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GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM

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CHAPTER 23

Gene expression in astrocytes during and after ischemia

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Introduction

Ischemia is a combination of substrate deprivation, severe hypoxia and failure to remove toxic metabolic products. No aspect of metabolism is spared in ischemic injury. The degree of injury is dependent on the duration and extent of ischemia. The damage may be transient, possibly reversible to some extent or irreversible and sufficient to cause tissue death. The key biochemical determinants of irreversible cell damage are not completely known. However, the interrelated factors of energy failure, lactic acidosis, and calcium imbalance are presently viewed as critical steps leading to ischemic cell damage or death (Kaplan et al., 1987). The expression of specific genes during cerebral ischemia and post-ischemia also plays an important, determinant role in the damaging and recovering processes (Takeda et al., 1992).

The overall goal of this chapter is to understand the expression of some specific genes in astrocytes during and after ischemia. The hope is that understanding these events will make it possible to augment responses that protect neural cells from injury and to inhibit deleterious responses that damage cells in cerebral ischemia and various pathological conditions.

Gene expression during cerebral ischemia

Cerebral ischemia increases the expression of several groups of genes in the central nervous system (CNS). Dempsey et al. (1991) and Muller et al. (1991) noticed an increase in ornithine decarboxylase mRNA levels after transient ischemia. Lindvall et al. (1992) studied the expression of mRNAs for members of the nerve growth factor family in forebrain ischemia. They found that the level of brain-derived neurotrophic factor mRNA and nerve growth factor mRNA increased but neurotrophin-3 decreased after the insults. This suggested that brain ischemia triggers dynamic changes in gene expression which may involve the cellular response to growth, differentiation, stress and cause functional and/or morphological changes (Takeda et al., 1992).

The expression of the late response genes that produce long-term changes in cells is regulated by the immediate early genes (IEGs) (Jorgensen et al., 1989; Nowak et al., 1990; Kindy et al., 1991; Uemura et al., 1991a,b; Blumenfeld et al., 1992; Combs et al., 1992; Welsh et al., 1992) is greatly influenced by cerebral ischemia. Induction of IEG mRNA is correlated with the duration of is-

chemia, i.e., the longer the time of ischemia, the greater the increase in certain gene expression. Nuclear IEGs form the third messenger system which converts cytoplasmic signals into long-term changes of gene expression. The IEG transcription and translation is very tightly controlled and could be activated rapidly and transiently within minutes of stimulation.

It has been shown that the c-fos gene is activated by transient cerebral ischemia (Nowak et al., 1990). The translation of the c-fos genes into fos protein following 2 minutes of ischemia seems to protect cells from dying in the hippocampus. c-Fos mRNA is induced but without translation into fos protein would lead to the death of CA1 pyramidal neurons (Nowak et al., 1990; Uemura et al., 1991a). This demonstrated that up-regulation of certain gene and protein expressions may signify a specific genetic response to ischemia which affects tolerance to ischemia and ultimate cell survival (Combs et al., 1992).

Nowak (1985) was the first to describe induction of the heat shock protein (hsp) -70 gene following global ischemia biochemically and at the cellular level using immunocytochemistry. Similar studies by Dienel et al. (1986) and others (Jacewicz et al., 1986) confirmed that hsp-70 and other hsps were induced in brain by ischemia. Though the function of many hsps is not known, most of them appear to bind to and regulate other proteins. Welsh et al. (1992) has reported that the expression of hsp-70 mRNA is prolonged in regions undergoing injury, but its expression is transient in surrounding regions that recover. Hsp-70 expression showed a longer time course than c-fos expression. This demonstrated an overlapping in the distribution of hsp-70 and c-fos expression after ischemia. The prolonged time course of hsp-70 mRNA expression supports the view that transient expression of stress proteins in cells exposed to adverse environmental conditions may provide a survival advantage.

The alteration in the cytoskeleton gene expressions and the rearrangement of their proteins may be important requisites in the process of the activation of astrocytes after injury. Reactive astrocytes play a major role in the homeostatic maintenance of CNS in response to ischemia and other insults (Eng et al., 1995). Kindy et al. (1992) and Petito et al. (1990) reported an increase in glial fibrillary acidic protein (GFAP) and vimentin, the two astrocytic intermediate filament proteins, in the area of the ischemic lesion. GFAP mRNA increased after ischemia (Kindy et al., 1992; May et al., 1992) but remained expressed at low levels in non-ischemic cerebellum (Kindy et al., 1992). Forebrain ischemia also up-regulated vimentin mRNA and protein levels in the cortex (Kindy et al., 1992). Xie et al. (1989) showed a transient increase of β-actin mRNA in ischemic brain after 8 hours of recirculation, but Dempsey et al. (1991) could not demonstrate any alterations in β-actin mRNA level after ischemia and post-ischemia.

The mineral oil induced ischemic model

The complexity of the brain and the systematic adaptation always lead to some uncertainties and discrepancies in interpretation of the experimental observations from animal models. To alleviate these problems, the cell culture system is being used to develop and establish models for studying ischemia (Yu et al., 1992b). Although cell culture models lack complete mimicry of the *in vivo* situations, this shortfall is offset by the simplicity of the system and the control gained over the extracellular environment (Kimelberg, 1983; Murphy and Horrock, 1993). In this study, primary culture of cerebral cortical astrocytes from new-born rats (Yu et al., 1991, 1993) were used to study the gene expression in astrocytes under an experimental condition simulating *in vivo* ischemia and post-ischemia.

The ischemia condition was created by sealing the neural cell culture with a layer of mineral oil before draining the culture medium (Fig. 1). This model has been used to investi-

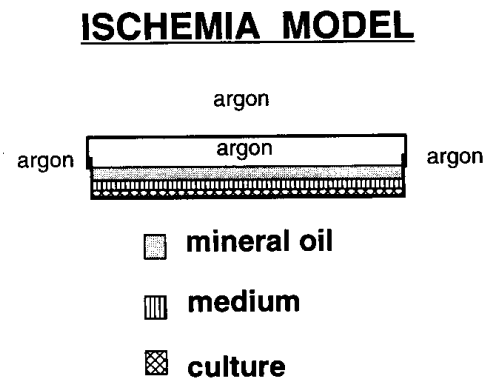

Fig. 1. Schematic diagram of the mineral oil induced ischemia model. For details, see text.

gate the uptakes of energy substrates and neurotransmitters in cultured astrocytes during and after ischemia (Yu et al., 1992b). Mineral oil is commonly employed in microbiology laboratories to create anaerobic conditions (Hugh and Leifson, 1953; Koneman et al., 1988). Mineral oil dissolves oxygen, but delivers it across the oil–aqueous interface only very slowly, perhaps because of its high viscosity. When the lowest possible level of oxygen contamination is desired, oxygen in the mineral oil should first be removed by degassing under vacuum, then saturated with nitrogen by bubbling for 30 minutes with pure nitrogen. The deoxygenated and nitrogen-saturated mineral oil was stored under argon in a tightly closed bottle. HEPES buffer containing medium was used in the ischemia studies. This would avoid the buffering problem caused by the loss of carbon dioxide in culture system buffered with only sodium bicarbonate.

For the ischemic condition, culture medium is drained with a syringe after a layer of mineral oil is added. Accumulation of toxic metabolites does not occur in the culture system because of the large volume of extracellular medium. This protocol eliminates the dilution of the toxic metabolites by reducing the volume of culture medium to a minimum. It also limits the amount of nutrients (e.g., glucose, amino acids, etc.) available to the cultured cells. Cultures under this condition are exposed to an

TABLE 1

Comparison of the mineral oil induced ischemia model in culture to the physiological ischemia

Ischemia components	Physiological ischemia	Mineral oil model
Severe hypoxia	Lack of oxygen supply from fresh blood	Limited amount of dissolved oxygen in remaining medium and insulation of the culture from air (source of oxygen) by mineral oil and argon
Substrate deprivation	Lack of supply of glucose and other nutrients from fresh blood	Limited amount of glucose and other nutrients in remaining medium
Accumulation of toxic metabolites	Accumulation due to no blood circulation	Accumulation due to limited volume of incubating medium

artificial ischemic environment simulating the *in vivo* ischemic condition where blood supply is interrupted (Table 1). The culture is then incubated at 37°C for the desired time up to a maximum of 4 hours in an argon atmosphere. Argon is much heavier than air, therefore it is more effective than the commonly used nitrogen in resisting air currents and vortices that can carry air to the sample. At the end of the incubation, mineral oil is drained and the culture is washed with ice-cold phosphate buffer solution. The post-ischemia condition is created by the addition of normal feeding medium containing fetal calf serum to the ischemic cultures before the removal of mineral oil. Mineral oil is easily removed as it always floats on the surface of the incubating and washing medium.

Gene expression in ischemic astrocytes

Although astrocytes have been viewed traditionally as supportive cells for neurons in the CNS, mounting evidence indicates that astro-

TABLE 2

The sequences of primers used for the RT-PCR studies. HPRT (a housekeeping gene) is used as control gene for comparison among experimental conditions to assure equal RNA loading (refer to Murphy et al., 1993; Eng et al., 1995)

Primer	Sequence		Predict bp
GFAP	5′-GCT,CAA,TGC,TGG,CTT,CAA,GG	20 NT	654
	3′-GGA,GGA,GCT,CTG,CGT,TGC,GG	20 NT	
Vimentin	5′-ACT,CAC,CTG,TGA,AGT,GGA,TGC	21 NT	177
	3′-TGG,TAT,TCA,CGA,AGG,TGA,CG	20 NT	
β-Actin	5′-GTG,GGC,CGC,TCT,AGG,CAC,C	19 NT	539
	3′-CTC,TTT,GAT,GTC,ACG,CAC,GA	20 NT	
c-Fos	5′-CTT,GAA,GAC,GAG,AAG,TCT,GCG	21 NT	226
	3′-GGT,CAT,TGA,GAA,GAG,GCA,GG	20 NT	
Heat Shock 70	5′-TGA,TCA,AGC,GCA,ACT,CCA,C	19 NT	209
	3′-GTT,GGC,ATC,GAT,GTC,GAA,G	19 NT	
HPRT	5′-GTA,ATG,ATC,AGT,CAA,CGG,GGG	21 NT	211
	3′-CCA,GCA,AGC,TTG,CAA,CCT,TAA,CC	23 NT	

cytes have a wide range of function other than maintaining a balanced homeostatic environment in the CNS (Hertz et al., 1982, 1990; Eng et al., 1995). Several lines of evidence have indicated the important roles of astrocytes in the regulation of neuronal functions. The neuron–astrocyte interaction is an important issue in neuroscience research (refer to Yu et al., 1992a; Fedoroff et al., 1993). In this study, quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) (for details, see Murphy et al., 1993; Eng et al., 1995) was utilized to quantify the induction of the mRNAs encoding c-fos, hsp-70, GFAP, vimentin, and β-actin in cultured astrocytes under ischemia and post-ischemia. The sequences of the primers used for this study are given in Table 2.

The measurement of c-fos gene expression in ischemic astrocytes provides significant evidence demonstrating the induction of response to the injury. The detection of hsp-70 gene would reflect the late responses in cellular protection. A change in the expression of cytoskeleton protein genes in the ischemic astrocytes may reflect the activation of astrocytes to ischemia. Understanding the chronology of these gene expressions would provide some early markers for detecting injury and reactivation of astrocytes under these pathologic conditions. All the genes studied appeared to be activated in this experimental system, though the time course and degree of expression were different.

c-Fos and heat shock protein gene expression

c-Fos gene expression in astrocytes under ischemia was followed for 2 hours (Fig. 2). In 30 minutes of ischemia, the c-fos level has risen to ten-fold above the control level. It ascended to twenty-fold at 1 hour of ischemia but dropped to approximately seven-fold at 2 hours of ischemia. Post-ischemia reactivated the c-fos

gene expression in astrocytes. Fifteen minutes of post-ischemia after 2 hours of ischemia reactivated the c-fos gene level to twenty-fold above the control. It further ascended to over thirty-fold at 30 minutes of post-ischemia. This level dropped to almost twenty-fold of the control at 1 hour of post-ischemia incubation and further came down to less than ten-fold at 3 hours. At 6 hours, it was at five-fold and remained at around this level throughout the rest of post-ischemia incubation.

Heat shock gene expression in cultured astrocytes under ischemia was activated within 30 minutes under ischemia (Fig. 2). Its ratio of expression did not elevate to above six-fold of the control throughout the 2 hours of ischemic incubation. Astrocytes reperfused after 2 hours of ischemia showed a further activation of hsp-70 gene. Within 15 minutes of post-ischemic incubation, the level of hsp-70 gene was elevated to ten-fold above the control. It remained at levels between six to nine-fold from 30 minutes to 6 hours in post-ischemia. The level of the hsp gene fell to below five-fold at 24 hours of post-ischemia incubation.

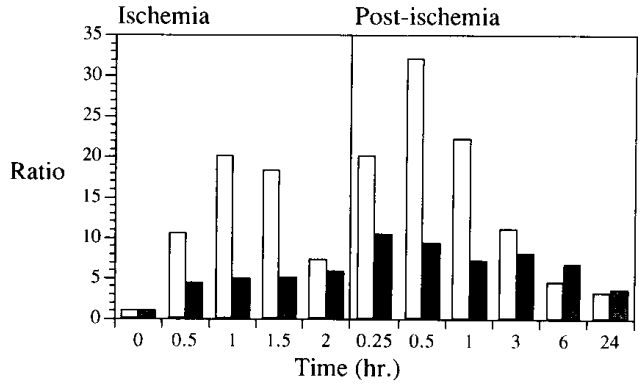

Fig. 2. The c-fos (□) and heat shock protein (■) gene expressions in cultured astrocytes under ischemia and post-ischemia. The expression levels were expressed as ratios of the densitometric units from ischemic and post-ischemic astrocytes to the controls. Controls were cultures without exposure to ischemia. All cultures in post-ischemia study were under ischemia incubation for 2 hours.

Cytoskeleton protein gene expressions

The time courses for the mRNA expression of GFAP, vimentin, and β-actin were followed (Fig. 3) under similar ischemic and post-ischemic conditions. GFAP mRNA expression in astrocytes under 30 minutes of ischemia went up to five-fold above the control (Fig. 3). It further went up to over ten-fold of the control at 2 hours of ischemia. In cultures reperfused after 2 hours of ischemia, the level of GFAP mRNA in astrocytes under post-ischemia dropped to slightly below three-fold of the control value in the first 15 minutes. It returned to slightly above six-fold and retained at this level for up to 6 hours. Then the levels slightly dropped to between four- and five-fold in the remaining post-ischemia incubation period.

Ischemia did not activate vimentin gene as the other genes (Fig. 3). The levels of vimentin

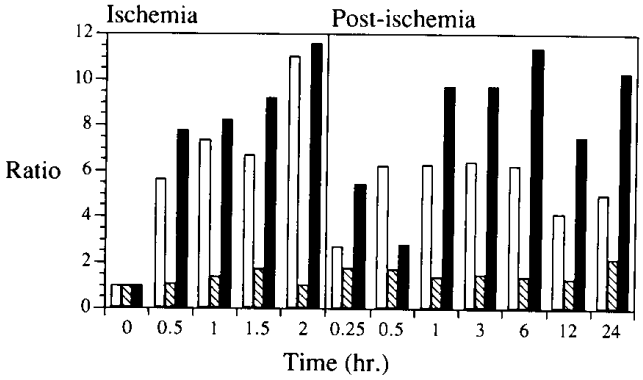

Fig. 3. The GFAP (□), vimentin (▨) and β-actin (■) gene expressions in cultured astrocytes under ischemia and post-ischemia. The expression levels were expressed as ratios of the densitometric units from ischemic and post-ischemic astrocytes to the controls. Controls were cultures without exposure to ischemia. All cultures in post-ischemia study were under ischemia incubation for 2 hours.

mRNA fluctuated but rarely over two-fold of the control throughout the ischemia and post-ischemia incubation. Within 1.5 hours of ischemia incubation, the vimentin mRNA level slowly ascended to two-fold of the control, but it returned to the control level at 2 hours of ischemia. Although the level was two-fold of the control at the initial stage and at the end of

post-ischemia, the changes in vimentin gene level did not seem significant.

The expression of β-actin mRNA was activated to a higher degree than the expression of GFAP and vimentin mRNA under similar incubation conditions. Thirty minutes of ischemia raised the level of β-actin mRNA in astrocytes to above seven and a half-fold of the control. It further reached a level slightly higher than eleven and a half-fold in 2 hours of ischemia. In the first 30 minutes of post-ischemia phase, the level of β-actin mRNA was descended to less than three-fold of the control. Its expression was reactivated and reached to ten-fold in the next 30 minutes and remained high in the rest of post-ischemic incubation up to 24 hours.

Summary

Involvement of the IEGs in brain injury and ischemia is under intensive investigation (Gubits et al., 1993). There are several families of the IEGs. They include the fos, jun, and zinc finger genes that encode transcription factors. Products of the fos family (c-fos, fra-1, fra-2, and fos B) bind to members of the jun family (c-jun, jun B, jun D) via leucine zippers, and this dimer then binds to the AP-1 site (consensus sequence –TGACTCA–) in the promoter of target genes, which in turn regulate the expression of late response genes that produce long-term changes in cells. For example, c-fos may regulate the long-term expression of preproenkephalin, nerve growth factor, dynorphin, vasoactive intestinal polypeptide, tyrosine hydroxylase and other genes with AP1 sites in their promoters (Curran and Morgan, 1987; Sheng and Greenberg, 1990). It is likely that the c-fos gene up-regulation observed in ischemic astrocytes leads to the changes observed in the expressions of hsp and cytoskeleton protein genes in this experimental model. This is supported by the findings of Sarid (1991) and Pennypacker et al. (1994) who have shown that AP-1 DNA binding activity in hippocampus recognized an AP-1 sequence from the promoter region of the GFAP which is a potential target gene. van de Klundert et al. (1992) also suggested the involvement of AP-1 in transcriptional regulation of vimentin.

IEGs can be induced within minutes by extracellular stimuli including transmitters, peptides, and growth factors. In this study, we have shown that c-fos induction by ischemia was rapid and transient. Ischemia induces c-fos in nerve cells is believed to be related to calcium entry into the cells. It has been shown by Greenberg et al. (1986) and Morgan and Curran (1986) that c-fos induction by all stimuli, except the growth factors, required calcium flux through voltage sensitive calcium channels (VSCCs). Sheng et al. (1988) reported that calcium stimulates c-fos transcription through a calcium/cAMP response element in the c-fos promoter. Growth factors induce c-fos transcription through a serum response element (SRE) in the c-fos promoter (Sheng and Greenberg, 1990; Treisman, 1990). Kainic acid can also induce c-fos in brain (Sonnerberg et al., 1989) by stimulating kainic acid/alpha-amino-3-hydroxyl-5-methyl-4-isoxazole proprionate receptors. These stimulated receptors elevate intracellular calcium either by depolarizing cells and subsequent calcium entry through VSCCs (Lerea et al., 1992) or by direct calcium entry through the receptors (Hollmann et al., 1991; Barnes and Henley, 1992). Therefore, it is important to elucidate whether similar receptors which induce calcium release are involved with the ischemia/post-ischemia induced c-fos transcription in astrocytes.

The mechanisms of hsp induction are still being explored. It has been shown that heat, heavy metals, glutamate, and low pH can induce hsps in cultured astrocytes and neurons (Dwyer et al., 1991; Nishimura et al., 1991). It has been proposed that denatured proteins activate heat shock factors whose proteins bind to heat shock elements to stimulate hsp-70 transcription (Pelham, 1986). Heat shock elements are sequences found in the promoters of all heat shock genes. Many studies have also

shown that hsps protect a variety of cells from injury. The hsp-70 gene is not expressed in most normal cells. Following stress, however, massive amounts of hsp-70 protein can be induced. Increasing expression of the hsp-70 family members confers thermotolerance (Li et al., 1992). CNS studies have also shown that prior hyperthermia of a moderate degree protects the brain from subsequent lethal hyperthermia (Brown, 1990) and ischemia (Kitagawa et al., 1991). Prior modest ischemia also appears to mitigate against subsequent lethal ischemic injury (Kirino et al., 1991). Though the function of this hsp gene is still unknown, it has been hypothesized that it's protein could bind to partially denatured proteins and preserve their tertiary structure (Pelham, 1986). Therefore, it is important to elucidate whether hsp exerts such protective effect in astrocytes under ischemia and post-ischemia. At the moment, the translation of c-fos and hsp-70 genes is under investigation in our laboratory. Preliminary results have demonstrated that both c-fos protein and hsp are up-regulated in astrocytes under mineral oil induced ischemia (Fu and Yu, unpublished data).

Although GFAP and β-actin genes were up-regulated in this model, it is not clear whether they play any functional role in the ischemic damages and astrocytic response to the injury. The expression of these cytoskeleton proteins are also under investigation in our laboratory. It is important to elucidate the interactions of IEGs products and the expression of cytoskeleton proteins in the astrocytic response to ischemic insult.

The major brain damage induced in cerebral ischemia occurs within the first few hours of cerebral ischemia. Powerful new research tools have demonstrated that some of the damage are due not only to the loss of blood supply but also to many secondary changes within brain cells. Several deleterious factors already have been found, including hypoxia, lactic acidosis, release of neurotransmitters, influx of calcium, activation of phospholipase A2, release of excitatory amino acids, excess of free radicals, and nerve cell metabolic paralysis (decrease of oxygen and glucose consumption)(Kaplan et al., 1987). These factors are believed to injure cells immediate but the injury becomes permanent only after a delay. We believed that these specific genes are important factors involved with the delay. Understanding mechanisms and targets of their induction can provide insights into the earliest biochemical events in the astrocytic response to ischemia. The hope is that understanding these events will ultimately make it possible to augment responses that protect cells from ischemia and to inhibit deleterious responses. Furthermore, the precise knowledge of the chronology of the events involved with the delay of the permanent damage will be helpful in creating a window for the application of cell-saving therapy. This mineral oil induced ischemia model appears to be suitable system with which to dissect the molecular mechanisms of ischemic induced injury in astrocytes and other nerve cells.

References

Barnes, J.M. and Henley, J.M. (1992) Molecular characteristics of excitatory amino acid receptors. *Prog. Neurobiology*, 39: 113–133.

Blumenfeld, K.S., Welsh, F.A., Harris, V.A. and Pesenson, M.A. (1992) Regional expression of c-fos and heat shock protein-70 mRNA following hypoxia-ischemia in immature rat brain. *J. Cerebral Blood Flow Metab.*, 12: 987–995.

Brown, I.R. (1990) Induction of heat shock (stress) genes in the mammalian brain by hyperthermia and other traumatic events: a current perspective. *J. Neurosci. Res.*, 27: 247–255.

Combs, D.S., Dempsey, R.J., Donaldson, D. and Kindy, M.S. (1992) Hyperglycemia suppresses c-fos mRNA expression following transient cerebral ischemia in gerbils. *J. Cerebral Blood Flow Metab.*, 12: 169–172.

Curran, T. and Morgan, J.I. (1987) Memories of fos. *Bioessays*, 7: 255–258.

Dempsey, R.J., Carney, J.M. and Kindy, M.S. (1991) Modulation of ornithine decarboxylase mRNA following transient ischemia in the gerbil brain. *J. Cerebral Blood Flow Metab.*, 11: 979–985.

Dienel, G.A., Kiessling, M., Jacewicz, M. and Pulsinelli, W.A. (1986) Synthesis of heat shock proteins in rat brain cortex after transient ischemia. *J. Cereb. Blood Flow Metab.*, 6: 505–510.

Dwyer, B.E., Nishimura, R.N., Clegg, K.B. and de Vellis, J. (1991) Regulation of heat shock protein synthesis in rat astrocytes. *J. Neurosci. Res.*, 28: 353–358.

Eng, L.F., Lee, Y.L., Murphy, G.M. and Yu, A.C.H. (1995). An RT-PCR study of gene expression in a mechanical injury model. In: A.C.H. Yu, L.F. Eng, K.J. McMahan, H. Schulman and E.M. Shooter (Eds.), *This volume*, pp. 219–229.

Fedoroff, S., Juurlink B.H.J. and Doucette, R. (Eds.) (1993) *Biology and Pathology of Astrocytes-Neuron Interactions.* Plenum Press, New York.

Greenberg, M.E., Ziff, E.B. and Greene, L.A. (1986) Stimulation of neuronal acetylcholine receptors induced rapid gene transcription. *Science*, 234: 80–83.

Gubits, R.M., Burke, R.E., McIntosh, C., Bandele, A. and Munell, F. (1993) Immediate early gene induction after neonatal hypoxia-ischemia. Brain Res. (Molec. Brain Res.), 18: 228–238.

Hertz, L. (1982) Astrocytes. In: A. Lajtha (Ed.), *Handbook of Neurochemistry, Vol. 1*, 2nd edn. Plenum, New York, pp. 319–355.

Hertz, L. (1990) Regulation of potassium homeostasis by glial cells. In: G. Levi (Ed.), *Development and Function of Glial Cells.* Alan R. Liss, New York, pp. 225–234.

Hollmann, M., Hartley, M. and Heinemann, S. (1991) Ca^{++} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science*, 252: 851–853.

Hugh, R. and Leifson, E. (1953) The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J. Bacteriol.*, 66: 24–26.

Jacewicz, M., Kiessling, M. and Pulsinelli, W.A. (1986) Selective gene expression in focal cerebral ischemia. *J. Cereb. Blood Flow Metab.*, 6: 263–272.

Jorgensen, M.B., Deckert, J., Wright, D.C. and Gehlert, D.R. (1989) Delayed c-fos proto-oncogene expression in the rat hippocampus induced by transient global cerebral ischemia: an in situ hybridization study. *Brain Res.*, 484: 393–398.

Kaplan, J., Dimlich, R.V.W., Biros, M.H. and Hedges, J. (1987) Mechanisms of ischemic cerebral injury. *Resuscitation*, 15: 149–169.

Kimelberg, H.K. (1983) Primary astrocyte culture — a key to astrocyte function. *Cell. Mol. Neurobiol.*, 3: 1–16.

Kindy, M.S., Bhat, A.N. and Bhat, N.R. (1992) Transient ischemia stimulates glial fibrillary acid protein and vimentin gene expression in the gerbil neocortex, striatum and hippocampus. *Brain Res. (Molecular Brain Research)*, 13: 199–206.

Kindy, M.S., Carney, J.P., Dempsey, R.J. and Carney, J.M. (1991) Ischemic induction of protooncogene expression in gerbil brain. *J. Molec. Neurosci.*, 2: 217–228.

Kirino, T., Tsujita, Y. and Tamra, A. (1991) Induced tolerance to ischemia in gerbil hippocampal neurons. *J. Cereb. Blood Flow Metab.*, 11: 299–307.

Kitagawa, K., Matsumoto, M., Tagaya, M., Kuwabara, K., Hata, R., Handa, N., Fukunaga, R., Kimura, K. and Kamada, T. (1991) Hyperthermia-induced neuronal protection against ischemic injury in gerbils. *J. Cereb. Blood Flow Metab.*, 11: 449–452.

Koneman, E.W. et al. (1988) *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Co., Philadelphia, PA, pp. 169–170.

Lerea, L.S., Butler, L.S. and McNamara, J.O. (1992) NMDA and non-NMDA receptor-mediated increase of c-fos mRNA in dentate gyrus neurons involves calcium influx via different routes. *J. Neurosci.*, 12: 2973–2981.

Li, G.C., Li, L., Liu, R.Y., Rehman, M. and Lee, W.M.F. (1992) Heat shock protein hsp70 protects cell from thermal stress even after deletion of its ATP-binding domain. *PNAS*, 89: 2036–2040.

Lindvall, O., Ernfor, P., Bengzon, J., Kokaia, Z., Smith, M.L., Siesjo, B.K. and Persson, H. (1992) Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemia coma. *Proc. Natl. Acad. Sci. USA*, 89: 648–652.

May, P.C., Robison, P., Fuson, K., Smalstig, B., Stephenson, D. and Clemens, J.A. (1992) Sulfated glycoprotein-2 expression increases in rodent brain after transient global ischemia. *Brain Res. (Molecular Brain Research)*, 15: 33–39.

Morgan, J.I. and Curran, T. (1986) Role of ion flux in the control of c-fos expression. *Nature*, 322: 552–555.

Muller, M., Cleef, M., Rohn, G., Bonnekoh, P., Pajunen, A.E., Bernstein, H.G. and Paschen, W. (1991) Ornithine decarboxylase in reversible cerebral ischemia: an immunohistochemical study. *Acta Neuropathologica*, 83: 39–45.

Murphy, E.J. and Horrocks, L.A. (1993) Mechanisms of hypoxic and ischemic injury. Use of cell culture models. *Mol. Chem. Neuropathol.*, 19: 95–106.

Murphy, G.M., Jr., Jia, X-C., Yu, A.C.H., Lee, Y.L., Tinklenberg, J.R. and Eng, L.F. (1993) Reverse transcription and polymerase chain reaction techniques for quantification of messenger RNA in primary astrocyte cultures. *J. Neurosci. Res.*, 35: 643–651.

Nishimura, R.N., Dwyer, B.E., Cole, R. and de Vellis, J. (1991) Comparison of heat shock protein synthesis in rat astrocytes. *J. Neurosci. Res.*, 28: 352–358.

Nowak, T.S. Jr., Ikeda, J. and Nakajima, T. (1990) 70-kDa heat shock protein and c-fos gene expression after transient ischemia. *Stroke*, 21 (11 suppl): III107–111.

Nowak, Y.S. Jr. (1985) Synthesis of a stress protein following transient ischemia in the gerbil. *J. Neurochem.*, 45: 1635–1641.

Pelham, H.R.B. (1986) Speculation on the functions of the major heat shock and glucose-regulated protein. *Cell*, 46: 959–961.

Pennypacker, K.R., Thai, L., Hong, J.S. and McMillian, M.K. (1994) Prolonged expression of AP-1 transcription factors in the rat hippocampus after systemic kainate treatment. *J. Neurosci.*, 14: 3998–4006.

Petito, C.K., Morgello, S., Felix, J.C. and Lesser, M.L. (1990) The two patterns of reactive astrocytosis in postischemic rat brain. *J. Cereb. Blood Flow Metab.*, 10: 850–859.

Sarid, J. (1991) Identification of a cis-acting positive regulatory element of the glial fibrillary acidic protein gene. *J. Neurosci. Res.*, 28: 217–228.

Sheng, M., Dougan, S.T., McFadden, G. and Greenberg, M.E. (1988) Calcium and growth factor pathways of c-fos transcriptional activation require distant upstream regulatory sequences. *Mol. Cell. Biol.*, 8: 2787–2796.

Sheng, M. and Greenberg, M. (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. *Neuron*, 4: 477–485.

Sonnenberg, J.L., Mitchelmore, C., Macgregor-Leon, P.F., Hempstead, J., Morgan, J.I. and Curran, T. (1989) Glutamate receptor agonists increase the expression of Fos, Fra and AP-1 DNA binding activity in the mammalian brain. *J. Neurosci. Res.*, 24: 72–80.

Takeda, A., Onodera, H., Yamasaki, Y., Furukawa, K., Kogure, K., Obinata. M. and Shibahara, S. (1992) Decreased expression of neurotrophin-3 mRNA in the rat hippocampus following transient forebrain ischemia. *Brain Res.*, 569: 177–180.

Treisman, R. (1990) The SRE: a growth factor responsive transcriptional regulator. *Semin. Cancer Biol.*, 1: 47–58.

Uemura, Y., Kowall, N.W. and Beal, M.F. (1991a) Global ischemia induces NMDA receptor-mediated c-fos expression in neurons resistant to injury in gerbil hippocampus. *Brain Res.*, 542: 343–347.

Uemura, Y., Kowall, N.W. and Moskowitz, M.A. (1991b) Focal ischemia in rats causes time-dependent expression of c-fos protein immunoreactivity in widespread regions of ipsilateral cortex. *Brain Res.*, 552: 99–105.

van de Klundert, F.A., van Eldik, G.J., Pieper, F.R., Jansen, H.J. and Bloemendal, H. (1992) Identification of two silencers flanking an AP-1 enhancer in the vimentin promoter. *Gene*, 122: 337–343.

Welsh, F.A., Moyer, D.J. and Harris, V.A. (1992) Regional expression of heat shock protein-70 mRNA and c-fos mRNA following focal ischemia in rat brain. *J. Cereb. Blood Flow Metab.*, 12: 204–212.

Xie, Y.X., Herget, T., Hallmayer, J., Starzinski-Powitz, A. and Hossmann, K.A. (1989) Determination of RNA content in postischemic gerbil brain by in situ hybridization. *Metabolic Brain Disease*, 4: 239–251.

Yu, A.C.H., Hertz, L., Norenberg, M.D., Sykova, E. and Waxman, S.G. (Eds.) (1992a) *Neuronal–Astrocytic Interactions: Implications for Normal and Pathological CNS Function.* Progress in Brain Research, Vol. 94. Elsevier, Amsterdam.

Yu, A.C.H., Lee, Y.L. and Eng, L.F. (1992b) Glutamate as an energy substrate in neuronal-astrocytic interactions. In: A.C.H. Yu, L. Hertz, M.D. Norenberg, E. Sykova and S.G. Waxman (Eds.), *Neuronal–Astrocytic Interactions: Implications for Normal and Pathological CNS Function.* Progress in Brain Research, Vol. 94. Elsevier, Amsterdam, pp. 251–259.

Yu, A.C.H., Lee Y.L. and Eng, L.F. (1991) Inhibition of GFAP synthesis by antisense mRNA in astrocytes. *J. Neurosci. Res.*, 30: 72–79.

Yu, A.C.H., Lee, Y.L. and Eng, L.F. (1993) Astrogliosis in culture. I. The model and the effect of antisense oligonucleotides on glial fibrillary acidic protein synthesis. *J. Neurosci. Res.*, 34: 295–303.