INCREASED BIOLOGICAL ACTIVITY OF EXTRACTS OF THE HYPOTHALAMUS IN THE EARLY STAGES AFTER EXPOSURE TO IONIZING RADIATION

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One of the manifestations of the change in the functional state of the hypothalamus after exposure to ionizing radiation is a disturbance of the neurosecretory function, demonstrable by histological and histochemical methods [1, 3, 5, 8-11, 13]. These investigations were carried out on different species of animals in different conditions of whole-body and local irradiation. However, during analysis of the data in the literature, two phases of the change in the neurosecretory function of the hypothalamic nuclei can be clearly distinguished: an initial phase (the first hours after exposure), characterized by the accumulation of neurosecretion in the cells of the supraoptic and paraventricular nuclei, and a subsequent phase (3rd-6th days) in which the content of neurosecretory granules is reduced. According to recent findings, the neurosecretory granules detectable histochemically are not identical with the neurohormones oxytocin and vasopressin, produced in the neurons of the nuclei of the anterior hypothalamus, but rather characterize the substance carrying the hormone [2]. In addition, there is a disagreement regarding the interpretation of the accumulation of neurosecretory material in the cells; whether this takes place in fact as a result of stimulation of function or of prevention of the entry of the hormone into the blood.

The object of the present investigation was to study the biological activity of extracts of the hypothalamus in the first phase after irradiation.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out to determine the biological activity of extracts of the hypothalamus obtained from animals 3 h after whole-body irradiation with γ-rays from Co^{60} on a type ÉGO-2 apparatus with a total dose of 400 R for rabbits and 600 R for rats. Meanwhile the biological activity of extracts of the hypothalamus of control animals was determined. The antidiuretic, vasopressor, and oxytocic activity of the hypothalamic extracts of the rabbit and rat were assessed. The experiments were carried out during the spring and summer.

To prepare the extracts the rabbits were killed by air embolism and the rats by decapitation; the brain was quickly extracted and the region of the diencephalon without the pituitary was removed. In the experiment on rats, the material thus obtained from 3 animals was cooled and ground in 5 ml of 10% trichloroacetic acid, left to stand in the cold for 30 min, and then centrifuged at 2500 rpm for 15 min at 0°. The supernatant was alkalified with 5 N K_2CO_3 solution to pH 7.2-7.4. The total volume of extract was 5.0-5.5 ml, and the weight of the sample of brain tissue was 400-600 mg.

The antidiuretic activity was determined from the change in the permeability of the bladder wall of a frog (Rana temporaria). Sawyer's method [12], in Yu. V. Natochin's modification [7], was used. Extracts obtained from rabbits of both sexes were investigated. After destruction of the spinal cord, the frog's bladder was filled with Ringer's solution diluted 1 : 10, through the cloaca, carefully separated, and placed in a vessel with constantly aerated Ringer's solution (20 ml) to which 1 ml of the test extract or 0.2 ml (1 unit) of a commercial preparation of pituitrin P was added. The quantity of water in milligrams passing through 1 cm^2 of bladder surface per minute (q) (the shape of the bladder was assumed to be spherical) or the change in weight of the bladder as a percentage of its initial value during the period of 60 min for which it stayed in the test solution was investigated.

The numerical results were subjected to statistical analysis by Student's method and are shown in Table 1.

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TABLE 1. Changes in Biological Activity of Extracts of the Hypothalamus 3 h After Irradiation

Experimental conditions	Activity									
	Antidiuretic			Vasopressor			Oxytocin			
	No. of expts.	q	%	No. of expts.	Amplitude (in mm)	%	No. of expts.	Amplitude (in mm)	Attainment of maximum (in min)
Control	13	0,697±0,075	13,6	38	19,7±2,3	22,5	15	16,4±1,2	11,8
Irradiation	17	1,039±0,079	21,6	47	28,7±2,4	32,3	15	26,7±2,9	14,6
Pituitrin (0.5 i.u.)	—	—	—	28	50,0±2,6	62,5	—	—	—
Pituitrin (1 i.u.)	17	—	20,8	—	—	—	12	17,8±2,5	12,0
Pituitrin (2 i.u.)	—	—	—	—	—	—	12	28,8±2,8	19,2
Ringer's solution	21	—	3,9	—	—	—	—	—	—
P (irradiational control)	—	<0,005	<0,005	—	<0,02	—	—	<0,01	—

It is clear from Table 1 that 3 h after irradiation an increase in the antidiuretic activity of the hypothalamic extracts was present: at this period it corresponded roughly to the activity of 1 i.u. (0.2 ml) of a standard preparation of pituitrin P.

The vasopressor activity was assessed from the reaction of the blood pressure recorded in the abdominal aorta of the rat under ether anesthesia. In these experiments the extract was obtained from rats (males and females) and was injected in a dose of 0.1-0.2 ml into one of the abdominal veins. The activity of each extract was tested in 2-4 rats. Control and experimental extracts were tested in the same rat, and the order of administration was alternated. In each experiment the pressor effect to intravenous injection of 0.1 ml (0.5 i.u.) of pituitrin P was also recorded.

Intravenous injection of the extract caused a transient fall of blood pressure in the rat, accompanied by slowing of the heart rate, after which the pressure was restored and a second (pressor) phase developed. Sometimes the pressure again fell slightly immediately after this phase. In some cases the initial fall of pressure, which evidently must be attributed to some toxic side-effects of the extracts, was very considerable. The pressor effect did not develop and the rat died with a progressive fall of pressure and cardiac arrest. In individual experiments an initial fall of pressure was also observed in response to injection of pituitrin P. The magnitude of the main pressor stage of the effect in mm Hg and of a percentage of the initial level of the blood pressure was determined. It is clear from Table 1 that the extract obtained from the irradiated animals possessed stronger vasopressor activity than the controls.

The oxytocic activity of the extract was determined from their effect on the spontaneous motor activity of the rat's uterus (weight of animals 130-200 g). In these experiments extracts obtained from rabbits were used. The motor activity of the isolated cornu of the rat's uterus in a constantly aerated Tyrode solution at 38° was used. The extract was added to the Tyrode solution in a volume of 1 ml. The effect was estimated from the amplitude of the reaction in millimeters and the time taken to reach the maximum of the reaction in minutes.

As Table 1 shows there was a clear increase in the oxytocic activity of the hypothalamic extracts from the irradiated animals. The activity of the control extracts corresponded to the activity of approximately 1 i.u. of a standard preparation of pituitrin P, and the activity of the extracts obtained from the irradiated animals corresponded to 2 i.u.

Hence, 3 h after irradiation, an increase was observed in all types of biological activity of the hypothalamic extracts, thus confirming and supplementing the histochemical data indicating stimulation of the neurosecretory function of the hypothalamic nuclei. As previously demonstrated histochemically [3], this reaction may be suppressed by the preliminary adminstration of chlorpromazine. Similar results were obtained when the antidiuretic activity of the hypothalamic extracts of rabbits receiving chlorpromazine intravenously in a dose of

TABLE 2. Effect of Chlorpromazine on Antidiuretic Activity of Extracts of Hypothalamus (in % of Change of Weight of Bladder in 60 min)

Experimental conditions	No. of rats	M ± m
Control	8	13.4±0.88
Irradiation	10	28.4±2.31
Chlorpromazine + irradiation	11	20.5±1.29
Chlorpromazine	2	16.0

5-10 mg/kg 15 min before irradiation was determined. The antidiuretic activity of the extract from the individual animals in these circumstances corresponded to the control value.

As Table 2 shows, injection of chlorpromazine before irradiation led to a statistically significant decrease in production of the antidiuretic factor by comparison with that found in the irradiated animals not receiving chlorpromazine.

The initial reaction described above is possibly nonspecific in character, and may be a manifestation of the stress effect of ionizing radiation. As a possible trigger mechanism for this reaction, the central effect of adrenalin may be suggested, for this substance is disseminated at these periods of radiation sickness [4, 6] to the adrenergic structures of the recticular formation of the brain stem.

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