THE EFFECT OF TEMPERATURE ON THE FUNCTION OF THE PREOPTIC NUCLEI IN THE AMPHIBIAN HYPOPHYSIS

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The great adaptive powers of cold-blooded animals and the conditions of variable environment temperature is brought about by some regulatory mechanisms, of which the principal one is governed by the hypothalamus. In our previous studies [4] we have shown that in amphibia an experimentally induced temperature change markedly affects the neurones of the preoptic region. Thus in spring frogs, the effect of high temperature is to cause an increased neurosecretion of a substance from cells of the preoptic nucleus in the hypothalamohypophyseal tract, but at low temperatures, the opposite picture is observed microscopically.

In a new set of experiments on 28 adult lake frogs and 44 green toads, and on 234 toad tadpoles we have studied functional changes in the preoptic nuclei under different temperature conditions, seven animals being kept in the cold at 9°, and 26 in warm surroundings at 27°. Sexually mature frogs, and tadpoles before the start of natural metamorphosis (end of June and first half of July) were collected from inland waters, and kept in groups in the laboratory at different temperatures for three days.

The shortness of the time for which the animals were exposed to the influence of these temperatures was the result of what we found in the preliminary experiments, which showed that a three day exposure caused definite changes in the microscopical structure of the hypothalamic region. A more prolonged sojourn at the high temperature had an adverse effect on the frogs, and in the tadpoles it stimulated metamorphosis.

The tissue of the preoptic region, and the anterior lobe of the hypophysis were tested on toad tadpoles. The tadpoles which were to serve as recipients were first carefully selected. Previously it had been shown that the influence of the active rudiment of the preoptic nucleus on tadpole development is by no means the same when it is transplanted into different regions.

When the thyroid glands were tested in a similar way, we transplanted thyroid tissue into the abdominal cavity [2], though for active hypothalamic rudiments transplantation into this region was less effective than was the brain. Uniformity of the test conditions was ensured by removing the natural preoptic region in the tadpole recipients, which arrested metamorphosis. We have previously described the method of partial decerebration [4]. Two kinds of operations were performed. In the first we removed the diencephalon and forebrain (Dtd) in 58 larvae, and in the second we removed only the forebrain (Dt) from 110 larvae. In the first group, metamorphosis was completely prevented; however in the second group, the operated animals did not show any essential differences from normal ones. In both groups direct contact between the graft and the brain existed, because all the transplantations were made into the cranial cavity. It is known that after such a partial decerebration there is a change in the reactivity of the tadpole tissue with respect to hormonal substances [4], but under the conditions of our experiments this circumstance was not relevant, because it was manifested to the same extent in all the series to be compared.

In experiments to test the larval material, the tadpole recipient received a complete preoptic region, or a complete anterior hypophyseal lobe from one individual. When corresponding material from adult specimens was tested both the preoptic region and the hypophyseal lobe were cut with a fine blade in the saggital and frontal planes so as to form four portions, and they were then immediately transplanted separately into four different tadpoles. For comparison, and to reveal the effect of stimulation, we used 26 intact specimens, and tadpoles which had been operated but which had not received grafts. All the experimental and control animals were killed six days after transplantation.

A longer period of test was not desirable, because the experiments were carried out under complete starvation. At the end of our observations, in all the tadpoles we measured the length and determined the weight of the body, tail, intestine, and hindlimbs. The mean values for these quantities for all groups of the main experiments are shown in Tables 1 and 2.

TABLE 1. Results of Testing the Hypothalamus and Hypophysis from Adult Lake Frogs on Tadpoles from which the Forebrain (Endbrain) and Diencephalon (Dtd) or Only the Forebrain (Dt) were Removed

Type of operation	Series	Length (in mm)			Weight (in mg)		
		Of body	Of tail	Of intestine	Of body	Of tail	Of limb
Removal of forebrain and diencephalon	Normal tadpoles	72	48	232	3641	1054	117
	Controls (without a graft)	76	51	316	4557	2282	134
	Transplantation of hypothalamus (cold)	64	43	244	3540	762	155
	Transplantation of hypothalamus (heat)	74	52	289	4602	1205	148
	Transplantation of hypophysis (cold)	71	48	206	3845	1020	136
	Transplantation of hypophysis (heat)	65	38	149	3060	688	158
Removal of forebrain (Dtd)	Control (without graft)	66	42	193	3479	908	112
	Transplantation of hypothalamus (cold)	61	40	140	3068	616	127
	Transplantation of hypothalamus (heat)	69	43	164	3817	857	116
	Transplantation of hypophysis (cold)	68	45	182	3745	1025	176
	Transplantation of hypophysis (heat)	60	38	127	3165	751	185

RESULTS

Before proceeding to analyse the results obtained we must first consider a fact we had established previously, that the substance of the preoptic nuclei are able to stimulate the development both of intact and of partially decerebrate tadpoles [3].

The rate of metamorphosis is usually judged in terms of such indices as change of tail length, the intestine, and body weight, as well as the increase in weight of the hindlimbs. Therefore the more advanced the stage of metamorphosis of a tadpole, the shorter was its tail and intestine, and the greater the weight of the hindlimbs.

TABLE 2. Results of Testing the Hypophyses and Hypothalamic Region from Tadpoles at Different Stages of Development, Previously Maintained at Different Temperatures

Organ transplanted	Series	Stage of development of tadpole	Length (in mm)			Weight (in mg)		
			Of body	Of tail	Of intestine	Of body	Of tail	Of limb
Hypothalamus	Normal		74	50	245	3720	1124	119
	Control		71	48	223	3516	365	115
	Cold	III	63	37	157	2982	814	129
	Heat	III	65	42	215	3408	912	110
	Heat	IV	75	54	251	3915	1168	102
Hypophysis	Cold	III	69	44	196	3307	962	134
	Heat	III	59	36	132	2735	610	198
	Heat	IV	64	40	165	2964	823	160

From the results of Table 1, from the different groups it could be seen that metamorphosis occurring after the removal of the diencephalon (Dtd – control) receives further activation from the transplanted frog diencephalon. The stimulating influence is specifically shown in the greater resorption of the intestine by the recipient tadpoles (tail length 316 mm in the controls – Dtd, against 244 and 289 mm in the tadpoles with transplantation of the hypothalamus), a greater reduction of body weight (4557 mg in the control animals and 3540 and 4602 mg under the influence of the transplant), by an increase in weight of the limbs (134 mg in the controls, and 155 and 148 mg in the experimental groups.

The influence was more significant in experiments with transplantation of the hypothalamus of frogs kept at low temperature. We interpret this result as indicating that in the cold the previously formed neurosecretion is deposited in the region of the preoptic nuclei, and its outflow into the neurohypophysis reduced. The reduction in the activity of the transplants from frogs kept in the warmth is to be attributed to the strong stimulation by the higher temperature of neurosectretory into the hypothalamohypophyseal tract. A different relationship of the indices of the biological activity was found by comparing the results of the tests of the anterior hypophyseal lobe. From the degree of resorption of the intestine and tail, and from the growth of the hindlimbs by the recipient tadpoles, there appeared to be less thyrotropic substance in the hypophyses of animals kept at low temperature. On this account, such hypophyses exerted less stimulating influence on metamorphosis than did those of animals kept at a high temperature.

Precisely similar results were obtained from corresponding tests on the tadpoles from which only the forebrain was removed, and which subsequently showed no marked suppression of metamorphosis (see lower half of Table 1 – Dt – control). It must also be noted that in our experiments, when an arbitrary amount of the graft was used, the hypophyseal tissue exerted a far greater stimulating effect on the development of the recipient than did fragments of the hypothalamic region. Naturally, such a comparison is extremely arbitrary, and there is no foundation for drawing any far-reaching conclusions on the degree of stimulation exerted by tissues differing in their origin and in their functional significance. Nevertheless, it is important to emphasize that together with the manifestly thyrotropic action of the hypophyseal transplants, a similar morphogenetic effect was observed under the influence of transplants of the preoptic nuclei. Considerable stimulation occurred when these transplants were made into the brain, and it took place both in animals in which development had ceased after partial decerebration, as well as in tadpoles capable of completing their metamorphosis. Tests of the region of preoptic nuclei and hypophyses from toads gave similar results.

Experiments to test the hypothalamic region and hypophyses of tadpoles gave results close to those which have already been described. When these experiments were conducted at high temperature, we had to place two groups of tadpoles at different stages of development together. One series was identical with the group which was kept at the low temperature (the so-called third stage). Tadpoles of the second series kept in the warmth were at an earlier (second) stage of development. Simultaneous separation of the two groups for the high-temperature conditions was made so that at the moment of testing the previously retarded tadpoles had advanced to a developmental stage identical with that at which the other group had been arrested by the low temperature [1]. Naturally, under the influence of heat the tadpoles at the later stage of development advanced developmentally even in a short time. Tests were carried out on one group of tadpoles from whom the forebrain (Dt) had previously been removed. Into each was transplanted the whole preoptic region, or the hypophysis of a donor. The results of the tests are shown in Table 2.

A comparison of the mean values of Table 2 shows that in tadpoles kept at the low temperature the hypothalamic region contains more active substance than it does in tadpoles kept in the warmth. Accordingly, larvae which received hypothalamic tissue from tadpoles kept at a low temperature proceeded considerably farther in their metamorphosis, attaining a tail length of 37 mm, intestinal length of 157 mm, and weight of hindlimbs 129 mg, than did those receiving a graft from larvae kept in the warmth; in the latter group the tail length was 42 mm, intestinal length 215 mm, and weight of the hindlimbs 110 mg. A reduction of the morphogenetic action of the hypothalamus was found in tadpoles of both groups kept at the high temperature, but there was no stimulating influence from the graft when transplantation was made from tadpoles at a late stage, although they had actually advanced further developmentally. The reverse relationship was found with tests of hypophyses for thyrotropic substance. The most active were the hypophyses of tadpoles which had been kept in the warmth, but had not started metamorphosis. In tadpoles which had begun to metamorphose there was some reduction in the amount of thyrotropic substance, which is apparently normal in activity metamorphosing larvae. When we carried out special tests of the hypophyses from intact tadpoles of different stages of development, we found a high thyrotropic activity before and at the onset of metamorphosis. Later the activity was reduced, and not until the tadpoles changed into the young frogs was there any increase in the content of thrytropic substance in the glandular tissue of the hypophysis.

Therefore the high temperature, by increasing the outflow of neurosecretion from the preoptic nuclei of the hypothalamus, favors the formation of the thyrotropic substance in the cells of the anterior lobe of the hypophysis, and during metamorphosis this substance is rapidly liberated, a process which in turn may be further accelerated by an increase of environmental temperature.

SUMMARY

The state of the hypothalamic preoptic nuclei was investigated in two species of anura and in their tadpoles varied according to the temperature conditions. Cold favors the accumulation of neurosecretion and increases the

capacity of the preoptic cells to stimulate the metamorphosis of intact and partly decerebrate tadpoles; heat accelerates the flow of hypothalamic secretion to the neurohyphysis and decreases the effect of the transplant under test. The thyrotropic substance of the hypophysis is affected by temperature in the opposite way, so that the amount is reduced by cold, and increased by heat.

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