EXPERIMENTAL BIOLOGY

THE ACTION OF CORTISONE ON THE OVARIES OF THE LOACH

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The functional connection between the adrenal cortex and the ovaries has been shown by several experimental investigations carried out on mammals [8, 15]. In the adrenal cortex of sexually mature females cyclic changes are observed which are synchronous with the changes taking place in the ovaries during the sexual cycle. After castration hypertrophy of the adrenal cortex takes place. The adrenal cortical hormones alter the sensitivity of the ovaries to the gonadotropic hormone [11]. Nevertheless the question of the presence of similar functional connections in members of other classes of vertebrates has hardly been investigated at all. Only in the viviparous cartilaginous fishes has an increase in the size of the interrenal gland been described during pregnancy [17].

The physiological mechanism of the transformation of female fishes into the spawning state has received little study. The majority of experimental research has been devoted to the study of only one of the links of this mechanism – the action of the gonadotropic hormones of the hypophysis on the ovaries [1]. It was found that the luteinizing hormone is the "spawning hormone" of the teleost fishes, which stimulates maturation of the oocytes and ovulation [7], i.e. the change from stage IV to stage V of maturity, according to the accepted terminology of ichthyologist [9]. The follicle-stimulating, lactogenic and adrenocorticotropic hormones themselves do not cause this reaction, but they increase the sensitivity of the ovaries to the luteinizing hormone [7]. It was also shown that certain steroid hormones (methyltestosterone, progesterone and desoxycorticosterone) may also cause maturation of oocytes and ovulation in female loach [6], but the mode of action of these hormones is not yet explained.

The hypophysis of the bony fishes contains biologically active substances whose action on mammals and birds is similar to that of the follicle-stimulating [4], luteinizing [4, 19], lactogenic [14], somatotropic [16], thyrotropic and adrenocorticotropic [18] hormones. After intramuscular injection of female loach with a suspension of these substances from the hypophysis of fish, maturation of oocytes and ovulation (at a temperature of 19-20°) usually begin on the average after 24 hours [3]. After injection of luteinizing hormone or chorionic gonadotropin, however, or of the blood or urine of pregnant women, this reaction appears much later, usually 39-48 hours after the injection. The earlier reaction of the female loach to the injection of the substances from the hypophysis of fish, compared with the reaction to the purified luteinizing hormone, may depend on the fact that the hypophysis stimulates not only the function of the ovaries but also the activity of the other endocrine glands, hormones from which act synergistically with the luteinizing hormone on the ovaries. The adrenocorticotropic hormone in particular, evidently stimulates the activity of the interrenal gland, which secretes some form of glycocorticoid into the blood stream. It was shown that this glycocorticoid is present in considerable quantity in the blood plasma of fishes during the spawning period [12].

It was therefore decided to study the action of cortisone on the ovaries of bony fishes and to investigate its action on the sensitivity of the ovaries to luteinizing hormone, since this would enable the part played by glycocorticoids in the transformation of female fishes into the spawning state to be established.

EXPERIMENTAL METHOD

Experiments were carried out on adult female loach (Misgurnus fossilis), caught in January 1958 in the neighborhood of the town of Stanislav. In winter the ovaries of the female loach are in stage IV of maturity, i.e. they contain large, immature oocytes, which are completing their period of growth and deposition of lutein. Their transformation to stage V of maturity, i.e. the appearance of mature oocytes and of ovulation is never accomplished under laboratory conditions spontaneously, but may easily be brought about by injection of material from the hypophysis of fish [3, 10] or mammals [5], and also by human tissue and tissue fluids containing chorionic gonadotropin [5]. From 40 to 48 hours after the intramuscular injection of a preparation of luteinizing hormone, or of a suspension or extract of tissues containing this hormone or chorionic gonadotropin, the female loach begins to lay mature spawn in the water. If the mature females are taken in the hand or gently massaged with the finger along the abdomen from head to tail, the spawn flows freely from the reproductive orifice of the fish. Without injection of gonadotropic hormones or, if the dose of hormone injected is below threshold, the ovaries remain in stage IV of maturity and no spawn emerges in response to abdominal massage.

Loach were injected with the following hormone preparations:

1) Cortisone acetate, manufactured by Biddle, Sawyer and Co. (London). 1 ml of an aqueous suspension of this preparation contained 25 mg of cortisone acetate. Before injection, the suspension was diluted with distilled water.

2) A purified preparation of luteinizing hormone from pigs' hypophyses, produced in the experimental manufacturing laboratory of the All-Union Institute of Experimental Endocrinology (1 unit of activity of the hormone was contained in 3 mg of the dry powder). The preparation was dissolved in distilled water immediately before injection.

Before the experiments the loaches were kept in a large tank with frequently changed tap water. During the experiments they were kept in glass tanks containing water but no sand or plants, at a temperature of 18-20°, and were given no food. The loaches were examined 24, 48 and 72 hours after the injection, and in cases when it was necessary to know exactly the time of onset of the reaction, they were examined every 1-2 hours for certain periods of time. If 72 hours after the injection no spawn had been expelled from the genital aperture, the fish was opened up so that the condition of its ovaries could be determined. In several experiments in which cortisone acetate was injected without other hormones, the fishes were opened up 48 hours after the injection.

Results of the Simultaneous Action of Cortisone and Luteinizing Hormone on Female Loach

Series of experiments	Dose of cortisone acetate, in mg	Dose of luteinizing hormone, in mg	Number of female loach	Number of females		
				laying mature ova	in which maturation of oocytes only took place	in which ovulation of immature follicles alone took place
1	1	–	5	0	5	0
2	1	1	5	5	0	0
3	1	0.5	5	5	0	0
4	1	0.2	5	2	2	0
5	0.5	–	5	0	3	0
6	0.5	0.5	5	3	2	0
7	0.5	0.2	5	0	5	0
8	–	1	6	0	0	1
9	–	0.5	5	0	0	0
10	–	0.2	6	0	0	1

EXPERIMENTAL RESULTS

After a single intramuscular injection of 1-5 mg of cortisone acetate into the female loaches, maturation of all or almost all the large oocytes in their ovaries took place. Thanks to the fusion of the droplets of lutein into a homogeneous, translucent mass, these oocytes themselves became translucent. No ovulation developed, however. After a few days the mature oocytes which remained inside their follicles showed degenerative changes and underwent resorption.

If these mature oocytes were artificially extracted and fertilized with sperm obtained by compressing the testes of the loach between two watch glasses, they developed normally. In one large female loach, opened up 48 hours after injection of 7 mg of cortisone acetate, several hundred mature ova were artificially extracted and fertilized. 77.9% of these ova developed normally, and from them larvae were hatched, after 3-4 days, which survived in the laboratory without food for about two weeks.

After injection of 0.5 mg of cortisone acetate, maturation of almost all the large oocytes took place in only a few female loach. In the remainder, the ovaries stayed in stage IV of maturity, although among the mass of immature oocytes inside the follicles, here and there translucent mature oocytes were encountered, singly or in groups.

In order to discover the result of the simultaneous action of cortisone and luteinizing hormone, female loaches weighing from 35 to 45 g were given injections of 0.5-1.0 mg of cortisone acetate into the muscles of the left side of the body, and 5-10 minutes later they were injected, in the muscles of the right side of the body, with various subthreshold doses of luteinizing hormone. Control females of the same weight were given injections of cortisone acetate alone or subthreshold doses of luteinizing hormone without cortisone. The results of these experiments are shown in the table.

As may be seen from the table, after the injection of subthreshold doses of luteinizing hormone soon after cortisone acetate, a valid transformation of the ovaries of the female loach from stage IV to stage V of maturity took place, leading to the excretion of mature spawn. The smaller the dose of cortisone acetate injected, the more luteinizing hormone was it necessary to give in order to obtain a valid reaction. In their action on the ovaries of the loach, cortisone and luteinizing hormone were synergists.

Furthermore, if the female loaches were injected with 1 mg of cortisone acetate and 1 mg of luteinizing hormone, they began to excrete mature ova only 22-28 hours after injection, i.e. at the same time as after injection of the hypophyseal substances of the loach, and 12-18 hours earlier than after injection of even large doses of purified luteinizing hormone without cortisone.

For an explanation of the mechanism of action of cortisone on the ovaries of the loach, we carried out experiments to study the action of this hormone on pieces of the ovaries in vitro.

B. N. Kazanskii [2] showed that if from female sturgeon or salmon, in the last 2-3 hours befores maturation, pieces of ovary are removed and removed in ovarian fluid, maturation of the oocytes and ovulation are effected in vitro. He postulated that ovulation and maturation of ova outside the body are possible from the time when morphologically visible changes have taken place in the relationships between the oocytes and the cells of the follicular epithelium.

We have repeatedly observed that if, a short time after injection of a suspension of hypophysis, luteinizing hormone, chorionic gonadotropin or the urine of pregnant women, the female loaches are opened up and pieces of their ovaries are placed in Ringer's or Holtfeter's solution [13], then normal maturation of the oocytes and ovulation take place in these pieces at approximately the same times as in the female body (sometimes even after existence outside the body for many hours). However, our numerous attempts to achieve maturation of oocytes and ovulation in pieces of ovary from the loach by the action on them of hypophyseal substances from the loach or of preparations of gonadotropic hormones, added to them in vitro, have never yielded positive results. Only when the suspension of hypophyseal substances of gonadotropic hormone was injected into the body of the fish, and the pieces of ovary were excised and placed in Ringer's solution not sooner than 3 hours after injection, did maturation of the oocytes take place in them; if they were excised after 12 hours or more, ovulation also took place. Evidently the gonadotropic hormone must act on the ovaries for a definite period of time within the body of the fish, and only at the conclusion of this time can the processes which it has stimulated be continued outside the body – in isolated pieces of ovary placed in physiological saline.

The results of the experiments with cortisone were interesting. Small pieces of the ovaries, excised from female loaches in stage IV of maturity, were placed in porcelain dishes with 20 ml of Ringer's solution. To this solution in the different dishes were added 1 mg of cortisone acetate, a suspension of the hypophyseal substance of one or two loaches or 1-3 mg of luteinizing hormone. The hypophyseal suspension and the luteinizing hormone caused no visible changes in the pieces of ovary. After the addition of cortisone acetate to the Ringer's solution, in every case after various times, from 26 to 32 hours, the greater part of the large oocytes had matured and become translucent. Hence cortisone acts directly on the ovaries and causes maturation of oocytes of the loach in vitro in the absence of any other hormone.

The addition to the Ringer's solution of a suspension of the substance of one or two hypophyses of the loaches at the same time as the cortisone acetate did not lead to the development of ovulation in the pieces of ovary in vitro. Maturation of the oocytes in these pieces took place at the same times as after the action of cortisone acetate alone. Addition of the hypophyseal suspension to the solution at various intervals of time after the cortisone acetate, whether before or after the onset of maturation of the oocytes in vitro, also did not cause ovulation.

The experimental findings suggest that some glycocorticoid, close in its properties to cortisone, takes part in the physiological mechanism of transformation of female bony fishes from stage IV to stage V of maturity. This hormone probably has an effect on the metabolism of the fish during its transformation to the spawning state, and at the same time acts directly on the ovaries as a synergist of the luteinizing hormone.

SUMMARY

Injection of cortisone to loaches (Misgurnus fossilis) caused maturation of oocytes without ovulation. The same could be observed in in vitro experiments. With the administration of cortisone and subliminal doses of purified luteinizing hormone the ovulation and oviposition of mature ova occurred 12 to 18 hours earlier than in the instances of large doses of luteinizing hormone without cortisone.

Evidently a certain glucocorticoid, similar to cortisone, takes part in the physiological mechanism causing the fish to change into conditions of spawning. It acts directly on the ovaries and is a synergyst of the luteinizing hormone.

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