Changes in Ovarian Venous Blood Flow Following Cannulation; Effects of Luteinizing Hormone (LH) and Antihistamine[1] (36042)

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Ovarian venous cannulation has been a frequently used technique to estimate the secretion rates of ovarian steroids (1–9). Estimates of flow rates as determined by the total collected volume have exhibited considerable variation (3–9 ml/hr), depending on the length of the bleeding time and the endocrine status of the animal. Continuous measurements of flow rates following cannulation are not available. Determination of intact flow rates by Wurtman (10) indicates that administration of luteinizing hormone (LH) results in ovarian hyperemia and that this is the result of release of a vasoactive substance by LH from the ovary rather than a direct effect of LH. Further evidence for such a vasoactive substance is provided by the studies of Szego and Gitin (11) and more recently by Lipner (12).

The present investigation was undertaken to determine, through continuous measurement, changes in ovarian blood flow following cannulation and to evaluate the effects of LH and antihistamine treatment on these changes.

Materials and Methods. Mature female rats (Holtzman Co., Madison, WI), 60–70 days of age, were kept in 14 hr photoperiods (5 a.m. to 7 p.m.) at 24 ± 0.5°. Feed (Wayne Lab. Blox, Allied Mills, Chicago, IL.) and water were administered *ad libitum*. Daily vaginal smears were taken to establish cyclicity. Only animals in diestrus were used throughout the experiment.

All animals were injected via tail vein with 0.8 ml of test solution 20 min prior to cannulation. Animals were grouped into four categories according to treatment as follows; (group 1) 0.9% saline solution only; (group 2) 50 μg/kg of NIH-LH-S15 in saline; (group 3) 50 μg/kg of NIH-LH-S15 and 4.0 mg/kg of promethazine hydrochloride (Phenergan, Wyeth Labs.); (group 4) 4.0 mg/kg of promethazine hydrochloride only.

At the time of cannulation, animals were anesthetized lightly with ether followed by 18–22 mg/kg of sodium pentobarbital (Abbot Labs.). Each animal also received 0.8 mg of heparin intravenously.

Laparotomy was performed, and the left utero-ovarian vein was isolated. A 23 gauge needle attached to PE-50 polyethylene tubing (0.584 mm i.d.) was inserted and tied securely with silk. The uterine branch was immediately ligated with silk placed in position prior to cannulation. The polyethylene end of the cannula was directed over a Narco Model 705-0014 drop flow counter which was attached to a Narco Model DMP-4A Physiograph. In order to determine if the effects of LH were specific with respect to the ovarian vein, in some animals the femoral vein was cannulated as described above. All cannulas were calibrated with blood immediately after use to determine the number of drops per milliliter.

Flow rates were calculated on the basis of 3 min intervals and reported at the middle of each 3 min interval as follows: 1.5, 4.5, 7.5, 10.5, 13.5, 16.5, and 19.5 min after the beginning of each bleeding. In the case of femoral veins, the bleeding period was extended to 30 min. Due to variations in bleeding rate between individual animals, flow rates were expressed as percentage of the initial (0–3 min) observation. All values at 1.5 min were recorded as 100%.

Results. 1. Flow rates from ovarian vein (Fig. 1). Flow rates from the ovarian vein of control animals (group 1) decreased significantly to 73% of initial values by 7.5 min and to 38% by 16.5 min. The values obtained at 19.5 min were not significantly lower than those observed at 16.5 min. Administration of LH (group 2) resulted in an

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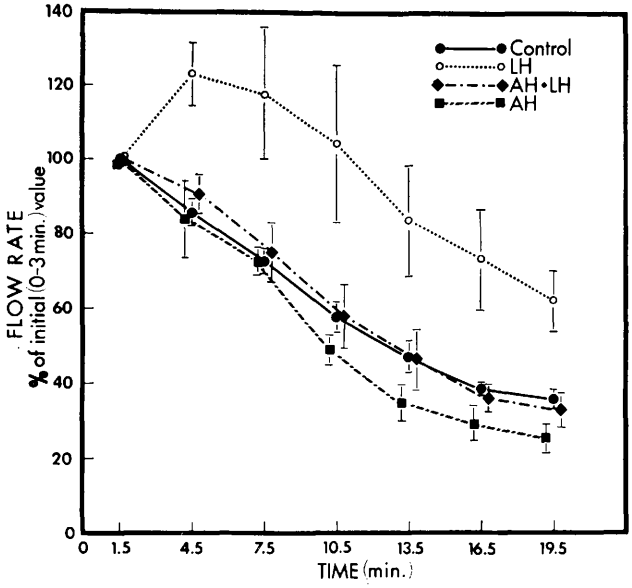

FIG. 1. Ovarian venous blood flow rates (mean and SE) in control, LH, LH-AH (promethazine hydrochloride), and AH treated rats. Flow rates are expressed as a percentage of the initial (0–3 min) reading. All values at 1.5 min were recorded as 100%.

increase in the flow rate of 123% of initial value by 4.5 min, and at 10.5 min, the flow rate was still over 100%. Lowest value measured was found at 19.5 min after the initiation of bleeding, but this was still 63% of the initial value. Analysis of variance and comparison by Student's *t* test indicated that LH produced significantly higher flow rates at each time interval.

Administration of antihistamine (AH) completely prevented the stimulatory effect of LH. No significant differences could be observed between the control (group 1) and the antihistamine–LH treated (group 3) rats. Flow rates were reduced to 36% by 16.5 min and did not change significantly thereafter. Final measured flow rate was 34% of the initial rate. Analysis of variance revealed a significant interaction between LH and AH. Administration of antihistamine alone (group 4) produced a small decrease in comparison to control (group 1) rates, but this decrease was not significant with the exception of the value obtained at 19.5 min after initiation of bleeding. Final flow rates were 26% of initial rates.

2. *Flow rates from femoral vein* (Fig. 2). Flow rates from the femoral vein of control animals decreased to 70% of initial value by 16.5 min and fluctuated between 70 and 85% until the end of the 30 min bleeding period. None of the fluctuations after 16.5 min were significant.

Administration of LH had no significant effect on the flow rates from the femoral vein at any time during the bleeding period. Flow rates at 16.5 min averaged 69.5% and did not diverge significantly thereafter until the end of the 30 min collection period. Lowest value (59%) was recorded at 28.5 min.

Discussion. Results of the present experiments indicate that blood flow from the ovarian vein decreases rapidly following cannulation and hemorrhage from the vein. In control animals, flow rates dropped to 50% of initial values by 13 min following initiation of bleeding. This explains the variation in reported flow rates (1–9) when calculated from the total collected volume and the elapsed collection time. Reported collection periods have varied from 20 to 90 min. The reduced flow rate is apparently due to reduced circulating blood volume as a result of hemorrhage, rather than a local change induced by the presence of the cannula, since replacement of the lost fluid volume restores flow rates to almost 100% of initial values [(13); Piacsek and Huth, unpublished data].

The present results also show that LH can

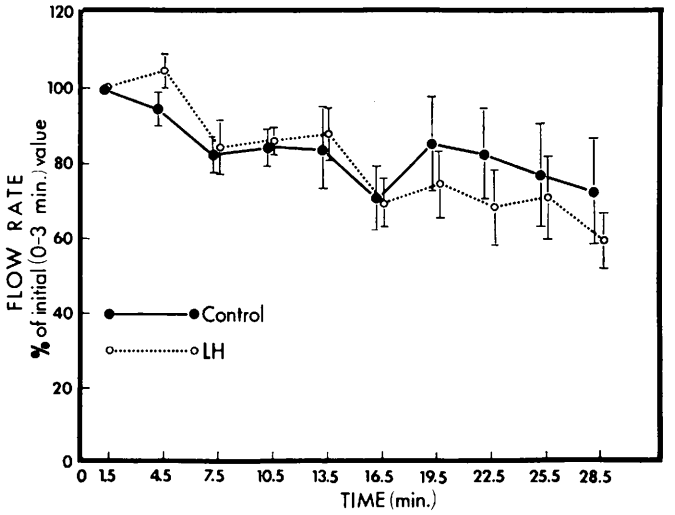

Fig. 2. Blood flow rates (mean and SE) from femoral vein of control and LH treated rats. Flow rates are expressed as a percentage of the initial (0–3 min) reading. All values at 1.5 min were recorded as 100%.

significantly increase blood flow through the ovary and that this effect is not abolished by cannulation of the ovarian vein. This increased flow rate should be taken into consideration when the ovarian venous cannulation technique is used to estimate steriod secretion. Values at 4.5, 7.5, and 10.5 min were above 100% of initial values. This may have been due to an abnormally low initial reading, as a result of an handling artifact. This effect was observed only in the LH treated animals.

Since the administration of promethazine hydrochloride did not produce a significant change in blood flow (with the exception at 19.5 min) in the absence of LH, but completely prevented the LH-induced increase, the action of LH must be exerted through the release of histamine or some histamine-like substance. This is in good agreement with previous studies (10–12). The small decrease in group 4 animals at 19.5 min may have been due to blocking the effect of histamine released by endogenous LH.

The histamine-releasing activity of LH appears to be restricted to ovarian tissue, since no change in blood flow through the femoral vein was observed following LH administration.

Summary. The present results indicate that cannulation of the ovarian vein and subsequent hemorrhage results in rapid decline of blood flow from the cannula. This decline is significantly retarded by the administration of LH. The stimulatory effect of LH is apparently histamine-mediated, since simultaneous administration of promethazine hydrochloride completely blocks the LH-induced increase in blood flow.

1. Eto, T., Masuda, H., Shruki, Y., and Hosi, T., Jap. J. Anim. Reprod. **8**, 34 (1962).

2. Hashimoto, I., and Wiest, W. G., Endocrinology **84**, 873 (1969).

3. Hashimoto, I., Henricks, D. M., Anderson, L. L., and Melampy, R. M., Endocrinology **82**, 333 (1968).

4. Hashimoto, I., and Wiest, W. G., Endocrinology **84**, 886 (1969).

5. McDonald, D. M., Sciki, K., Prizant, M., and Goldfien, A., Endocrinology **85**, 1137 (1969).

6. Uchida, K., Kodowaki, M., and Miyake, T., Endocrinol. Jap. **16**, 227 (1969).

7. Goldman, B. D., Kamberi, I. A., Siiteri, P. K., and Porter, J. C., Endocrinology **85**, 1137 (1969).

8. Barraclough, C. A., Collu, R., Massa, R., and Martini, L., Endocrinology **88**, 1437 (1971).

9. Piacsek, B. E., Schneider, T. C., and Gay, V. L., Endocrinology **89**, 39 (1971).

10. Wurtman, R. J., Endocrinology **75**, 927 (1964).

11. Szego, C. M., and Gitin, E. S., Nature (London) **201**, 682 (1964).

12. Lipner, H., Proc. Soc. Exp. Biol. Med. **136**, 111 (1971).

13. Ichikawa, S., Morioka, H., and Sawada, T., Bull. Univ. Osaka Prefect. Ser. B **22**, 1 (1970).

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