Effect of Human Chorionic Gonadotropin on Reproductive Organ Blood Flow in Cycling Rats (43957)

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Abstract. **Recent characterization of luteinizing hormone (LH)/human chorionic gonadotropin (hCG) receptors in uterine vascular tissue, evidence that expression of these receptors is cyclic in nature, and demonstration of a correlation between hCG level and uterine vascular resistance lead us to investigate the effect of hCG administration on blood flow in reproductive organs of cycling and ovariectomized Sprague-Dawley rats. Blood flow (ml/min/g dry wt/cardiac output ± SEM) was determined by microsphere spectroscopy (^{57}Co, ^{113}Sn, ^{95}Nb, ^{141}Ce). Baseline uterine (0.5842 ± 0.1037) and cervical (0.7785 ± 0.1199) blood flows were greater in diestrus-2 rats than in every other group. Diestrus-2 (0.4530 ± 0.0584) and estrus (0.4692 ± 0.0848) rats had greater baseline ovarian blood flow than proestrus rats (0.2521 ± 0.0279). A single intraperitoneal injection of 50 IU hCG on each day of the 4-day estrus cycle decreased uterine flow by more than 30% within 20 min ($P < 0.05$), but did not alter uterine flow in ovariectomized rats. This dose of hCG also decreased ovarian flow in diestrus-2 rats (0.5219 ± 0.0857 to 0.4207 ± 0.0753), decreased liver flow in diestrus-2 (0.0282 ± 0.0060 to 0.0231 ± 0.0051) and estrus (0.0301 ± 0.0029 to 0.0203 ± 0.0038) rats, and increased liver flow in ovariectomized rats (0.0279 ± 0.0054 to 0.0325 ± 0.0050). Injection of 0.10 IU hCG did not alter blood flow to reproductive organs in any group, but decreased liver flow in estrus rats (0.0469 ± 0.0121 to 0.0326 ± 0.0088). Neither dose of hCG altered cervical, kidney, or skeletal muscle flow in any group. Our results indicate an organ specific, dose-dependent blood flow response to hCG in cycling rats, which appears, in the case of uterine flow, to be attenuated by removal of the ovaries. The present findings suggest high doses of hCG given clinically may decrease uterine flow and potentially lead to implantation failure.** [P.S.E.B.M. 1996, Vol 211]

Within the past decade, human chorionic gonadotropin (hCG)/luteinizing hormone (LH) receptors have been identified in nongonadal reproductive tissues, such as the uterine tubes, umbilical cord, uterus, and brain of many species (1–7). More recently, hCG/LH receptors have been localized in vascular smooth muscle and endothelial cells of uterine blood vessels, with these receptors being more numerous in small intramyometrial vessels than in large extramyometrial vessels (8, 9). Density of uterine hCG/LH receptors changes with the estrus/menstrual cycle. Rat studies reveal that uterine hCG/LH receptor number is highest either on diestrus or metestrus, and lowest either on estrus or proestrus, varying inversely with serum LH levels (10, 11). Human studies have shown that uterine vascular hCG/LH receptor mRNA and protein are highest during the secretory phase of the menstrual cycle (8), and that ovulatory women have more uterine hCG binding capacity than do anovulatory women (7).

The presence of hCG/LH receptors in the uterus and the cyclic nature of the receptor density suggest that, in some species, hCG/LH regulates uterine func-

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tion. A documented quiescent effect of hCG on porcine and human uterine electrical activity and contractility indicates that one function of hCG/LH in the uterus is to act as a muscle relaxant (12, 13). The effect of hCG on myometrial contraction appears to be mediated by decreased intracellular free calcium levels and down regulation of gap junctions (13, 14). Exogenous hCG also acts to release humoral agents, directly affecting tissue levels of progesterone and cAMP in the rat uterus (15) and levels of prostaglandins and thromboxanes in the human uterine arteries (9).

Localization of hCG/LH receptors in uterine blood vessels, as well as evidence that hCG increases nonluteal ovarian blood flow (16, 17) and either increases (18, 19) or decreases (20) testicular blood flow, have prompted investigation of the role of hCG in control of rat uterine blood flow. Although the rat does not produce hCG (21–23), hCG has been used rather than LH in many previous investigations for two main reasons. First of all, the large quantity of highly purified hormone needed for these studies is more easily obtained in the case of hCG. Secondly, both hormones bind to the same receptors (24). Initial human studies have shown a correlation between hCG level and decreased uterine vascular resistance in pregnancy (25), during stimulated ovarian cycles (9, 26), and in vessels *in vitro* (9). Studies in pseudopregnant rats conversely reveal that administration of hCG decreases uterine blood flow (27). To date, no studies have investigated the effect of hCG/LH administration on uterine blood flow in cycling rats. The objective of the current study was to determine the rat uterine vascular response to hCG administration during the 4-day estrus cycle and after ovariectomy.

Materials and Methods

Animals. Female Sprague-Dawley rats (176–200 g; Charles River Laboratories, Stower Ridge, NY) were housed in a temperature-controlled room with a shifted light cycle (lights on 12 AM–2 PM) and were fed standard rat chow and water *ad libitum*. Rats were housed under these conditions for approximately 2 weeks prior to experiments, and weighed 233.59 g (±3.59 g) at the time of use. Rats demonstrating regular 4-day cycles and ovariectomized (ovx) rats were used. Rats were staged using vaginal cytology, a widely accepted method known to correlate well with steroid hormone and gonadotropin levels (28). Ovariectomies were performed 10 days prior to experiments. Experiments were initiated at 10 AM.

Experimental Procedure. For terminal experiments, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip; Butler, Columbus, OH) and the trachea, the left femoral artery, and the aorta via the right carotid artery were cannulated (PE-240 and PE-50). Rats received 0.20 cc porcine heparin (1000 units/ml, ia; Elkins-Sinn, Cherry Hill, NJ). Throughout surgery and experiments, rectal temperature was maintained at 37°C with a heating pad. Aortic blood pressure was recorded using a Narco Biosystems (Houston, TX) Physiograph CPM and Statham P23D6 transducer (Stoelting, Wood Dale, IL) and was used to verify proper placement of the aortic cannula, at or near the left ventricle, for injection of microspheres.

Following a 20-min stabilization period, radioactive-labeled microspheres (15 ± 3 μm diameter; 100,000 cpm/volume; DuPont NEN, Boston, MA) suspended in physiological saline with Tween 80 (0.01%) were injected through the aortic cannula. The microspheres in a given injection were tagged with one of the following isotopes: ^{57}Co, ^{113}Sn, ^{95}Nb, ^{141}Ce. Beginning 10 sec before isotope injection and continuing 1.5 min after injection, a pre-hCG treatment reference blood sample was collected at a rate of 0.118 ml/min from the femoral artery using a Harvard Apparatus Constant Withdrawal Pump (Mills, MA) with BD 2 cc Multifit syringe (Rutherford, NJ).

Intraperitoneal injection of either human chorionic gonadotropin (hCG) or saline immediately followed. hCG from stock solution (lot CR = 127; 14,900 IU/mg; National Hormone and Pituitary Program, Rockville, MD) was diluted to 50 IU/0.50 cc and to 0.10 IU/0.50 cc. Rats from each day of the estrus cycle and ovx rats received one of the two doses. The high, 50 IU dose of hCG was based on the dose used for superovulation in rats. Intermediate doses of 10, 5, and 0.5 IU were tested, but these groups were neither completed nor reported in the manuscript because the initial response observed was similar to that seen in rats injected with 50 IU. The low, 0.10 IU dose was chosen for study because at this dose the response disappeared. Proestrus animals served as controls, receiving 0.50 cc saline. Following a 20-min waiting period, intra-aortic injection of a second (post-treatment) microsphere suspension was performed and withdrawal of a post-hCG treatment reference blood sample was completed. The 20-min experimental time was chosen for two reasons. First of all, a previous study in pseudopregnant rats demonstrated decreased uterine blood flow in response to intra-arterial injection of hCG within 20 min (27). Secondly, the plasma half-life of LH has been demonstrated to be very short (21 min) (29). While the half-life of hCG is known to be considerably longer (30), the rat produces only LH, so one would expect any effect of hCG to occur relatively rapidly.

The ovaries, uterus, cervix, and both kidneys were obtained for whole organ blood flow determination. The right and left kidneys were used as indicators of equal distribution of microspheres; only animals showing less than 30% difference in right versus left kidney flow were retained for analysis. This was the

maximum amount of difference between the kidneys when the location of the cannula in the heart ventricle was verified by blood pressure tracings. In addition, tissue samples of gastrocnemius muscle and liver were harvested. All organ and tissue samples were blotted dry, placed in test tubes, weighed, and counted for radioactivity (Beckman Industries 8500 gamma counter; Irvine, CA). Samples were desiccated in a Nasco 420 oven (Houston, TX) for 24 hr to obtain dry weights.

Blood Flow Measurements. The reference blood sample method allowed for simultaneous measurement of cardiac output and regional blood flow. This is a widely accepted technique for which the calculations are well validated (31–33). Pre- and post-treatment cardiac outputs were calculated by the following equation:

$$\text{Cardiac Output} = \frac{\text{Total Injected Counts}}{\text{Blood Counts} \times \text{Rat Body Weight}} \times \text{Withdraw Rate}$$

Comparisons of counts in the pre- and post-treatment reference blood samples to counts in the organs and tissues allowed for determination of the pre- and post-treatment blood flows to each organ using the following equation:

$$\text{Blood Flow} = \frac{\text{Organ Count}}{\text{Blood Count}} \times \text{Withdraw Rate} \times \frac{1}{\text{Dry Weight} \times \text{Cardiac Output}}$$

Statistics. One way ANOVA followed by Bonferroni t tests were used to compare baseline cardiac outputs, as well as baseline blood flows to each organ, among the various estrous cycle days. Pre- and post-hCG cardiac outputs, as well as pre- and post-hCG blood flows to each organ, were compared within each estrous cycle day using paired t tests. A significance level of $P < 0.05$ was used.

Results

Baseline cardiac outputs did not differ among animal groups (Table I), and cardiac outputs were not altered by injection of hCG in any group. As shown in Table II, baseline uterine and cervical blood flows were greater in diestrus-2 animals than in diestrus-1, proestrus, estrus, or ovx animals ($P < 0.05$); and diestrus-2 and estrus animals exhibited greater baseline ovarian blood flow than proestrus animals ($P < 0.05$). Baseline kidney, liver, and skeletal muscle blood flow did not differ among the groups.

As shown in Table III, injection of 50 IU hCG decreased uterine blood flow on all cycle days ($P < 0.05$), but did not decrease uterine blood flow in ovx rats. In addition, this dose of hCG decreased ovarian blood flow in diestrus-2 animals, decreased liver blood flow in diestrus-2 and estrus animals, and increased liver blood flow in ovx animals ($P < 0.05$). Injection of 0.10 IU hCG had no effect on blood flow in reproductive organs of cycling or ovx rats (data not shown). Liver blood flow in estrous animals was decreased at the 0.10 dose (from 0.0469 ± 0.0121 to 0.0326 ± 0.0088, $P < 0.05$). Neither dose of hCG affected blood flow to the cervix, kidney, or skeletal muscle, and injection of saline in proestrus (control) rats did not alter blood flow to any organ (data not shown).

Table I. Mean Cardiac Outputs (ml/min ± SEM) in Cycling and Ovariectomized Rats

Estrus cycle day				ovx
Diestrus-1 (n = 16)	Diestrus-2 (n = 16)	Proestrus (n = 23)	Estrus (n = 16)	(n = 16)
35.39 (±3.07)	36.44 (±2.30)	31.49 (±2.40)	35.77 (±3.38)	34.73 (±2.06)

Discussion

Baseline Blood Flow. Our finding that baseline uterine flow is greatest on diestrus and lowest on estrus is consistent with earlier studies utilizing alternative techniques, such as inert gas clearance (34), injection of radioactive saline (35), and *in vivo* microscopy (36). Similarly, the current finding that baseline ovarian flow is greater on estrus and diestrus than on early proestrus is supported by results from an earlier study utilizing venous outflow measurements (37). In contrast, a previous microsphere study found uterine and ovarian flow were greatest on proestrus and lowest on metestrus (38), and a study utilizing electromagnetic flow probes found ovarian flow to be greater on proestrus and estrus than during diestrus (39).

Differences between the current and earlier microsphere studies may be partially accounted for by the size of microspheres used. Smaller diameter microspheres employed in our study (15 ± 3 μm vs 25 ± 5 μm) have the advantage of occluding less of the vascular bed, being less variable in size, being safer to use in larger numbers, and, in turn, providing more reliable measures of flow in smaller regions (33). Some differences may also be accounted for by the time of day at which the studies were performed. Ovarian flow during early proestrus is significantly lower than ovarian flow during late proestrus (37). Our studies were performed just before the anticipated onset of the LH surge.

Baseline kidney, liver and skeletal muscle blood flows did not differ among animals on various estrous cycle days or ovx animals, indicating cyclic baseline blood flow differences are limited to reproductive tis-

Table II. Mean Baseline Organ Blood Flow (ml/min/g dry wt/CO ± SEM) in Cycling and Ovariectomized Rats

Organ	Estrus cycle day				ovx
	Diestrus-1 (n = 16)	Diestrus-2 (n = 16)	Proestrus (n = 23)	Estrus (n = 16)	(n = 16)
Uterus	0.2070 (±0.0336)	0.5842[a] (±0.1037)	0.1367 (±0.0278)	0.0786 (±0.0093)	0.0872 (±0.0259)
Cervix	0.2931 (±0.0491)	0.7785[a] (±0.1199)	0.2605 (±0.0463)	0.1820 (±0.0473)	0.0994 (±0.0663)
Ovary	0.3769 (±0.0408)	0.4530[b] (±0.0584)	0.2521 (±0.0279)	0.4692[b] (±0.0848)	—
Left kidney	0.7354 (±0.0408)	0.5858 (±0.0493)	0.7084 (±0.0493)	0.6507 (±0.0537)	0.5407 (±0.0331)
Liver	0.0397 (±0.0039)	0.0292 (±0.0048)	0.0424 (±0.0080)	0.0385 (±0.0064)	0.0277 (±0.0031)
Sk Musc	0.0062 (±0.0011)	0.0088 (±0.0015)	0.0153 (±0.0037)	0.0081 (±0.0009)	0.0070 (±0.0008)

[a] Flow greater versus all other groups ($P < 0.05$).
[b] Flow greater versus proestrus ($P < 0.05$).

Table III. Mean Organ Blood Flow (ml/min/g dry wt/CO ± SEM) Before and After 50 IU hCG in Cycling and Ovariectomized Rats

Organ	Estrus cycle day				ovx
	Diestrus-1 (n = 8)	Diestrus-2 (n = 8)	Proestrus (n = 8)	Estrus (n = 8)	(n = 8)
Uterus					
Before	0.1973 (±0.0569)	0.6077 (±0.1472)	0.1284 (±0.0126)	0.0730 (±0.0076)	0.0482 (±0.0072)
After	0.1194[a] (±0.0288)	0.3597[a] (±0.0872)	0.0837[a] (±0.0192)	0.0497[a] (±0.0071)	0.0446 (±0.0072)
Ovary					
Before	0.4194 (±0.0706)	0.5219 (±0.0857)	0.2894 (±0.0443)	0.5228 (±0.1508)	—
After	0.3167 (±0.0506)	0.4207[a] (±0.0753)	0.4066 (±0.1034)	0.6487 (±0.1679)	—
Liver					
Before	0.0455 (±0.0056)	0.0282 (±0.0060)	0.0526 (±0.0198)	0.0301 (±0.0029)	0.0279 (±0.0054)
After	0.0518 (±0.0087)	0.0231[a] (±0.0051)	0.0885 (±0.0504)	0.0203[a] (±0.0038)	0.0325[b] (±0.0050)

[a] Decrease versus pre-hCG ($P < 0.05$).
[b] Increase versus pre-hCG ($P < 0.05$).

sues and are likely dependent upon the influence of sex hormones.

Baseline liver blood flow was found to be an order of magnitude below baseline blood flow to the reproductive organs. This may be explained by the fact that, in the current study, liver flow represented only hepatic arterial flow. The portal flow was not included because microspheres which would otherwise enter the liver via the portal vein get caught in the gastrointestinal circulation. Researchers who have calculated portal flow by adding flows to the stomach, intestine, spleen, pancreas, and mesentery have found that it is an order of magnitude greater than hepatic arterial flow (31), on par with flow to other organs.

Effect of hCG on Ovarian Blood Flow. Previous studies have shown that hCG/LH increases ovarian blood flow in cycling rats (37), immature gonadotropin-primed rats (40), and pseudopregnant rats (27). We found no stimulatory effect of hCG on ovarian blood flow in any of our groups, and, in fact, the high dose of hCG decreased ovarian flow in diestrus-2 rats. The fact that we pooled both ovaries and measured flow in both luteal and nonluteal tissue may have masked flow change occurring in nonluteal tissue, as studies have shown hCG effects blood flow only in nonluteal tissue (16, 17). It would have been more conclusive to assay the individual ovaries, as well as luteal and nonluteal tissues, separately.

Effect of hCG on Uterine Blood Flow. Consistent with results reported for pseudopregnant rats, rats

on all days of the estrus cycle showed decreased uterine blood flow after 50 IU of hCG was administered. The fact that hCG did not decrease uterine flow in ovariectomized rats suggests either: (i) that the effect is an indirect one, with some ovarian factor as the direct mediator; or (ii) that uterine pre-exposure to estrogen and/or progesterone is required for the effect of hCG to occur. Preliminary results indicate that when hCG is applied directly to diestrus uterine arterioles *in vivo*, circumventing the intact ovaries, vasodilation, not vasoconstriction, occurs (41).

Effect of hCG on Liver Blood Flow. Glycosylated, biologically active hCG has been found in liver tissue (42, 43); however, receptors for hCG have not been located in rat liver (11). Thus, the findings that hCG decreased liver blood flow in estrus (both doses) and diestrus-2 animals (high dose), and increased liver blood flow in ovx animals (high dose) were unexpected. The liver, together with the kidneys and ovaries, is a principle tissue involved in metabolizing hCG (44). Some of the liver blood flow changes seen with hCG administration may therefore be the result of changing metabolic objectives. It has also been shown that, as hCG levels increase, the production of hepatic lipase increases. The lipase then migrates to ovarian blood vessel endothelium, where it is thought to have a role in the delivery of sterol for ovarian steroid synthesis (45).

Conclusions

Results from the current study indicate high doses of hCG decrease uterine blood flow in rats. Previous studies in rats have shown that high doses of hCG can also decrease vascular permeability, leading to implantation failure (46). The decreased uterine blood flow demonstrated in the current study could potentially be an additional mechanism of implantation failure.

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