THE NATURE OF THE REGULATORY INFLUENCES EXERTED BY THE HYPOTHALAMUS ON THE ANTERIOR LOBE AND PARS INTERMEDIA OF THE HYPOPHYSIS

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In reports from our laboratory [3, 4, 9] we have described how removal of the preoptic neuclei of the hypothalamus from larvae of the anuria causes three kinds of disturbances: suppression of metamorphosis, hyperpigmentation of the skin, and a marked hydrophilia of the tissues. It is known that the hypothalamus exercises its regulatory influence on physiological processes not only by means of the nervous connection but also by a humoral neurosecretion carried by the blood. The three phenomena we have described are so intimately associated that the impression is obtained that the the connection is due to a single common factor. It is known that metamorphosis is controlled by the thyrotropic rudiment of the anterior hypohyseal lobe of the hypophysis; the melanophore response depends upon the pars intermedia; however, regulation of water metabolism is associated with the hormonal rudiments of the posterior lobe. It has been shown that removal of the preoptic nuclei brings about changes in the microstructure of all parts of the neuro- and adenohypophysis of tadpoles [3, 8].

The object of the present investigation has been to distinguish between the nervous and neurosecretory influences of the hypothalamus on the hypophysis, and hence on morphogenesis.

EXPERIMENTAL METHOD

The experiments were carried out on Rana esculenta larvae at various ages and stages of development. The preoptic nuclei Dtd [1, 2, 3, 5, 6, 9] were removed from 208 tadpoles at various stages, and from 98 young frogs (aged 1, 2, or 3 years), and from 48 adult frogs. In 64 tadpoles and 38 young frogs, a portion of the anterior hypothalamus containing neurosecretory nuclei was taken from other larvae or frogs and grafted into the extirpated region. For comparison, in 86 tadpoles and 18 frogs in their first year we removed only part of the hypothalamic region or else made a vertical incision by passing a sharp spatula between the preoptic recess and the chiasma. In this way we were able to destroy the nervous connections between the preoptic nuclei and the hypophysis, and yet enable the neurosecretion to reach the neurohypophysis. As a control we used 62 adult and 38 young frogs of different ages, all of them intact.

During our observations on the development and pigmentation we made records of the times and degrees of change in the pigmentation. In many cases we made total preparations of portions of the skin and back and of the web between the digits; from them we counted the number of melanophores, determined the number of granules in the different categories of pigment cells, whose diameter we measured. We also studied the pigment response of portions of skin from normal and from hypophysectomized tadpoles reared in an extract of hypothalamus, or of the pars intermedia of the hypophysis which was collected both from intact and from experimental frogs three and five days after the operation.

To prepare the extract, on every occasion we removed portions of the diencephalon, weighing 12-15 mg, from two adult frogs; these portions were triturated with 0.25 ml of physiological saline. In the same way we prepared extracts of tissue of the pars intermedia of the hypophyses. In other experiments we placed tadpoles which had developed excess pigmentation as a result of the operations in an extract made from 10 hypothalami mixed with 100 ml of physiological saline. In supplementary experiments we confirmed the influence of the grafts of the posterior hypophysis on the pigment response of the experimental tadpoles.

Length and Body Weight of Certain Organs of Larvae on Which Operations Have Been Performed at Various Stages of Development (mean values)

Stage of development	Operative interference	Length (in mm)		Weight (in mm)		
		whole larva	intestine	whole larva	tail	hind-limb
First	Control	59.0	121.6	2.124	548.0	31.0
	Removal of Dtd	66.8	344.8	3.788	698.5	10.7
	Incision of hypothalamus	63.1	235.0	3.377	582.8	14.8
Second	Control	52.0	134.6	1.366	502.0	28.0
	Removal of Dtd	66.1	240.6	2.683	538.3	20.0
	Incision of hypothalamus	60.5	160.0	2.270	493.3	25.6
Third	Control	54.8	112.2	1.560	304.0	42.0
	Removal of Dtd	64.2	231.7	3.074	442.8	28.5
	Incision of hypothalamus	60.0	143.5	1.605	305.8	35.7
Fourth	Control	46.4	78.2	1.324	284.0	63.0
	Removal of Dtd	51.4	127.2	1.660	302.0	52.5
	Incision of hypothalamus	48.2	97.0	1.444	260.0	62.4

EXPERIMENTAL RESULTS

Results illustrating the development of the tadpoles after the double experimental treatment are shown in the table.

The results of the experiments on tadpoles at stages before metamorphosis (stages I, II, and III), and during active metamorphosis (stage IV) were all of the same type, because removal of the anterior part of the diencephalon (Dtd) caused the arrest (at stages I and II) or suppression of development (at stages III and IV). An incision of the hypothalamus produces a certain inhibitory influence on tadpole development, but metamorphosis is by no means prevented. The difference between the control and the experimental series was particularly small in larvae at stages III and IV, as appeared from a comparison of indices such as the length of resorbed intestine and the weight of the growing hindlimbs. These results resemble those obtained by transplantation of diencephalic tissue from larvae at various stages into the operated tadpoles: the hypothalamic transplants from tadpoles at stages III-IV stimulated metamorphosis by far the most strongly.

Therefore extirpation of the preoptic nuclei is associated with suppression of metamorphosis and with an enhanced hydrophilia of the tissues of the tadpole, and with the formation of an increased amount of melanin. However, an incision into the hypothalamus leads only to an increased pigmentation. This response is shown very clearly in frogs of various ages. The color of the dorsum of the trunk becomes dark 20-30 min after removal of the diencephalon. In tadpoles this response developed rather more slowly, and the sharp contrast of color between the experimental and control groups was apparent 24 h after the operation. Apparently here the physiological condition of the melanophores in the skin of the larvae and frogs is related to the state of adaptation to the environment, and is of some significance. The natural color of the tadpoles living in water is quite dark, due to branching melanophores. On the other hand, in the green frogs under natural conditions the melanophores are constricted, and appear as dots or lumps; therefore the range of their response is greater.

The replacement homo- or heterografts of diencephalon to some extent stimulated metamorphosis, but exerted no influence on the melanophores of decerebrate animals. Their ineffectiveness was shown by transplantation of portions of the posterior hypophyseal lobe into the body cavity or into the space left by the extirpated diencephalon. In all these cases the animals remained dark even when placed in vessels where they were kept on a light background for up to 20-25 days.

These results enable us to distinguish to some extent between the different influences of the hypothalamic region. Although at the present time the humoral influence of the neurosecretion on metamorphosis (a morphogenetic process) is self-evident, nevertheless the nature of the regulation of the pigment reactions requires some clarification. Our results indicate that these responses are not related to neurosecretion. This conclusion was confirmed by the following experiments.

Immediately after extirpation of the preoptic nuclei from tadpoles at stage II the animals were placed in an extract of hypothalamic tissue of sexually mature frogs. The second group of operated tadpoles was kept in water. It was found that in the first group metamorphosis was prolonged to exceed somewhat the period taken by the second group, but there were no differences in skin color. We then carried out a number of experiments in vitro. Pieces of skin from normal and hypophysectomized tadpoles were placed in an extract of the hypothalamus of an adult frog. As a control we used pieces of skin placed in physiological saline. In neither case was there any change in the condition of the melanophores.

In further experiments pieces of skin from hypophysectomized tadpoles were placed in extracts of the pars intermedia of normal adult frogs, and of hyperpigmented frogs from which the diencephalon had been removed. Pieces of skin in the two extracts were kept in the dark, but no difference in the response of the cutaneous melanophores to the different extracts was found. However, pieces of skin in physiological saline remained unchanged. Therefore the onset of neurosecretion of the cells of the preoptic nucleus exerts no direct influence on amphibian pigment cells.

To sum up we may say that the stimulating influence of the hypophysis on metamorphosis mediated by the thyroid gland is maintained even when the nervous connections between the hypothalamus and the neurohypophysis have been severed. Destruction of the nervous connections causes an increased cutaneous pigmentation. Under the influence of transplanted hypothalamic tissue metamorphosis may be restored, but then hyperpigmentation is maintained. The increased deposition of melanin in the skin occurs also in cases when the hypothalamus is merely damaged, i.e., when the source of neurosecretion is preserved so that metamorphosis is not prevented.

The influence of the hypothalamus on the pars intermedia, as far as the secretion of intermedin is concerned, must therefore be exerted along nervous pathways, and is not directly related to a nervous secretion. The nervous influence of the hypothalamus is exerted through the pars intermedia itself, as is confirmed by the results of similar operations in which the hypophysis was removed at the same time. There was then a regular pallor of the integument, just as was found typically for the hypophysectomized tadpoles [7, 9].

It is known that the numerous fibers of the preopticohypophyseal tract terminate in two main regions of the neurohypophysis and that a few of them extend as far as the pars intermedia. It is in this respect that the pars intermedia differs from the anterior lobe. Dawson and Green [10,12] have described nerve fibers running between the cells of the pars intermedia in the part occupied by granules of neurosecretion. It is important to note that these fibers have no connection with blood vessels, in fact, there are no blood vessels in the pars intermedia of the amphibia [8].

Note that our experiments were carried out on tadpoles in which the thyreotropic function of the hypophysis was at its zenith. Such experiments were carried out later on three-year-old and adult frogs, whose hypophysis exerts a gonadotropic function. At the same time we experimented on frogs aged one or two years, i.e., in a period of development when thyreotropic function is largely suppressed but when gonadotropic function has not yet started. Under these conditions the stimulation of melanization by the pars intermedia may also be related to the adrenocorticotropic function of the anterior lobe, and deserves attention on account of recent findings concerning the interference between these hormonal origins [6].

The function of the pars intermedia in stimulating melanocytes after extirpation of the source of neurosecretion does not change appreciably, as was shown by experiments on the reaction of skin fragments. The action of the neurosecretion of the hypothalamus and of the posterior hypophyseal lobe is not equivalent to the hormonal influence of the pars intermedia.

The influence of the hypothalamus on the pars intermedia is therefore exerted through nervous pathways. Here our findings were similar to recent results by Etkin [11] who divided the hypophyseal stalk in amblystoma larvae. Whether the larvae metamorphosed or not they remained hyperpigmented for the whole of the experiment, which was continued for 40 days. Subsequent histological investigation showed a vascular connection between the hypothalamus and hypophysis of the metamorphosed larvae. However, despite the maintenance of a vascular connection the animals remained dark, thus providing a further proof of the division of the nervous connections.

Apparently the involvement of the hypothalamus in nervous regulation of the pigment reaction is exerted by suppression of secretion of intermedin by the pars intermedia of the hypophysis. When the inhibitory influence of the hypothalamic region was eliminated by destruction of the hypothalamo-hypophyseal nervous connections, more intermedin entered the bloodstream, leading to an expansion of the melanophores. At this stage the pigment reaction of the operated animals is hormonal in nature, because it develops gradually, reaching a maximum in both tadpoles and frogs after a relatively long time.

SUMMARY

The preoptical center of tadpoles and frogs was ablated, or nervous connections with the hypohysis were severed. Hyperpigmentation was not eliminated by homo- or heterografts of the hypothalamus, which stimulated metamorphosis. The melanophores of skin grafts of hypophysectomized amphibians were resistant to the hypothalamic neurosecretion, but sensitive to the extract of pars intermedia. Whereas the effect of the hypothalamus on the anterior lobe and on its thyreotropic function is humoral, the regulation of pigment reactions is effected via the nervous pathways, and is apparently produced by inhibition of the secretion of intermedin in the pars intermedia.

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