on a long list of ischemia-affecting factors, two particularly important factors include the background genetic heterogeneity and presence or absence of reperfusion in a model. In general, genetic heterogeneity originates from three sources: (1) The embryonic stem cells used for “knocking out” the gene are generally from 129/Sv (Gerlai 1996); (2) the founder mouse is typically C57BL/6; and (3) in many studies, the strain used for generating mice for experimental analysis is of another strain (e.g., BALB/c). When animals with multiple genetic backgrounds are used (assuming that offspring from heterozygotic animals are not used for cross-comparison), it is difficult to eliminate the possibility that differences in experimental animals versus controls is due to heterogeneity from either strain’s genetic carryover.

The results described above are particularly salient in studies of focal ischemia in mice because genetic background has been shown to play a significant role in determining the extent of ischemic damage produced (Barone et al. 1993; Hara et al. 1997; Majid et al. 2000). Colleagues and we have recently established a database allowing for cross-comparison among three of the most commonly used strains of mice in their susceptibility to damage by both reversible focal ischemia (Aronowski et al. 2000) and permanent ischemia (original data; Figure 1). We used 129/SvJ, C57BL/6J, and BALB/cJ mice for cross-comparison. Focal ischemia was induced by a tandem unilateral distal MCA/CCA occlusion either permanently for 24 hr (permanent ischemia) or for 150 min followed by 21.5 hr of reper-

<sup>1</sup>Abbreviations used in this article: CCA, common carotid artery; CP, cerebral perfusion; MCA, middle cerebral artery; PKC, protein kinase C;  $\gamma$ PKC-KO,  $\gamma$  isoform of protein kinase C.



**Figure 1** Infarct volume determined with triphenyltetrazolium chloride in 129/SvJ, BALB/cJ, and C57BL/6J mice after 150 min of unilateral middle cerebral artery/common carotid artery occlusion followed by 21.5 hr of reperfusion (white bars) or 24 hr of permanent occlusion (black bars). N = number of mice per group. \*  $p < 0.05$  from permanent ischemia in the same strain.

fusion (reversible ischemia) (Aronowski et al. 2000; Waxham et al. 1996). The damage was demarcated using 2,3,5-triphenyltetrazolium chloride staining, and infarct volume was determined morphometrically using a computer-based image analyzer operated by “Brain” software (Drexel University, Philadelphia, PA), as previously described (Aronowski et al. 2000). All animals were kept in a 12:12 hr light:dark cycle with free access to food and water. All procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals* (NRC 1996) and were approved by the institutional Animal Welfare Committee.

Our results are summarized in Figure 1. We initially observed that there is no significant difference in the infarct volume among the three strains of mice subjected to reversible ischemia (Aronowski et al. 2000). The average infarct volumes (mean  $\pm$  standard deviation) in the reperfusion group were  $16.7 \pm 6.6$  mm<sup>3</sup>,  $22.6 \pm 7.4$  mm<sup>3</sup>, and  $20.9 \pm 6.3$  mm<sup>3</sup> for 129/SvJ, BALB/cJ, and C57BL/6J mice, respectively. In contrast to reversible ischemia, the amount of damage was strikingly different among mice subjected to permanent ischemia. The average infarct volumes were  $10.4 \pm 6.7$  mm<sup>3</sup>,  $38.3 \pm 8.6$  mm<sup>3</sup>, and  $22.3 \pm 5.3$  mm<sup>3</sup> for 129/SvJ, BALB/cJ, and C57BL/6J mice, respectively (Figure 1).

We are intrigued to observe how different strains of mice responded differentially to permanent versus reversible focal ischemia. BALB/cJ mice displayed progressive development of damage in response to increasing ischemic durations. Infarct volume after 150 min of reversible ischemia was approximately half the volume after permanent ischemia. Interestingly, in C57BL/6J mice, no significant difference in infarct volume was detected between permanent and reversible ischemia. In contrast to BALB/cJ, 129/SvJ mice displayed up to 60% more damage after reversible

ischemia than 129/SvJ mice following permanent ischemia, indicating that in this particular strain, reperfusion contributes to overall ischemic damage. This unique susceptibility of 129/SvJ to reperfusion injury, compared with BALB/cJ and C57BL/6J, most likely cannot be based solely on the difference in cerebral blood flow reduction. All three strains of mice have similar absolute blood flow (Majid et al. 2000).

Based on our cerebral perfusion (CP<sup>1</sup>) measurement determined within the ischemic core, using laser-Doppler flowmetry, the CP reduction in all three strains were indistinguishable and ranged between 5.8 and 6.9% of the baseline perfusion values. These results of CP are in close agreement with the values of hemodynamic changes in the same strains of mice, previously reported by others (Majid et al. 2000). However, to exclude cerebral blood flow conclusively as a factor underlying cross-strain stroke susceptibility, we recommend comprehensive studies of CBF throughout the penumbral tissue ischemia in all of the strains. Nevertheless, at present, a role for some intrinsic factor(s) contributing to the distinct vulnerability to ischemic reperfusion damage among mice can be hypothesized.

### Role of $\gamma$ -Isoform of Protein Kinase C (PKC<sup>1</sup>) in Reperfusion Injury

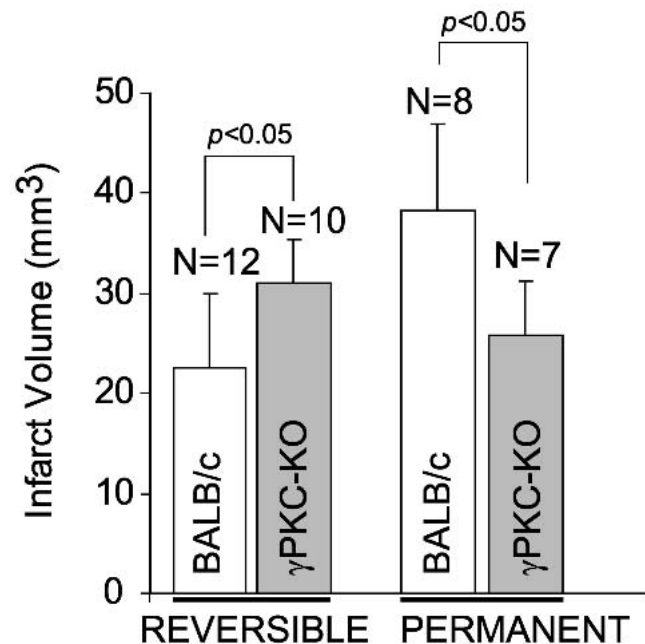
Protein phosphorylations mediated by protein kinases represent essential steps in a variety of vital neuronal processes that could affect susceptibility to ischemic stroke. PKC, a member of the family of at least 12 serine-threonine kinases, is one of the most important multifunctional protein kinases in brain (Tanaka and Nishizuka 1994). It is implicated in many vital aspects of central nervous system physiology, including synaptic plasticity, excitability, growth, proliferation, gene expression, and apoptosis (Battaini 2001; Tanaka and Nishizuka 1994). A great body of evidence proposes that changes in PKC during ischemia are an important factor regulating neuronal susceptibility to damage (Aronowski et al. 1992; Cardell et al. 1990; Domanska-Janik and Zalewska 1992; Hara et al. 1990; Louis et al. 1988; Onodera et al. 1989; Wieloch et al. 1991). An important role of PKC in ischemia/reperfusion injury in the heart was postulated for many years (Meldrum et al. 1996; Numaguchi et al. 1996). However, no information on the role of PKC in ischemia/reperfusion in the brain is available to date. The amount and activity of PKC in lung tissue varies significantly among various mice strains (Dwyer-Nield et al. 2000). Therefore, the existence of potentially similar differences in PKC in brain tissue makes PKC a possible candidate for the regulation of strain-dependent susceptibility to reperfusion damage.

To determine whether PKC in fact plays a causal role in ischemia/reperfusion damage, we studied mice that were genetically deficient in the  $\gamma$ -isoform of PKC ( $\gamma$ PKC-KO<sup>1</sup>). The  $\gamma$ PKC represents the neuronal specific isoform of the enzyme (Huang et al. 1988). The  $\gamma$ PKC-KO animals used in this study had been characterized previously by Abeliovich et al. (1993) and our group (Aronowski et al. 2000). The

breeding stocks used in this study were back-crossed into the BALB/cJ, as previously described (Aronowski et al. 2000). BALB/cJ wild-type mice obtained from the Jackson Laboratory (Bar Harbor, ME) were used as the control. Ischemia was produced by 150 min or permanent unilateral MCA/CCA occlusion, and infarct volume was determined with triphenyltetrazolium chloride, as described above. We reported earlier that  $\gamma$ PKC-deficient mice subjected to reversible ischemia developed significantly larger infarction than wild-type (BALB/c) mice after the same duration of ischemia ( $22.6 \pm 7.4$  vs.  $31.1 \pm 4.2$  mm<sup>3</sup>) (Aronowski et al. 2000). Data referring to reversible ischemia in Figure 2 is adopted from Aronowski et al. 2000. We used these data to conclude that  $\gamma$ PKC mediates neuroprotection. In this article, we provide additional evidence to illustrate that this neuroprotective role of  $\gamma$ PKC is mediated primarily through reduction of the detrimental effects of reperfusion. Such evidence is based on experimental data showing that  $\gamma$ PKC-KO mice subjected to permanent ischemia (with no reperfusion) developed significantly smaller infarct volume than BALB/c ( $25.8 \pm 5.4$  vs.  $38.3 \pm 8.6$  mm<sup>3</sup>). This result indicates the predominantly deleterious role of  $\gamma$ PKC during permanent ischemia.

## Conclusion

The combined results described above demonstrate that  $\gamma$ PKC may play a contrasting role in regulating the vulner-



**Figure 2** Infarct volume after 150 min of unilateral middle cerebral artery/common carotid artery occlusion followed by 21.5 hr of reperfusion (REVERSIBLE) or 24 hr of permanent occlusion (PERMANENT) in the  $\gamma$  isoform of protein kinase C ( $\gamma$ PKC-KO) and wild-type (BALB/cJ) mice. N = number of mice per group.  $p < 0.05$ , difference between the indicated groups.

ability of tissue to ischemia/reperfusion-induced damage. It functions first, as a deleterious factor during evolution of intras ischemic neuronal damage, and second, as a neuroprotective factor during post ischemic reperfusion.

In addition to determining a potential role for  $\gamma$ PKC in reperfusion damage, at least two important general conclusions can be reached from this study:

1. To avoid misleading conclusions about the role of gene mutation on ischemic susceptibility, it is important that we used both the reversible and permanent ischemia models in all studies utilizing mutant animals.
2. Studies assaying both permanent and reversible ischemia may provide unique and useful guidance regarding pharmacological approaches to optimize therapeutic effect (e.g., use of a  $\gamma$ PKC inhibitor [once available] as a treatment for stroke appears logical if applied during ischemia, but not during reperfusion).

In summary, reperfusion injury following ischemic stroke represents a true phenomenon that should be addressed in all studies evaluating mechanisms of damage produced by focal stroke. This phenomenon is especially applicable now that appropriate models to study reperfusion-induced damage are available.

## Acknowledgment

Work described in this article was supported in part by National Institutes of Health, National Institute of Neurological Disorders and Stroke grant 1 R01 NS40974.

## References

- Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S. 1993. Modified hippocampal long-term potentiation in PKC gamma-mutant mice. *Cell* 75:1253-1262.
- Aronowski J, Cho KH, Strong R, Grotta JC. 1999. Neurofilament proteolysis after focal ischemia: When do cells die after experimental stroke? *J Cereb Blood Flow Metab* 19:652-660.
- Aronowski J, Grotta JC, Strong R, Waxham MN. 2000. Interplay between the gamma isoform of PKC and calcineurin in regulation of vulnerability to focal cerebral ischemia. *J Cereb Blood Flow Metab* 20:343-349.
- Aronowski J, Grotta JC, Waxham MN. 1992. Ischemia-induced translocation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II: Potential role in neuronal damage. *J Neurochem* 58:1743-1753.
- Aronowski J, Ostrow P, Samways E, Strong R, Zivin JA, Grotta JC. 1994. Graded bioassay for demonstration of brain rescue from experimental acute ischemia in rats. *Stroke* 25:2235-2240.
- Aronowski J, Strong R, Grotta JC. 1997. Reperfusion injury: Demonstration of brain damage produced by reperfusion after transient focal ischemia in rats. *J Cereb Blood Flow Metab* 17:1048-1056.
- Barone FC, Knudsen DJ, Nelson AH, Feuerstein GZ, Willette RN. 1993. Mouse strain differences in susceptibility to cerebral ischemia are related to cerebral vascular anatomy. *J Cereb Blood Flow Metab* 13: 683-692.
- Battaini F. 2001. Protein kinase C isoforms as therapeutic targets in nervous system disease states. *Pharmacol Res* 44:353-361.

- Buchan AM, Xue D, Slivka A. 1992. A new model of temporary focal neocortical ischemia in the rat. *Stroke* 23:273-279.
- Bulkley GB. 1987. Free radical-mediated reperfusion injury: A selective review. *Br J Cancer* 8(Suppl):66-73.
- Cardell M, Bingren H, Wieloch T, Zivin J, Saitoh T. 1990. Protein kinase C is translocated to cell membranes during cerebral ischemia. *Neurosci Lett* 119:228-232.
- Crumrine RC, Thomas AL, Morgan PF. 1994. Attenuation of p53 expression protects against focal ischemic damage in transgenic mice. *J Cereb Blood Flow Metab* 14:887-891.
- del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. 2000. Inflammation and stroke: Putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol* 10: 95-112.
- Domanska-Janik K, Zalewska T. 1992. Effect of brain ischemia on protein kinase C. *J Neurochem* 58:1432-1439.
- Dugan LL, Lin TS, He YY, Hsu CY, Choi DW. 1995. Detection of free radicals by microdialysis/spin trapping EPR following focal cerebral ischemia-reperfusion and a cautionary note on the stability of 5,5-dimethyl-1-pyrroline N-oxide (DMPO). *Free Radic Res* 23:27-32.
- Dwyer-Nield LD, Paigen B, Porter SE, Malkinson AM. 2000. Quantitative trait locus mapping of genes regulating pulmonary PKC activity and PKC-alpha content. *Am J Physiol Lung Cell Mol Physiol* 279:L326-L332.
- Farber E. 1973. ATP and cell integrity. *Fed Proc* 32:1534-1539.
- Ferriero DM, Holtzman DM, Black SM, Sheldon RA. 1996. Neonatal mice lacking neuronal nitric oxide synthase are less vulnerable to hypoxic-ischemic injury. *Neurobiol Dis* 3:64-71.
- Gerlai R. 1996. Gene-targeting studies of mammalian behavior: Is it the mutation or the background genotype? *Trends Neurosci* 19:177-181.
- Guegan C, Onteniente B, Makiura Y, Merad-Boudia M, Ceballos-Picot I, Sola B. 1998. Reduction of cortical infarction and impairment of apoptosis in NGF-transgenic mice subjected to permanent focal ischemia. *Brain Res Mol Brain Res* 55:133-140.
- Hara H, Ayata C, Huang PL, Waeber C, Ayata G, Fujii M, Moskowitz MA. 1997. [3H]L-NG-nitroarginine binding after transient focal ischemia and NMDA-induced excitotoxicity in type I and type III nitric oxide synthase null mice. *J Cereb Blood Flow Metab* 17:515-526.
- Hara H, Onodera H, Yoshidomi M, Matsuda Y, Kogure K. 1990. Staurosporine, a novel protein kinase C inhibitor, prevents posts ischemic neuronal damage in the gerbil and rat. *J Cereb Blood Flow Metab* 10:646-653.
- Huang FL, Yoshida Y, Nakabayashi H, Young WS III, Huang KP. 1988. Immunocytochemical localization of protein kinase C isozymes in rat brain. *J Neurosci* 8:4734-4744.
- Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME. 1997. Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. *J Neurosci* 17:9157-9164.
- Kamii H, Kinouchi H, Sharp FR, Koistinaho J, Epstein CJ, Chan PH. 1994. Prolonged expression of hsp70 mRNA following transient focal cerebral ischemia in transgenic mice overexpressing CuZn-superoxide dismutase. *J Cereb Blood Flow Metab* 14:478-486.
- Kaplan B, Brint S, Tanabe J, Jacewicz M, Wang XJ, Pulsinelli W. 1991. Temporal thresholds for neocortical infarction in rats subjected to reversible focal cerebral ischemia. *Stroke* 22:1032-1039.
- Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH. 1991. Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. *Proc Natl Acad Sci U S A* 88:11158-11162.
- Kloner RA, Przyklenk K, Whittaker P. 1989. Deleterious effects of oxygen radicals in ischemia/reperfusion. Resolved and unresolved issues. *Circulation* 80:1115-1127.
- Louis JC, Magal E, Yavin E. 1988. Protein kinase C alterations in the fetal rat brain after global ischemia. *J Biol Chem* 263:19282-19285.
- Majid A, He YY, Gidday JM, Kaplan SS, Gonzales ER, Park TS, Fenstermacher JD, Wei L, Choi DW, Hsu CY. 2000. Differences in vulnerability to permanent focal cerebral ischemia among 3 common mouse strains. *Stroke* 31:2707-2714.
- Matsumoto K, Lo EH, Pierce AR, Halpern EF, Newcomb R. 1996. Secondary elevation of extracellular neurotransmitter amino acids in the reperfusion phase following focal cerebral ischemia. *J Cereb Blood Flow Metab* 16:114-124.
- Meldrum DR, Cleveland JC Jr, Mitchell MB, Sheridan BC, Gamboni-Robertson F, Harken AH, Banerjee A. 1996. Protein kinase C mediates Ca2(+)-induced cardioadaptation to ischemia-reperfusion injury. *Am J Physiol* 271:R718-R726.
- Memezawa H, Smith ML, Siesjo BK. 1992. Penumbra tissues salvaged by reperfusion following middle cerebral artery occlusion in rats. *Stroke* 23:552-559.
- NRC [National Research Council]. 1996. Guide for the Care and Use of Laboratory Animals. 7th ed. Washington DC: National Academy Press.
- Numaguchi K, Shimokawa H, Nakaïke R, Egashira K, Takeshita A. 1996. PKC inhibitors prevent endothelial dysfunction after myocardial ischemia-reperfusion in rats. *Am J Physiol* 270:H1634-H1639.
- Onodera H, Araki T, Kogure K. 1989. Protein kinase C activity in the rat hippocampus after forebrain ischemia: Autoradiographic analysis by [3H]phorbol 12,13-dibutyrate. *Brain Res* 481:1-7.
- Saluja I, O'Regan MH, Song D, Phillis JW. 1999. Activation of cPLA2, PKC, and ERKs in the rat cerebral cortex during ischemia/reperfusion. *Neurochem Res* 24:669-677.
- Saluja I, Song D, O'Regan MH, Phillis JW. 1997. Role of phospholipase A2 in the release of free fatty acids during ischemia-reperfusion in the rat cerebral cortex. *Neurosci Lett* 233:97-100.
- Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. 1999. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. *Nat Med* 5:554-559.
- Soriano SG, Coxon A, Wang YF, Frosch MP, Lipton SA, Hickey PR, Mayadas TN. 1999. Mice deficient in Mac-1 (CD11b/CD18) are less susceptible to cerebral ischemia/reperfusion injury. *Stroke* 30:134-139.
- Soriano SG, Lipton SA, Wang YF, Xiao M, Springer TA, Gutierrez-Ramos JC, Hickey PR. 1996. Intercellular adhesion molecule-1-deficient mice are less susceptible to cerebral ischemia-reperfusion injury. *Ann Neurol* 39:618-624.
- Tanaka C, Nishizuka Y. 1994. The protein kinase C family for neuronal signaling. *Annu Rev Neurosci* 17:551-567.
- Uematsu D, Greenberg JH, Reivich M, Hickey WF. 1989. Direct evidence for calcium-induced ischemic and reperfusion injury. *Ann Neurol* 26: 280-283.
- Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA. 1998. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nat Med* 4:228-231.
- Watson BD, Prado R, Veloso A, Brunschwig JP, Dietrich WD. 2002. Cerebral blood flow restoration and reperfusion injury after ultraviolet laser-facilitated middle cerebral artery recanalization in rat thrombotic stroke. *Stroke* 33:428-434.
- Waxham MN, Grotta JC, Silva AJ, Strong R, Aronowski J. 1996. Ischemia-induced neuronal damage: A role for calcium/calmodulin-dependent protein kinase II. *J Cereb Blood Flow Metab* 16:1-6.
- Wieloch T, Cardell M, Bingren H, Zivin J, Saitoh T. 1991. Changes in the activity of protein kinase C and the differential subcellular redistribution of its isozymes in the rat striatum during and following transient forebrain ischemia. *J Neurochem* 56:1227-1235.
- Yang GY, Zhao YJ, Davidson BL, Betz AL. 1997. Overexpression of interleukin-1 receptor antagonist in the mouse brain reduces ischemic brain injury. *Brain Res* 751:181-188.
- Yoshimoto T, Houkin K, Tada M, Abe H. 1997. Induction of cytokines, chemokines and adhesion molecule mRNA in a rat forebrain reperfusion model. *Acta Neuropathol (Berl)* 93:154-158.