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PIRACETAM (2-PYRROLIDINONE ACETAMIDE) INDUCED MODIFICATIONS OF THE BRAIN POLYRIBOSOME PATTERN IN AGEING RATS

ROBERT E. BURNOTTE, JEAN G. GOBERT and JACQUES J. TEMMERMAN

U.C.B.-Pharmaceutical Division, Biochemical Research Department, Rue Berkendael, 68, 1060 Bruxelles

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Abstract—The cerebral polyribosome:ribosome ratio decreases during the ageing of rats. A significant increase in the ratio was observed in aged Piracetam-treated animals.

AMONG the main parameters representative in the loss of efficiency of the nerve cells in the course of ageing, the polyribosomes are of particular interest because they are a measure of cellular activity and there is a direct relation between the polyribosome content of a cell and its activity in protein synthesis. Consequently changes in the brain polyribosome content may be directly related to the loss of activity of the cells during maturation from the embryonic to the adult state in rodents (Lerner *et al.*[6] and Zomzely *et al.*[9] Previous experiments demonstrated a stimulatory action of Piracetam on cellular ATP-producing systems*.[7]

MATERIAL AND METHODS

Animals. Homogeneous groups of rats (males) of the Wistar strain, weighing 85 ± 5 g, 120 ± 10 g, 400 ± 20 g and 520 ± 20 g were formed. Under our conditions of breeding the ages of these animals were 3–4 weeks, 5–6 weeks, 25 weeks and 41 weeks.

The animals received a daily dose of 100 mg/kg of Piracetam in an aqueous solution. The drug was given each day at 10 a.m. and the animals were killed on the 4th day, 4 hr after administration.

Extraction and analysis of the polyribosomes. The animals were killed and the brains immediately excised and chopped in an ice-cold buffer containing sucrose 0·32 M, Tris 10 mM, $MgCl_2$ 2 mM and KCl 10 mM, pH 7·4. The tissue was disrupted in a Potter homogenizer, 4 gentle strokes were sufficient. The homogenate was centrifuged for 10 min at 10,000 *g* (refrigerated Servall centrifuge, SS 34 rotor). The supernatant was treated with sodium deoxycholate (DOC) 1% final concentration, and allowed to stand for 10 min at 0°. Under these conditions, only free ribosomes were obtained. Three ml of the supernatant were placed on the top of a 17–40% sucrose gradient containing Tris 10 mM, $MgCl_2$ 2 mM and KCl 10 mM, pH 7·4.

* J. G. Gobert and A. J. Van de Walle, unpublished results.

The gradients were centrifuged for 4·5 hr at 24,000 *g* (Beckman Spinco L2, 65 centrifuge, SW 25·2 rotor). after which the fraction distribution was analysed in an ISCO gradient fractionator connected to a flow cell spectrophotometer (Uvicord, 0·4 cm light pathway, wave length: 254 nm). The optical densities were automatically recorded.

Determination of polyribosome content. The fractions of the gradient containing the ribosomes (zone 1) and the polyribosomes (zone 2) were collected. Optical densities were measured at 260 and 280 nm. The ribonucleic acid content of each fraction was determined with Adams' normograph.[8]

Ribonuclease treatment. Ribonuclease (Ribonuclease A, Worthington) was added to the post-mitochondrial supernatant in a final concentration of 0·5 γ/ml and the mixture was incubated for 2·5 min at 37°. It was then immediately chilled and centrifuged.

RESULTS

Ribonuclease treatment. To check the polyribosomal content of the cerebral extracts the effect of a mild ribonuclease treatment was examined.

As seen in Fig. 1, the area corresponding to the polyribosomal structures disappeared after this treatment. The mean polyribosome:ribosome ratio was almost zero in the ribonuclease treated animals and 1·31 in the young untreated animals (group A).

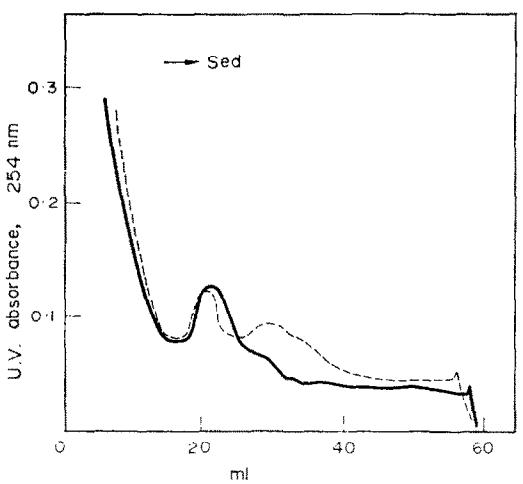

FIG. 1. Sucrose gradient sedimentation pattern of young rat brain polyribosomes: effect of a mild ribonuclease treatment. For details see Material and Methods. Solid line: ribonuclease-treated polyribosomes, Dotted line: untreated polyribosomes.

Modification of the cerebral polyribosome : ribosome ratio in rats during ageing. Four groups of rats were set up according to age and the ratios between their polyribosome and ribosome contents were measured. The results are plotted in Fig. 2. A progressive and statistically significant decrease of the polyribosome:ribosome ratio was observed as the animals grew older.

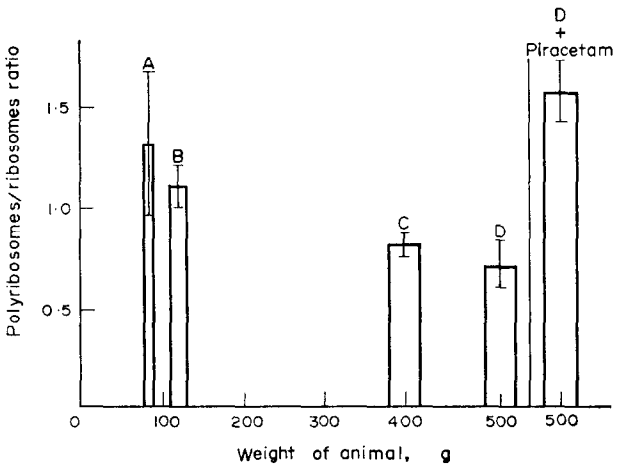

FIG. 2. Modification of the brain polyribosome: ribosome ratio in rats of increasing age and the effect of Piracetam treatment on the aged animals (group D). For details, see text.

Statistical analysis of the results showed that the differences between groups B, C and D are significant ($P < 0{\cdot}01$), whereas the difference between the groups A and B was not significant ($P < 0{\cdot}5$).

Enhancement of the polyribosome: ribosome ratio in the aged Piracetam-treated rats. When aged animals receive Piracetam a substantial increase in the polyribosome: ribosome ratio was observed. It must be emphasized that the values measured in the aged Piracetam-treated animals were statistically higher than those observed in young untreated animals (Fig. 2). Statistical analysis of the results indicated that the

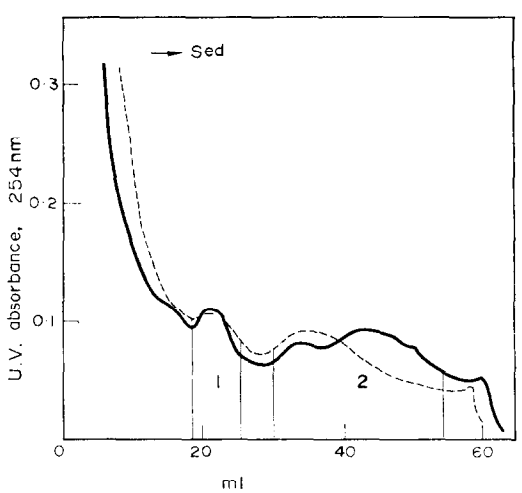

FIG. 3. Sucrose gradient sedimentation patterns of rat brain polyribosomes from aged Piracetam-treated and aged untreated animals. Zones 1 and 2 in the gradient correspond to the parts of the gradient collected for the spectrophotometric determination of RNA in the monosomes and the polysomes, respectively. Other details are given in Material and Methods. Solid line: Piracetam-treated animals. Dotted line: untreated animals.

difference between the treated and the untreated aged animals was significant at $P < 0{\cdot}001$. Moreover, the sedimentation pattern of the polyribosomes was modified in the treated animals. A greater amount of heavy polyribosomes was found in the Piracetam-treated aged animals than in those untreated (Fig. 3). An identical Piracetam treatment performed on younger animals (120 ± 20 g) did not induce any increase in the polyribosome:ribosome ratio.

DISCUSSION

Polyribosomes are cytoplasmic structures responsible for the biosynthesis of proteins and the amount pressed in cells is directly related to the protein synthesis in the cell. In the present study, the polyribosomes were chosen as indicators of cell ageing because the polyribosome content of the post-mitochondrial supernatant was defined by a mild treatment with ribonuclease, an enzyme which in very low concentrations selectively destroys the mRNA strand and transforms the polyribosomes into ribosomes.

The influence of the ageing process on the polyribosome:ribosome content was checked in four groups of rats of increasing age and a significant and progressive decrease of the polyribosome:ribosome ratio was observed in aged animals.

Secondly, rats were treated with Piracetam and the same parameter examined. A significant increase in the polyribosome:ribosome ratio was observed in the treated animals. The measured values were even higher than those observed in young animals.

Moreover, a comparison of the sedimentation profiles of the polyribosomes extracted from Piracetam-treated and untreated animals indicated that a significantly larger amount of polyribosomes occurred in the treated rats.

It is well established today that the storage of long term memory is dependent on the synthesis of one or several proteins in the brain cells.[1] Moreover, an increase in the polyribosome:ribosome ratio was also observed during the training of old rats.[2]

The Piracetam-induced stimulation of the protein synthesis machinery thus gives support to the neuropharmacological experiments which show an increase in many cereberal performancs and protection against posthypoxic amnesia in rats.[3–5]

REFERENCES

1. S. H. BARONDES, in *International Review of Neurobiology* (Eds. C. C. PFEIFFER and J. R. SMYTHIES) Academic Press, New York (1970).
2. H. DELLWEG, R. GERNER and A. WACKER, *J. Neurochem.* **15,** 1109 (1968).
3. C. GIURGEA, *Actualités Pharmacologiques*, in press (1972).
4. C. GIURGEA, D. LEFEVRE, C. LESCRENIER and M. DAVID-REMACLE, *Psychopharmacologia* **20,** 160 (1971).
5. C. GIURGEA and F. MOURAVIEFF-LESUISSE, *Archs Int. Pharmacodyn. Thér.* **191,** 279 (1971).
6. M. P. LERNER, F. O. WETTSTEIN, H. R. MERSCHMAN, J. G. STEVENS and B. R. FRIDLANDER, *J. Neurochem.* **18,** 1495 (1971).
7. J. P. PEDE, L. SCHIMPFESSEL and R. CROCKAERT, *Archs Int. Physiol. Biochem.* **79,** 28 (1971).
8. WARBURG and CHRISTIAN, *Biochem. Z.* **310,** 384 (1942).
9. C. E. ZOMZELY, S. ROBERTS, S. PEACHE and D. M. BROWN, *J. biol. Chem.* **246,** 2097 (1971).