NEUROPAT. POL., 1984, 22, 3
PL ISSN 0028-3894

BARBARA GAJKOWSKA, MIROSŁAW J. MOSSAKOWSKI,
ANNA ZARĘBA-KOWALSKA

# ACTION OF INDOMETHACIN ON NUCLEOLAR FINE STRUCTURE
## Preliminary report

Laboratory of Electron Microscopy and Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences

In the recent years indomethacin, a well known inhibitor of fatty acid cyclo-oxygenase, has been widely used in experimental studies on brain ischemia as a factor preventing the damaging effects of prostaglandins, the level of which was shown to increase remarkably at the time of cerebral ischemia and during the postischemic recovery (Ruszczewski 1977; Gaudet, Levine 1979; Gaudet et al. 1980; Iannotti et al. 1981; Bhakoo et al. 1982). As demonstrated by Abdel-Halim et al. (1980) indomethacin in a single dosis of 3 mg/kg body weight reduces the cerebral synthesis of prostaglandins to 20 percent for at least 8 hours. Furlow and Hallenbeck (1978), Hallenbeck and Furlow (1979) and Boulu et al. (1982) noticed that indomethacin premedication of animals subjected to temporary cerebral ischemia remarkably reduced postischemic abnormalities of the regional cerebral blood flow. Indomethacin was also claimed to prevent development of the postischemic brain edema (Crockard et al. 1980; Iannotti et al. 1981; Bhakoo et al. 1982) and protein extravasation to the brain tissue in experimental arterial hypertension, induced by intravenous administration of adrenaline (Johanson 1981). In our own studies, it was shown that the indomethacin administered prior to carotid arteries ligation in Mongolian gerbils prevented appearance of postischemic microcirculatory abnormalities and reduced to a great extent nerve tissue lesions, due to ischemic incident (Mossakowski, Kwiatkowska-Patzer 1982, 1983). However, electron microscopic studies besides beneficial effect on the brain tissue, revealed some striking abnormalities in the ultrastructure of nucleoli of nerve and glial cells being unusual for the ischemic nerve tissue alterations (Brierley et al. 1973; Garcia et al. 1978). This inclined us to study the direct effect of indomethacin on the nerve tissue in *in vivo* and *in vitro* conditions.

## MATERIAL AND METHODS

The studies were performed on organotypic cultures of the newborn rats cerebellum and on animals given intraperitoneal injection of indomethacin in a dosis identical with that used in experiments on the temporary brain ischemia (Mossakowski, Gajkowska 1984).

Tissue cultures. Organotypic cultures were prepared from newborn Wistar rat cerebellar tissue on collagen-coated coverslips and mantained in Carrel flasks. Nutrient medium, renewed twice weekly, consisted of 50% human serum, 40% balanced salt solution (BBS) and 10% embryo saline extract, supplemented with glucose to a final concentration of 600 mg/100 ml of medium. The pH of the medium ranged from 7.0 to 7.3. Selected 14 day cultures were subjected for three days to the action of indomethacin. This drug was added into the incubating medium in two doses: 4 mg% and 8 mg%. Cerebellar cultures of the same age *in vitro* carried out in a routine manner with no addition of indomethacin served as control ones.

For electron microscopy the cultures were briefly rinsed in Locke solution. *In situ* fixation was carried out in buffered glutaraldehyde, pH 7.2, the postfixation in osmium tetroxide, the embedding in Epon 812. The cultures were cut on an ultramicrotome, counterstained with uranyl acetate and lead citrate and examined under JEM 7 A electron microscope.

Animals. Experiments were performed on 4-month-old Mongolian gerbils (*Meriones unguiculatus*) of both sexes which were given intraperitoneal injection of indomethacin (Merck-Sharp and Dohme Res. Lab. USA) dissolved in Krebs-Ringer solution in a dose of 10 mg/1 kg. The drug was administered either 45 min or 6 hrs prior to decapitation. The control animals were given intraperitoneal injection of the solvent.

The tissue blocks comprising cerebral cortex, hypothalamus, thalamus, striatum, septal nuclei and Ammon's horn were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.2 for 1 h, postfixed in osmium tetrachloride for 1 h, washed with Millonig's buffer, dehydrated in grading ethanol and embedded in Epon 812. The ultrathin sections were counterstained in grids with uranyl acetate and lead citrate and examined under JEM 7 A electron microscope.

## RESULTS

Studies on tissue cultures. Marked effects of indomethacin on the nucleoli of both nerve and glial cells were seen after 3 days incubation of cultures in nutrient medium containing the drug in a dose of 4 and

8 mg%. Remarkable variability of size and shape of nucleoli and their partial segregation were the most striking features (Figs 1, 2, 3, 4). The shape of nucleoli was more irregular than in normal untreated cultures. There was partial segregation of RNP granules and fibrils. Relatively large extranucleolar space of chromatin was visible (Figs 1, 2, 3). The

*Fig. 1.* Dose of 4 mg% indomethacin. Partial segregation of the nuclear masses. Note the granular and dense fibrillar areas (arrows) and large extranucleolar accumulation of chromatin (Chr). × 10 520

*Ryc. 1.* Dawka 4 mg% indometacyny. Jąderko wykazuje częściową segregację. Widoczna jest część ziarnista i gęste skupienia części włóknistej jąderka (strzałki) oraz obfite zewnątrz-jąderkowe nagromadzenie chromatyny (Chr). Pow. 10 520 ×

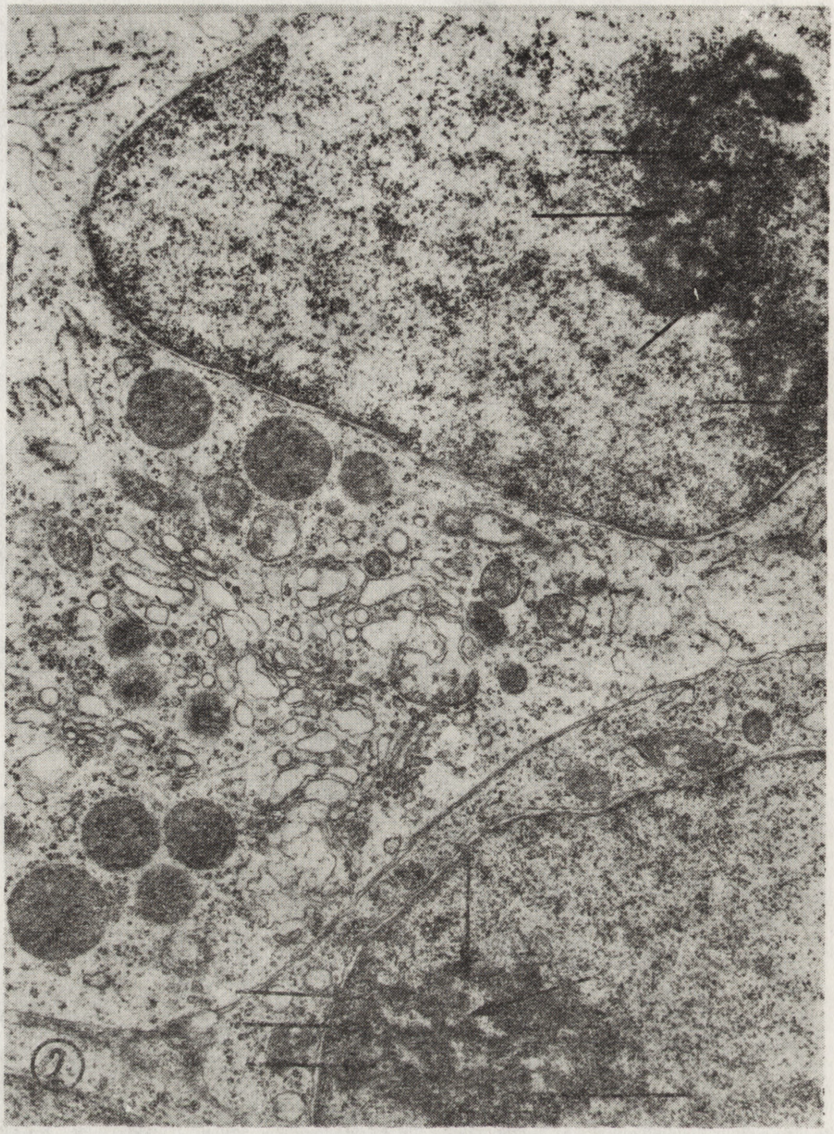

*Fig. 2.* Dose of 4 mg⁰/₀ indomethacin. In intranucleolar chromatin many perichromatin granules (arrows) are present. × 10 520

*Ryc. 2.* Dawka 4 mg⁰/₀ indometacyny. W chromatynie wewnątrzjąderkowej liczne ziarnistości (strzałki) perychromatynowe. Pow. 10 520 ×

number of perichromatin granules was very high, particularly at the inner surface of nucleolus-associated chromatin (Figs 2, 3). The granular portion of nucleoli was smaller than in cells in untreated cultures (Figs 1, 4). In most of the cells nuclei contained dispersed chromatin granules, but in some of them significant amount of chromatin condensed in irregular aggregates was seen. In the cytoplasm the Golgi complexes

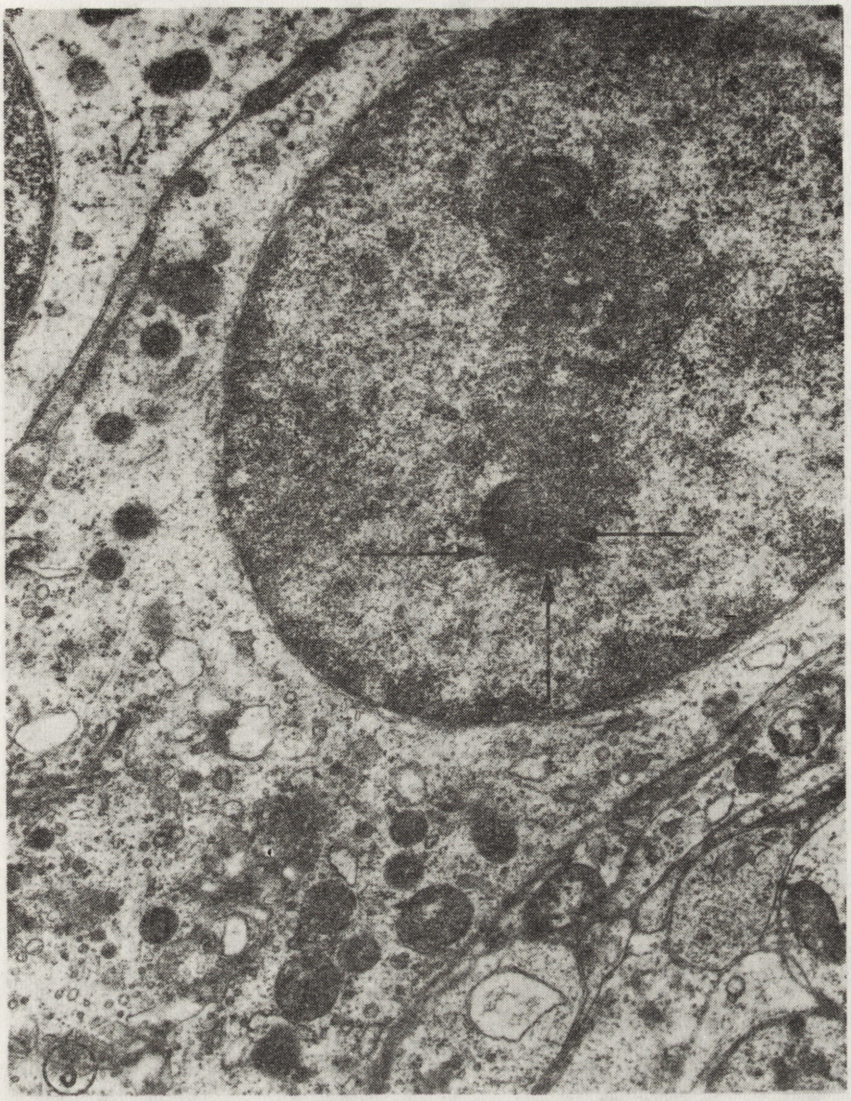

*Fig. 3.* Dose of 8 mg% indomethacin. Note close relationship of the perichromatin granules (arrows) with fibrillar part of nucleolus and intranucleolar chromatin. The fibrillar substance of the nucleolus forms irregular figures. × 10 520

*Ryc. 3.* Dawka 8 mg% indometacyny. Zwraca uwagę ścisły związek ziarnistości perychromatynowych (strzałki) z częścią włóknistą jąderka i wewnątrzjąderkową chromatyną. Część włóknista jąderka tworzy różnego kształtu figury. Pow. 10 520 ×

were well developed and numerous mitochondria and liposomes were present. In some cells channels of rough endoplasmic reticulum were widely distended (Figs 2, 4). The above described changes were present in cultures treated with both drug doses. No significant dose-dependent differences were noticed, although in some cultures with higher in-

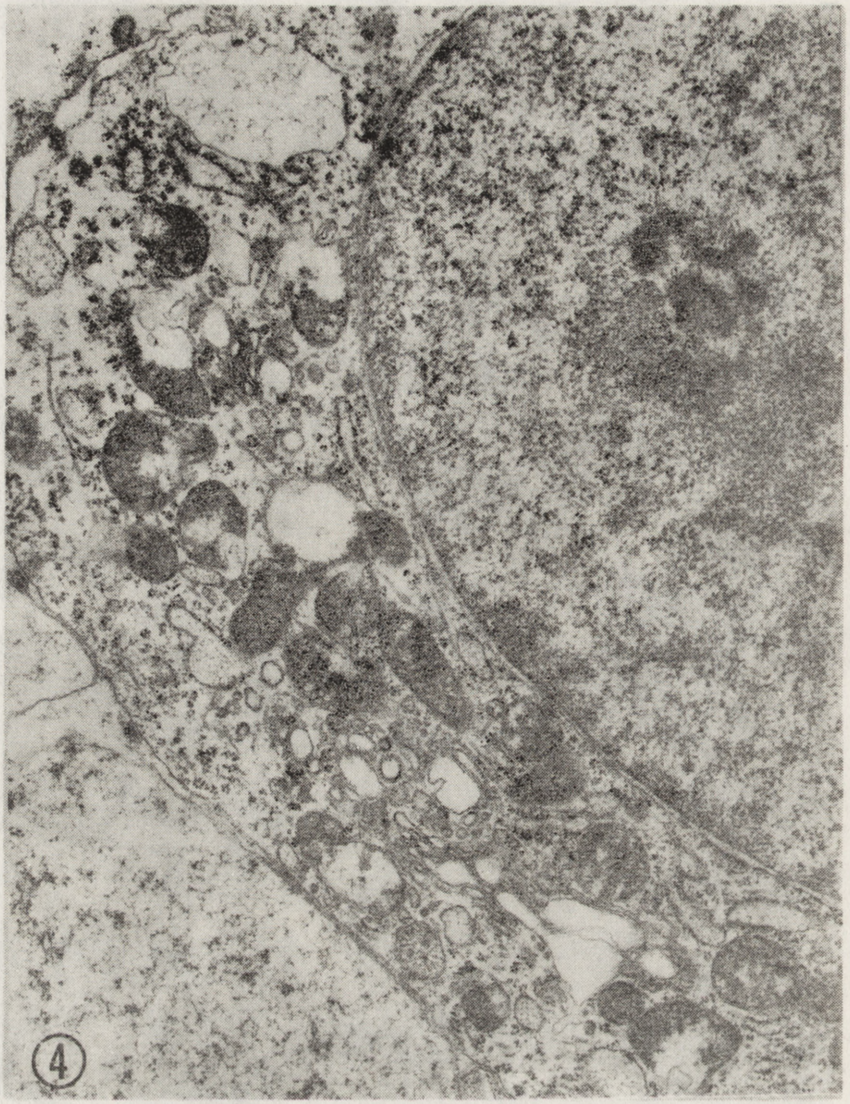

*Fig. 4.* Dose of 8 mg% indomethacin. Fragment of the nucleolus with typical microsegregation. × 10 520

*Ryc. 4.* Dawka 8 mg% indometacyny. Fragment jąderka z typowymi cechami mikrosegregacji. Pow. 10 520 ×

domethacin dose the nucleolar abnormalities seemed to be more pronounced.

Studies on animals. In indomethacin treated animals some of the nerve and glial cells revealed ultrastructural changes in the nucleoli and cytoplasm. They were more common in cortical and thalamic nerve cells. Nucleolar changes consisted in segregation of its components into two distinct zones: granular and fibrillar. The granular component was

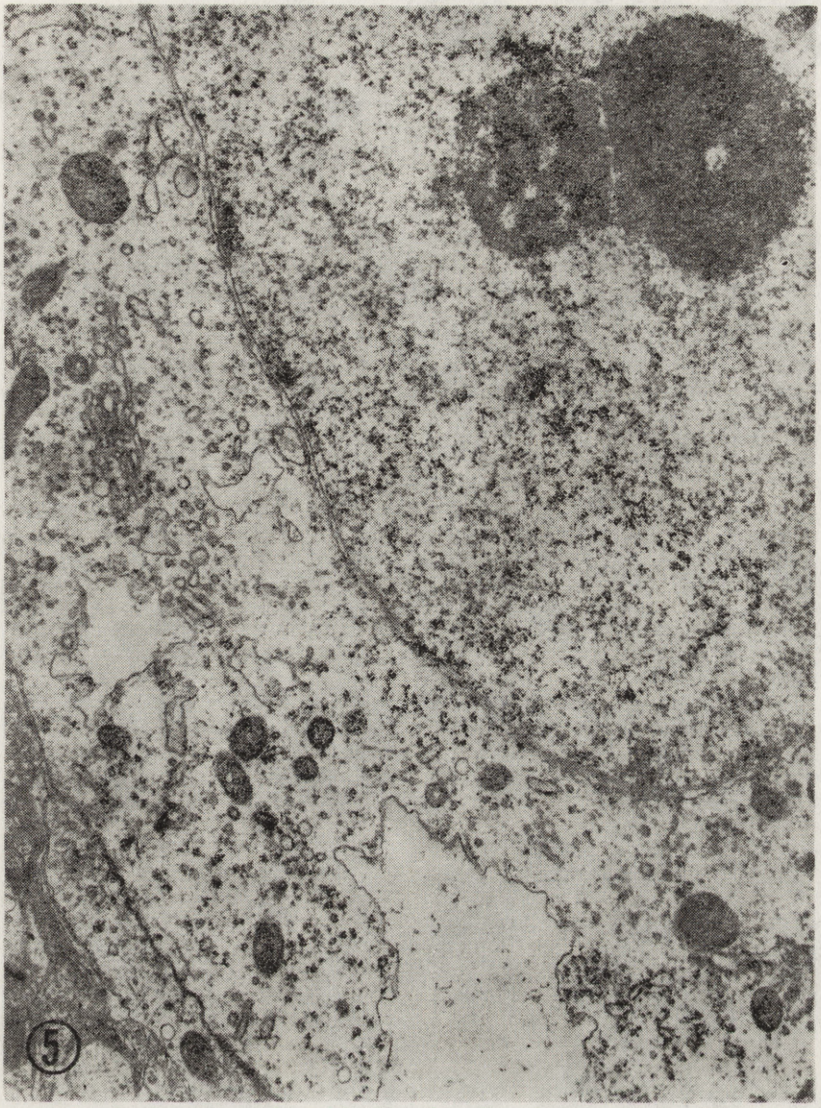

*Fig. 5.* Cerebral cortex of gerbil 45 min after indomethacin. Note the partial segregation of granular and fibrillar material. × 10 520

*Ryc. 5.* Kora mózgowa chomika mongolskiego. 45 min po podaniu indometacyny. Widoczne oddzielenie się części ziarnistej i włókienkowej jąderka. Pow. 10 520 ×

separated sharply from other elements (Fig. 5). The granular substance was usually located in central portion of nucleoli, while the fibrillary one, as a rule was extruded to their periphery. The extranuclear areas contained numerous perichromatin granules (Figs 5, 6). Amount of the perinucleolar chromatin was changeable. The chromatin in most of the nuclei was homogeneously distributed. In the cytoplasm of the cells some channels of rough endoplasmic reticulum and Golgi complexes were markedly distended. Most of the channels were electron lucent,

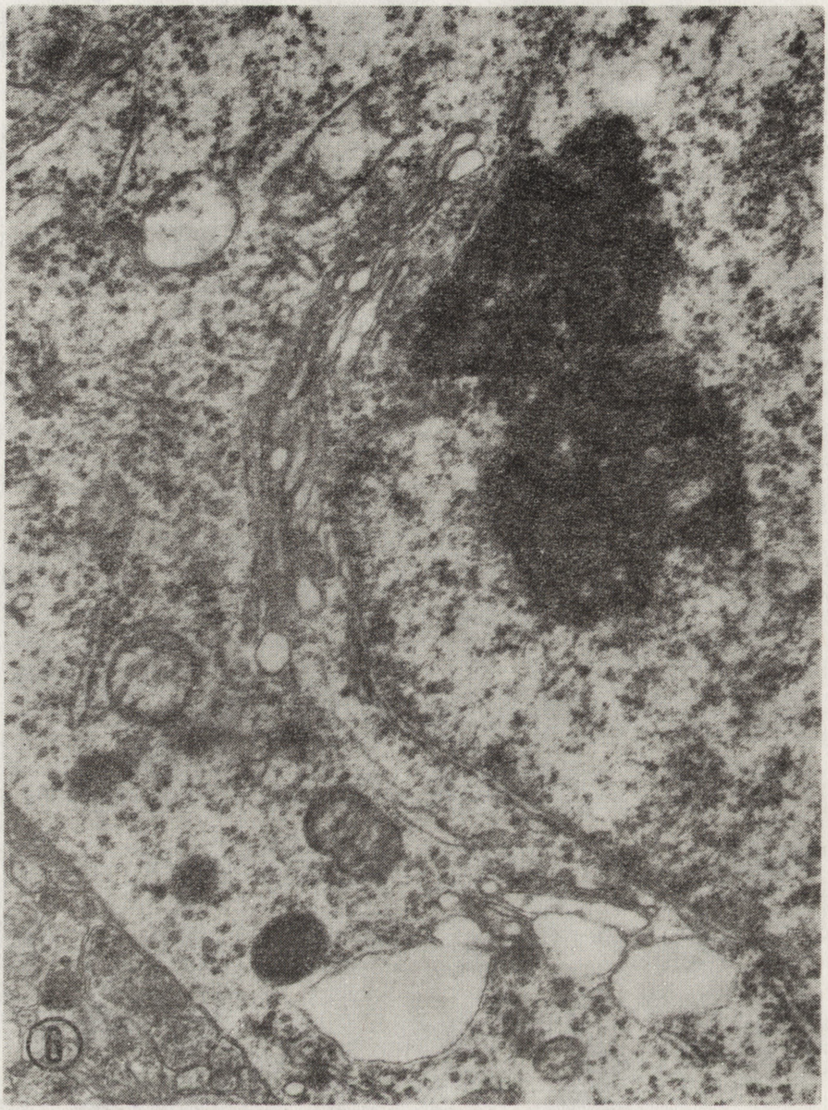

*Fig. 6.* Striatum of gerbil 45 min after indomethacin. Partial segregation of fibrillar and granular compartment of nucleolus and condensed perinucleolar chromatin (Chr). × 10 520

*Ryc. 6.* Striatum chomika mongolskiego 45 min po podaniu indometacyny. Widoczne częściowe oddzielenie się składników włóknistych i ziarnistych jąderka oraz zagęszczenie przyjąderkowej chromatyny (Chr). Pow. 10 520 ×

only some of them contained a small amount of flake-like material (Figs 5, 6).

The above described changes were present in animals sacrificed both 45 min and 6 hrs following drug administration. It seemed that in animals with longer exposition for the drug the nucleolar changes were more widely distributed.

## DISCUSSION

Our observations disclose of the influence of indomethacin on the cellular metabolism of the central nervous system. The drug induces atypical nucleolar segregation, known under the name of microsegregation. This is characterized by the juxtaposition of granular and fibrillar nucleolar compartments, without their segregation into completely separated zones. Similar nucleolar lesions were observed in other tissues after cordycepin and campthotecin action (Puvion et al. 1974; Gajkowska et al. 1977) and were considered as morphological evidence of disturbances in normal synthesis or processing of nucleolar RNA. The observed nucleolar segregation was concomitant with an accumulation of perichromatin granules not only within nuclear chromatin located in the extranuclear areas but also at the surface of the nucleolar RNA components. It was only recently, that cytochemical and electron microscopic-autoradiographic studies were undertaken to explain the functional significance of perichromatin granules (Kumar, Wu 1973; Puvion et al. 1976; Gajkowska et al. 1977). It was suggested that, with a great probability these abnormal granules corresponded to storage forms of either nontransported or abnormally processed ribosomal RNA. Our observations on nucleolar alterations induced by indomethacin *in vitro* and *in vivo* are in good agreement with previous studies on the action of some antimetabolites on the nucleic acids metabolism in non-neural tissue (Monneron, Bernhard 1969; Monneron et al. 1970; Vazquez-Nin, Bernhard 1971; Puvion et al. 1974; Nash et al. 1975). Microsegregation of nucleolar substances concomitant with abnormal perichromatin granules accumulation in the nerve tissue cellular elements under the action of indomethacin may suggest that the drug induces impairment in either nucleolar RNA processing or r-RNA transport. In that case remote effects of indomethacin may be harmful to the nerve tissue metabolism. This side effects of the drug, if confirmed in biochemical studies, will certainly limit its usefulness in the cerebro-vascular events, despite its protective role in the development of postischemic tissue alterations.

## WPŁYW INDOMETACYNY NA ULTRASTRUKTURĘ JĄDERKA

Doniesienie wstępne

S t r e s z c z e n i e

Prześledzono wpływ indometacyny na ultrastrukturę jąderka komórek nerwowych i glejowych w warunkach *in vivo* i *in vitro*.

Wykazano, że pod wpływem leku dochodzi do mikrosegregacji jąderka i do nieprawidłowego nagromadzenia ziaren perichromatynowych zarówno wśród, jak i na powierzchni okołojąderkowej chromatyny.

Spostrzeżenia te sugerują, że indometacyna, podobnie jak szereg substancji antymetabolicznych może prowadzić do zaburzenia dojrzewania jąderkowego RNA lub upośledzenia transportu r-RNA. Ten uboczny efekt działania leku, jeśli znajdzie potwierdzenie w badaniach biochemicznych, ograniczy niewątpliwie jego użyteczność w zapobieganiu tkankowym następstwom niedokrwienia mózgu.

## ВЛИЯНИЕ ИНДОМЕТАЦИНА НА УЛЬТРАСТРУКТУРУ ЯДРЫШКА

Краткое сообщение

### Резюме

Исследовалось влияние индометацина на ультраструктуру ядрышка нервных и глиальных клеток в условиях *in vivo* и *in vitro*.

Было показано, что под влиянием препарата наступает микросегрегация ядрышка и неправильное скопление перихроматиновых зерен, как внутри, так и на поверхности околоядрышкового хроматина.

Эти наблюдения показывают, что индометацин, как и ряд антиметаболических веществ может вести к нарушению созревания ядрышковой РНК или к ухудшению транспорта р-РНК. Этот побочный эффект влияния препарата, если будет подтвержден в биохимических исследованиях, несомненно ограничит его пригодность для предотвращения тканевых последствий ишемии мозга.

## REFERENCES

1. Abdel-Halim M., Lunden G., Anggard E.: Prostaglandin profiles in nervous tissue and blood vessels of the brain of various animals. Prostaglandins, 1980, 10, 249—258.
2. Bhakoo K. K., Lascelles P. T., Crockard H. A., Avery S. H.: Brain prostaglandins and cerebral edema following temporary vascular occlusion in gerbils. V. Intern. Conf. Prostaglandins. Florence, May 18—21, 1982. Abstracts 706.
3. Boulu R. G., Plotkin M., Guemau C., Sofeir M., Wiernsperger N.: Effect of indomethacin in experimental cerebral ischemia. Pathol., Biol., 1982, 30, 278—281.
4. Brierley J. B., Meldrum B. S., Brown A. W.: The threshold and neuropathology of cerebral anoxic, ischemic cell change. Arch. Neurol., 1973, 29, 367—373.
5. Crockard H. A., Iannotti F., Hanstock A. H., Smith R. D., Harris R. J., Symon L.: Cerebral blood flow and edema following carotid occlusion in gerbil. Stroke, 1980, 11, 494—498.
6. Furlow T. W. Jr., Hallenbeck J. M.: Indomethacin prevents impaired perfusion of the dog's brain awter global ischemia. Stoke, 1978, 9, 591—594.
7. Gajkowska B., Puvion E., Bernhard B.: Unusual perinucleolar accumulation of ribonucleoprotein granules induced by camptothecin in isolated liver cells. J. Ultrastruct. Res., 1977, 60, 335—347.
8. Garcia J. H., Lossinsky A. S., Kauffman F. C., Conger K. A.: Neuronal ischemic injury: light microscopy, ultrastructure and biochemistry. Acta neuropathol. (Berl.), 1978, 43, 85—95.
9. Gaudet A. J., Levine L.: Transient cerebral ischemia and brain prostaglandins. Biochem. Biophys. Res. Comm., 1979, 86, 393—401.
10. Gaudet R., Alam I., Levine L.: Accumulation of cyclo-oxygenase products of arrachidonic acid metabolism in gerbil brain during reperfusion after bilateral common carotid artery occlusion. J. Neurochem., 1980, 35, 653—658.

11. Hallenbeck J. M., Furlow T. W. Jr.: Prostaglandin $I_2$ and indomethacin in the dog. Stroke, 1979, 10, 629—637.
12. Iannotti W., Crockard A., Ladds G., Symon L.: Are prostaglandins involved in experimental ischemic edema in gerbils. Stroke, 1981, 12, 301—306.
13. Johanson B. B.: Indomethacin and cerebro-vascular permeability to albumin in acute hypertension and cerebral embolism in rat. Exp. Brain Res., 1981, 42, 331—336.
14. Kumar A., Wu R.: Role of ribosomal RNA transcription in ribosome processing in HeLa cells. J. Mol. Biol., 1973, 80, 265—276.
15. Monneron A., Bernhard W.: Fine structural organization of the interphase nucleus in some mammalian cells. J. Ultrastruct. Res., 1969, 27, 266—278.
16. Monneron A., Burglen M., Bernhard W.: Action of toyocamycin on nucleolar fine structure and function. J. Ultrastruct. Res., 1970, 32, 370—389.
17. Mossakowski M. J., Gajkowska B.: Influence of indomethacin on the ultrastructural pathology of the brain following temporary ischemia in mongolian gerbils. Neuropat. Pol., 1984, 22, 347—365.
18. Mossakowski M. J., Kwiatkowska-Patzer B.: Effect of indomethacin on the morphology of the brain vascular network in the postischemic period. In: Developmental and circulatory aspects of brain metabolism. Ed. B. B. Mrsulja. Raven Press. New York (in press).
19. Mossakowski M. J., Kwiatkowska-Patzer B.: Wpływ indometacyny na niedokrwienne uszkodzenia mózgu u chomika mongolskiego (*Meriones unguiculatus*). Neuropat. Pol., 1983, 21, 287—301.
20. Nash R., Puvion E., Bernhard W.: Perichromatin fibrils as components of rapidly labeled extranuclear RNA. J. Ultrastruct. Res., 1975, 53, 395—405.
21. Puvion E., Garrido G., Viron A.: Technique rapid d'isolement et de survie en monocouches d'hepatocytes de rat adulte. C. R. Acad. Sci. (Paris), 1974, 279, 509—512.
22. Puvion E., Moyne G., Bernhard W.: Action of 3'deoxyadenosine (Cordycepin) on the nuclear ribonucleoproteins of isolated liver cells. J. Microsc. Biol. Cell, 1976, 25, 17—32.
23. Ruszczewski P.: Poziom endogennych prostaglandyn w krwi żylnej mózgu u psa w warunkach hipo- i hiperkapnii, hipoksji i ostrego niedokrwienia. Praca doktorska. CMDiK PAN, Warszawa 1977.
24. Vazquez-Nin G., Bernhard W.: Comparative ultrastructural study of perichromatin and Balbiani ring granules. J. Ultrastruct. Res., 1971, 36, 842—860.

Authors' address: Medical Research Centre, Polish Academy of Sciences, 3, Dworkowa Str., 00-784 Warszawa.