CHANGES IN THE SECRETORY NEURONS OF THE HYPOTHALAMUS AND HYPOPHYSIS ASSOCIATED WITH SALT LOADING

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The role of neurons of the anterior hypothalamus in the regulation of water metabolism has been demonstrated experimentally. It was shown that dehydration of an organism causes a reduction in the neurosecretory material in all portions of the hypothalamo-hypophyseal tract [12, 14, 16]. In this case, hypertrophy of the neurons and their loss of the tigroid substance are regarded as signs of elevated function of the hypothalamus [8, 9, 11, 13, 15, 17]. Excess administration of water to the organism, on the other hand, leads to an accumulation of the neurosecretory material in the nuclei along the pathway of the axons from the hypothalamo-hypophyseal tract, as well as in the neurohypophysis [7, 10].

Apparently, the change in the neurosecretory activity of the hypothalamus is not the only result of a disturbance in the water metabolism, inasmuch as hypothalamic control extends to the function of many organs, in particular, the hypophysis. Therefore, shifts in the water balance, mediated across the hypothalamus, most probably influence the state of the endocrine glands. These considerations formed the basis for our investigation, the problem being to elucidate the relationship between the thyrotrophic function of the anterior lobe of the hypophysis and changes in the water regime caused by salt loading.

Acaudate amphibia served as the subjects in our investigations: we used 212 tadpoles of Rana esculenta and Pelobates fuscus, 16 yearling, and 20 adult Rana ridibunda, plus 30 white rats weighing 150—200 grams. In series of five-day periods, the amphibia were kept in distilled water, in dechlorinated tap water, and in isotonic (0.7%) and hypertonic (1%) NaCl solutions. The rats in the different series were given a 2.5% MaCl solution as their drinking water for periods of 5, 10, 15 and 20 days. The concentration of the solution was chosen on the basis of experimentation by other authors [12, 16]. Control rats received normal water. The drinking ration was the same for the experimental and control animals. The experiments on the rats were repeated twice. At the end of each experiment, the animals were sacrificed by etherizing. Fixation of the mid-brain, hypohysis, and thyroid gland (the latter only in rats) was carried out in Bouin's solution. The tissue was imbedded in paraffin, and 4-6 μ thick sections were stained: according to the method of Halmy-Dyban, with hematoxylin and phloxin according to the method of Gomori, with the Unn-Pappenheim reagent, with aldehyde-fuchsin, with PAS reagent, with azane according to Mallory, and with hematoxylin and eosin. At the same time that portions of material were fixed, tissues of the preoptic region, the hypophysis and the thyroid gland were tested on tadpoles of various species (648 specimens) in order to demonstrate the presence of biologically active substances in them.

EXPERIMENTAL RESULTS

Neurons of the preoptic neclei of amphibia, kept in normal, and especially in distilled, water, showed signs of obstructed neurosecretion. The cytoplasm of the neurons was densely filled with intensely staining secretory granules. A large number of granules and drops, sometimes very coarse, were located extracellularly, in the brain tissue (see figure 1). In the amphibia kept in isotonic, and especially in hypertonic, solutions, we observed a marked decrease in the number of granules in the neurons, related to an outflow of secretion from the cells and the brain tissue. The rare granules were distributed only at the periphery of the cytoplasm of certain cells. The majority of cells were devoid of neurosecretion (2). At the same time, we noted a pronounced reduction of neurosecretion material in the posterior lobe of the hypophysis in the amphibia that were kept in the hypertonic solution.

The results of testing the biological activity of tissues from preoptic regions on stage III tadpoles of different

species are presented in Table 1. For purposes of brevity, the numerical data in Table 1 represent mean data of the total effect achieved from testing the material taken from amphibia kept in distilled and normal water, compared to the data for the animals that were placed in the hypertonic solution.

TABLE 1. Data on the Metamorphogenic Activity of the Mid-Brain and Hypophysis of Amphibia After Salt Loading

Material studied	Tadpoles (recipients)	Medium	Length (in mm)			Weight (in mm)		
			Entire tadpole	Tail	Intestine	Entire tadpole	Tail	Extremity
Mid-brain of the adult frogs	Green toad	Water	24	13	60	250	33	16
		Sodium chloride solution	28	19	140	380	46	16
The same from yearling	Rana esculenta	Water	36	21	50	623	103	27
The same	Pelobates fuscus	Sodium chloride solution	38	26	146	664	122	21
The same								
Hypophysis of the tadpoles	Bombina	Water	67	44	141	3437	966	163
	The same	Sodium chloride solution	68	46	229	3626	973	117
		Water	27	16	41	320	46	17
		Sodium chloride solution	31	18	56	423	113	15
		Water	34	20	90	478	97	21
		Sodium chloride solution	34	18	27	375	64	21

Under the influence of the hypertonic solution, the concentration of material in the preoptic region of the amphibia capable of stimulating the metamorphosis decreased. A certain tendency toward reduced biological activity of the tissue substrate in the preoptic region was observed even in the amphibia that were placed in the normal water, as compared with those that were kept in distilled water. Testing the hypophyses of the tadpoles showed an elevation of their concentration of thyrotrophic substance under the influence of the hypertonic solution.

The changes in the neurons of the supraoptic and paraventricular nuclei of the rats were varied, depending on the length of exposure to the salt. The neurosecretion in the control was abundant both in the secretory neurons and in the hypothalamo-hypophyseal tract, in the form of diffuse grains, granules and drops. In the supraoptic nuclei, two types of neurons may be contrasted. The first are large cells, with a cytoplasm that is rich in diffusely disseminated, fine secretory granules, the larger portion of which is concentrated in the perinuclear region (3). The

TABLE 2. Mean Data (in micra) of the Diameter (d) of the Large Neurons Belonging to the First Type, and the Diameter (d) and Volume (V) of Their Nuclei, in the NSO, NPV, and β-Basophiles of the Hypophysis from Control and Experimental Rats

Duration of the experiment (in days)	NSO			NVP			β-basophiles		
	Neuron	Nucleus		Neuron	Nucleus		Cell	Nucleus	
	d	d	V	d	d	V	d	d	V
5	27.6	12.2	951	22.9	12.5	1.025	19.4	7.9	242
10	25.9	13.0	1.153	17.3	10.5	602	19.3	8.0	268
15	23.4	12.2	950	17.9	10.4	571	15.1	9.2	408
20	21.4	12.4	1.001	16.4	10.6	623	15.6	8.4	311
Control	17.1	10.6	623	15.7	9.7	460	14.9	7.6	230

second type is made up of elongated cells with smaller dimensions, with a compact cytoplasm that contains vividly staining granules (Table 2). The neurosecretion in these cells enters the axons, forming swellings that are filled with fine granules. Similar pictures of the manner in which the neurosecretion localizes in the nerve fibers were described earlier by A. L. Polenov [4, 5]. The neurons of the paraventricular nuclei are more uniform in shape, size, and granularity of the cytoplasm.

As can be seen from Table 2, under the salt loading conditions cells of the supraoptic nuclei underwent the greatest changes. After 5 days, the number of granules in the neurons of these nuclei decreased markedly. The cytoplasm was vacuolized, and decidedly swollen. The Gomori-positive substance was scant, and localized only about the periphery of the cells. In single neurons we observed diffusely disseminated, fine granules. The nuclei and nucleoli were enlarged. In areas of the supraoptic nuclei located nearer to the chiasma, there remained isolated stained, elongated cells, with homogeneous cytoplasm. The nuclei in these cells were small, deformed, and severely contoured. The cells were undergoing degenerative changes, preparatory to their conversion into secretory bodies. After ten days the secretory depletion of the cells in the supraoptic nuclei had progressed further, with considerable increase in vacuolization. The borders of the cells were poorly defined (4). Hypertrophy of the neurons, nuclei and nucleoli still remained. After 15 days of salt loading, the hypertrophy of the neurons was less apparent than in the previous intervals; the size of the nuclei and nucleoli decreased. In the neurons adjacent to the chiasma, secretory granules had begun to accumulate. The state of the neurons after the 20 day salt exposure was close to that of the controls, except that mild hypertrophy of the cytoplasm, nuclei and nucleoli still remained.

TABLE 3. Indices of Metamorphogenic Activity in the Mid-Brain, Hypophysis, and Thyroid Gland of the Rat

Duration of the experiment (in days)	Mid-brain	Hypophysis	Thyroid gland		
	resorption index		test data	diameter of the follicles	height of the epithelium
5	1.562	1.128	555	31.9	10.1
10	1.399	1.091	592	37.1	9.8
15	1.081	698	480	38.0	9.5
20	-	-	-	33.3	9.1
Control	1.220	868	521	26.1	11.7

The salt loading did not affect the paraventricular nuclei to the same degree as it did the supraoptics. The five day exposure caused a certain increase in the size of the neurons, nuclei and nucleoli, and a shift of the Gomori-positive substance to the periphery of the cytoplasm. After 10 days, the structure of the paraventricular nuclei was essentially normal.

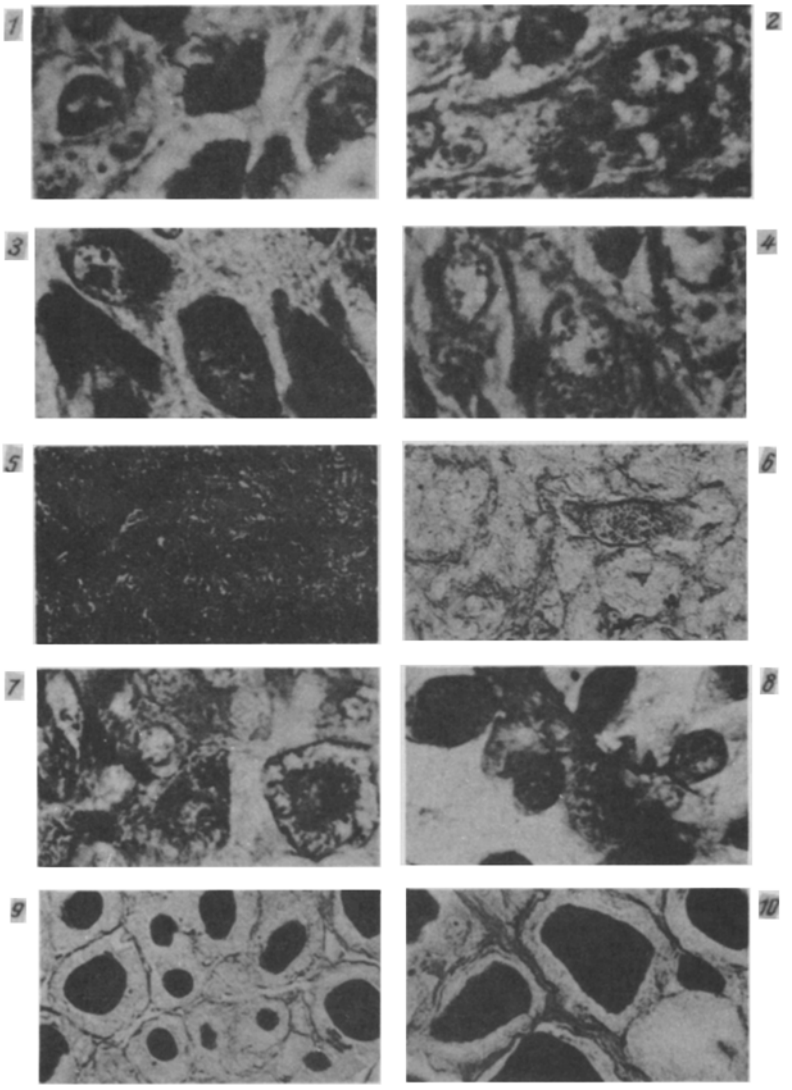
Under normal conditions, the posterior lobe of the rat hypophysis is so "clogged" with neurosecretion that it is impossible to differentiate individual cells (5). One finds numerous Herring bodies here, as well as coarse drops and granules of neurosecretion. Salt loading caused a loss of the neurosecretory material from the posterior lobe, with an increase in the dimensions of the organ and sings of marked hyperemia (6). After 15–20 days of the experiment, the hyperemia diminished, a small number of neurosecretory granules appeared, and single, small, stray cells were seen, with cytoplasm that took the aldehyde-fuchsin stain intensely.

In the control rats, the β-basophiles in the anterior lobe of the hypophysis localized mainly around the vessels in the central portion of the gland. Their cytoplasm was vacuolated, and there were rather few granules of aldehyde-fuchsin substance (7). The δ-basophiles were encountered in the peripheral portions of the gland, outside of the connection with the blood vessels.

Salt loading caused an increase in the number of basophile cells; these cells varied markedly in size, and contained densely granulated, or dark, homogeneous, cytoplasm. Following along the path of the capillaries, it was possible to trace all stages of specific granularity in the basophiles (8). Under the conditions of the experiment, no δ-basophiles were observed. This picture was preserved on the 5th and 10th days of the experiment. After 15 days, we noted a degranulation of the basophiles, and partial vacuolization of the peripheral portions of their cytoplasm, These phenomena were enhanced after 20 days, and hyperemia of the organ was observed.

The epithelium in the thyroid gland of the control was cuboidal in form: the colloid was homogeneous, and only in isolated follicles was it seen to be weakly vacuolated. In the experimental animals, the epithelium was depressed, and the amount of colloid was increased (see Table 2). In this case, the epithelial cells were depleted of polysaccharides, while the basal membranes and the interfollicular connective tissue yielded a more intense PAS reaction than the control (9, 12).

The results of testing tissue from the mid-brain, anterior lobe of the hypophysis and thyroid gland of control and experimental rats on the tadpoles are presented in Table 3. To simplify the table, we established the total index of mean resorption indices for all the tadpole organs in each series, as we did in one of our previous works [1].



Changes in the hypothalamic nuclei and the hypophysis of amphibia under conditions of salt loading. 1—neurons of the preoptic nucleus of a frog kept in distilled water; 2—the same neurons after 5 days of salt loading (aldehyde-fuchsin, counterstained with hematoxylin, magnified 1200x); 3—neurons of the supraoptic nucleus from a control rate; 4—the same after a 10 day salt loading (hematoxylin chromate and phloxin, according to Gomori, magified 1200x); 5—posterior lobe of the hypophysis from a control rat; 6—removal or the neurosecretion from the posterior lobe of the hypophysis following a 5 day salt loading (according to the method of Halmy-Dyban, magnified 280x); 7—β-basophiles in the anterior lobe of the hypophysis of a control rat; 8—accumulation of granules by the basophiles after 5 days of salt loading (according to the method of Halmy-Dyban, magnified 1200x); 9—thyroid gland of a control rat; 10—the same after a 20 day salt loading (PAS reaction, magnified 540x).

These data show that, under salt loading conditions, the metamorphogenic activity of the mid-brain tissue underwent marked weakening after 5 days; it then rose somewhat, increasing in strength up to the 15th day, which agrees with the histological picture of granule accumulation in the neurons of the supraoptic muclei.

The thyrotrophic activity of the anterior lobe of the hypophysis decreased in the first 10 days of the experiment, despite the fact that at that time the number of basophiles increased, and they became enriched with aldehyde-fuchsinophilic substance. By the 15th day, the thyrotrophic activity of the hypophyseal tissue increased markedly, although in this case, degranulation of the basophilic cells was noted. The thyroid glands from the animals that underwent prolonged salt exposure possessed the strongest effect on the metamorphosis of the tadpoles, which is in excellent agreement with the morphological signs of progressive hypofunction (see Table 2).

Thus, our experiments showed that a hypertonic solution leads to monotypic, morphological changes in the preoptic nuclei of the amphibian hypothalamus, and in the supraoptic and, partially, in the paraventricular nuclei of the rat hypothalamus. These changes are manifested by a reduction in neurosecretion, a hypertrophy of the neurons, an enlargement of the nuclei and nucleoli; they reflect a marked increase in utilization of the neurosecretion within the organism, resulting in decreased diuresis [2, 6]. Analogous changes were observed in the hypothalamo-hypophyseal system by I. A. Krasnovskaya and A. L. Polenov [3], using white mice in experiments involving injections of 5% NaCl solution.

The increase that we observed in the number of basophilic cells, and their hypertrophy, are in agreement with the data of other authors [8, 9]. However, biological testing of the tissue from the anterior lobe of the hypophysis showed that an increase in thyrotrophic activity, which would be expected with such a surplus of basophiles, is not observed. In turn, the results of microscopic study and biological testing showed that there was no stimulatory effect by the hypophysis on the thyroid gland. Apparently, this is explained by a disruption of the normal interrelationships between the hypophysis and the thyroid gland, since prior to our investigation it was shown that vasopressin, a component part of the neurosecretion, causes a suppression of thyroid gland function, acting on the anterior lobe of the hypophysis [6].

SUMMARY

A relationship was shown between the function of hypophysis and thyroid gland and the state of hypothalamic nuclei of amphibia and rats in conditions of salt loading. The number of neurosecretory granules dropped markedly in the neurones of the supraoptic nuclei; this drop was less significant in the paraventricular ones. Tests carried out on tadpoles point to the loss of biologically active substances by the diencephalon. The number of basophils increases in the anterior lobe, granulation of their cytoplasm becomes enhanced, but the thyroid gland function at that time does not undergo any stimulation. Prolonged constant salt action does not exclude the restoration of secretion in the neurones of hypothalamic nuclei and of the structure normalization of the anterior lobe of hypophysis.

LITERATURE CITED

1. A. A. Voitkevich, Izv. AN SSSR, Seriya biol. No. 1 (1946), p. 117.
2. A. A. Voitkevich, In the book: Contemporary Questions in Endocrinology [in Russian]. Moscow, (1960), p. 48.
3. I. A. Krasnovskaya and A. L. Polenov, Theses from the Reports of the 1st Conference of Morphologists-Endocrinologists [in Russian]. Moscow (1960), p. 27.
4. A. L. Polenov, Dokl. AN SSSR, Vol. 102 (1955), p. 365.
5. A. L. Polenov, Arkh. anat., gistol. i embriol., No. 2 (1957), p. 72.
6. Yu. B. Skebel'skaya, Probl. éndokrinol. i gormonoter., No. 6 (1956), p. 30.
7. B. Anderson and P. A. Jewell, Acta anat., Vol. 38, Nos. 1-2 (1958), p. 1.
8. D. Bachrach, K. Kovacs, A. Traub, et al., Kisérl. orvostud., v. 6 (1954), p. 209.
9. D. Bachrach and Z. Zellforsch., v. 46 (1957), p. 457.
10. R. J. Barrnett, Endocrinology, Vol. 55, (1954), p. 484.
11. D. Eichner and Z. Zellforsch, Bed. v. 40 (1954), p. 151.
12. A. Gastaldi, Arch. Sci. biol. (Bologna), Vol. 37 (1953), p. 380.
13. G. Gilbert, Anat. Rec. Vol. 132 (1958), p. 563.
14. W. Hild, Arch. Path. Anat., Bd. 319, S. 526 (1951).
15. R. C. Holland, B. A. Cross, and C. H. Sawyer, Am. J. Physiol., Vol. 196 (1959), p. 791.
16. F. Leveque, Anat. Rec., Vol. 117, (1953), p. 741.
17. S. Reichlin, Endocrinology, Vol. 60 (1957), p. 470.