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Long-Term Effect of Irradiation: Function of Hypothalamic-Thyroid Axis and Correction of Revealed Disorders by Neurotropin.

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Recently, in studies of the effects of relatively low doses of radiation, an increasing role has been assigned to the reactions of the nervous system and its distinct parts [2,3,8,9].

The noticed increase of the morbidity rate after the Chernobyl accident among the residents of the "stringent" radioactive control area, appears at first glance to be independent of the ionizing radiation action. However, in fact, the rising incidence rate results to a large extent from chronic radioactive stress. Underpinning this stress are disorders in the neurotransmitter adaptation processes, leading to the development of the diencephalic syndrome and corresponding changes in the cardiovascular, digestive and immune systems of the organism.

From an analysis of our previous results it follows, that the main reason for diencephalic disorders is the discoordination of transmitter relationships resulting from the long-term effect of irradiation in a variety of brain structures, especially in the hypothalamus. The manifestation of these changes depends not only on the radiation dose but also on the direct and indirect effects of radioiodine on the endocrine functions as a whole, and the hypothalamic-thyroid axis, in particular.

It is known that the medial zone of the hypothalamus, via nervous and hormonal mechanisms, respectively can regulate the action of the neuro- and adenohypophysis. Thus, this zone acts as an intermediate link between the nervous and endocrine systems. In its turn, the interconnection of the neurotransmitter and neurohormonal components central regulation allows compensatory-adaptive reactions to be formed. Disturbances of this balance provide a basis for the development of long-term postirradiation diencephalic disorders.

Both, the thyroid and glucocorticoid hormones are involved in adaptation to a variety of extreme conditions. First of all, they can affect energy exchange by mitochondrial enzyme induction, stimulate protein synthesis, and activate the oxidative degradation of fats and carbohydrates.

The aim of the present study was to investigate the neurotransmitter relationships in the medial zone of the hypothalamus and their connection with thyroid functional activity long-term after external and combined irradiation, as well as the possibility of correcting revealed disorders with neurotropin.

MATERIAL AND METHODS

Mature male Wistar rats weighting 180-220 g were used for the experiments. Thyroid function was estimated by radioimmunoassay kits according to the following blood serum indexes: T_3, the level of thriiodothyronine (nmol/litre), T_4, the level of thyroxine (nmol/litre), TG, thyroglobulin (ng/ml). The insulin concentration in the blood serum (pmol/litre) was also measured. To estimate a variety of neurotransmitter systems functional state, the intensity of neuronal uptake of ^3H- or ^{14}C-labeled transmitters (5-hydroxytryptamine, dopamine, norepinephrine, GABA, glycine, the stable precursor of acetylcholine - choline) in the left half of the mediobasal hypothalamus was studied. In addition, the specific binding of ^3H-corticosterone was determined in the same brain structure. A weighted sample of hypothalamus (10 mg) was obtained from cold brain by a microtrocar. The methods of determining neuronal uptake of neurotransmitters has been were described at length [7]. Radio labels from Amersham were used in the study; the homogenate (0.1%) was obtained in a calcium-free preparation medium. After incubation the synaptosome fraction was filtered through Sinpore No. 2 (Czechoslovakia) and Vladipore (Vladimir, Russia) filters (1.5 m). In the study of the receptor binding of corticosterone, the 500-fold excess of unlabeled hormone was used [5]. For the removal of

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unbound hormone 1 ml of gel (ACRYLEX P-10, Hungary) was added to each sample. The radioactivity was measured with a Mark-3 scintillation counter (Tracor, USA). The data were expressed as dpm/mg of fresh tissue.

The study was carried out in the following series together with the respective controls: 1) 6 months after whole-body X-irradiation (0.5 Gy) with RUM II apparatus (mode of operation: without filter, voltage 164 KV, current intensity 10 mA, skin-focus distance 100 cm); 2) 6 months after single whole-body X-irradiation (0.5 Gy) combined with intraperitoneal injection of ^{131}I (6.5 μCi/kg); 3) 6 months after combined irradiation whole-body X-irradiation (0.5 Gy) and injection of ^{131}I in 6,5 μCi/kg dosage) and 3 months after thrice-repeated intramuscular injection of neurotropin in therapeutic doses. Neurotropin is a non protein extract from vaccinated rabbit skin, containing bioactive compounds that are formed as a result of immune reaction. For about 30 years this drug has been used in clinical treatment. Among its actions the normalization effect on the immune reactivity and antistressor changes in neuroendocrine regulation mechanisms are most pronounced. The effect of neurotropin can be revealed only on an altered background, while it has a slight influence on the normal status of the organism. Given the fact that this drug is able to affect hypothalamic activity and taking into account our previous result [4] concerning its normalization effect on the irradiation-altered glucocorticoid hormone reception in the central nervous system (CNS) structures, including the hypothalamus, an attempt has been made to modulate the changes arising from chronic irradiation stress by neurotropin, i.e., to apply this agent as a therapeutic drug.

RESULTS

Previously, when studying the effect of X-irradiation (0.5 Gy) [8], an early activation (after 2 hrs.) of both the inhibitory and excitative neurotransmitter processes was found in the hypothalamic zone, as a marker of high functional activity of this structure. In a long-term investigation (6 months) a discoordination of the neurotransmitter relationships within the CNS structures was observed [9]. At the same time the receptor binding of corticosterone and activity of glycine-, dopamine- and cholinergic processes were decreased to some extent. On the other hand, the neuronal uptake of 5-hydroxytryptamine and GABA was elevated (Fig.1). An increasing level of corticosterone reception 6 months after combined action of external and incorporated (injections of labeled iodine) irradiation reflects the decreasing concentration of glucocorticoids in the blood serum and high requirements of brain structures for these hormones. Against this background, a reduction of activity of both the inhibitory and excitative neuromediatory processes, i.e., the depression of hypothalamus functional activity, was found. The administration of neurotropin in therapeutic doses, 3 months after irradiation, revealed a tendency to normalization of corticosterone reception in the hypothalamus, accompanied by activation of the cholin- and dopaminergic processes, and reduction of the glycinergic processes.

Earlier, when similar experiments were carried out, in the majority of the CNS structures studied, we noticed a steady activation of the processes. These investigations were performed to elucidate the effects of different extreme factors, including irradiation, in relatively remote periods, when the elements of neurotransmitter and neurohormonal discoordination could be found. An enhanced secretion of acetylcholine during neurogenic or another chronic stress type causes CNS and visceral organ dysfunctions [1]. The

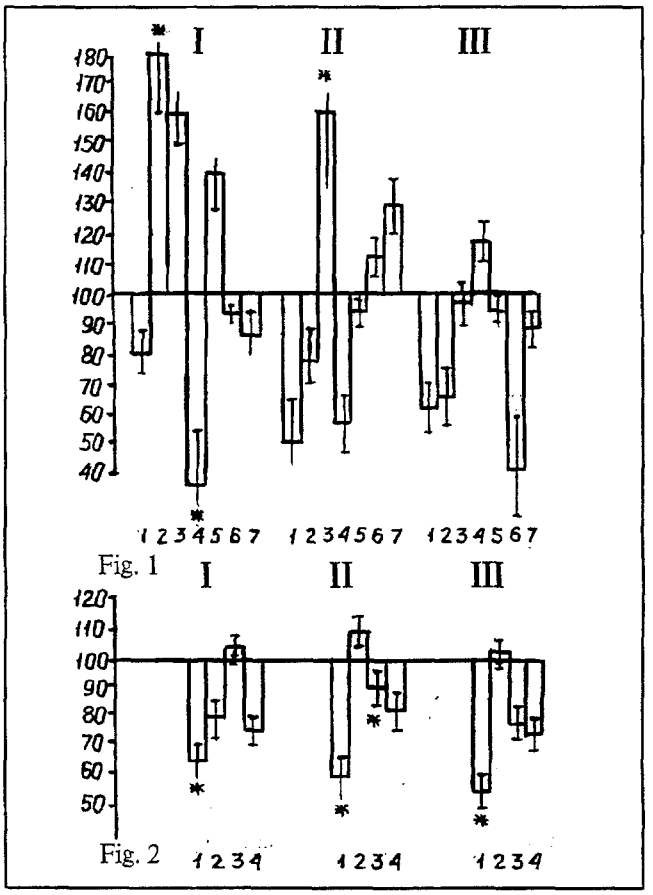

Fig. 1

Fig. 2

Fig.1 Rate of choline (CH), 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (D), GABA (G), and glycine (GL) neuronal uptake and corticosterone receptor binding (C) in rat hypothalamus (% of control). 1) 6 months after single whole-body X-irradiation (0.5 Gy); 2) 6 months after combined irradiation (whole-body X-irradiation (0.5 Gy) and injection of ^{131}I in 6.5 mCi/kg dosage; 3) 6 months after combined irradiation and 3 months after neurotropin injection. The data with $P<0.05$ are designated by an asterisk.

Fig.2 Triiodothyronine (T_3), thyroxine (T_4), thyroglobulin (TG), and insulin blood serum concentrations. Legend as in Fig.1.

intensity of neuronal reverse uptake of certain transmitter is believed to characterize the functional activity of the corresponding neuronal systems [10,11]. In agreement with this, our findings have shown that during chronic irradiation stress the inhibitory and excitative transmitter processes in the hypothalamic zone are depressed, but can be restored to some extent by neurotropin. The reduction of hypothalamic

functional activity affects the thyroid gland action as well. A noticeable decrease of the T_3 and T_4 blood serum concentration after external irradiation (Fig.2) and a significant lowering of the T_3 concentration after combined irradiation were observed. With the reduction of thyroid hormones level, the concentration of thyroglobulin was slightly but significantly decreased, indicating the disorders on the gland follicle level. Administration of neurotropin practically does not change these indexes. The ^{131}I action appears to cause pronounced alterations in the thyroid gland, lasting for 3 months. The prolonged hypofunction of the thyroid, connected with the direct action of ^{131}I, disorders on the follicle and hormonal secretion levels cannot be corrected by neurotropin through hypothalamus-mediated effects. Moreover, the pronounced drop of the insulin level, as well as the development of relative adrenal cortex insufficiency [6], support the proposals concerning disintegration of the central regulatory mechanisms. Thus, the results presented here indicate the development of stable and long-lasting disorders in the neurotransmitter and neurohumoral regulation processes when relatively low doses of external and, especially, combined irradiation were applied. These processes taking place in the CNS and, in particular, in the hypothalamus area provide the basis for formation of various autonomic disorders. In the case of additional ^{131}I administration the manifestation of the disorders is redoubled through direct and hypothalamus-mediated radioiodine action on the endocrine glands.

The normalization of the neurotransmitter adaptation processes, partially noted in the hypothalamus, is insufficient and, since the thyroid disturbances have already set in do not improve gland functional activity. In connection with the above, the correction of the revealed disorders must be carried out at earlier times after irradiation and supplemented with direct therapy of the affected organs and systems together with commonly accepted agents (adaptogens) used in the treatment of diseases characterized by a nonspecific component of pathogenesis.

LITERATURE CITED

1. N.I. Beller, B.K. Bolondinsdkii, I.I.Busygina, *et al.* Cholinergic mechanisms of visceral function regulation [in Russian] Leningrad (1986).
2. P.D.Gorizontov, Muscular activity and state of neuroendocrine regulation systems [in Russian] Moscow (1973).
3. Yu.G.Grigor'ev, The radiation safety of space flights [in Russian], Moscow (1975).
4. V.N.Gurin, M.Yu.Taits, T.V.Dudina, *et al. Byull.Eksp.Biol.*, **110**, No. 9. 261-263 (1990).
5. D.A.Zhukov, *Fiziol. Zh. SSSR*, **69**, No.11, 1463-1465 (1983).
6. M.Yu.Taits and T.V.Dudina, *Radiobiol.* **16**, No. 2, 301-303 (1976).
7. M.Yu.Taits, T.V.Dudina, T.S.Kandybo, *et al., Izvest. Akad. Nauk Beylorus. Sek. Biol. Nauk.* No. 5, 69-72, (1976).
8. M.Yu.Taits, T.V.Dudina, T.S.Kandybo, *et al. Radiobiol.* **28**, No. 5, 660-662.
9. M.Yu.Taits, T.V.Dudina, T.S.Kandybo, *et al. Ibid.* **30**, No. 2, 276-279 (1990).
10. L.L.Iversen, *Biochem. Pharmacol.* **23**, No. 14, 1927-1935 (1974).
11. B.Moisset, *Brain Res.*, **121**, 113-120 (1977).

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Myoclonic Hyperkinesia Induced in Rats by Repeated Injections of Picrotoxin Into The Neostriatum

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Using animal models, a number of symptoms occurring in neurological disorders of the human extrapyramidal system have been successfully reproduced by acting selectively upon neurotransmitter systems of the neostriatum. Most of the models are those of parkinsonism produced by causing malfunction of the

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