Physiol. Res. 42: 161 – 165, 1993

Effect of Postischaemic Recirculation on DNA Synthesis In Vulnerable Rat Brain Regions

M. GOTTLIEB, M. CHAVKO

Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

Received September 2, 1992
Accepted January 22, 1993

Summary

Incorporation of labelled thymidine into DNA of different rat brain regions was studied after 30 min of forebrain ischaemia and recirculation periods up to 24 hours. The amount of label incorporated into DNA showed a different pattern in the brain. After 1 hour of recirculation, the incorporation was reduced in the cortex and in the striatum, without any significant change in the hippocampus. After 3 to 9 hours the incorporation remained depressed in the cortex, diminished in the hippocampus, and increased by 2-2.5 fold in the striatum. Later, after 24 hours, the DNA labelling returned to control values in the striatum and in the cortex, but was increased in the hippocampus.

Key words

DNA Synthesis – Ischaemia – Reperfusion – Rat Brain Regions

Introduction

Various brain regions exhibit different metabolic responses to oxygen deprivation (Pulsinelli and Duffy 1983). Many of them have been experimentally studied. However, to our knowledge, few studies have been done with respect to nucleic acid metabolism. Cerebral ischaemia, anoxia or hypoxic-hypoxia are known to inhibit RNA polymerase activity (Yanagihara 1974, 1978, Gottlieb *et al.* 1981, Dienel *et al.* 1985, Giuffrida *et al.* 1985), and polypeptide chain initiation and elongation (Metter and Yanagihara 1979). Increased or reduced DNA polymerase activity was found after spinal cord ischaemia in neuronal and glial cell nuclei, respectively (Gottlieb *et al.* 1981). Decreased ^{3}H-thymidine incorporation into cortex DNA after brain hypoxia *in vivo* and *in vitro* was also observed (Serra *et al.* 1981). So far, none of the published results deal with a regional analysis of DNA metabolism following ischaemia and/or recirculation and its impact on regional vulnerability of the brain to ischaemia.

In the present study, we measured the incorporation of labelled thymidine into DNA extracted from the cortex, the hippocampus and the striatum following an early period of recirculation and 30 min forebrain ischaemia, because these brain regions are the most vulnerable to ischaemic damage (Pulsinelli and Brierley 1979).

Material and Methods

Male Wistar rats (Velaz, Prague) weighing 230-300 g were used for this study. The animals were allowed free access to food pellets and tap water during the experiments.

Induction of Brain Ischaemia

Transient forebrain ischaemia was induced by four blood vessel occlusion (Pulsinelli and Brierley 1979). Briefly, the animals were anaesthetized with pentobarbital (25 mg/kg), both vertebral arteries were cauterized through the alar foramina at the first cervical vertebrae and polyethylene cuffs were placed loosely around each common carotid artery without interrupting carotid blood flow. The animals were allowed to recover from anaesthesia for 24 hours. Ischaemia was produced by constriction of the carotids of conscious rats. Rats that did not become unresponsive within 60 seconds after clamping were excluded from the study. After 30 min, the carotids were released and the restoration of carotid artery

blood flow was verified visually. Physiological body temperature was maintained during ischaemia by a heating pad.

Implantation of Cannulas

For intraventricular administration of the labelled precursors the animals were prepared after the vertebral and carotid arteries have been made ready for occlusion. The control animals were intact rats with cannulas implanted into the lateral ventricles.

A midline incision was made on the scalp and a small hole was made in the skull over the lateral ventricles according to the coordinates from the stereotaxic atlas of Paxinos and Watson (1982). The stainless steel cannulas ending with polyethylene sleeve filled with saline and sealed by a flame were implanted into both ventricles and fixed in the skull using dental cement. The scalp incision was closed with surgical clips.

Injection of Radioactive Precursors

The labelled precursor (^{14}C- or ^{3}H-thymidine, specific activity 170 GBq/mmol or 1550 GBq/mmol, respectively, Amersham, Great Britain), 0.2 MBq/brain, was administered sixty minutes before the end of each period examined through reopened implanted cannules.

Extraction of DNA and Radioactivity Assay

The animals were killed by decapitation and the brains were rapidly removed and dissected into four regions: the anterior cortex, posterior cortex, striatum and hippocampus. The cerebral structures were homogenized in 10 volumes of phosphate-buffered saline (PBS) at pH 7.5 and 0-4 °C, and DNA was extracted according to the method of Schmidt and Tannhauser (1949) with minor modifications (Serra *et al.* 1981). The DNA content in homogenates was determined with ethidium bromide (Karsten and Wollenberger 1977). Radioactivity of the samples was measured on a liquid spectrometer Tri-Carb C2425 (Packard).

Statistical Analysis

All data are expressed as means ± S.E.M. Statistical significance was tested using Student's t-test.

Table 1
^{3}H-Thymidine distribution between TCA-insoluble and TCA-soluble fraction after 3 h of postischaemic recirculation.

	Control		Recirculation 3 h	
Brain region	TCA insoluble	TCA soluble	TCA insoluble	TCA soluble
Anterior cortex	53.9±15.2	33.4±5.7	34.0±2.3	35.4±5.2
Posterior cortex	55.6±11.2	47.0±3.8	51.8±7.0	47.0±5.5
Striatum	189.5±40.2	97.5±9.5	442.9±14.3*	94.3±27.6
Hippocampus	123.2±13.3	91.2±11.8	89.6±8.9	68.0±12.0

^{3}H-thymidine incorporation into TCA-insoluble fraction is expressed as Bq.10 μg DNA^{-1} and the incorporation into TCA-soluble fraction as Bq.mg tissue^{-1}. Data represent means ± S.E.M. for 6 rats in TCA-insoluble results and for 4 rats in TCA-soluble results. * $p<0.01$

Results

Incorporation of ^{3}H-thymidine into DNA from the rat brain after intraventricular injection of the precursor shows heterogeneous pattern of the distribution in control rat brains (Table 1). The heterogeneous pattern of radioactivity found in the brain DNA is apparently due to the gradient in precursor availability in the brain. Sixty minutes after the injection, the highest radioactivity was found in the striatum (189.5 Bq/10 μg DNA) followed by the hippocampus (123.2 Bq/10 μg DNA) and the cortex (54 Bq/ 10 μg DNA).

The amount of TCA-soluble thymidine radioactivity was found in descending order in the striatum, followed by the hippocampus and cortex (Table 1). The gradient in soluble thymidine radioactivity corresponds to the different distance of examined structures from the site of the precursor injection. On the other hand, the ratio between TCA-insoluble and TCA-soluble radioactivity in the striatum, which exceeds that in the hippocampus and

in the cortex indicates different metabolic DNA turnover. Our results are in accord with the data on concerning DNA labelling after intraperitoneal injection of ^{3}H-thymidine, assessed by autoradiography. The highest labelling was localized in the neighbourhood of the lateral ventricles in the subependymal layer (Mareš 1975, Messing *et al.* 1979).

Thymidine incorporation into DNA of the cerebral cortex, striatum and hippocampus after ischaemia and recovery is shown in Fig. 1. In the cortex and in the striatum the incorporation was decreased by 30-50 % after 1 hour's recirculation, without significant changes in the hippocampus.

After 3 hours of reperfusion, DNA radioactivity recovered to control values in the posterior cortex and increased more than 2-fold over the controls in the striatum. Following 6 and 9 hours of recirculation, the incorporation into DNA decreased in the posterior cortex and in the hippocampus. On the contrary, DNA labelling in the striatum still remained above the control values. Later, after 24 hours of reperfusion, the incorporation returned to the preischaemic value in the striatum and in the posterior cortex, while the incorporation into the hippocampus rose by 34 %.

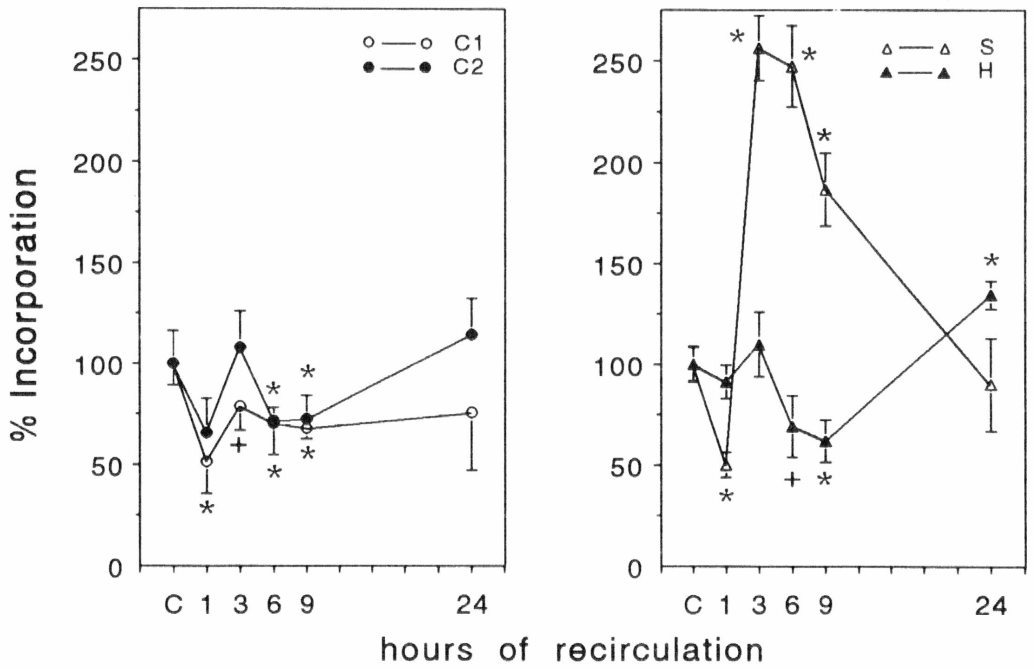

Fig. 1
Incorporation of labelled thymidine into rat brain regions after postischaemic recirculation. C - control (100 %), C1 - anterior cortex, C2 - posterior cortex, S - striatum, H - hippocampus. Data represent means ± S.E.M. from 6 rats. + $p<0.02$; * $p<0.01$

Discussion

The changes in thymidine incorporation in the brain could have been the result of changes in local precursor availability in ischaemic brain tissue compared to the controls. The time (60 min) of *in vivo* incorporation was chosen on the basis of preliminary experiments concerning the time course in different brain regions indicating the incorporation of the labelled precursors was linear up to 120 min.

No significant changes in thymidine incorporation between cannulated intact control animals and sham operated control were found, so that intact control rats were used as the control group.

For that reason we determined the ratio of soluble to insoluble radioactivity after ischaemia and 3 hours of recirculation when a significant rise in thymidine incorporation into striatal DNA occurred. As shown in Table 1, we failed to find any significant changes in the amount of TCA-soluble radioactivity in the striatum or in any other structure before or after ischaemia. It is unlikely that the results obtained might be due to changes in specific radioactivity of the precursor pool. The results do not support the possibility that the increase of thymidine incorporation into DNA in the striatum is due to different precursor availability. The rise of incorporation into striatal DNA following 3 hours postischaemia is unexpected

considering the lack of an increase in replicative DNA synthesis.

It has been repeatedly demonstrated that in spite of the very rare occurrence of mitosis, there is always a small number of non-neural cells and microvessel cells that are able to synthetize DNA and multiply. Hypertrophy and proliferation of astrocytes in central nervous system lesions have long been recognized, and increased numbers of astrocytes have been demonstrated quantitatively in experimentally induced stab wounds (Latov *et al.* 1979) and lesions induced by kainic acid (Murabe *et al.* 1981). Cellular proliferation also occurs following ischaemic infarction in the gerbil brain (Du Bois *et al.* 1985). As assessed by thymidine autoradiography, the proliferation took place from 2 to 7 days after occlusion and may be consistent with the astrocyte hyperplasia and macrophage accumulation reported for human infarcts. The stimuli for post-infarction cell-proliferation are supposingly released from degenerating nerve cells or from serum extravasation (Heldin *et al.* 1981). Our finding of increased thymidine incorporation into striatal DNA as early as 3 hours postischaemia is reminiscent of increased incorporation into DNA of the rat striatum after acute morphine administration or deafferentation (Gall *et al.* 1979, Messing *et al.* 1979). The stimuli for this very early proliferation may be coupled to receptor mediated processes. The different time course and the degree of postischaemic DNA labelling in vulnerable brain regions could contribute to the mechanism of their different response to ischaemic attack. However, the precise role of the postischaemic increase in DNA synthesis remains to be investigated.

Acknowledgment
We would like to thank Mrs. Margita Salgari for her technical assistance.

References

DU BOIS M., BOWMAN P.D., GOLDSTEIN G.W.: Cell proliferation after ischaemic infarction in gerbil brain. *Brain Res.* **347:** 245–252, 1985.

DIENEL G.A., CRUZ N.F., ROSENFELD S.J.: Temporal profiles of proteins responsive to transient ischaemia. *J. Neurochem.* **44:** 600–610, 1985.

GALL C., ROSE G., LYNCH G.: Proliferative and migratory activity of glial cells in the partially deafferented hippocampus. *J. Comp. Neurol.* **183:** 39–55, 1979.

GIUFFRIDA A.M., ALBERGHINA M., SERRA I., VIOLA M.: Biochemical changes of lipid, nucleic acid and protein metabolism in brain regions during hypoxia: Effect of CDP choline. In: *Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine.* V. ZAPPIA, E.P. KENNEDY, B.I. NILSSON, P. GALLETTI (eds), Elsevier, Amsterdam, 1985, pp. 217–238.

GOTTLIEB M., CHAVKO M., MARŠALA J.: Effect of ischaemia on the activity of DNA-dependent RNA polymerase and DNA polymerase. *J. Neurochem.* **37:** 255–257, 1981.

HELDIN C.H., WESTERMARK B., WASTESON A.: Specific receptors for platelet-derived growth factor on cells derived from connective tissue and glia. *Proc. Natl. Acad. Sci. USA* **78:** 3664–3668, 1981.

KARSTEN U., WOLLENBERGER A.: Improvements in the ethidium bromide method for direct fluorometric estimation of DNA and RNA in cell and tissue homogenates. *Anal. Biochem.* 77: 464–470, 1977.

LATOV N., NILAVER G., ZIMMERMAN E.A., JOHNSON W.G., SILVERMAN A., DEFENDINI R., COTE L.: Fibrillar astrocytes proliferate in response to brain injury. A study combining immunoperoxidase technique for glial fibrillary acidic protein and radioautography of tritiated thymidine. *Dev. Biol.* **72:** 381–384, 1979.

MAC DONNELL P., HUFF K., GROUSE L. GUROFF G.: Brain nucleic acids. In: *Biochemistry of Brain.* S. KUMAR (ed.), Pergamon Press, London, 1980, pp. 211–240.

MAREŠ V.: An autoradiographic study of regional differences in DNA synthesis in the brains of young adult mice. *Acta Histochem.* **53:** 70–76, 1975.

MESSING R.B., DODGE C., WAYMIRE J.C., LYNCH G.S., DEADWYLER, S.A.: Morphine induced increases in the incorporation of ^{3}H-thymidine into brain striatal DNA. *Brain Res. Bull.* **4:** 615–619, 1979.

METTER E.J., YANAGIHARA T.: Protein synthesis in rat brain in hypoxia, anoxia and hypoglycemia. *Brain Res.* **161:** 481–492, 1979.

MURABE Y., IBATA Y., SANO Y.: Morphological studies on neuroglia. II. Response of glial cells to kainic acid-induced lesions. *Cell Tissue Res.* **216:** 569–580, 1981.

PAXINOS G., WATSON C.: *The Rat Brain in Stereotaxic Coordinates.* Academic Press, Sydney, 1982.

PULSINELLI W.A., BRIERLEY J.B.: A new model of bilateral hemispheric ischaemia in the unanasthetized rat. *Stroke* **10:** 267–272, 1979.

PULSINELLI W.A., DUFFY T.E.: Regional energy balance in rat brain after transient forebrain ischaemia. *J. Neurochem.* **40:** 1500–1503, 1983.

SCHMIDT G., TANNHAUSER S.J.: A method for a determination of deoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissue. *J. Biol. Chem.* **161:** 83–89, 1945.

SERRA I., ALBERGHINA M., VIOLA M., GIUFFRIDA A.M.: Effect of hypoxia on nucleic acid and protein synthesis in different brain regions. *Neurochem. Res.* **6:** 595–605, 1981.

YANAGIHARA T.: Cerebral anoxia: Effect on transcription and translation. *J. Neurochem.* **22:** 113–117, 1974.

YANAGIHARA T.: Experimental stroke in gerbils: Effect on translation and transcription. *Brain Res.* **158:** 435–444, 1978.

Reprint Requests

Dr. M. Gottlieb, Institute of Neurobiology, Slovak Academy of Sciences, Šrobárova 57, 040 01 Košice, Slovak Republic.