An Effect of Luteinizing Hormone on the Fractional Perfusion of the Rat Ovary

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ABSTRACT. The ^{42}K method of Sapirstein has been modified to estimate the effect of gonadotropins on ovarian fractional blood flow. Microgram doses of luteinizing hormone (LH) cause a rapid increase in the proportion of the cardiac output delivered to the ovaries; the maximum increase obtainable, about 75%, is present about 20 min after the injection of the hormone. An effect similar to that of LH is produced by histamine, but not by FSH, prolactin, serotonin, epinephrine, or norepinephrine. The fractional perfusion of the mature rat ovary is greatest during the estrous phase of the estrous cycle. Since LH administration also enhances ovarian ^{42}K uptake in the mature rat, it is possible that one function of this hormone involves the cyclic regulation of ovarian blood flow. (*Endocrinology* **75:** 927, 1964)

WHEN immature rats are treated with microgram doses of a partially purified preparation of luteinizing hormone (LH), they develop ovarian hyperemia (1). This increase in the intravascular space of the ovary may be quantified by measuring the amount of ^{131}I-labeled albumin present in the ovary at equilibrium. It has been shown that the maximal effect obtainable, a doubling of the size of the vascular bed, is present about two hours after the administration of the gonadotropin.

There is no necessary relationship between the magnitude of the intravascular space of an organ and the proportion of the cardiac output which the organ receives. The fractional blood flow of an organ (the percentage of the cardiac output which perfuses it) is determined largely by the relative arteriolar resistances of the organ and the remainder of the peripheral vascular bed. The region of the vascular bed which enlarges in hyperemia ordinarily contributes little to vascular resistance. Hence, an increase in the ovarian ^{131}I-albumin space following LH administration could represent reactive hyperemia, a consequence of *decreased* ovarian blood flow, as well as unchanged or increased ovarian perfusion.

Techniques are now available for the estimation of organ blood flow in the intact, unanesthetized rat (2). Since changes in the proportion of the cardiac output which perfuses an organ may be of great importance in regulating how much of certain circulating substances becomes available to it (3), studies were performed on the effects of LH on ovarian fractional blood flow estimated by ^{42}K uptake. It has been found that small doses of partially purified LH produce a very rapid increase in this index of ovarian perfusion. This increase appears to be both organ- and hormone-specific, and can be demonstrated in both mature and immature animals; it may thus participate in the changes in ovarian blood flow which occur during the estrous cycle.

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Materials and Methods

Mature 160–180 g or immature 80 g Sprague-Dawley female rats were immobi-

lized in a restraining cage and given multiple intravenous injections with No. 25 needles into the tail veins. To measure the effect of LH on ovarian blood flow, partially purified ovine LH (NIH-LH-S6 or -S7) was dissolved fresh in a physiologic saline solution and injected rapidly in a total volume of 0.2 ml. At various intervals later (from 6 sec to 24 hr), animals were given about 1 μc of ^{42}KCl (obtained from the Isoserv Co., Cambridge, Mass.) in a total volume of 0.3 ml. The ^{42}KCl was dissolved in saline, in a final concentration of 4 mEq K/l. Rats were killed by neck fracture 15 sec after receiving the potassium. The aortic arch was then rapidly transected to stop the circulation, and the organs to be studied were dissected free of fat and connective tissue, weighed, and counted in a gamma scintillation counter. A solution containing 1% of the injected dose of ^{42}K was counted periodically along with the tissue samples, and tissue ^{42}K content was expressed as the percentage of the administered dose. In this manner, a correction was made for the rapid decay of the isotope.

Partially purified ovine follicle-stimulating hormone (NIH-FSH-S2) and prolactin (NIH-P-S4) were dissolved in saline and administered as described above for LH.[2]

Serotonin creatinine sulfate, histamine dihydrochloride, *l*-epinephrine bitartrate and *l*-norepinephrine bitartrate were dissolved in 0.001N HCl, and administered as described for LH. Control rats received the diluent.

To study the relation between ovarian ^{42}K uptake and the estrous cycle, rats received a single injection of ^{42}K at 9:00–10:00 AM, and were killed 15 sec later. A vaginal smear was then taken, stained and classified as described before (4), and the ovaries were removed for ^{42}K assay.

Results

Content of ^{42}K in the Rat Ovary at Various Times After Administration. The ability of ^{42}K to serve as a measure of organ blood flow arises from the fact that it is very rapidly cleared from the circulation. Subsequently it is retained by most organs, probably mixing with the intracellular potassium pool. Certain organs such as brain are unable to conserve the potassium delivered to them; their ^{42}K content falls rapidly during the first minute after injection as the isotope returns to the circulation. Other organs such as heart maintain a relatively constant ^{42}K level during the first minute after injection, indicating that their extraction coefficient for the recirculating isotope is the same as that of the body in general. Hence the ^{42}K method may be used to estimate the fractional perfusion of the rat heart, but not the brain (2).

To determine whether the ^{42}K method was applicable to ovary, groups of ten mature rats were killed at 5, 15 and 45 seconds after injection of the tracer substance. Ovarian ^{42}K content remained essentially unchanged during this period (Fig. 1).

Each pair of adult ovaries received about 0.075% of the cardiac output, or about 1.25%/g of tissue. Ovaries of immature animals showed behavior toward circulating ^{42}K similar to that of adult rats. Each pair of immature rat ovaries took up about 0.045% of the administered ^{42}K, or about 3%/g of tissue.

Effect of Ovine LH on the Fractional Perfusion of the Immature Rat Ovary. Immature rats were given varying doses of ovine LH, and ovarian fractional blood flow was estimated by measuring ^{42}K uptakes 20 minutes or two hours later. Twenty minutes after its administration, as little as 30 μg/kg (2.5 μg/rat) of the gonadotropin produced a 66% increase in ovarian ^{42}K uptake (Fig. 2). The administration of four times as much LH (120 μg/kg) produced an effect only slightly greater. Two hours after the administration of 120 μg/kg of LH, the net increment in ovarian fractional blood flow was slightly less than that observed after 20 minutes, but still significant.

[2] All the pituitary hormones used were a gift of the Endocrine Study Section, National Institutes of Health, Bethesda, Maryland.

Smaller doses of LH were ineffective at two hours; much larger doses (up to 5 mg/kg) produced blood flow changes that were only slightly greater.

To examine the possibility that the increased ^{42}K content found in ovaries of LH-treated rats resulted from a change in the relative efficiency of their mechanism for extracting and retaining the circulating isotope, ovarian ^{42}K was measured at various times after the administration of the tracer substance, as in Fig. 1. It was found that, following the administration of LH, the level of ^{42}K in the ovary still did not change appreciably in the first minute after its injection. This demonstrated that Sapirstein's criteria (2) for the applicability of the ^{42}K method were still satisfied.

Neither FSH nor prolactin produced consistent alterations in ovarian ^{42}K uptake, even in doses 20 to 40 times greater than the minimum effective dose of LH (Fig. 2).

To determine whether the effect of LH on ovarian blood flow was also manifest in other pituitary target organs, adrenal ^{42}K uptake was measured in groups of six control rats and in animals which had received 120 μg/kg of the gonadotropin two hours earlier. Untreated animals took up $0.147 \pm 0.012\%$ of the administered isotope per pair of glands; adrenals from rats pretreated with LH received essentially the same fraction of the cardiac output ($0.136 \pm 0.013\%$). Treatment with LH also did not influence cardiac ^{42}K uptake.

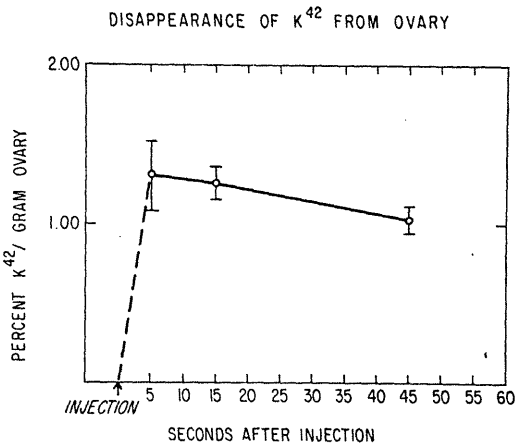

FIG. 1. Ovarian ^{42}K content during the first minute after injection. Mature rats were given 1 μc of ^{42}KCl by tail vein and were killed 5, 15 or 45 sec later. Ovaries were weighed and assayed for ^{42}K. There was no significant depletion of ovarian ^{42}K during the period of study. (Bars represent the standard errors of the means.)

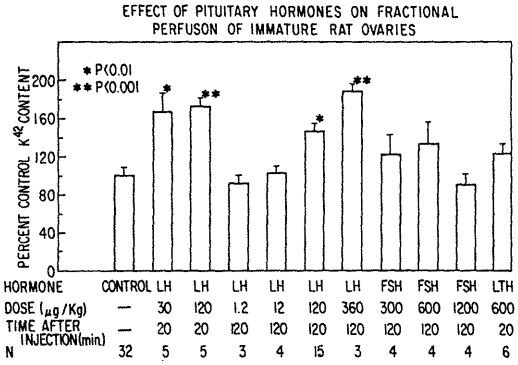

FIG. 2. Effect of varying doses of pituitary gonadotropins, given 20 or 120 min before assay, on ovarian ^{42}K uptake.

Time Course of Effect of LH on Ovarian ^{42}K. Groups of six immature rats were given 120 μg/kg (10 μg/rat) of LH, and ovarian blood flow was measured at various time intervals from six seconds to 24 hours later. LH produced a slight increase in ovarian ^{42}K uptake within six seconds of its administration (Fig. 3). This effect of LH became statistically significant after two minutes. It was maximal after 20 minutes (170% of control values), still present after six hours, but gone after 24 hours.

Relation Between Ovarian Blood Flow and the Vaginal Estrous Cycle. The relation between the state of vaginal estrus and ovarian blood flow was examined in 52

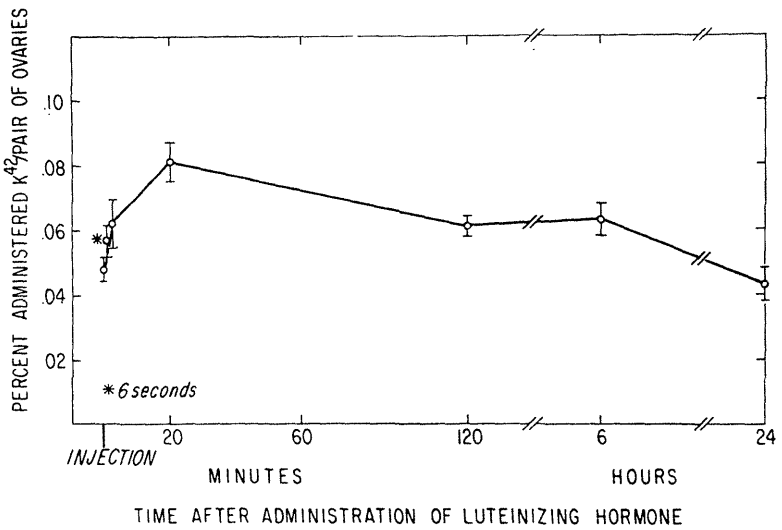

FIG. 3. Time course of the response of ovarian fractional blood flow to LH administration. Groups of 6 immature rats were given 120 μg/kg of LH, and ovarian blood flow was measured 6 sec to 24 hr later.

mature rats, taken at random from ten litters which had been maintained for ten days in controlled diurnal lighting (7:00 AM–7:00 PM). Ovarian ^{42}K content was significantly higher during the estrous phase of the cycle than during metestrus, diestrus, or proestrus, whether expressed per pair of organs or per unit weight of tissue (Table 1).

Effect of LH on the Fractional Perfusion of the Mature Rat Ovary. Vaginal smears were taken on a large number of mature rats, and two groups each consisting of eight animals in diestrus were selected. One group received 120 μg/kg (20 μg/rat) of LH 20 minutes before ovarian blood flow was estimated by ^{42}K uptake; the other group was pretreated with only the LH diluent. Ovaries of control rats received $0.068 \pm 0.003\%$ of the cardiac output, while those of rats pretreated with LH contained $0.102 \pm 0.007\%$ of the tracer substance. The dose of LH used thus caused an increment in the fractional blood flow to the adult ovary (+50%) of approximately the same magnitude as that produced in the immature animal.

Effect of Certain Biogenic Amines on Ovarian Fractional Blood Flow. Szego and Gitin have recently demonstrated that there is a significant decrease in the content of histamine in the rat ovary two hours after the animal is treated with LH (5). Experiments were therefore performed to determine whether the effects of LH on ovarian blood flow might be mediated by the release of histamine or of other physiologically active amines. Mature rats were given 30 μg/kg (5 μg/rat, calculated as free base) of histamine, serotonin, *l*-epinephrine, or *l*-norepinephrine, and ovarian blood flow was estimated 15 seconds later. Both epinephrine and norepinephrine markedly decreased ovarian fractional blood flow (Table 2). Serotonin was associated with a slight increase, which was not statistically significant. Histamine, however, rapidly increased ovarian ^{42}K uptake by a factor similar to that maximally obtainable with LH. In subsequent experiments it was shown that a significant increase in ovarian fractional blood flow could be produced following the administration of as little as 0.5 to 1.0 μg of histamine.

TABLE 1. Relation between ovarian fractional perfusion and the estrous cycle

Stage	No. of animals	Ovary weight (mg/pair)	% Cardiac output (per g)
Proestrus	6	55.8 ±4.6	0.89 ±0.068
Estrus	10	52.2 ±4.1	1.17 ±0.059*
Metestrus	9	56.0 ±4.1	0.92 ±0.071
Diestrus	27	46.2 ±2.7	0.96 ±0.050

Rats were given 1 μc of ^{42}KCl by tail vein and were killed 15 sec later. A vaginal smear was taken, and the ovaries were weighed and assayed. "% Cardiac output" is taken to be percentage of administered ^{42}K taken up per g of ovary. Data are given as mean ±SE.
* Differs from proestrus ($p < 0.01$), metestrus ($p < 0.05$) and diestrus ($p < 0.001$).

To rule out the possibility that histamine influenced ovarian fractional perfusion indirectly, by releasing pituitary LH, the effect of histamine on ovarian ^{42}K was studied in hypophysectomized rats (obtained from the Hormone Assay Laboratories, Chicago, Illinois). Mature, 160–180 g Sprague-Dawley female rats were hypophysectomized and maintained for five weeks; during this time the animals did not gain weight. Groups of five rats were then given 30 μg/kg of histamine, or only its diluent, and ovarian ^{42}K was measured as described above. It was found that the atrophic ovaries of control hypophysectomized rats received 1.27 ±0.14% of the cardiac output, per gram of tissue, while gonads of rats pretreated with histamine took up significantly more ^{42}K (2.06 ±0.29%). Thus, this effect of histamine on the rat ovary does not require an intact pituitary gland.

Discussion

The demonstration that the rat ovary behaves toward ^{42}K in the same way as does the body in general, for the first minute after its injection, provides a potentially useful tool for the study of the role of circulatory factors in ovarian physiology. LH has been found to increase the fraction of the cardiac output which is delivered to the ovary. This fraction may be of great importance in determining what proportion of at least two types of circulating substances is made available to the ovary: 1) substances with a very short circulatory half-life, and 2) substances whose clearance in a single circulation through the ovary is very great.

The amount of intravenously administered epinephrine which is taken up by the heart is approximately a linear function of the fraction of the circulating blood which perfuses this organ (3). This is probably a consequence of the rapidity with which epinephrine is cleared from the blood: it is available to the heart for only a few circulations. On the basis of the data reported here, it would be anticipated that LH administration would, by enhancing ovarian fractional perfusion, increase the percentage of a given dose of epinephrine which is taken up by ovary. Preliminary observations suggest that this is indeed the case. It is not un-

TABLE 2. Effect of biogenic amines on ovarian fractional blood flow

Amine	No. of animals	% Cardiac outut (per g)
Control	15	1.19 ±0.06
l-Epinephrine	10	0.60 ±0.08*
l-Norepinephrine	10	0.52 ±0.10*
Serotonin	8	1.45 ±0.25
Histamine	12	2.04 ±0.25*

Rats were given 5 μg of the amine 15 sec before ovarian blood flow measurements were made. "% Cardiac output" is taken to be percentage of administered ^{42}K taken up per g of ovary. Data are given as mean ±SE.
* Differs from control ($p < 0.001$).

likely that, under conditions of stimulation, the ovary might clear the blood traversing it of a major fraction of its content of particular substrates or amino acids. Under these conditions, the blood flow to the ovary would be an important determinant of how much of the substrate the ovary could possibly receive. Ovarian *blood flow* is equal to the product of ovarian "fractional blood flow," estimated by its ^{42}K uptake, and the cardiac output. Since LH has not been shown to influence the cardiac output, it seems likely that its administration also increases ovarian blood flow (in proportion to ^{42}K uptake). LH could thereby enhance the uptake by ovary of any substance which this organ efficiently clears from the circulation. The changes in ovarian ^{42}K uptake described here may represent one physiological mechanism of action of luteinizing hormone. The hypothesis that one general mechanism of hormone action involves expansion of the microcirculation of the target organ has been suggested by other authors (6).

The effect of LH on ovarian fractional blood *flow* is manifest within minutes of its administration, and reaches a peak about 20 minutes later; the maximal increment in ^{42}K uptake which LH can produce is about 75%. The effect of luteinizing hormone on ovarian blood *content* reaches a peak after two hours, when large doses of the hormone double the ^{131}I-albumin space in the ovaries (1). The studies described here indicate that this ovarian hyperemia is actually associated with increased ovarian perfusion. This is in contrast with the proestrous rat uterus, which, though it may appear hyperemic, receives a share of administered ^{42}K, or, presumably, of the cardiac output, which is no greater than that delivered to the uterus of the diestrous animal (7).

Changes in the fractional perfusion of an organ are the consequence of alterations in the relative resistances in that organ's vascular bed and in the remainder of the extrapulmonic circulation. Local vascular resistance may be changed by neural, humoral and local factors. It is thus possible that LH could increase ovarian blood flow by: 1) influencing the activity of vasoconstrictor or vasodilator nerves to the ovary; 2) exerting a direct hormonal effect on the ovarian vascular bed, or causing the release of a non-ovarian hormone which might have this effect; or 3) changing the ovarian milieu, such as by releasing bound vasoactive substances within the ovary or by causing the accumulation of vasoactive metabolites. The speed with which LH changes ovarian ^{42}K uptake, and the fact that this hormone has not been shown to regulate autonomic nervous tone or to release extraovarian hormones, suggests that it acts either by a direct effect, or by rapidly releasing a vasoactive substance ordinarily bound in ovary in an inactive form. Such a substance could be histamine: Szego and Gitin have shown that LH administration causes a depletion of ovarian histamine (5), and the data presented above indicate that this amine produces rapid changes in ovarian fractional perfusion similar to those which follow the administration of LH.

The anterior pituitary gland contains fairly large amounts of histamine (8). To rule out the possibility that free histamine of pituitary origin was present in the partially purified LH preparation used, and that this amine was responsible for the changes observed in ovarian blood flow, NIH-LH-S6 and NIH-LH-S7 preparations were assayed for histamine by a bio-assay procedure: An adult rat was anesthetized with urethane; a carotid artery was cannulated; and blood pressure was recorded continuously through a strain-gauge manometer system. Mul-

tiple injections of histamine or the LH preparations were administered by tail vein, and changes in blood pressure were noted. It was found that, whereas 300 nanograms of histamine produced a consistent fall in blood pressure in this preparation, as much as 1 mg of LH had no effect. This indicates that the smallest dose of LH which will enhance ovarian ^{42}K uptake (2.5 μg/rat) must contain less than 1 nanogram of histamine, or less than one five-hundredth the minimum effective dose of this amine.[3]

It is not possible at this time to choose between the hypotheses that LH exerts a direct effect on the vascular smooth muscle of the rat ovary, or that LH changes ovarian perfusion by the release of histamine bound in this organ. It would be of interest to determine whether LH still produced its ovarian circulatory effects in animals pretreated with antihistaminic or histamine-depleting drugs, or to chart the time course of the release of isotopically labeled ovarian histamine following the administration of LH. The possibility must also be considered that ovary normally extracts less than half the ^{42}K delivered to it, and that LH enhances ^{42}K uptake solely by virtue of its effect on this extraction process. This seems unlikely: ovary contains a large potassium "sink"; moreover, so dramatic a change in ^{42}K extraction would be expected to alter the slope of the ^{42}K time curve. However, the data now available do not allow this possibility to be discarded.

Since LH also enhances ovarian fractional perfusion in the mature rat, it is possible that this hormone participates in the changes in ovarian blood flow which are found during the estrous cycle. The level of LH in blood of the rat reaches a peak on the evening of proestrus, about 12 to 18 hours before changes in ovarian blood flow (in estrus) are manifest (9). It is possible that endogenously released LH, operating continuously over a period of hours, produces an ovarian vascular response with a different time course from that associated with the single injection of exogenous material used here.

[3] Dr. Clara M. Szego, of the University of California, Los Angeles, California, has assayed NIH-LH-S4 for histamine by the standardized *in vitro* guinea pig ileum method. As much as 20 μg of LH gave no response in a preparation which was clearly affected by 2 nanograms of histamine.

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References

1. Ellis, S., *In* Albert, A. (ed.), Human Pituitary Gonadotropins, Charles C Thomas, Springfield, Ill., 1961, p. 289.
2. Sapirstein, L. A., *Amer J Physiol* **194:** 161, 1958.
3. Wurtman, R. J., I. J. Kopin, and J. Axelrod, *Endocrinology* **74:** 63, 1963.
4. Wurtman, R. J., E. W. Chu, and J. Axelrod, *Nature (London)* **198:** 547, 1963.
5. Szego, C. M., and E. S. Gitin, *Nature (London)* **201:** 682, 1964.
6. Szego, C. M., and D. A. Lawson, *Endocrinology* **74:** 372, 1964.
7. Kopin, I. J., and R. J. Wurtman, *Nature (London)* **199:** 386, 1963.
8. Feldberg, W., *In* Ciba Foundation Symposium on Histamine, 1956, p. 6.
9. Ramirez, V. D., and S. M. McCann, *Endocrinology* **74:** 814, 1964.

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