Hormonal Effects on Egg Transportation in Rana pipiens

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=국문초록=

Rana pipiens에서 호르몬이 난자운반에 미치는 영향

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Rana pipiens의 난자운반은 섬모운동에 의하여 이루어지며 섬모운동은 배란시에 분비되는 난소호르몬에 의존하는 것으로 알려져 왔다. 저자는 이러한 난자운반이 순전히 난소호르몬에 의존하는가 난소호르몬 이외의 기타 호르몬이 관여하는 것인가 또는 난소호르몬 없이도 수행될 수 있는가를 알아보려고 본 실험을 시도하였다. 난관 적출된 개구리에 배란을 유도시켜 얻어진 복강난자를 난소적출을 실시한 암 Rana pipiens의 복강내에 이전시킨 대조군과 이전 직후 뇌하수체, 뇌하수체와 progesterone, progesterone, FSH, LH 또는 estrogen 등을 각각 주사시킨 각 호르몬 투여군의 6군으로 나누고 이식한 난자들에 대한 각 호르몬의 효과를 관찰하여 아래와 같은 결과를 얻었다.

1. 난소적출 Rana pipiens에 배란된 난자를 이식시키면 이식체내에서 난자운반이 진행되었다.

2. Progesterone, estrogen, FSH, LH 및 progesterone과 뇌하수체 호르몬 투여군의 난자운반은 대조군과 비슷한 현상을 보여 주었으므로 이들이 섬모운동에 직접 관여하는 것으로 보여지지 않았으나 뇌하수체 호르몬은 섬모운동과 난관투입에 유효한 영향을 미치었다.

3. 난자의 난관으로의 진입은 복강내에 난자수가 일정량(약 200)에 다달아야 이루어졌다.

4. 난소적출 개구리에서의 난자운반 진행상황으로 보아 난소가 섬모운동, 난자운반을 촉진하는 것으로 보이지 않았으며 섬모운동을 일으키는 요인은 복강내에 있는 난자 자체가 어떤 액성물질을 분비하는 것으로 고찰하였다.

I. INTRODUCTION

The transport of the ova from the ovary to the ostium tuba requires two hours at laboratory temperatures. The abundant supply of cilia of the female abdominal cavity serve to carry the eggs ovulated from any surface of the ovary anteriorly toward and into one or another of the ostia. These cilia are believed to be produced because of a possible response to an ovarian hormone and are, therefore, considered to be a secondary sex characteristic (Rugh, 1951).

The effects of steroids in the induction of ovulation and maturation was studied, and performed by injecting progesterone intraperitoneally into adult Rana pipiens females (Smith and Ecker, 1970). The results of these experiments were quite striking; ovulation was rarely obtained, but essentially all the unovulated oocytes, examined about two days after injection, were in the second meiotic metaphase and were activatable. Additional studies were performed by immersing ovarian fragments in various hormone preparations in vitro (Subtelny, et al., 1968). Under optimal concentrations of either

pituitary suspension or the mammalian steroids, progesterone and deoxycorticosterone acetate, essentially all the oocytes could be stimulated to mature to the second meiotic metaphase while still within their ovarian follicles. Maturation was demonstrated by the fact that these oocytes could be artificially activated, a response shown only by eggs which are physiologically mature (Subtelny and Bradt, 1961). It was observed that steroids were much more efficient inducers of oocyte maturation, while pituitary suspensions were better inducers of ovulation (Smith and Ecker, 1970).

It has been clearly demonstrated that maturation can be induced with some steroid hormones in vitro (Schuetz, 1967; Masui, Smith, et al., 1968). In these cases, the hormone works equally well with ovarian fragments or with cocytes dissected from their ovarian follicles prior to stimulation (Smith, 1968). In addition, unlike pituitary hormones, progesterone is effective after only a few minutes exposure.

However, in all of the foregoing studies emphasis was placed on the induction of ovulation itself and the stage of maturation of either the ovulated or the unovulated eggs with little or no attention being paid to the egg transportation. The purpose of this study was to determine whether this egg transportation response to only ovarian hormone or the other hormones beside of it, or was devoid of any ovarian hormone. The separate effect of the pituitary hormone and gonadotrophic hormones (or other factors) was also investigated on the ciliary current in Rana pipiens.

Ⅱ. MATERIALS AND METHODS

Mature female frogs, Rana pipiens, body length measured of at least seventy millimeters from snout to anus, were used for this experiment. For each of the six series of experiments, fourty-eight frogs were obtained from a commercial biological supply company.

The ovariectomy was performed to avoid any ovarian hormonal effects on the egg transportation, and the oviductectomy was done for collection of the body cavity eggs. The method which Rugh described in 1935 for obtaining body cavity eggs was followed fourty eight hours after the oviductectomized frogs' recovery. Ovulation was induced by injecting two fresh frogs' pituitary glands into the abdominal cavity and injecting 0.2 cc proluton (10 mg) into the dorsal lymphatic sac. Twenty-four hours after the injection for induced ovulation, the frogs were sacrificed. The abdomen was opened and if ovulation had occurred, the liberated eggs found in the coelomic cavity were collected by the wide-monthed eye dropper. About two hundred body cavity eggs were gently transferred through the incision of the ovariectomized frog via the eye dropper.

In each experimental series, the following hormones, four pituitary glands and 0.2 cc proluton (10 mg) together, 0.3 cc proluton (15 mg) alone, 0.2 cc FSH (1 unit), 0.2 cc LH (1 unit), and 0.3 cc estrogen (15 mg) were injected into the dorsal lymphatic sac for the desired effect of the egg transportation immediately after suturing the incision. The control was not treated with hormones after egg transplantation. Twenty-four hours after the injection of these hormones, the ovariectomized frogs' abdomen was carefully opened surgically and checked the eggs' position of the abdomen or of the oviduct for each hormone's effect on the egg transportation.

Ⅲ. RESULTS

The amount of jellyless eggs collected depended upon the animals' condition. A large number of liberated eggs (about one thousand eggs)

could be collected from a single ovulating female but many of them did not liberate sufficient eggs for the transplantation.

For ovariectomy, both sides of the abdomen were surgically opened to remove the ovaries at the firsttime. However, the second time both ovaries were removed through only one opening on one side of the abdomen. The results of this procedure were of benefit to this experiment; for example quicker operation, less bleeding, and fast recovery. The ovariectomized frogs were successfully operated and the incisions were perfectly healed after two weeks, but some of the animals became very weak. Many of them died within five to seven days after the operation.

Meanwhile, the egg transportation was demonstrated by opening the body cavity of ; 1) an actively ovulating frog, 2) a non-ovulating adult female, and 3) an adult male. 2 and 3 were removed from the refrigerator twenty four hours before the demonstration. A strip of the ventral abdominal wall was excised from each of the three frogs. Body cavity eggs were placed on each of the abdominal stripes. After five minutes, the eggs placed on the ovulating female's abdominal wall were carried along by the ciliary mevement in the original direction of the ostium, but there appeared to be no movement of the eggs on the others.

During the time of this study, October to January, four different amounts of the hormones were used in the oviductectomized frogs for inducing ovulation: 1) two pituitary glands and 0.1 cc proluton (5 mg), 2) two pituitary glands and 0.2 cc proluton (10 mg), 3) three pituitary glands and 0.1 cc proluton, and 4) three pituitary glands and 0.2 cc proluton. It was found that 1) and 4) did not sufficiently induce ovulation, and 2) and 3) were almost one hundred per cent effective in inducing ovulation. To conserve on the number of frogs sacrificed for hypophysectomies groups, 2) was injected to produce ovulation in this experiment.

About 50, 100, 150, and 200 body cavity eggs respectively were transplanted into the ovariectomized frogs in each experiment series. The injection of the hormones immediately followed completion of the egg transplantation.

Table 1. The effects of the hormones with different amounts of eggs in the body cavity on egg tansportation.

Amount eggs / Hormones	50	100	150	200
Pituitary glands(4)	Several eggs in the upper part of abdomen	Few eggs in ostium or upper part of abdomen	Upper part of abdomen	Lots of eggs in both side of oviduct
Pituitary glands(2) + Progesterone(0.2 cc)	Few eggs in upper part of abdomen	Eggs in upper part of abdomen	Eggs in upper part of abdomen	Several eggs in one side of oviduct
Progesterone(0.3 cc)	—	—	Few eggs in ostium	Few eggs in oviduct
F.S.H. (0.2 cc)	—	—	Eggs in upper part of abdomen	Eggs in oviduct
L.H. (0.2 cc)	—	—	Eggs in upper part of abdomen	Lots of eggs in oviduct
Estrogen (0.3 cc)	—	—	Eggs in upper part of abdomen	Several eggs in oviduct
Control	—	—	Eggs in upper part of abdomen	Several eggs in oviduct

They were checked for the effect of egg transportation after twenty four hours.

Table 1 shows the effects of the hormones with different amounts of eggs in the body cavity on the egg transportation. When fifty eggs were transplanted, the pituitary glands, and pituitary glands and proluton together produced the transportation of several eggs to the upper part of the abdomen or near the ostium. With 100 transplanted eggs, a few eggs were in the ostium with the injection of the pituitary glands. It was also shown that control produced same results as the other hormones when 150 and 200 eggs were transplanted. Pituitary glands with 200 eggs resulted in many eggs filling both sides of the oviduct. Twenty four hours following the egg transplantation, all of the animals were either dead or unconcious in all of the experiment series. It was found that the frogs died as a result of too many operations performed within a short period of time rather than the dosage of the hormones, because the control animals had the same effect. Also the parasitic infections had a detrimental effect on the health of the frogs.

More encouraging results were obtained from the frogs in which 150 eggs were transplanted. All hormones effected the transport of the eggs into the oviduct when 200 eggs or more were transplanted(Fig. 1). The pituitary glands especially, showed tremendous effects on the egg transportation with this amount of eggs.

Several eggs were usually in the upper third of the oviduct on the side the egg transplantation was done when 200 eggs were transplanted in control group(Fig. 2). Eggs filled both sides of the entire oviduct with the injection of four pituitary glands. To determine why eggs were only in the upper third of the oviduct with the other hormones, the frogs were sacrificed after

Fig. 1. A dissection of the ovariectomized frog injected with progesterone.

Fig. 2. A dissection of the control ovariectomized frog.

fourty-eight hours instead of twenty-four hours to reach the ovisac. It was found that the egg transportation in the upper third of the oviduct was caused by the frogs' weakness.

Their death was carefully investigated to determine whether too many operations or too many injections of urethane within a short period of time, the amount of the hormones, or parasitic infections resulted in the death of the animals. To solve the first question, the ovariectomy and the egg transplantation were simultaneously done and then the hormones were injected. They were unconciouse after twenty-four hours and showed the same results as before. The second question was easily solved when the controls were compared with the other frogs. It was found that the controls were in the same physical condition without any injection of hormones. Therefore, the reason of their death seems to be the parasitic infections.

Ⅳ. DISCUSSION

When ovulation occurs in the frog, eggs are released from the ovary into the body cavity of the adult female. The eggs are devoid of any jelly coats at this time but are enclosed in a thin, transparent, vitelline membrane. The body cavity of the female is almost entirely lined with cilia, each cilium having its effective beat or stroke in the general direction of one of the ostia. Rugh (1951) believed that these cilia are produced because of a possible response to an ovarian hormone and are secondary sex characteristic. On the contrary, it was found that the egg transportation occured in ovariectomized frogs of this study. It was suggested that the ovary plays no role in stimulating ciliary currents of egg transportation.

The abundant supply of cilia of the female abdominal cavity serve to carry the ovulated eggs from any surface of the ovary anteriorly toward and into one or another of the ostia. Egg transportation was well demonstrated by the placing of body cavity eggs on the ovulated abdominal wall and non-ovulated abdominal wall. The eggs were carried along in the original direction of the ostium by the ciliary movement only on the ovulated frogs' abdominal wall.

It is also of interest that controls showed a similar result on the egg transportation as the hormone injected animals. Thus, the gonadotrophic hormone which were used in this study do not seem to have any significant effects on the egg transportation. The pituitary gland hormones tremendously stimulate the ciliary movement and entrance of the eggs into the oviduct. It was determined that a certain amount of eggs secrete some materials or some chemicals themselves in the body cavity to induce the cililary currents without the presence of the ovary.

The results also showed that the eggs easily entered into the ostium because it was very elastic and was always opened. The pressure exerted by an accumulation of a certain number of eggs, at least two hundred, was necessary in the body cavity for entranceof eggs from the ostium to the oviduct.

One problem was encountered in that it was impossible to check for ovulation without killing the animals in the early experiment. But fortunately a suitable amount of pituitary glands and proluton was found for induction of ovulation, and the percentage of successful ovulation was very high, about ninety-five percent.

The amount of body cavity eggs liberated after ovulation was dependent on the animal's condition. Only about seventy liberated eggs were collected from many frogs because of weakness from parasitic infections and operations. But one out of four frogs could liberate about one thousand eggs. At least four oviductectomized frogs are recomm-

ended for induced ovulation in order to get sufficient liberated eggs for each experimental series.

The procedures for collecting body cavity eggs, preparation of hormones and pituitary glands, egg transplantation, and hormone injection, should be performed within a short time. The eye dropper was used for collecting body cavity eggs in the early experiment, but for faster collection of the eggs were washed out with spring water into a petridish after opening of the abdomen. There were about eighty to ninety eggs at the half line of the eye dropper for the transplantation. When the eggs were transferred, care must be taken to avoid the transference of too much spring water into the abdomen of the ovariectomized frog. It is recommended to perform the ovariectomy and egg transplantation simultaneously for this study. Results were uniform for all hormones and the control on examination.

Further study will be necessary to determine the chemicals or the materials which the eggs themselves secrete when they gather together in the body cavity.

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