ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. IX Fasc. 2 Szczecin 1979

Marek K. JURKOWSKI, Michał STACHOWIAK, Jacek W. BIAŁOWĄS

Physiology

EFFECTS OF CERTAIN STRESS SITUATIONS AND OF DBS ON ACETYLCHOLINETRANSFERASE, ACETYLCHOLINESTERASE, AND MONOAMINEOXIDASE ACTIVITIES IN VARIOUS REGIONS OF BRAIN OF JUVENILE CARP, (*Cyprinus carpio* L.)

WPŁYW NIEKTÓRYCH SYTUACJI STRESOWYCH I DETERGENTU (DBS) NA AKTYWNOŚĆ ACETYLOCHOLINOTRANSFERAZY, ACETYLOCHOLINOESTERAZY I MONOAMINOOKSYDAZY W POSZCZEGÓLNYCH CZĘŚCIACH MÓZGOWIA NARYBKU KARPIA (*Cyprinus carpio* L.)

Institute of Medical Biology
Academy of Medicine in Gdańsk
Dept. of Physiology and Dept. of Anatomy

Effects of stress situations and of a detergent (DBS) on activities of the cholinergic system enzymes, i.e., acetylocholinetransferase (AChT) and acetylcholinesterase (AChE) as well as on the activity of monoamineoxidase (MAO) in fore-, mid-, and hindbrain of carp juveniles were studied. Both the thermal and immobilisation stresses as well as the detergent were found to cause a slight increase in the AChT activity in midbrain accompanied by a decline of the AChE activity in mid- and hindbrain; on the other hand, the MAO activity increase, brought about by the detergent, occurred in hindbrain only.

The results show the fish cholinergic system activity to be affected by the two stress situations and the detergent contained in the fish habitat. Thus the detergent, apart from its known toxic effects, may act as a stressor to fish.

INTRODUCTION

As shown by several papers describing the influence of toxic substances on aquatic organisms, many chemical compounds, detergents included, may – apart from their being toxic – exert a stressing influence as well (Łukianienko, 1974). The brain microdissection technique, developed in the recent years, allowed to observe defined changes in the enzymatic activity and in the level of adrenergic system neurotransmitters in certain mammals, particularly in hypothalamus (Palkovitz a. al., 1975, Kvetansky a. al., 1977, Białowąs a. al., 1978, Stachowiak a. al., 1978). However, the available literature provides no data on effects of stress situations upon changes in the enzymatic activity in any region of the fish central nervous system.

Histochemical studies (Wächtler, 1974, Yamane a. al., 1974, Yamamoto a. al., 1977) show various brain centres in fish to reveal differentiated acetylcholinesterase (AChE) and monoamineoxidase (MAO) activities.

A quantitative determination of the activity of neurotransmitter-synthesising and -degrading enzymes provides an indirect evidence of their level in brain under defined experimental conditions.

The common occurrence of the cholinergic system in animals (Fischer, 1971, Wächtler, 1974) as well as farmacological data on the role of the system in higher vertebrates (Koelle, 1963) stimulated the authors' interest in the problem of the extent to which the activities of acetylcholinetranssferase (AChT), acetylcholinesterase (AChE) and – additionally – monoamineoxidase (MAO) in various regions of juvenile carp brain were affected by altered environmental conditions.

MATERIAL AND METHDOS

Autumn carp juveniles supplied by the State Fish Farm, Wdzydze were tested. Fishes with no discernible pathological changes were acclimatised for 2 weeks in a 200 l aquarium with running water heated to 20 ± 1°C; the oxygen content of the water ranged within 4–6 mg/l, no ammonia being detected. The conditioned fishes were divided between four 50 l aquaria with water aerated up to 5 mg0_2/l; three aquaria with water of 20°C housed the control individuals, those tested for immobilisation, and for detergent effects, the individuals to experience thermal stress being placed in the fourth aquarium with water heated to 32°C. The fishes were immobilised by enmeshing them in a netting stretched on a specially constructed frame in a respective aquarium. In the detergent test, the DBS concentration was 5 mg/l SA. The experimental tanks measured 50X30X33 cm. Each experiment was run for 5 hrs after which time the fishes were stunned by striking a blow at their heads, and heart blood was taken from the control individuals in order to assess their condition from protein contents in the serum. Then the fishes were decapitated, heads being placed in dry ice. After taking the brain out it was discected on a freeze microtome at –20°C to separate fore-, mid-, and hindbrain. These brain parts were homogenised in re-distilled water (1:10 v/v). Proteins were determined according to

Lowry et al. as described by Mejbaum-Katzenellenbogen and Mochnacka (1969); the AChE activity was determined using Ellman's colorimetric technique as described previously (Jurkowski, 1977). Isotop techniques were employed to the assessment of activities of AChT (Fonnum, 1975) and MAO (Wurtman and Axelrod, 1963). In all determinations, the analytic grade reagents were used as well as the Amerscham isotope substrates: (G^3H) tryptamine and ($1-^{14}C$) acetyl-coenzyme A of the specific activities of 870 and 58 mCi/mM, respectively. The results obtained were expressed in terms of μg protein per 5 min. incubation (AChE) and μg protein per hr incubation (AChT, MAO). The results are presented as arithmetic means from eight separate replicated measurements.

RESULTS AND DISCUSSION

Fig. 1 shows the position of cuts on brain dissection and those parts assumed to represent fore-, mid-, and hindbrain. Owing to technical reasons, both thalamus and hypothalamus were included into midbrain.

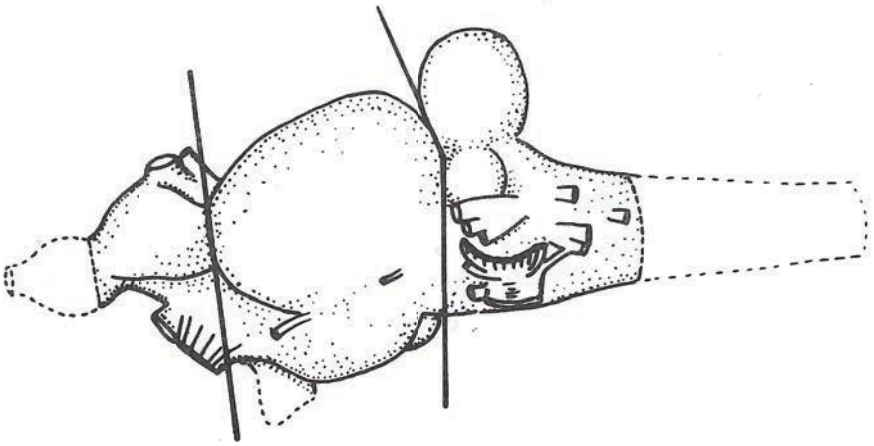
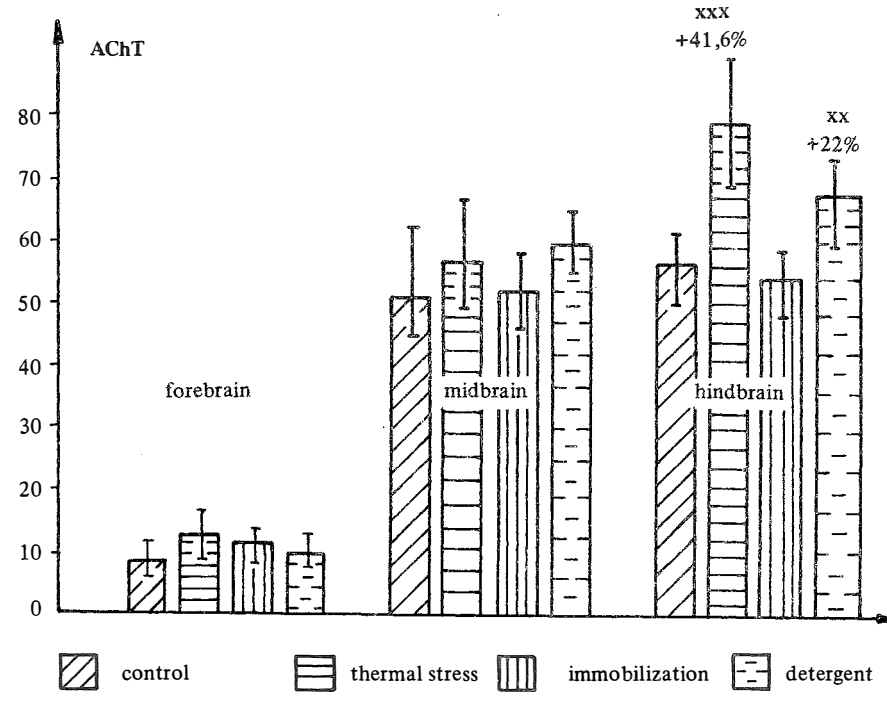


Fig. 1. Planes of brain dissection (after Jasiński. 1973)

The serum protein content analysis performed in the control individuals showed the range of 2.8–3.7 g%, which is indicative of a good condition according to the criteria set by Dombrowski (quoted after Amlacher, 1972).

Figs. 2, 3, and 4 illustrate changes in the activities of AChT, AChE, and MAO, respectively, in the experimental groups of fishes.

The control individuals showed differences in the enzymatic activity between the parts of brain examined. The highest MAO activity was recorded in midbrain, the values for fore- and hindbrain being lower by a factor of 2.3. AChT showed a low activity in forebrain, while the activities of this enzyme in mid- and hindbrain were 6 times as high.



Rys. 2. Changes in acetylocholinetransferase activity in stresses studied (N = 8 ±SD)

The AChE activity, too, was at its lowest in forebrain, the values found for mid- and hindbrain being about 3 and 1.5 times higher, respectively.

Under the thermal stress, a slight increase in fish locomotoric activity, consistent with the temperature effect on fish locomotion (Sigmund and Vogel, 1977) was observed. The analysis of enzymatic changes showed fluctuations in the MAO activity, the differences being, however, statistically insignificant. On the other hand, the hindbrain AChT activity increased by about 41.6% ($p \leqslant 0.001$) and the AChE activity dropped by about 48% ($p \leqslant 0.001$). A similar (by ca 31%, $p \leqslant 0.01$) drop in the AChE activity under the thermal stress was observed in midbrain.

The fishes tested for the effect of immobilisation made repeated attempts to release themselves from the nets. Their MAO and AChT activities showed no statistically significant changes. On the other hand, considerable changes were found in the AChE activity in all the brain regions examined: and increase by about 47% ($p \leqslant 0.05$) in forebrain and decreases in mid- and hindbrain by ca 41.4 and 28% ($p \leqslant 0.001$), respectively.

The fishes kept in the DBS-containing water showed a high locomotor activity, frequently remaining just beneath the surface. Hindbrain revealed an increase in its MAO and AChT activity by ca 56% ($p \leqslant 0.001$) and 22% ($p \leqslant 0.01$), respectively. The AChE

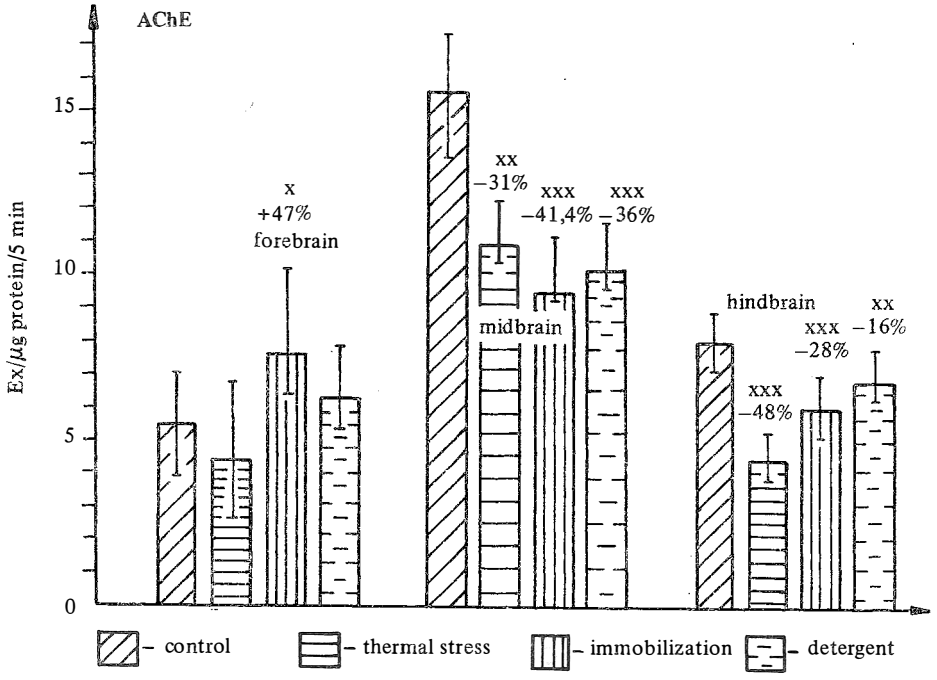


Fig. 3. Changes in acetylcholinesterase activity in stresses studied (N = 8 ±SD)

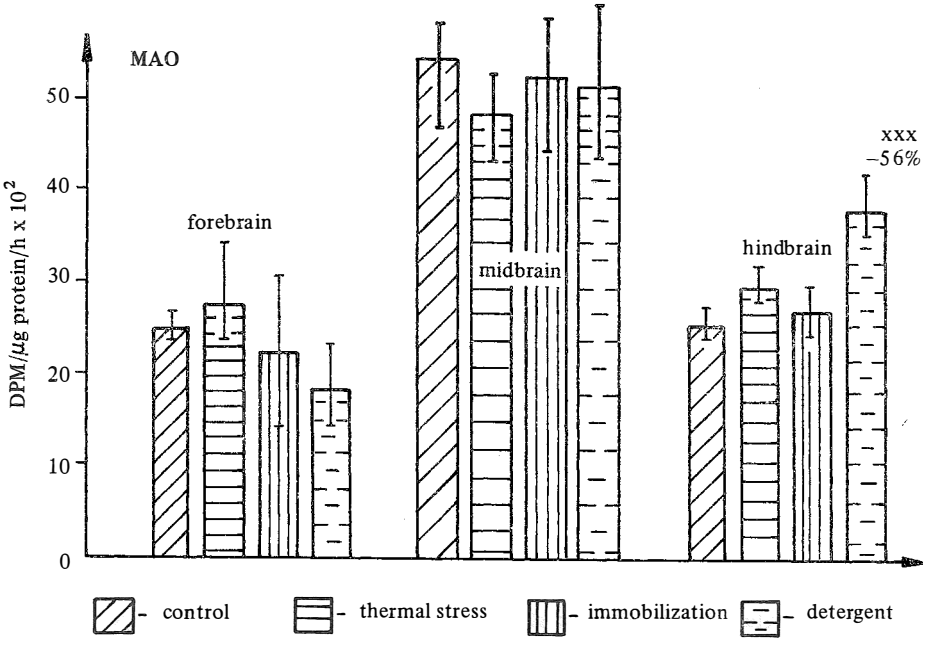


Fig. 4. Changes in monoamineoxidase activity in stresses (N = 8 ±SD)

activity, however, declined by about 36% ($p \leqslant 0.001$) and 16% ($p \leqslant 0.01$) in mid- and hindbrain, respectively.

The activity of each enzyme determined in the present studies seem consistent with the histochemical data. The low activities of the forebrain enzymes are presumably associated with the fact that this brain region is in fishes only at its initial stage of development, the principal neural centres being placed in caudal brain parts. Also, a lack of important neural centres in forebrain, the role of which in fish behaviour is still questioned (Brown, 1957; Pučkov, 1962), amy be responsible for the slightest changes in the enzymatic activities found in the experimental situations.

The largest differences in enzymatic activities were found in mid (including thalamus and hypothalamus) within which significant differences between the activities during a number of intoxications were revealed by Łukianienko (1974); this part of fish brain plays – due to its neural connections – a considerable part in fish behaviour.

The increase in the hindbrain AChT activity both under the thermal stress and in the detergent-containing water is presumably associated with an intensified locomotor activity of a fish in those situations since no similar changes were found in the immobilised fishes.

A high thiamine concentration in fish brain evidences a high activity of acetylcholine-synthesising enzymes (Pučkov, 1962). Also, an increase in the Mg^{++} -dependent ATP-ase in the fishes affected by the detergent may – indirectly – give evidence of an intensified synthesis processes, the acetylcholine synthesis inluded (Jurkowski, 1977). On the other hand, although the increase in the acetylcholine-synthesising enzyme activity is observed in the entire brain, the statistical significance of this increase was found in hindbrain and only under the influence of the detergent and thermal stress. Higher vertebrates may use some part of the energy supplied by ATP transformations for the resorption of acetylcholine and binding the latter in large storage vesicles. This mechanism seems to be operative in fishes experiencing stresses, particularly in view of the fact that the acetylcholine-degrading enzyme activity decreases markedly in all the situations studied.

It is difficult to conclude, basing on the results obtained, on a role of the adrenergic system in the stress situations studied as catecholamines are degraded extraneurally, too, with an active participation of catechol-O-methyltransferase (COMT) occurring in fish brain where its activity was found (Mazeaud, 1974) to be exceede by the MAO activity by the factor of 2. As indicated by earlier data (Jurkowski, 1977), an increase in the activity of Na^+, K^+-dependent ATP-ase may provide an indirect evidence of the role of MAO in brain of the fishes affected by the detergent. The present data confirm this fact to a certain extent since the MAO activity was found to have increased in hindbrain only.

Studies on mammalian neurotransmitters show changes in the cholinergic system to give rise to changes in the adrenergic one, and vice versa. The fish central nervous system seems to be a site of a similar phenomenon. However, the question of a role of the adrenergic system in fishes under stress can be answered only via the direct examination of the tyrosine hydroxylase activity and catecholamines content, the latter being the subject of our project currently in progress.

REFERENCES

Amlacher E., 1972: Taschenbuch der Fischenkrankheiten für Veterinarmediciner und Biologen, VEB G. Fischer Verlag, Jena.

Białowąs J., Jurkowski M.K., Stachowiak M., Dytkowska A., 1978: The influence of food deprivation, exposure to cold and immobilization on monoamine oxidase activity in some hypothalamic nuclei of the rat brain. – Acta Neurobiol. Exp. 38: 283–288.

Brown M.E., 1957: The Physiology of Fishes, II, Academic Press Inc., New York.

Fischer H., 1971: Vergleichende Pharmakologie der Überträgersubstanzen in tiersystematischer Darstellung. Handbuch der experimentallen Pharmakologie, 629, Springer, Berlin-Heidelberg-New York.

Fonnum F., 1975: A rapid radiochemical method for the determination of choline acetyltransferase. – J. Neurochem. 24: 407–409.

Jurkowski M.K., 1972: Effect of DBS detergent on activity of some enzymes of young carp (Ciprinus carpio L.) brain, gills and serum. – Acta Ichthyol. Piscat. (Szczecin). VII, 2: 47–53.

Koelle G.B., 1963: Cholinesterases and anticholinesterase agents. Handbuch der experimentellen Pharmakologie, 15, Springer Verlag, Berlin.

Kvetansky R., Palkovitz M., Mitro A., Torda T., Mikulaj L., 1977: Catecholamines in individual hypothalamic nuclei of acutely and repeatedly stressed rats. – Neuroendocrinology 23: 257–267.

Łukianienko I.W., 1974: Toksykologia ryb [Toxicology of Fisches] PIWRiL, Warszawa.

Mazeaud M.M., 1974: Monoamine oxidase and catechol-O-methyl transferase in tissues of reinbow trout (Salmo gairdneri Rich.). – Comp. Gen. Pharmac. 5 : 251–253.

Mejbaum-Katzenellenbogen W., Mochnacka L., 1969: Kurs praktyczny z biochemii [Practic Biochemistry], PWN, Warszawa.

Palkovitz M., Kobyashi R.M., Kizer J.S., Jakobowitz D.M., Kopin I.J., 1975: Effects of stress on catecholamines and tyrosine hydroxylase activity of individual hypothalamic nuclei. – Neuroendocrinology. 18: 144–153.

Pučkov N.V., 1962: Fizjologia ryb [Physiology of Fishes], PWN, Warszawa.

Sigmund R., Vogel J., 1977: The dependence of hearth rate and locomotor activity on water temperature in the carp. – Experientia. 33: 1607.

Stachowiak M., Białowąs J., Kurkowski M.K., 1978: Catecholamines in some hypothalamic and telencephalic nuclei of food deprived rats. Acta Neurobiol. Exp. 38: 157–165.

Wächtler K., 1974: The distribution of acetylcholinesterase in the Cyclostome brain. 1. Lampetra planeri (L.). – Cell Tiss. Res. 152: 259–270.

Wurtman R.J., Axelrod J., 1963: A sensitive and specific assay for the estimation of monoamine oxidase. – Biochem. Pharmacol. 12: 1439–1440.

Yamamoto K., Tohyama M., Shimizu N., 1977: Comparative anatomy of the topography of catecholamine containing neuron system in the brain stem from birds to teleosts. – J. Hirnforsch. 18: 229–240.

Yamane S., Kino A., Teshima S., 1974: Histochemical demonstration of cholinesterase activity in tissues of the carp and effect of DDVP on its activity in situ. – Acta Histochem. Cytochem. 7: 167–174.

Translated: mgr Teresa Radziejewska

WPŁYW NIEKTÓRYCH SYTUACJI STRESOWYCH I DETERGENTU (DBS) NA AKTYWNOŚĆ ACETYLOCHOLINOTRANSFERAZY, ACETYLOCHOLINOESTERAZY I MONOAMINOOKSYDAZY W POSZCZEGÓLNYCH CZĘŚCIACH MÓZGOWIA NARYBKU KARPIA (*Cyprinus carpio* L.).

Streszczenie

Zbadano aktywność enzymów układu cholinergicznego, tj. acetylocholinotransferazy (AChT) i acetylocholinoesterazy (AChE) oraz aktywność monoaminooksydazy (MAO) w przodo-, śród- i tyłomózgowiu jesiennego narybku karpia oraz wpływ 5-godzinnego przebywania ryb w podwyższonej temperaturze (32°C), immobilizacji i dodatku detergentu (DBS) w ilości 5 mg/l SA na aktywność tych enzymów. U zwierząt kontrolnych stwierdzono różnice w aktywności badanych enzymów pomiędzy poszczególnymi częściami mózgowia. Najwyższą aktywność MAO stwierdzono w śródmózgowiu, niższą ok. 2,3 raza w przodomózgowiu i tyłomózgowiu. AChT wykazuje niewielką aktywność w przodomózgowiu, zaś 6 razy większą w śród- i tyłomózgowiu. Natomiast AChE najniższą aktywność wykazuje w przodomózgowiu, ok. 3 razy większą w śród- i ok. 1,5 raza większą w tyłomózgowiu.

We wszystkich badanych sytuacjach w poszczególnych częściach mózgu nie stwierdzono zmian statystycznie istotnych w aktywności MAO z wyjątkiem tyłomózgowia, gdzie aktywność tego enzymu w przypadku działania detergentu wzrosła o 56%. W układzie cholinergicznym stwierdzono wzrost aktywności AChE w przodomózgowiu i znaczne spadki w śródmózgowiu i tyłomózgowiu we wszystkich rodzajach eksperymentów. Stwierdzono również niewielki wzrost aktywności AChT, który statystycznie istotny jest tylko w tyłomózgowiu w przypadku stresu cieplnego (wzrost ok. 42%) i pod wpływem detergentu (wzrost ok 22%).

Z uzyskanych rezultatów wynika, iż w badanych sytuacjach stresowych, jak również w ekspozycji na detergent zmienia się aktywność układu cholinergicznego. Wydaje się więc, że detergent może wywierać działanie stresowe na ryby, obok znanego już działania toksycznego.

М. Юрковски, М. Стаховяк, Й.В. Бяловенс

ВЛИЯНИЕ НЕКОТОРЫХ СТРЕССОВЫХ СИТУАЦИИ И ДЕТЕРГЕНТА (DBS) НА АКТИВНОСТЬ АЦЕТИЛХОЛИНТРАНСФЕРАЗЫ, АЦЕТИЛХОЛИНЭСТЕРАЗЫ И МОНОАМИНОКСИДАЗЫ В ОТДЕЛЬНЫХ ЧАСТЯХ ГОЛОВНОГО МОЗГА МАЛЬКОВ КАРПА (CYPRINUS CARPIO L.)

Р е з ю м е

Исследовали активность энзимов холинергической системы, т.е.: ацетилхолинтрансферазы (АХТ) и ацетилхолинэстеразы (АХЭ) а также активность моноаминоксидазы (МАО) в переднем-, среднем- и ромбовидном мозге осенних мальков карпа. Исследовали также влияние 5 часового пребывания рыб в повышенной температуре (32°С), иммобилизации и добавки детергента (DBS) в количестве 5 мг/л SA (СА) на активность названных энзимов. У рыб контрольных отмечено различия активности исследованных энзимов в отдельных частях головного мозга. Наиболее высокую активность МАО находили в среднем мозге, - низшую 2,3 раза в переднем- и ромбовидном мозге. АХТ проявляет небольшую активность в переднем мозге, но в 6 раз большую проявляет в сред-

нем и ромбовидном мозге. АХЭ самую низкую активность имеет в переднем мозге, примерно 3 раза высшую в среднем- и 1,5 раза высшую в ромбовидном мозге.

Во всех исследованных ситуациях в отдельных частьях головного мозга не отметили статистически существенных изменений в активности МАО, за исключением ромбовидного мозга, в котором активность этого энзима, при действии детергента увеличилась на 56%. В холинергической системе отмечено увеличение активности АХЭ в переднем мозге и заметное уменьшение в среднем и ромбовидном мозге во всех экспериментах. Отмечено также небольшое увеличение активности АХТ, которое является статистически существенным только в ромбовидном мозге при термическом воздействии (увеличение примерно на 42%) и под влиянием детергента (ув. примерно на 22%).

Из полученных результатов вытекает, что в исследованных стрессовых ситуациях а также в экспозиции на детергент, изменяется активность холинергической системы. Поэтому мы предполагаем, что детергент, кроме известного уже токсического влияния, может также иметь стрессовое действие на рыб.

перевод: др. Юзеф Домагала

Adress:

Instytut Biologii Medycznej
Akademii Medycznej w Gdańsku
80-211 Gdańsk
Polska–Poland

Zakład Fizjologii
Marek K. Jurkowski
Michał Stachowiak
Zakład Anatomii Prawidłowej
Jacek W. Białowąs
Polska–Poland

Received: 15 III 1979 r.