CHANGES IN RIBOSOMES FROM ISCHEMIC SPINAL CORD

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The paper deals with changes in polyribosomal profiles and with incorporation of amino acids into spinal cord ribosomes of dogs after ligature of their lumbar aortas. The polyribosomal profile was unchanged after 80 minutes' ischemia, but ischemia for two 40 min periods with 40 min recirculation in between resulted in disintegration of polysomes into their ribosomal subunits. Recirculation for 48 h was sufficient for return to control values. The incorporation activity of ribosomes *in vitro* was unchanged, but cell saps isolated from ischemic cells reduced incorporation of amino acids into ribosomes. The results are discussed in terms of concentration changes of adenine nucleotides under the given experimental conditions.

Lack of fresh blood supply to a nerve tissue leads to a rapid exhaustion of the macroergic substrates and thus to inhibition of proteosynthesis[1-4]. Total cerebral ischemia is accompanied by simultaneous blocking of initiation, elongation and termination of the polypeptide chain synthesis, while polyribosomes remain intact[5]. The disintegration of polyribosomes to their ribosomal subunits does not occur until after recirculation, which is interpreted as due to the blocking of initiation of proteosynthesis[5,6]. This blocking is caused by the presence of an unidentified factor, formed as a result of reduced concentration of ATP. From the point of view of energy supply, a partial ischemia and a total ischemia represent rather different conditions. The present paper compares results obtained under different conditions of blood supply to the nerve tissue.

EXPERIMENTAL

Animals. The experiments were performed upon 28 dogs of either sex, 2 to 6 years old. Using thiopental anaesthesia, ischemia was effected by ligature of abdominal aortas, closely above arteria coeliaca branching.

Isolation of ribosomes and cell saps. The spinal cord was taken out in the region L_5-S_2 and immediately placed into the physiological saline with glycerine (1 : 1), pre-cooled to −20°C. The tissue was then worked up at 4°C. After sheath removal the tissue was weighed and homogenized in three volumes of a medium which was 50 mM Tris-HCl buffer pH 7·6, 25 mM-KCl, 5 mM-$MgCl_2$ and 0·25M sucrose, and contained dextran sulphate, 100 μg/ml. The postmitochondrial supernatant was obtained by centrifugation of the homogenate at 12000 *g* for 15 min. For the isolation of ribosomes sodium deoxycholate was added to the supernatant to a final conc.

of 1%. Cell saps were isolated in the same way without the addition of sodium deoxycholate. The ribosomes and cell saps were obtained by centrifugation for 180 min at 105000*g* and 4°C (ultracentrifuge MSE Superspeed 65).

Polysomal profiles. The ribosomal pellet was homogenized in a medium which was 50 mM in Tris-HCl buffer 7·6, 250 mM in KCl, 5 mM in $MgCl_2$ and 12·5% in sucrose. The ribosomes in an amount of 4 to 8 optical units at A_{260} were applied[6] onto 20 ml of an exponential gradient of sucrose, covering a concentration range of 12·5–46% (w/v) in the same buffer and centrifuged at 130000*g* and 4°C for 4 h in the ultracentrifuge MSE Superspeed 65. The gradients were withdrawn from the bottom and their absorbances were measured in a through-flow UV analyser at 254 nm.

In vitro *incorporation of amino acids.* The incorporation of ^{14}C-labelled amino acids into proteins proceded in a mixture which was 50 mM in Tris-HCl, pH 7·6, 25 mM in KCl and 5 mM in $MgCl_2$: 1 ml of this mixture contained 2 μM of ATP, 0·2 μM of GTP, 5 μM of phosphoenolpyruvate, 50 μg of pyruvate kinase, 0·2 of A_{260} units of suspended ribosomes, one A_{260} unit of cell saps and 0·25 μCi of ^{14}C-labelled protein hydrolysate from *Chlorella* (specific activity 1 Ci/g, Institute for Research, Development and Production of Radioisotopes, Prague). The mixture was incubated at 37°C for 60 min. The protein concentration in the incubation mixture was determined according to Lowry and coworkers[7]. Samples of the mixture were worked up for measuring radioactivity on filtration paper according to Mans and Novelli[8]. The radioactivity was measured with an apparatus Packard Tri-Carb C 2425.

Determination of concentration of adenine nucleotides. The withdrawn spinal chord was immediately freezed in liquid nitrogen. The region L_5-S_2 was analysed for the contents of free adenine nucleotides AMP, ADP and ATP according to Pechán[9]. The results were evaluated statistically by the Student test.

RESULTS

Fig. 1 shows the polysomal profiles obtained from the individual experimental groups. Fractions 1, 2, 3 and 4 from control animals represent small ribosomal subunits, large subunits, monosomes and polyribosomes. The material in Fig. 1*A* was obtained from dogs after 80 min ischemia, which caused no serious damage to the polysomal profile. In contrast (Fig. 1*B*), ischemia imposed for two 40 min periods with a 40 min recirculation in between resulted in a practically complete disintegration of polyribosomes to the ribosomal subunits. Fig. 1*C* represents the polysomal profile from dogs which went through conditions of group *B*, followed by recirculation for 48 hours; as can be seen such a recirculation was sufficient for restoration of the complete polysomal profiile.

The data on *in vitro* incorporation of labelled amino acids into ribosomes are given in Table I. Three values are given for each group of dogs. The first was obtained by incubation of ribosomes and cell saps isolated from the same spinal cord, the second is a result of incubation of ischemic ribosomes and control cell saps and the third refers to control ribosomes and to ischemic cell saps. Table I shows that in the use of a whole system of spinal cord samples the incorporation activity was significantly affected in the group *B* only; in the "ribosome test" no marked alteration

TABLE I

Incorporation of ^{14}C-Labelled Amino Acids into Ribosomes of Canine Spinal Cord in Relation to Time of Ischemia

The results are expressed in dpm/mg protein ±S.E. *B* 40 min ischemia, 40 min recirculation and another 30 min ischemia; *C* as in group *B*, but followed by 48 h recirculation. *n* denotes the number of dogs.

Group	*n*	Ischemic ribosomes + ischemic cell sap	Ischemic ribosomes + control cell sap	Control ribosomes + ischemic cell sap
Control	9	2 832 ± 328	2 948 ± 67	3 100 ± 345
Ischemia (80 min)	7	3 048 ± 376	3 007 ± 369	2 604 ± 354
B	7	2 230 ± 281	2 772 ± 215	2 294 ± 322
C	5	2 709 ± 186	2 769 ± 362	2 884 ± 248

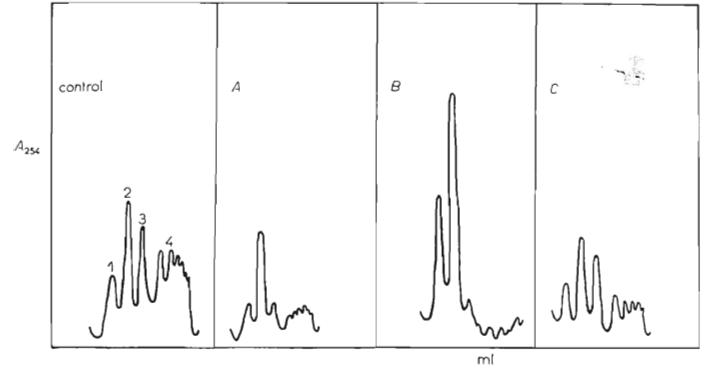

FIG. 1

Polysomal Profiles from Spinal Cords of Dogs Ischemized for Different Intervals

The small subunits 1, large ribosomal subunits 2, monosomes 3 and polyribosomes 4 were separated in an exponential density gradient of sucrose. *A* 80 min ischemia; *B* 40 min ischemia, 40 min recirculation and another 40 min ischemia; *C* conditions as in group *B*, followed by recirculation for 48 h.

was observed in any experimental group. The cell saps reacted most sensitively to experimental conditions by a marked inhibition of the amino acid incorporation in the group with the 80 min ischemia and in the group with two 40 min periods of ischemia. However, the differences in activity are not statistically significant.

The concentrations of AMP, ADP and ATP in the lumbosacral segments of spinal cord of control and ischemized dogs are given in Table II. They show that the greatest alterations occurred in the animals exposed to the 80 min ischemia, where the concentration of ATP was 30% of the control value. In the group with a 40 min ischemia followed by a 40 min recirculation and another 40 min ischemia (*B*) the level of ATP decreased by about 55% as against the control; after a subsequent recirculation for 48 h (group C) the decrease in ATP remained about the same. The total concentration of adenine nucleotides in all experimental groups decreased equally to about 50% of the control values.

DISCUSSION

Unlike the currently used complete ischemia of individual organs we induced only a partial ischemia of spinal cord in the region of the last lumbac and sacral segments by ligature of the abdominal aorta. A possible criterion of the ischemia degree is the decrease in concentration of a macroergic phosphates. The greatest decrease in concentration of ATP under our experimental conditions was observed after 80 minutes' ischemia (by 70%). In the group with 2 intermittent 40 minutes' ischemias and in the group where this treatment was followed by 48 h recirculation the decrease in ATP was 50%. Consequently, the ischemia was only partial and it must be as-

TABLE II

Concentration of Adenine Nucleotides in Canine Spinal Cord after Ligature of Abdominal Aorta and Recirculation

The values are expressed in nmol/g of wet spinal cord ± SE. The designation of groups is the same as in Table I and Fig. 1.

Group	*n*	AMP	ADP	ATP	AMP + ADP + ATP
Control	3	67 ± 6[a]	261 ± 20	2 019 ± 27[a]	2 347 ± 53
Ischemia (80 min)	5	347 ± 53[a]	280 ± 19	656 ± 88[a]	1 285 ± 159[a]
B	5	119 ± 6	214 ± 27	955 ± 89[a]	1 299 ± 122[a]
C	5	88 ± 7	276 ± 76	1 017 ± 77	1 380 ± 110[a]

[a] $P < 0.01$.

sumed that the various parts of the spinal cord were not affected evenly. The same can be stated as to the affection of the individual types of cell. Since even 48 h recirculation failed to restore both the ATP concentration and the sum concentration of adenine nucleotides to the original levels it can further be assumed that a part of the cells were damaged by the ischemia beyond repair.

Of interest from this point of view were the polysomal profiles, where the 80 min ischemia did not produce a marked disintegration of the polysomes, but after two 40 min periods of ischemia interposed with a 40 min recirculation disintegration of the polysomes to their subunits was almost complete. The same phenomenon was observed by Kleihues and coworkers[5] in total ischemia of brain. Their interpretation is that in a non-interrupted ischemia, as a result of the blocked production of ATP (95% decrease as against the control), there occurs simultaneous blocking of the initiation, elongation and termination, which keeps the polysomal profile preserved. The mentioned authors ascribe the disintegration of polysomes following recirculation to the formation of a soluble factor, similar to the repressor of translation described by Giloh and Mager[10].

In our experimental conditions, although proteosynthesis was not completely blocked (the maximum decrease of ATP after 80 min ischemia was 70%), the polysomal profile remained preserved. However, this decrease in ATP appears to be sufficient for the formation of a factor inhibiting the initiation of proteosynthesis, since recirculation led to disintegration of the polyribosomal fraction (experimental group with 40 min ischemia, 40 min recirculation and another 40 min ischemia). The blocking substance present after recirculation is probably formed in the part of spinal cord affected by the ischemia. However, it may also be formed in the blood vessel beyond the ligature, thus getting into the tissue by recirculation only. The latter possibility is indicated by the circumstances that the effect of the blocking substance on ribosomes in the course of limited circulation (partial ischemia) is too weak, so that the blocking does not occur.

The action of ribonuclease on polyribosomes is known to increase the portion of monosomes[11]. As in our experiments the disintegration of polyribosome was not accompanied by an increase in the content of monosomes, the postischemic disintegration of polyribosomes cannot be attributed to activation of ribonuclease, which occurs in some cases of hyperaminoacidemia[12].

In vitro incorporation of radioactive amino acids is a clear-cut proof that the decrease in proteosynthetic activity was caused by a soluble component cell sap, although in all experiments the incubation mixture had been saturated with the energy generating system. The activity of ribosomes in all the experimental groups was close to the control value. The activity of the cell saps was considerably reduced, especially in the group with 40 min ischemia – 40 min recirculation – 40 min ischemia, where it was 74% of the control value. Following the 80 min ischemia the decrease was lower (84%). It must be taken in mind, however, that in the latter

group the time elapsed from the first ligature to withdrawal of the spinal cord was 40 min longer. Forty-eight-hour recirculation after the ischemia was sufficient for proteosynthesis to return to control values.

The fact that the incorporation capability of control ribosomes was decreased in the presence of ischemic cell saps suggests that this decrease was not due to lack of mRNA or the initiation factors, with which the control ribosomes were well supplied. It appears that the disintegration of polysomes to the subunits in recirculation following ischemia was not caused by absence of a component or by failure of some of the proteosynthesis mechanismus, but was rather due to the presence of a new substance, formed in the period of ischemia. The decreased activity of supernatants from an ischemic spinal cord might also be caused by the acceptor sequence having been split off from t-RNA, occurring in lack of ATP.

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