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Uterine Serotonin and Receptor Blockade during Estrogen-Induced Uterine Hyperemia[1] (41199)

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Abstract. A role for serotonin in regulation of uterine blood volume (UBV) was investigated by testing estrogen-induced UBV changes in the presence of cyproheptadine and by measuring uterine serotonin concentration at 0, 65, and 125 min following estradiol administration. Rats, castrated on Day 0, were given maintenance doses of estradiol benzoate on Day 7 and 17β-estradiol, 0.5 μg/kg, iv, at time zero on Day 14. At 125 min doses of cyproheptadine which caused a 70% inhibition of the pressor responses to administered serotonin caused a significant reduction in UBV of estrogen-treated animals. Uterine serotonin content was determined by high-performance liquid chromatography with electrochemical detection. Uteri taken at 65 min after the intravenous estradiol showed no significant change in serotonin content or blood volume. At 125 min when the UBV of estrogen-treated animals was 172% of saline control UBV, uterine serotonin was significantly increased (541 ng/g vs 248 ng/g, $P = 0.05$).

Estradiol induces marked increases in uterine blood flow and blood volume following systemic administration or injection into the uterine arterial supply. Since the uterine vascular response characteristically has a time lag of 30–100 min between the estradiol injection and the elicitation of the hyperemia, numerous investigators have postulated that the estrogen does not produce vasodilation directly but triggers the release or action of a vasoactive mediator (1–4). Over the past several years our laboratories have evaluated various vasoactive compounds which are normally present in the uterus for characteristics which would implicate them in estrogen-induced uterine hyperemia (5–8). In consideration of each, experiments have been designed to determine whether the putative mediator fulfills the following criteria: The substance is found in the uterus and its concentration or turnover changes in response to estrogen administration; blockade of the receptor for the putative mediator blocks the estrogen-hyperemia; depletion of the stores or inhibition of mediator synthesis attenuates or prevents the estrogen response; and administration of the substance produces vasodilation similar to that produced by estrogen.

The present study examines the possibility that serotonin is the estrogen mediator by evaluating uterine hyperemia following serotonin blockade with cyproheptadine, and by measuring uterine serotonin concentrations during the estrogen hyperemia response. These studies are prompted by the earlier observations of Szego and Sloan (9) and Spaziani and Szego (10), that locally applied serotonin produces uterine hyperemia in the rat, and the work of McKercher *et al.* (5), which showed changes in uterine serotonin concentration following systemic administration of estradiol to rats.

Materials and Methods. *Drugs.* 17β-estradiol (Sigma Chemical Company, St. Louis, Mo.) was dissolved in 95% ethanol and diluted with phosphate-buffered saline (0.001 *M* phosphate buffer, pH 7.4, 0.15 *M* NaCl) to a concentration of 0.5 μg/ml. Vehicle consisted of phosphate-buffered saline containing an amount of ethanol equal to that found in the estradiol solution. All rats received 1 ml/kg of either estradiol or the saline vehicle. Cyproheptadine HCL (Merck, Sharp and Dohme, West Point, Pa.) was prepared at a concentration of 4 mg/ml in distilled water by gently heating and stirring. A dose of 4 mg/kg body weight was given iv

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immediately prior to the injection of estradiol or vehicle on the day of the hyperemia experiment. A supplemental dose of 5 mg/kg body wt was given subcutaneously 45 min after the estrogen or vehicle. This dose of cyproheptadine was sufficient to provide, at 15 min, an 80% attenuation of the systemic blood pressure increase in response to an intraarterial injection of 5 μg serotonin (Sigma Chemical Co., St. Louis, Mo.). With the supplemental dose of cyproheptadine, a 70% blockade of the blood pressure response to serotonin was maintained for the 2 hr duration of the estrogen hyperemia experiment. Control rats received an injection of distilled water in place of cyproheptadine.

Hyperemia model. Female Sprague–Dawley rats (Bio-Labs, Madison, Wisc.) weighing 175–200 g were bilaterally ovariectomized and housed in cycling light (12 hr light, 12 hr dark). On the seventh day following ovariectomy, each rat received a subcutaneous injection (0.1 ml/100 g body wt) of estradiol given as estradiol benzoate (Sigma Chemical Company, St. Louis, Mo.) dissolved in corn oil (1 μg estradiol/ml). On the 14th day the animals were anesthetized lightly with ether and the femoral vein exposed. The drug preparations described above were injected directly into the femoral vein, the incision was closed with a wound clip, and the animals were allowed to regain consciousness.

Uterine blood volumes were determined using ^{131}I-human serum albumin (^{131}I-RISA) (E. R. Squibb & Sons, Inc., Princeton, N.J.). Two hours following injection of the drug preparations, the animals were lightly anesthetized with ether and 10 μCi of ^{131}I-RISA in 0.1 ml saline were injected into the contralateral femoral vein. After an equilibration time of five minutes, the animals were killed by cervical dislocation and partially immersed in liquid nitrogen. The abdominal contents were exposed via a midline incision and the vena cava clamped. Blood was drawn from the vena cava with a heparinized syringe and 0.1 ml was pipetted into 2 ml of distilled water in a counting tube. The uterus was removed by sectioning at the cervical–vaginal junction and excess mesometrium and fat trimmed from the uterine surface. One horn was placed in a tared counting tube and the radioactivity determined using a Micromedic 588 gamma counter. A section of small intestine was also removed, freed of its contents, placed in a second tared counting tube, and counted in a similar manner. After counting, all tissue samples were dried for 48 hr at 100°, allowed to cool for 30 min and immediately weighed. Blood volume was determined on a dry weight basis using the following formula:

$$\mu\text{l blood/g tissue} = \frac{\text{counts per minute/g tissue}}{\text{counts per minute/1000 } \mu\text{l blood}}.$$

In the experiment defining serotonin concentration, additional groups of animals were sacrificed at 0 and 65 min following estrogen or saline treatment. One uterine horn was taken for counting; the other uterine horn was wrapped in aluminum foil, frozen in liquid nitrogen, and stored at −20° for up to 7 days prior to assay for serotonin; blood volumes were calculated on both a wet and dry weight basis to confirm that serotonin concentration expressed in terms of uterine wet weight could be compared to previous blood volume data.

Serotonin (5-HT) determination. Each uterine horn was immersed in liquid nitrogen, pulverized in its foil wrapper, then transferred to a tared 0.3-ml polypropylene conical vial containing 200 μl 1 *N* glacial acetic acid +0.1 μCi [^{3}H]serotonin. The tissue–glacial acetic acid suspensions were sonicated for 30 min at 4°, centrifuged 5 min in a Beckman microfuge, and the supernatant was filtered through a 0.2-μ Nucleopore polycarbonate membrane. From each filtrate, a 10-μl aliquot was transferred to a counting vial for assessment of recovery of [^{3}H]serotonin.

5-HT determinations were performed on 25-μl aliquots of each sample by a modification of the high-performance liquid chromatography with electrochemical detection (HPLC-EC) method of Sasa and Blank (11). Using a 3 × 500-mm column packed with Dupont Zipax SCX cation-exchange

resin and a 0.4 *M* acetate–citrate buffer (1 ml/min), 5-HT was eluted at 7 min. Other biogenic amines were present in the solvent front and were not measured. Figure 1 shows the response of the glassy carbon detector to graded doses of 5-HT. Replicate analyses of standard or of unknowns resulted in less than 3% variation in peak height, a degree of error attributable to microsampling devices. The 5-HT value of each sample was corrected for recovery and divided by the weight of the tissue to obtain 5-HT concentrations in nanograms per milligram of uterus. Each sample was analyzed the day of preparation and, where possible, on a later day. Assay of samples stored at 4° for as long as 2 days continued to give reproducible results.

Blood pressure determination. Blood pressure in animals anesthetized with sodium pentobarbital (50 mg/kg) was determined by femoral artery catherization with PE 50 tubing connected to an Ailtech (City of Industry, Calif.) pressure transducer which was coupled to a Beckman (Irvine, Calif.) R-611 dynograph.

Statistical analysis. Blood volumes were compared using a two way analysis of variance. In the cyproheptadine study, this test looked for interaction and main effects of drug (saline or estradiol) and treatment (cyproheptadine or vehicle). In the second study, this test assessed the blood volume effects of drug (saline or estradiol) and time (0, 65, and 125 min after estradiol administration). In each case, both uterine and small intestine blood volumes were examined. The serotonin concentration determinations were also analyzed for effects of drug and time. All analyses of variance were followed by Duncan's multiple comparison procedure (alpha level = 0.05) on the means of the experimental groups.

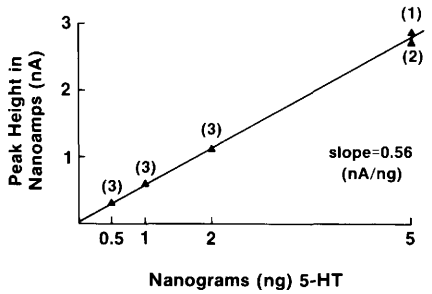

FIG. 1. Response of the glassy carbon electrochemical detector to graded doses of serotonin. The number of determinations at each point is shown in parentheses.

Results. Figure 2 shows the uterine blood volume responses to estradiol and saline in control and cyproheptadine-treated animals. In the control group, uterine blood volume of saline-treated animals was 223 ± 29 μl/g whereas uterine blood volume of estradiol animals was 469 ± 25 μl/g at 2 hr. In cyproheptadine pretreated animals, uterine blood volume of saline-treated animals was 256 ± 25 μl/g at 2 hr and that of estradiol-stimulated animals was 364 ± 22 μl/g. Comparisons among the experimental groups showed no statistical difference between the uterine blood volume of the saline-treated animals in the control and the cyproheptadine-treated groups. In contrast, cyproheptadine pretreatment caused a significant reduction in the uterine blood volume observed after estradiol administration. Blood volume of gut segments from saline- and estradiol-treated animals showed no effect of drug or treatment. (Control saline 167 ± 33 μl/g, estrogen 188 ± 53 μl/g; cyproheptadine/saline 160 ± 76 μl/g, cyproheptadine/estrogen 206 ± 98 μl/g.)

Table I shows serotonin concentrations

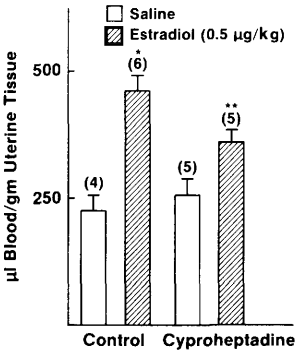

FIG. 2. Uterine blood volume response to estradiol or saline in control or cyproheptadine-treated animals. The number of animals in each group is shown in parentheses. *Denotes significant elevation of blood volume with respect to all other groups. **Denotes significant elevation with respect to saline-treated animals (alpha level = 0.05).

TABLE 1. SEROTONIN (5-HT) CONCENTRATIONS, UTERINE BLOOD VOLUME–WET WEIGHT DETERMINATIONS (UBV-W) AND UTERINE BLOOD VOLUME–DRY WEIGHT DETERMINATIONS (UBV-D) DURING THE RESPONSE OF HYPEREMIA MODEL RATS TO SALINE OR ESTRADIOL (0.5 μg/kg) ADMINISTRATION

Saline					Estradiol				
Time in minutes	N	5-HT (ng/g)	UBV-W (μl/g)	UBV-D (μl/g)	Time in minutes	N	5-HT (ng/g)	UBV-W (μl/g)	UBV-D (μl/g)
0	6	180.5 ± 70.0[a]	61.0 ± 6.2[c]	259.8 ± 29.3[c]	0	5	248.3 ± 129.5[a,b]	57.9 ± 6.1[c]	260.8 ± 24.2[c]
65	6	147.1 ± 67.9[a]	51.6 ± 4.5[c]	215.0 ± 26.3[c]	65	4	202.0 ± 84.1[a]	54.5 ± 9.2[c]	243.7 ± 38.1[c]
125	6	232.5 ± 87.2[a]	60.4 ± 7.2[c]	283.3 ± 40.2[c]	125	5	541.3 ± 155.0[b]	96.1 ± 17.3[d]	447.7 ± 99.9[d]

Note. Values represent mean ± SEM. Mean values with the same superscript are not different (alpha level = 0.05).

and uterine blood volumes of tissues taken at selected times following administration of estrogen and saline. Levels of uterine serotonin in saline-treated animals did not differ at the times tested. Uterine serotonin in estrogen-treated animals did not differ from controls at 0 and 65 min; however, they were significantly greater than controls at 125 min. Uterine blood volumes were also elevated 2 hr following estradiol. Intestinal blood volumes, although not shown, did not vary with time or estradiol treatment.

Discussion. The present study, in conjunction with earlier work, suggests that serotonin plays a role in uterine vascular response to estrogen. Previous investigations have shown that serotonin is present in the uterus and that it can produce vasodilation when applied to the rat uterus via a topical or intraluminal route (5, 9, 10). The present experiment using cyproheptadine to block serotonin receptors further supports the possibility that serotonin plays a role in estrogen-induced uterine hyperemia. The second portion of this study evaluates the uterine content of serotonin and shows that uterine serotonin increases in concert with uterine blood volume following administration of estradiol.

Animals pretreated with cyproheptadine showed diminished vascular responses to estradiol. Although a total blockade of the estrogen response was not achieved, the attenuation of the uterine blood volume response was of similar magnitude to the attenuation of systemic pressor response to injected serotonin produced by this dose of cyproheptadine. Higher doses of the drug approximating complete serotonin blockade were not used in this experiment due to a disproportionate increase in side effects with increased doses. The attenuation of the estrogen-hyperemia can be reasonably ascribed to a blockade of a serotonin-mediated process which leads specifically to uterine hyperemia. This is supported by the lack of an estrogen or cyproheptadine effect on sections of gut taken from each of the experimental animals. The attenuation can also be attributed to the antiserotonin, rather than the antihistamine, actions of

cyproheptadine, since Clark *et al.* (6), showed that H1, H2, or H1 + H2 receptor blocking drugs are without effect on the uterine responses to estradiol. Finally, it is unlikely that the depressed estrogen hyperemia response in cyproheptadine-treated animals is a result of the systemic blood pressure changes associated with the drug, since we have previously shown normal estrogen-hyperemia in animals with marked hypotension due to pretreatment with ganglionic blocking drugs (7).

The studies of uterine serotonin concentration are also consistent with a role for serotonin in the estrogen-hyperemia response. The uterine serotonin levels observed in the present study using an HPLC-EC method are in general agreement with the earlier data of McKercher *et al.* (5). Both studies show no significant change in serotonin concentration for the first 1 hr following estrogen administration. The present study shows a uterine serotonin increase at 2 hr when the uterine blood volume response to estradiol is elevated, and McKercher showed that uterine serotonin concentration was depressed at 4 hr when Clark (12) found blood volume (in this hyperemia model) to be returning to baseline. These data suggest that uterine content of 5-HT varies in parallel with blood volume after estradiol administration. McKercher's microfluorometric studies provide evidence that these changes are occurring in nonmast cell serotonin pools. He could detect no change in either the number or the serotonin content of uterine mast cells following estradiol; more importantly, he showed that estrogen hyperemia proceeds normally after total destruction of mast cells with 48/80 (14). It is possible that serotonin content is increasing passively with increases in serotonin-containing blood at the peak of the estrogen response. However, at the height of the hyperemia, rat uterine blood volume has increased from a baseline of approximately 5 to 10 μl. At the usual circulating serotonin levels of 200 pg/μl (13), this 5-μl increase would fall short of the 20 ng/horn increment in the uterine serotonin occurring at the time of hyperemia. Therefore, increases of serotonin in the uterus in response to estradiol are most likely of nonmast cell origin and are not a result of increased blood flow to the organ.

There are conflicting reports concerning uterine vascular responses to administered serotonin. The early work of Szego and Sloan (9) showed serotonin to be a uterine vasodilator whether administered via the uterine lumen or applied topically to the entering blood vessels. Furthermore, vasodilation was noted regardless of whether the experimental animals were intact rats or ovariectomized rats. These results are in contrast to the recent reports of Hammer and Mitchell (15), who showed a significant reduction in uterine blood flow of pseudopregnant rats following subcutaneous administration of the amine. In addition, Clark (16) reported that, in the sheep, intraarterial doses of serotonin produced uterine vasoconstriction in estrogenized nonpregnant ewes and pregnant ewes, and that blockade of serotonin receptors by methysergide produced marked reduction in uterine blood flow in both pregnant and nonpregnant ewes. Thus, we must await further work to determine the reason for these conflicting results. It is possible that hormonal differences in these animal models could be regulating uterine responses to serotonin or metabolic conversion of serotonin to a substance devoid of constrictor effects. 5-Hydroxyindole-acetaldehyde, for example, has none of the smooth muscle constricting activity of its parent compound, serotonin, at concentrations 100-fold greater (17).

Taken as a group, the present and previous observations of serotonin involvement in the estrogen hyperemia are reminiscent of our findings with the noradrenergic system. In each case, intraarterial administration of the agonist produces vasoconstriction, the uterine levels of the putative mediators change in response to estrogen administration, and receptor blocking drugs attenuate or ablate the estrogen hyperemia. Nevertheless, removal of the major uterine source of each mediator has no effect on the estrogen hyperemia. The results of receptor blockade imply that both 5-HT and norepinephrine are important for elicitation of the

estrogen response; however, the direct vasoconstrictor effects of these materials suggest that they are not the local mediators of estrogen-induced uterine vasodilation. One possible explanation for this is that the systemically administered blocking drugs are interfering with extrauterine norepinephrine and serotonin receptors controlling a step intermediate between estrogen administration and uterine vasodilation. Killam's report (18) that unilateral administration of estradiol into the uterine artery elicits only a unilateral vasodilation would appear to negate involvement of an extrauterine system. However, his findings would be compatible with involvement of an extrauterine factor if it were necessary to have local priming by the high concentration of injected estradiol as well as activation of the extrauterine control by the lower estradiol concentration reaching the general circulation. This possibility is currently being investigated in our laboratories.

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