Serotonin levels in fish brain: effects of hydrostatic pressure and water temperature

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Summary. The contents of serotonin (5 HT) and its metabolite 5 hydroxy indoleacetic acid (5 HIAA) have been measured (HPLC technique) in the brains of eels exposed to different conditions of hydrostatic pressure and temperature (HP = 1 or 101 ATA in winter, Tw = 14 °C, and in summer, Tw = 19 °C). It appears that an increase of Tw induces a significant increase of the 5 HT/5 HIAA ratio. In contrast, eels exposed at 101 ATA of HP for 1 h do not exhibit any modification in the 5 HT/5 HIAA brain ratio at a given temperature. The involvement of 5 HT under the conditions studied is discussed.
Key words. Fish; serotonin; hydrostatic pressure; temperature.

Amid the numerous factors of the fish environment which exhibit some changes, two have been studied in the laboratory: water temperature, Tw, and per se hydrostatic pressure, HP. A strong artificial increase of at least one of these factors induces some perturbations in fish behavior: hyperactivity, hyperexcitability, motor incoordination, disequilibrium (Tw, see Crawshaw[3]; HP, see Belaud et al.[2]. Some modifications in different functions are associated with these perturbations for example hyperventilation, tachycardia, changes in EEG and visually evoked responses[2]. Considering the importance of serotonin (5 HT) in the arousal states and behavior[1], and its relation with motor activity cycles not only of mammals but also of fish[4] and invertebrates[7], the question arises whether the observed perturbations are related to changes in the 5 HT content of the brain.

Material and methods. 20 eels (*Anguilla anguilla* L.) were used in the experiments, the aim of which was to study the effects of HP exposure (1 h at 101 ATA) at two metabolic levels: in winter (Tw = 14 °C, $\dot{M}O_2 = 0.8 \pm 0.16$ mmoles·h^{-1}·kg^{-1}) and in summer (Tw = 19 °C, $\dot{M}O_2 = 1.3 \pm 0.27$ mmoles·h^{-1}·kg^{-1}). The eel was chosen because of its resistance to HP exposure[2].

Fish were obtained from a regional fishery and stored in a polyethylene tank (300 l) where tap water was continuously renewed and aerated. This tank was in a room open to the outside, allowing a natural temperature and day/night period. One day before the experiment, fish were placed in the experimental tank with air bubbling and fed with tap water. The experiments were always performed at the same hour of the day, bearing in mind the nycthemeral variations of the relationship between 5 HT and locomotor activity[4].

On the day of the experiment, the experimental tank (entirely closed and gas-proof) was completely filled with water. One wall of the tank was made of a gas-proof soft rubber membrane to allow the pressure to be transmitted to the fish without modification in the mass of dissolved gases. The experimental tank was put into a 130-l hyperbaric chamber fed by compressed air at a rate of 10 atm/min to 101 ATA; decompression was carried out at the same rate after 60 min at the experimental pressure. When the decompression was achieved, fish were quickly frozen in liquid nitrogen and the brains were removed in the following 5 min. Control fish at atmospheric pressure were simultaneously studied.

Measurements of 5 HT and 5 HIAA were performed with the technique of high pressure liquid chromatography (HPLC) and electrochemical detection. The frozen brains were sonicated in 500 µl of perchloric acid 0.1 M (containing 10^{-2} M of pargilin). Chromatography was performed on a Nucleosil C18, 5 µm column.

The statistical significances of the results was evaluated at the 5% level with a two-way ANOVA.

Results. The results are shown in the table. In reference conditions (Tw = 14 °C, HP = 1 ATA) the 5 HT and 5 HIAA contents in eel brain are 157+16.7 ng·g^{-1} and 41 ± 2.1 ng·g^{-1} respectively.

1) Temperature effects. The table shows that at atmospheric pressure of 1 ATA, an increase of Tw, and thus of the metabolism, does not induce a significant change in 5 HT but 5 HIAA is significantly reduced; consequently, the 5 HT/5 HIAA ratio is increased ($p < 0.01$). The same results are observed at 101 ATA of HP.

2) Pressure effects are shown in the table. It appears that at a given Tw value (14 °C or 19 °C), exposure to 101 ATA of HP for 1 h does not modify significantly the content of 5 HT and 5 HIAA in eel brain: consequently, the 5 HT/5 HIAA ratio is not changed.

Moreover, the ANOVA analysis shows that no interaction exists between HP and Tw affecting the levels of 5 HT and 5 HIAA in the brain, or the ratio between them.

Discussion. To our knowledge very little work has been done on the serotonin levels in fish brain[4], and this study is the first to use the HPLC technique, which is actually the best method for this measurement[10]. Consequently, the values obtained at 1 ATA and TW = 14 °C can be considered as reference values for serotonin in eel brain (with the HPLC technic).

In general, results from this work are in agreement with those previously described for the catecholamines, CA[9]. In fact, increase of Tw or pressure does not modify significantly the brain 5 HT content, but when Tw increases, 5 HIAA is significantly lowered, whereas pressure does not modify brain 5 HIAA. Consequently an increase in Tw induces a significant increase of the 5 HT/5 HIAA ratio which is not modified by exposure to HP at a given temperature. These results are in contrast with those from Koblin et al.[5] who have observed, in mice, a decrease in the 5 HT/5 HIAA ratio under pressure. However, it is necessary to make it clear that these authors have attributed this change, at least in part, to the stress induced by restraint of the animal in the hyperbaric chamber. From the above it is possible 1) to state the hypothesis that exposure to 101 ATA of HP for 1 h is not a stress for the eel, 2) to assume that the results obtained by Koblin et al.[5] are due, at least in part, to the specific effects of the gas mixture (inert gases) inhaled under hyperbaric conditions and not to the per se hydrostatic pressure effects. Moreover, comparing the results obtained at 1 and 101 ATA of HP it appears that synthesis and degradation of 5 HT are in a steady equilibrium (the ratio 5 HT/5 HIAA is not modified).

To define the meaning of the 5 HT/5 HIAA ratio and of its variations is not easy if levels of the 5 HT precursor (the 5 HTP) are not known. However, Koblin et al.[5] consider it as a good estimation of the 5 HT turnover. Mefford et al.[6] studying the

Mean values (ng·g^{-1} ± SEM) of serotonin (5 HT) and 5 hydroxy indoleacetic acid (5 HIAA) contents in eel brains (n = 20). Brain weight = 100 ± 2.3 mg

	HP = 1 ATA			HP = 101 ATA		
	5 HT	5 HIAA	5 HT/5 HIAA	5 HT	5 HIAA	5 HT/5 HIAA
Tw = 14 °C	157 ± 16.7	41 ± 2.1	3.9 ± 0.35	187 ± 24.0	55 ± 5.4	3.5 ± 0.21
Tw = 19 °C	188 ± 10.6	29 ± 1.9	6.7 ± 0.38	248 ± 30.1	35 ± 4.7	7.2 ± 0.67

distribution of biogenic amines and associated metabolites in dog brain, have concluded that brain areas with lower 5 HT concentrations had a higher efficiency of utilization of this amine as evidenced by a lower 5 HT/5 HIAA ratio. In other words, from these authors[6], it seems that a low 5 HT/5 HIAA ratio means a high efficiency of 5 HT utilization. In the present work the fact that, when compared to reference values (HP = 1 ATA; TW = 14 °C), the ratio is increased with Tw but not with HP can mean that in fish, a high efficiency of 5 HT utilization is more dependent on temperature than pressure. From the results it may be supposed that the changes in behavior and the motor hyperactivity (which correlates well with 5 HT levels in fish brain[4]) observed under pressure are not due to changes in the content of indolamine neurotransmitter in brain but perhaps to an HP effect on receptor sites[8].

1 Abramson, H.A., Gettner, H.H., and Rolo, A., J. Asthma Res. *17* (1980) 175.
2 Belaud, A., Mabin, D., Barthelemy, L., and Peyraud, C., J. Physiol. (Paris) *72* (1976) 639.
3 Crawshaw, L.I., Am. Zool. *19* (1979) 225.
4 Genot, G., Conan, G.Y., Barthelemy, L., and Peyraud, C., Biochem. Physiol. *79C* (1984) 189.
5 Koblin, D.D., Little, H.J., Green, A.R., Daniels, S., Smith, E.B., and Paton, W.D.M., Neuropharmacology *19* (1980) 1031.
6 Mefford, I.N., Foutz, A., Noyce, N., Jurik, S.M., Handen, C., Dement, W.C., and Barchas, J.D., Brain Res. *236* (1982) 339.
7 Pandey, A., and Habibulla, M., Experientia *38* (1982) 946.
8 Sebert, P., and Barthelemy, L., IRCS Med. Sci. *12* (1984) 919.
9 Sebert, P., Lebras, Y., Barthelemy, L., and Peyraud, C., Aviat. Space envir. Med. *55* (1984) 931.
10 Straub, H., and Kuhlmann, D., Comp. Biochem. Physiol. *78C* (1984) 319.

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Selective protection of cells against X-irradiation. Isoproterenol protects only those cells that possess β-adrenoreceptors

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Summary. In mixed culture of Chinese hamster fibroblasts, clone 431, and transformed murine L fibroblasts, clone B-82, isoproterenol was found to protect only 431 cells against ionizing radiation. It was shown that 431 cells, in contrast to B-82 cells, possess β-adrenoreceptors, and the radioprotective effect of isoproterenol can be realized only if this agent interacts with β-adrenoreceptors coupled with the cAMP system. Since malignization often causes the disappearance of β-adrenergic and other hormone receptors, the combined culturing and irradiation of the cells studied can be regarded as a model of the growth of malignant cells (B-82) among normal tissue cells (431 cells) under conditions of radiation therapy. A possibility of selective protection against radiation damage of normal tissue cells, with retention of the former radiosensitivity of tumor cells, is discussed.
Key words. Radioprotection; cell cultures; isoproterenol; β-adrenoreceptor; cellular cAMP concentration.

At present methods for the enhancement of the efficacy of radiation therapy are chiefly based on the knowledge and application of the fact that oxygenation in the tumor is reduced as the result of an imbalance between cell growth and vascularization. The development of hypoxic conditions in the tumor offers a possibility of using radiosensitizers of hypoxic cells, such as metronidazol, misonidazol, etc. However, the efficacy of applying these radiosensitizers is diminished by the fact that only 10–20% of the tumor cells are hypoxic, and also because the fractionated irradiation commonly used in clinical practice sharply reduces the effect of radiosensitizers[9]. Thus, it is necessary to investigate other peculiarities differentiating tumor cells from normal cells[12]. It is well known that cell malignization often leads to the disappearance or 'masking' of receptors of some hormones and to the loss of the response of the cell adenylate cyclase system to these hormones[6,7,18,25]. We have demonstrated earlier that adenylate cyclase stimulation through the hormone receptors leads to a decrease in the cell radiosensitivity[4,5,22], and that the radioprotective effect is connected with activation of the cAMP system[23]. It follows from the results of these works that the chain of events listed below:

1) binding of the adenylate cyclase activator to the receptor;
2) adenylate cyclase stimulation;
3) cAMP accumulation;
4) intensification of cAMP-dependent phosphorylation,

leads to the increase in cell radioresistance. The absence of one required unit, that of the receptor binding to the agonist, serves as an obstacle for reducing the cell radiosensitivity under the effect of this agonist. It is suggested that introduction of the agonist into the mixture of two types of cells:

1) possessing a receptor to it and capable of increasing the cAMP concentration in response to this agonist and
2) devoid of receptor

would lead to selective protection of cells of the first type. This suggestion was experimentally confirmed in this work for mammalian cells (cultured in vitro) possessing and devoid of β-adrenergic receptors.

Materials and methods. Cells. Chinese hamster fibroblasts B 11 dii FAF-28, clone 431, were obtained from the Institute of Developmental Biology, the USSR Academy of Sciences, and murine fibroblasts L, clone B-82, from the Institute of Molecular Biology, the USSR Academy of Sciences. The cells were grown as monolayers at 37 °C in a medium containing 45% Eagle's medium, 45% medium No. 199, 10% bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). When they reached the late log growth phase the cells were washed off with 0.02% EDTA solution and suspended in Hanks' solution.

Determination of cell radiosensitivity. The cell suspension (500 cells in 1 ml in case of separate irradiation or 400 cells of 431 clone plus 700 cells of clone B-82 in 1 ml in case of combined irradiation) were subjected to X-irradiation in glass vials in 0.5–6.0 Gy doses (0.5 and 1.0 Gy/min, 200 kV, 15 mA, filters: 0.5 mm Cu + 1.0 mm Al). Following the irradiation, we added a growth medium containing 30% bovine serum. Cell survival was determined by the colony-forming ability[17]. Macrocolonies were counted 10 days after irradiation. 431 and B-82 cells form quite different macrocolonies. Macrocolonies of B-82 cells are flat and of a regular round shape; the cells are triangular or sometimes rhomboid. Macrocolonies of 431 cells are convex, multilayered of irregular shape; the cells are elongated, spindle-shaped. Due to these differences between the cell colonies it is possible to determine the radiosensitivity of these cells after their combined irradiation and culturing. The radioprotective capacity of the agent under study was judged by the changes in D_0 dose of the