CHANGES IN THE HYPOPHYSIS OF AMPHIBIAN LARVAE AFTER REMOVAL OF THE PRE-OPTIC NUCLEI

A. A. Voitkevich

Department of Histology and Embryology (Head, Corresponding Member AMN SSSR Professor A. A. Voitkevich) Voronezh' Medical Institute

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 10, pp. 93-95, October, 1961
Original article submitted January 16, 1961.

Unitl recently a variety of methods have been used to study the relationship between the neural and glandular portions of the hypophysis. They have included special staining methods for the hypothalamic nuclei, hypothalamic tract, and neurohypophysis [8, 15], transplantation of the anterior lobe of the hypophysis into a substrate either containing or not containing the neurosecretion [2, 14], the injection of extracts of various portions of the hypothalamic region [2, 13, 14], electrocoagulation of the paired neurosecretory nuclei [11, 12], and section of the supraoptico-hypophysial tract at a number of different levels [2, 9, 11]. From the sum total of all the experimental evidence obtained in this way it is clear that the gonadotrophic and adrenocorticotrophic functions of the anterior hypophysial lobe are influenced by the secretion of a substance formed in the hypothalamic nuclei. Evidence of a similar relationship for the thyrotropic is contradictory, and further studies in this direction are required.

Tadpoles are the most convenient object on which to test the thyrotropic properties of the hypophysis. In our laboratory, T. M. Ivanova [6, 7] showed that removal of the pre-optic region of the diencephalon from tadpoles arrests metamorphosis, or, if the operation is performed earlier, prevents it. The effect occurs when the thyroid and hypophysis are both intact.

In some further studies we have obtained similar results on tadpoles of other tailless amphibia which differ from the frog tadpole in many developmental features, and in their times and rate of metamorphosis [1, 4, 5]. Later we studied neurosecretion in the pre-optic nuclei in tadpoles of the green toad and in the spadefoot toad [3]. A further step was to study microscopical changes in the hypophysis, and alterations in its thyrotropic properties, both under normal conditions, and after elimination of the source of neurosecretion.

EXPERIMENTAL METHOD

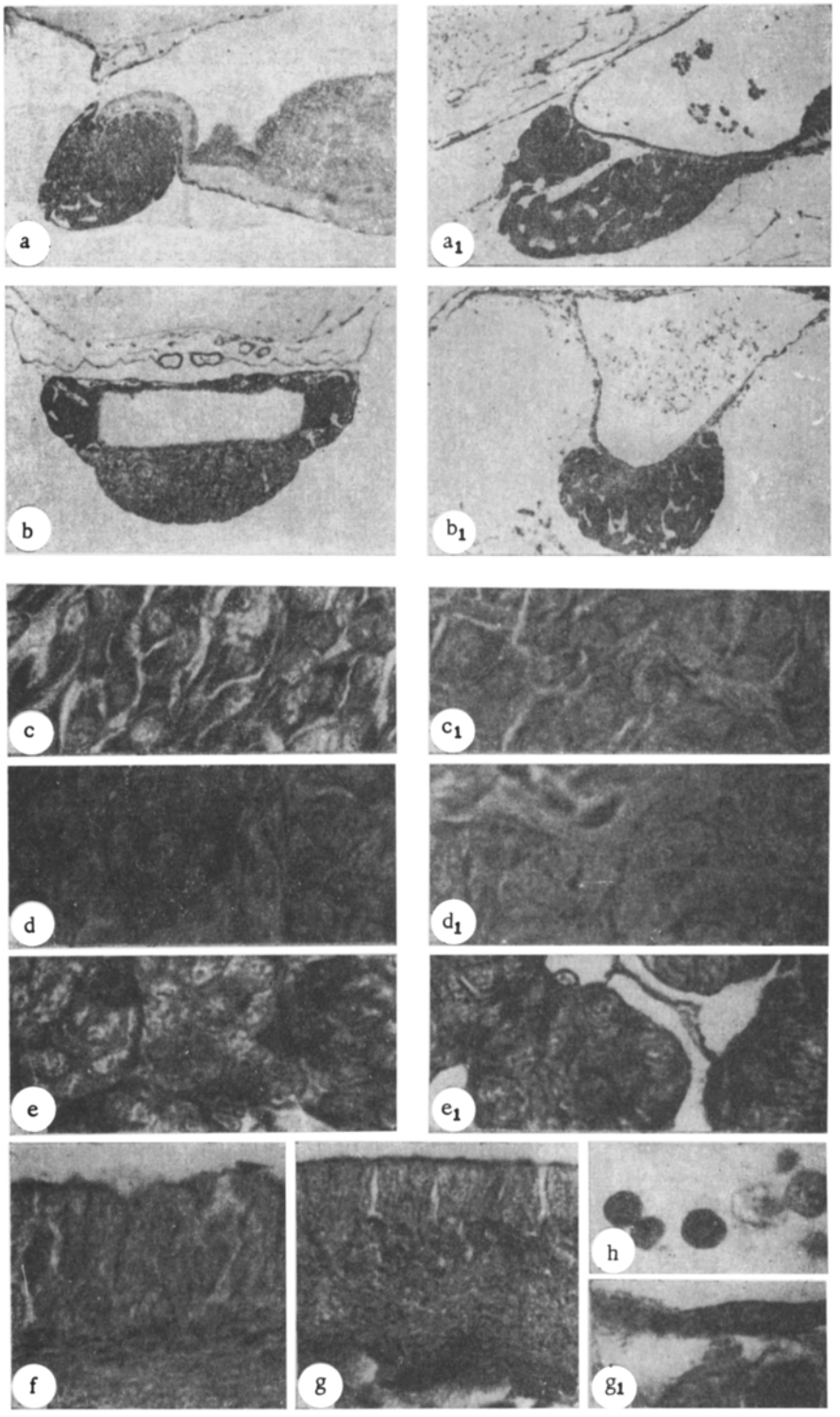
During the summer of 1960 we removed the hypothalamic region from tadpoles of the spadefoot toad, green frog, Bombina, and from the green toad. Altogether operations were made on more than 1,000 tadpoles of the four species, and most were made on those of the spadefoot toad and green frogs. The operative procedure and the conditions under which the experimental and control tadpoles were kept have been described previously [1, 6, 7].

Tadpoles were killed at various times after the operation, and the weight and size of many of the organs determined. At the same time, the head region was fixed in Bouin or Zenker. Saggital and frontal sections were cut and stained in Heidenhain's azan, in Gomori's chrome-alum with floxine, in Khélmi-Dyban's aldehyde-fuchsin, or in pironin and Schiff's reagent.

In several cases, a biological assay of the hypophyses was made for thyrotropic hormones. The telencephalon was removed from several tadpoles, and the hypophysis transplanted to take its place. We found that when a hypophysis was transplanted into a part of the brain adjacent to the source of neurosecretion, the thyrotropic effect was greater than when it was placed in the abdominal cavity. The number of spadefoot toad hypophyses implanted into a single tadpole varied from one to ten. Of course, all the tadpoles selected were of the same size, and at the stage immediately preceding metamorphosis. The hypophyses to be transplanted were taken from normal spadefoot toad tadpoles at different stages of development, either from previously operated animals or from control tadpoles of the same species at the same stage.

EXPERIMENTAL RESULTS

Differentiation of the secretory cells of the anterior lobe of the hypophysis, the basophil and oxyphil cells was clearly apparent at the onset of metamorphosis. The specific granules of the basophil cells stained well with aldehyde-fuchsin and with Schiff's reagent. In the tadpoles of all species, these cells were localized chiefly in the central cone-shaped lobe along the path of the capillaries which supply the anterior lobe with blood from the portal


a
a1
b
b1
c
c1
d
d1
e
e1
f
g
h
g1

vessels of the median eminence of the neurohypophysis. At this time, species differences in the amount of basophil cells corresponded accurately with the rate of subsequent metamorphosis: there were few of them in spadefoot toad tadpoles whose metamorphosis was slow, and more in the rapidly metamorphozing tadpoles of the green toad. During metamorphosis, the number of basophil cells showed no appreciable change, their cytoplasm lost its granules, and a large number of stained granules were present between the cells and in the cytoplasm of the capillary endothelium.

Removal of the source of hypothalamic secretion led to structural changes in all parts of the hypophysis, particularly the neural portion. These changes are well shown in a series of saggital and frontal sections taken 1, 3, 5, 10, 12, and 15 days after the operation. During the first few days after removing the pre-optic nuclei, in the operated region, and caudal to the third ventricle and infundibulum a very large number of wandering cells appeared and various methods of staining indicate large amounts of neuro secretion in their cytoplasm (see Figure h).

Reduction of the neurohypophysis begins from its caudal end which becomes much smaller and lighter and loses the previously-stored neurosecretion. Atrophic changes proceed centrally and extend to the median eminence (see Figure a, a_1). After 10, 12, and 15 days, the nerve fibers containing the neurosecretion have become completely reduced, and the cells of the ependyma of the neurohypophysis are very much flattened (see Figure f, g, g_1). The two independent blood supply systems which are normally well developed in the region of the posterior lobe and the median eminence are absent entirely from these parts, and have become displaced into the tissues adjacent to the adenohypophysis – the diencephalon and the anterior hypophysial lobe. This type of atrophic change in the neurohypophysis is particularly well shown by comparing the saggital and frontal sections (see Figure b, b_1).

Unlike the neurohypophysis, the adenohypophysis does not atrophy. On the contrary, both the pars intermedia and the anterior lobe hypertrophy (see Figure a_1, b_1), the effect being due to an edema of the tissue. In the adenohypophysis the capillaries are greatly dilated and the secretory cells are swollen and have lost their specific staining properties. Normally, before metamorphosis occurs the greatest accumulation of polysaccharides occurs in the pars intermedia. After operation, the cytoplasm of these cells takes up a lot of water, polysaccharides are lost, and most of the cells swell, losing their typical spindle or rhombic shape (see Figure c, c_1). Similar changes occur in the anterior lobe. Normally, the cytoplasm of the basophil cells, which usually occupy the central region of the anterior lobe and part of the region in contact with the neurohypophysis, stains strongly with Schiff's reagent. After the operation, there are fewer basophil cells, but they are greatly hypertrophied and contain small Schiff-positive granules in the peripheral cytoplasm, or outside the cells (see Figure d, d_1). When stained with Khêlmi-Dyban, it can be seen at a glance that there is not great difference in the cytological appearance of the anterior hypophysial lobe (see Figure e, e_1). We have previously pointed out that before metamorphosis the hypophysis of the tadpoles of the spadefoot toad contains few basophil cells, and at first sight it would appear therefore that the operation has not caused any essential changes. In fact, the condition of the basophil cells is altered. The cytoplasm of the remaining basophil cells stains intensely, but homogeneously; there are no granules either in the cytoplasm or in the intercellular spaces. There is little apparent change in the oxyphil cells, which usually occupy chiefly the zone bordered by the part of the anterior lobe developing in contact with the pars intermedia. After operation, the distribution of the oxyphil cells is somewhat disturbed, but they are present in the proximal portion of the anterior lobe.

The results of transplanting the hypophysis from spadefoot toad larvae of different stages into uniformly small tadpoles of Bombina and of the green frog showed in both cases: firstly, that before and during metamorphosis the hypophysis contains a thyrotropic rudiment, and secondly that its amount does not change appreciably during metamorphosis. There is a characteristic balance between the liberation of thyrotropic hormone into the body and the progressive differentiation of new basophil cells. At the end of metamorphosis, in the hypophysis of the young frogs there is an increase in the number of basophil cells, and on test they show an increased thyrotropic action. Facts of this kind, and in particular the increased thyrotropic activity of the newly metamorphozed frogs indicates the deposition of the thyrotropic hormone in the basophil cells.

It should be noted that Étkin and Lehrer [10] using an improved method, obtained results very similar to ours.

The results presented here can be interpreted as a demonstration of the direct dependence of thyrotropic function of the adenohypophysis on hypothalamic influences mediated through a neurohumoral factor in the neurohypophysis. Our results have been obtained while the infundibulum remained intact, as did also the main portions of the hypophysis, and the circulation of the two portal systems.

SUMMARY

A microscopical study was made of the changes occurring in the hypophysis at different times after removal of the anterior hypothalamus (including the pre-optic nuclei); numerous larvae of four anuran species were used.

Simultaneous assays of the pituitary glands for thyrotropic hormone were made. The removal of the source of neurosecretion caused a great reduction in the pars posterior and median eminence of the neurohypophysis, and a hypertrophy of the pars intermedia and pars anterior of the adenohypophysis. The basophil cells took up large amounts of water into the cytoplasm, their staining properties changed, and their polysaccharide content was reduced; these changes are attributed to the absence of the thyrotropic effect.

LITERATURE CITED

1. A. A. Voitkevich, Probl. Éndokrinol. i Gormonoter., No. 3 (1959) p. 26.
2. A. A. Voitkevich, Dokl. AN SSSR, 135, No. 1 (1960) p. 221.
3. A. A. Voitkevich, in the book: Modern Problems of Endocrinology [in Russian] No. 1 (Moscow, 1960) p. 48.
4. A. A. Voitkevich, Dokl. AN SSSR, 138, No. 1 (1961) p. 235.
5. A. A. Voitkevich, and A. V. Negovskaya, Dokl. AN SSSR, 90, No. 4 (1953), p. 689.
6. T. M. Ivanova, Shurn Obshchie Biol., 9, No. 3 (1948) p. 245.
7. T. M. Ivanova, Shurn. Obshchie Biol., No. 3 (1952) p. 182.
8. A. L. Polenov, Arkh. Anat., Gistol. i Émbriol., 35, No. 6 (1958) p. 114.
9. A. L. Polenov, Uchen. Zapiski Leningradsk. Med. In-ta, 3 (1959) p. 176.
10. W. Etkin, and R. Lehrer, Endocrinology, 67 (1960) p. 457.
11. G. W. Harris, Neural Control of the Pituitary Gland (London, 1955).
12. A. B. Rothballer, Excerpta Med., Sect. III, 11, No. 11 (1957) p. 3.
13. G. Sayers, E. Redgate, and P. C. Royce, Ann. Rev. Physiol., 20 (1958), p. 243.
14. E. and B. Sharrer, in the book: Recent Progress in Hormone Research, 10 (New York, 1954) p. 183.
