SOME CONDITIONS AFFECTING THE MORPHOLOGICAL DIFFERENTIATION OF THE PARS INTERMEDIA OF THE PITUITARY GLAND

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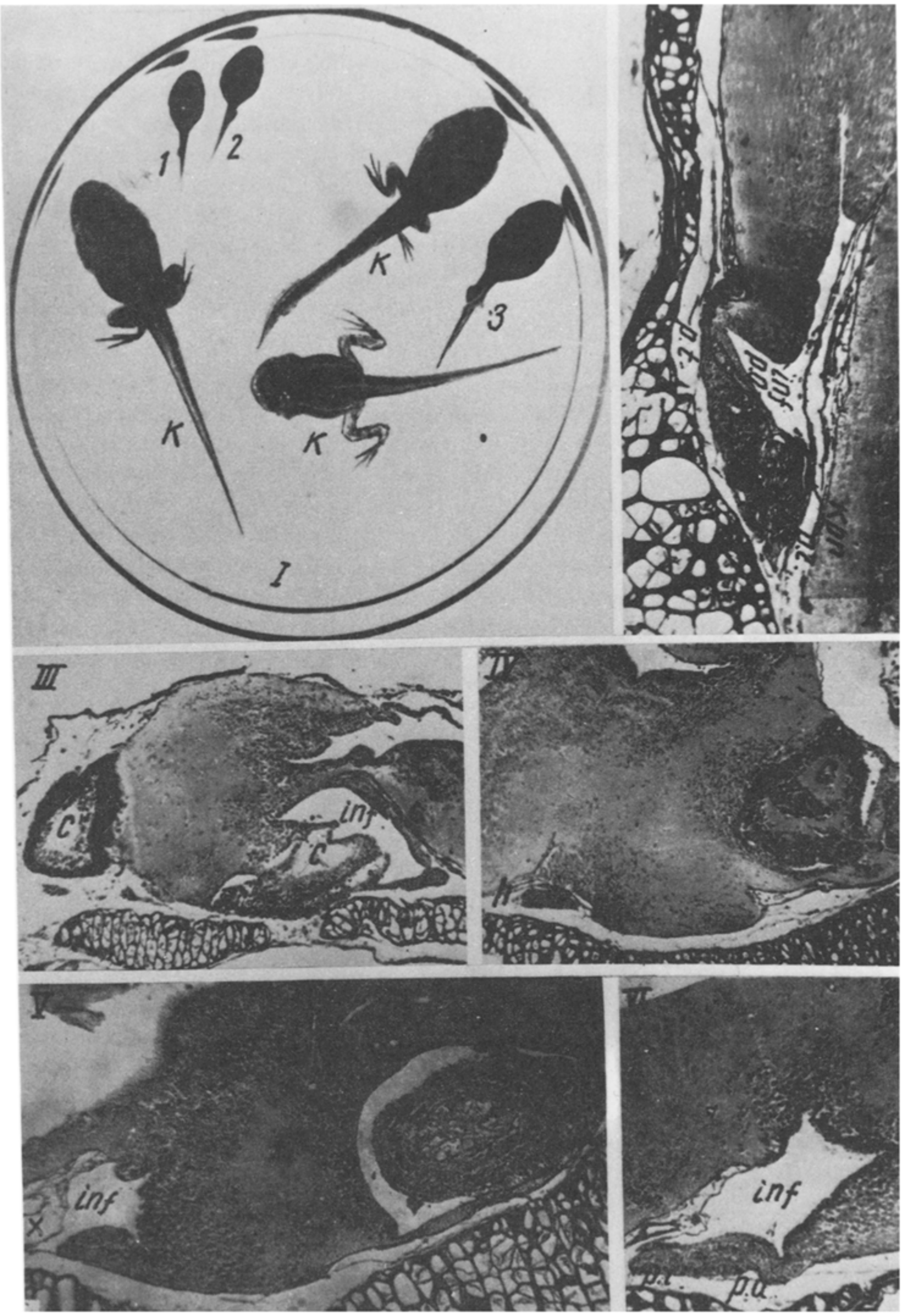
Interest in the neuroendocrine control of melanocyte-stimulating hormone (MSH) secretion has recently increased as a result of the discovery of the phenomenon of neurosecretion and of the biochemical nature of MSH and ACTH. It has been demonstrated that the hypothalmic regulation of the pars intermedia, which produces MSH, differs in its nature from the control of hormonal function in the pars anterior [12]. Extirpation of the preoptic nucleus in amphibia leads to a suppression of hormone formation in the pars anterior and marked activation of MSH in the pars intermedia of the pituitary [1, 2, 4, 10, 13]. Experimental influences which invoke changes in the activity of the neurosecretory cells are not only capable of affecting the function of the pars anterior but also of the cells of the pars intermedia [5]. The establishment of the various hormonal functions during the course of ontogeny is directly dependent on the sequence of differentiation adopted by the various zones of the adenohypophysis and this has recently been shown to depend on the level of neurosecretory activity [14]. We have demonstrated a similar relationship in preliminary experiments using small numbers of frog embryos which were subjected to removal of the basal portion of the pituitary rudiment and removal of the preoptic region of the midbrain [3].

This present article deals with research into certain factors affecting the morphological differentiation of the pars intermedia of the pituitary in two species of Anura—Pelobates fuscus and Rana ridibunda.

METHODS

Spawn from natural waters was cultured in large aquaria containing non-chlorinated water which was periodically renewed. The experimental and control tadpoles were given optimal rearing and feeding conditions. The method of operating on the embryo in the tail-bud stage has been described previously [3, 6, 12]. Single embryos of various groups were operated on, using the standard technique of removing one of the following rudiments: the adenohypophysis, the whole hypophysis, the 2 anterior cephalic vesicles (including the preoptic region). At the same time we carried out auto- and homo-transplantation of the excised adenohypophysial rudiment to the tail bud in one series of experiments, thus transferring the rudiment to a site some distance from the source of neurosection.

Larvae frequently died during the first few days after the operation. Periodic examination of the surviving larvae enabled us to follow the growth of both experimental and control tadpoles and the development of the latter did not appear to differ much from larvae living under natural conditions. We found that 345 experimental larvae (2 months after operation) survived until the end of metamorphosis; these were mainly Rana ridibunda larvae (323 individuals). The survival rate among different experimental groups varied between 14 and 18%. The success of the operation which we had carried out was evaluated from the results of histological examinations. Part of the head was removed from larvae which had been operated on, from intact (and unsuccessfully operated) control larvae, as were portions of the tail from transplant-recipient larvae,and all these tissues were fixed in Bouin's fluid. After fixation, serial paraffin wax sections (5-6 μ) were prepared and subsequently stained using Helm's method or Gomori's chrome alum hematoxylin technique.



I) 70 Day larvae of Rana ridibunda (k - control; 1, 2, 3, with adenohypophysis removed and transplanted in tail body; II, III, IV, V, VI) paramedial sagittal sections through brain and hypophysis of Rana ridibunda larvae (inf - infundibulum, h - hypophysis; pa - pars anterior; pi-pars intermedium; c) cyst; II) control; III, IV, V) cyst in neurosecretory preoptic region; III) second neurosecretory cyst in proximal region of hypophysis; IV) presence of neurosecretion in posterior lobe; V) complete hypophysectomy (X); VI) composition of the hypophysis after removal of preoptic region. Stained by Helm's method; III, IV magnified × 40; II, V and VI magnified × 60.

RESULTS

We observed a considerable lag in the growth of all those larvae which histological investigation had confirmed as having suffered complete removal of the parts selected for operation; this retardation of growth lasted 2-3 weeks. At the end of 2 months the most intense retardation was found in hypophysectomized tadpoles of green frogs (of which the mean weight was only one 20th that of the controls); all these tadpoles were of an albinotic silvery color. Larvae, which had undergone removal of the adenophysis rudiment, remained dwarfs but were somewhat larger than the hypophysectomized tadpoles. Somewhat larger still were the tadpoles which had been deprived of the preoptic region of the midbrain; in terms of weight they were twice as large as the hypophysectomized group and differed from these latter in being black in color. All the larvae with transplanted adenohypophyses were also black in color. No general, uniform, morphogenic reaction was observable among these latter as was found in larvae of the other experimental series.

All individuals with transplants were larger, but they varied very much in size and weight. They were considerably retarded in their general development and had not undergone differentiation of the posterior extremities even in those isolated cases where their weight was similar to that of the controls (cf. Fig. 1, 3). The melanocyte reaction was so strongly expressed among these individuals that merely touching the tadpole left a black spot on the finger of the research worker. This particular visual manifestation suggested that transplants secrete MSH in excess and that to a lesser extent they secrete somatotrophic hormone (STH), but that they are unable to secrete thyrotrophic hormone (TTH). We have shown, in earlier experiments involving the removal of the preoptic region in later tadpoles, that substitutive transplantation of the mid brain, in the first instance, leads to a stimulation of the thyrotrophic function of the hypophysis but has a less marked effect on the secretion of STH and does not change the hyperproduction of MSH [1].

Microscopical examination of the transplants has revealed the presence of a differentiated and somewhat hypertrophied pars intermedia and also of a pars anterior which is variously developed in different individuals; the pars anterior consists only of reserve cells and polytypic oxyphils. Our findings are similar to those obtained by other research workers with transplants of adenohypophysial rudiments [11, 14]. Such workers have, however, described cases of gigantism in certain hyperpigmented tadpoles [11]. Among 36 examples in which successful transplantations had been carried out, we were unable to observe a single case of excessive growth or of growth exceeding that of the controls. Experiments involving transplantation of adenohypophyses from tadpoles at various consecutive stages of development may shed some light on the reasons for the differences between our own findings and those of the above-mentioned authors [14]. These experiments have revealed that transplants taken from embryos in early and later stages of tail bud formation and inserted into the tail region of another tadpole did not differentiate to the point where they were able to ensure the secretion of STH. Transplants taken at later stages of development were, however, able to attain the condition in which increased STH secretion became possible even in the absence of direct contact with the brain.

Hyperpigmented dwarf tadpoles, subjected to removal of the preoptic region during embryonic life, possessed a characteristic "embryonic" type of hypophysis with a relatively large pars intermedia and an extremely undeveloped pars anterior (cf. figure II, VI). Such an "in line" position of the adenohypophysial lobe is reminiscent of the normal gland in cyclostomes, i.e., at an earlier phylogenetic stage of hypophysial development [12]. Further consideration of the phenomena described above caused us to turn our attention to larvae in which the operation for the removal of the preoptic region had proved unsuccessful but which had been accompanied by damage to the second cephalic vesicle. In certain individuals, spherical cysts with concentrically arranged nerve and neurogleal cells (cf. figure III, IV, V) were formed as a result of damage to the preoptic rudiment. A basal row of large, spherical, pyriform, or more rarely fusiform cells situated along the outer gleal membrane was found to be extremely rich in substances with an affinity for aldehyde fuchsin; these assumed the form of small particles and conglomerations of various sizes.

This type of anomolous structure, which we have occasionally observed in the past and described in previous publications [3], now assumes importance in that it is localized in a definite region associated with the formation or deposition of neurosecretory materials. That these spherical cysts are neurosecretory in nature is a supposition based on the results of their investigation in 13 larvae. In the brain of the larva depicted in the figure III, one cyst is situated in the preoptic region, the other at the base of the infundibulum. Both cysts are surrounded by a dense envelope, which has not, however, prevented the penetration of some neurosecretory material into the caudal portion of the neurohypophysis which occurs above the small compact pars intermedia. In the figure 5, there is depicted the situation in another dwarf larva with a single cyst in the preoptic region and a considerable accumulation of neurosecretory material in the posterior lobe of the hypophysis. In both cases the larvae were light-colored. Even more lightly-colored was the larva with a solid cyst in the preoptic region but without any hypophysis at all (cf. figure V); it was a dwarf. The larva, of which the brain is depicted in figure III and IV was also a dwarf.

Data of this kind serves as a basis for our conclusion that neurosecretory material is able to diffuse out of isolated cysts into the cerebral fluid and subsequently becomes partially concentrated in the caudal portion of the neurohypophysis. The possibility of neurosecretory material diffusing into the cerebral fluid as a result of damage to the hypothalamo-hypophysial tract has been suggested by other authors [9, 10]. Consequently, it is possible that the influence of the neurosecretory material is restricted to the pars intermedia of the hypophysis. In such cases the pars anterior of the hypophysis, being directly provided with neurosecretory material from another source (median prominence),proves to be poorly differentiated and unable to form adequate amounts of STH or to secrete any TTH at all.

The nature of the relationship between hypothalamic regulation and each of the components of the hormonal triad (MSH, STH, and TTH) is quite different. MSH remains under the control of the nervous system [7] facilitated by axons of the cells in the preoptic nucleus, so that the neurosecretory substance in this case assumes the role of mediator, and merely prolongs the inhibitory effect of the nervous impulses. The secretion of STH and TTH is controlled by a purely hormonal mechanism which is intensified with increasing vascularization, as this provides for the direct transfer of neurosecretory material to the pars anterior of the hypophysis. Contact induction from the side of the brain determines the initial differentiation of the pars intermedia; subsequent differentiation appears to coincide with a high gradient of function activity as judged by histochemical indices. Prevention of such contact in early embryogenesis [8] or the utilization of the adenohypophysial rudiment for transplantation at the same early stages of development [14] indicates the impossibility of autodifferentiation of the pars intermedia. The conditions for initial induction and subsequent functional control of this organ appear to be different and far from identical with those for other sections of the adenohypophysis.

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