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Mechanism of Protein Synthesis Inhibition in CNS During Postischaemic Reperfusion*

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Summary

We have used brain (dog, rat) and spinal cord (dog, rabbit) cell-free systems to study early postischaemic inhibition of protein synthesis. Ischaemia alone produced a relatively small decrease in activity of all subcellular systems used. When 15 min of normoxic reperfusion was used, more than 30 % decrease ($p<0.01$) in [^{14}C]-leucine incorporation was detected. A translational inhibitor that appeared in the postribosomal supernatant fraction at the early stage of reperfusion reduced translational capacity of an initiating cell-free system. It also phosphorylated the small (38 kDa) subunit of eukaryotic initiation factor 2 (eIF-2) *in vitro*. Effect of the inhibitor can be reversed by addition of partially purified intact eIF-2 and/or high concentration (2 mmol/l) of GTP. A prevention of postischaemic free oxygen radical formation by the reoxygenation with hypoxaemic blood, containing 37.5 mm Hg O_2 at 0–5 min and 56 mm Hg O_2 at 6–10 min of recirculation, that was followed by 5 min of normoxic reperfusion, resulted in a significant increase ($p<0.02$) of polypeptide chain synthesis *in vitro* when compared with normoxic reperfusion.

Key words:
Ischaemia – Protein synthesis – Reperfusion injury – Graded reoxygenation

Alterations in protein synthesis during ischaemia and subsequent reperfusion may be evaluated in terms of the capacity for protein synthesis or the integrity of the protein synthesizing apparatus, namely, the ribosomes and the initiation and elongation factors.

Ischaemia

Reduction in energy metabolism exerts its negative effect on various synthetic reactions requiring sufficient supply of ATP and GTP. Ischaemia, *per se*,

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has relatively little effect on the integrity of the protein synthesizing apparatus. Neither the enzymes found as a part of the cell sap nor ribosomes "frozen" as a consequence of the energy failure lose their activity (Kleihues and Hossmann 1971, Kleihues *et al.* 1975, Burda *et al.* 1980).

It is interesting, in this regard, that the activity of ribosomes isolated from ischaemic tissue by means of sodium deoxycholate (solubilisation of membranes) remained at the control level or was slightly increased (Burda *et al.* 1980). The ribosomal preparations containing larger portions of accessory proteins and/or membranes are rather more sensitive to ischaemic attack. The activity of a cell-free system can be diminished when gel filtration is used for removing the internal amino acid pool. Control postmitochondrial supernatants (PMS) are more sensitive to this procedure than the ischaemic and especially the reperfused ones. It is necessary to stress that the procedure of preparation of "intact" ribosomes and PMS might influence their activity and the internal controls must be done very carefully to distinguish the real effects of ischaemia.

Reperfusion

Available data indicate a different rate of the recovery between energy metabolism and protein synthesis after ischaemia (Kleihues *et al.* 1975, Cooper *et al.* 1977). The importance of protein synthesis renewal in complete functional and metabolic recovery of brain in the postischaemic period is evident from the finding that in selectively vulnerable regions of brain persistently decreased protein synthesis occurs prior to neuronal necrosis (Bodsch *et al.* 1985, Thilmann *et al.* 1986). The functional recovery of brain has only been observed in cases when protein synthesis was restored (Kleihues *et al.* 1975, Morimoto and Yanagihara 1981).

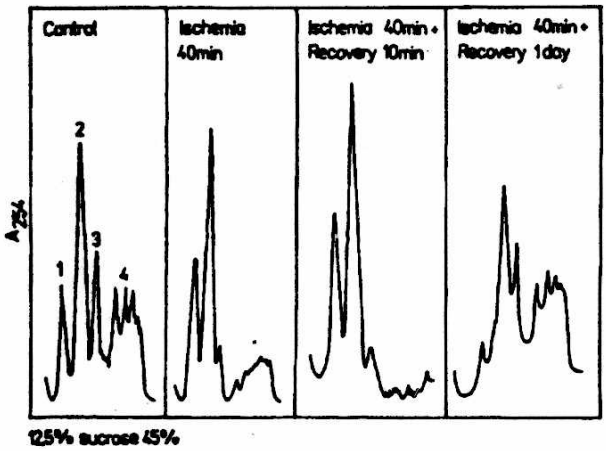

Fig. 1
Polyribosomal profiles from the spinal cord of rabbit subjected to 40 min of ischaemia and subsequent reperfusion. 1 – small subunits; 2 – large subunits; 3 – monosomes; 4 – polysomes (data from Chavko *et al.* 1987).

Early periods of recirculation after ischaemia are accompanied by almost complete breakdown of polyribosomes to their subunits (Fig. 1). This is also manifested by inhibition of labelled amino acid incorporation into proteins *in vitro*. Postischaemic inhibition of translation in CNS seems to be independent from the experimental model and animal species used (Kleihues and Hossmann 1971, Cooper *et al.* 1977, Burda *et al.* 1980, Dienel *et al.* 1980, Morimoto and Yanagihara 1981).

Disaggregation of polyribosomes in CNS was observed also in other experimental models including electroconvulsive shock (Metafora *et al.* 1977, Wasterlain 1977), administration of L-DOPA or *d*-amphetamine (Weiss *et al.* 1971, Moskowitz *et al.* 1975, 1977), administration of *d*-lysergic acid diethylamide (Holbrook and Brown 1976, Heikkila and Brown 1979), and starvation for some amino acids (Roberts and Morelos 1976).

Our studies have been focused on the investigation of the possibility that an inhibitor is present in the nervous tissue during early periods of postischaemic reperfusion and on partial characterization of this inhibitor. The most significant changes in protein synthesis occur in the initial period of reestablishment of blood circulation characterized by reactive hyperaemia. Dissociation of polyribosomes to subunits indicates the inhibition of initiation while elongation and termination of the translation seem to be rather unaffected.

Ischaemia alone produced a relatively small decrease (up to 17 %) in the activity of all subcellular systems used (total ribosomes, free ribosomes, membrane bound ribosomes, PMS). When 15 min of normoxic reperfusion was used, a significant decrease in [^{14}C]-leucine incorporation was detected. We investigated possible effect of metabolic waste products or substances formed by the degradation of blood components during stagnation in an ischaemic focus. The results showed that the effect of blood serum obtained after 40 min ischaemia did not differ from that of the control sera (Burda and Chavko 1986).

The notion of the postischaemic suppression of protein synthesis in CNS is based on experiments revealing that addition of poly(I), a specific inhibitor of initiation, results in a decrease of [^{14}C]-amino acid incorporation into proteins in a cell-free system based on PMS obtained from control and ischaemic brains, whereas the effect of poly(I) is not manifest in preparations from brains after short-term recirculation (Cooper *et al.* 1977). Evidence for the presence of initiation inhibitors formed in rabbit brain after LSD administration and their partial characterization has been published by Fleming and Brown (1986, 1987).

Most data on regulation of protein synthesis initiation in CNS have been obtained in experiments with reticulocyte lysates, in which the absence of hemine or presence of double-stranded RNA (dsRNA) resulted in an activation of HRI (heme-regulated inhibitor) or DAI (dsRNA-activated inhibitor), respectively. These inhibitors, just as some others found in other tissues, are cAMP-independent protein kinases able to phosphorylate a small (38 kDa) subunit of eIF-2 (Ochoa 1983, Delaunay *et al.* 1977, Paniers and Henshaw 1984, Ranu 1980. Wu *et al.* 1983, Proud and Pain 1982). The inhibition of ternary complex formation can be eliminated by adding intact eIF-2 or by increasing the concentration of GTP (Ranu 1980, Ranu 1982, Safer *et al.* 1982, Konieczny and Safer 1983). The same effect is obtained by the addition of several proteinaceous factors, some of which seems to be identical: ESP (eIF-2 stimulating protein) (De Haro and Ochoa 1978, De Haro *et al.* 1978),

cofactor eIF-2A (Dasgupta *et al.* 1976, 1978), cofactor eIF-2B (Majumdar *et al.* 1977, Konieczny and Safer 1983), GEF (guanine nucleotide exchange protein) (Ochoa 1983), stimulating factor (Ranu and London 1979) or antiinhibitor (Amesz *et al.* 1979) that enable recycling of eIF-2.GDP to eIF-2.GTP. The mechanism of inhibition at the early period of reperfusion was studied using several GTP concentrations. A concentration of 2 mmol/l GTP (5 fold higher than the optimal one in control PMS) completely eliminated postischaemic inhibition of amino acid incorporation *in vitro* (Chavko *et al.* 1987). The results presented by Ranu (1982) show that GTP blocks the phosphorylation of eIF-2 and also promotes the dephosphorylation of phosphorylated eIF-2. Thus, nonphosphorylated eIF-2 is available for resumption and maintenance of protein chain initiation cycle.

Table 1
Postischaemic inhibition of protein synthesis in rabbit spinal cord

Ribosomes	Cytosols	Addition	Inhibition (%)
Control	control	none	–
Ischaemia 40 min recovery 10 min	control	none	–
Control	control	inhibitor (3 μg)	36
Control	control	inhibitor + eIF-2	8
Control	ischaemia 40 min recovery 10 min	none	26
		eIF-2 (5 μg)	8
		GEF (5 μg)	10
		GTP (2 mmol/l)	4

(data from Chavko et al. 1987)

Table 2
Basic characteristics of postischaemic inhibitor

Activation	first minutes of reperfusion
Source	postribosomal supernatant
Precipitation at pH 5.3	supernatant
DEAE-cellulose	130 mmol/l KCl
Phosphocellulose	270 mmol/l KCl
Heparine Sepharose 4B	550 mmol/l KCl

The inhibitor itself (Tab. 1 and 2) is a protein present in the control cytosol as a proinhibitor that, like HRI (HCI), can be irreversibly activated by N-ethylmaleinimide. The presence of a substance with an inhibitory effect on protein synthesis and ability to phosphorylate the small subunit of eIF-2 as well as the possibility to eliminate its effect by the addition of intact eIF-2 and GTP, enables us to propose that, like in many other tissues, phosphorylation and dephosphorylation of eIF-2 represents an important regulatory mechanism occurring in the postischaemic nervous tissue (Chavko *et al.* 1987).

It is interesting, that cytosols from ischaemic tissues had no inhibitory effect during *in vitro* incubation in the presence of ATP enabling phosphorylation of eIF-2 (Burda and Chavko, 1986).

Graded reoxygenation

Proinhibitor activation should be explained by changes occurring immediately after the restoration of oxygenated blood supply into the ischaemically injured tissue. The reintroduction of oxygen together with its replenishment in ischaemic tissue result in an abrupt burst of free oxygen radicals (Granger *et al.* 1986).

Free oxygen radicals are an integral part of normal metabolism, but their concentrations are usually carefully controlled by complex intracellular reductive systems. Ischaemic damage to tissue may derange normal cellular functions and create suitable conditions for free oxygen radical formation while concomitantly reducing cellular defenses (Braughler and Hall 1989). The formation of superoxide radical by xanthine oxidase (McCord and Fridovich 1968) and the generation of other free radicals can be blocked in the ischaemic-reperfusion experiments by superoxide dismutase, catalase, allopurinol (specific xanthine oxidase inhibitor), their modifications or by some antioxidants (Chambers *et al.* 1985, Adkinson *et al.* 1986, Lim *et al.* 1986, Beckman *et al.* 1989).

Some of our results indicate that suppression of oxygen abundance during the early reoxygenation period by graded postischaemic reoxygenation improved the recovery, in terms of high energy phosphate recovery and neurohistopathological and neurological outcomes (Maršala *et al.* 1989, Danielisová *et al.* 1990). Kelly (1988) and Fraga *et al.* (1989) suggested a negative role of hyperoxia and free oxygen radicals in proteosynthesis. It was thought reasonable to use the same postischaemic regimen solving the problem of "recirculatory inhibition" of protein synthesis initiation. Reoxygenation with hypoxaemic blood containing 37.5 mm Hg O_2 at 0–5 min and 56 mm Hg O_2 at 6–10 min of recirculation followed by 5 min of normoxic reperfusion resulted in significant increase (25%, $p < 0.02$) of polypeptide chain synthesis *in vitro* when compared with immediate normoxic reperfusion.

The results obtained by this experimental approach suggest that graded postischaemic reoxygenation could be a simple and effective method, diminishing the secondary postischaemic damage of nervous tissue, including the newly synthesized proteins.

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