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Preservation of Estrogen-Induced Increase of Uterine Blood Volume Following Catecholamine and Mast Cell Histamine Depletion[1] (38756)

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Estrogen produces profound changes in uterine hemodynamics. Within hours after administration of the hormone, uterine blood flow is increased (1–3), uterine capillary permeability is enhanced (4) and the uterus becomes engorged with fluid (5). Since estrogenic substances are probably devoid of any direct vasoactive property it has been assumed that uterine hyperemia produced by estrogen is mediated indirectly by the release or production of a uterine vasodilator.

There now exists a considerable amount of evidence supporting the view that the mediator of estrogen-induced uterine hyperemia might be histamine. Evidence favouring this view includes the fact that histamine content of the uterus is decreased at the maximum of the hyperemic response (6, 7), the response to estrogen can be mimicked by histamine (5), several antihistamines appear to reduce the hyperemic response (5), and the effect can be mimicked by administration of the histamine liberator, compound 48/80 (5).

A simple technique for demonstrating that estrogen increases uterine blood volume has previously been described (8). The technique, utilizing radiolabelled human serum albumin (RIHSA), provides a reliable index of the altered uterine hemodynamic state produced by estrogen (8). The present study was undertaken to determine whether depletion of uterine norepinephrine or mast cell histamine in uterus would in any way alter the uterine blood volume response to estrogen.

Methods. Experiments were performed on female albino mice (NMRI strain) weighing approximately 25 g. All animals used were ovariectomized through bilateral lumbar incisions under ether anesthesia 2 wk prior to experimentation.

Mice were pretreated with either compound 48/80 (Burroughs, Wellcome Corporation), 6-hydroxydopamine (Hässle, Sweden) or 0.9% saline. The animals given 48/80 received the following dosage ip beginning 4 days prior to blood volume determination: Day 1: 2 mg/kg free base administered morning and afternoon; Day 2: same as Day 1; Day 3: 3 mg/kg administered morning and afternoon; and Day 4: 4 mg/kg administered morning and afternoon. One week prior to the blood volume determination another group received 6-hydroxydopamine ip during 2 days (150 mg/kg free base each day). Control animals were given saline injections in volumes (0.2–0.4 ml) equivalent to the above-mentioned groups.

The three animal groups were divided into two parts, one receiving estrogen (1 μg estradiol-17β), and the other receiving the solution in which estradiol was dissolved (1% ethanol in saline). Estrogen or its solvent was administered in a volume of 0.1 ml into the tail vein of the conscious animals. Since the maximum blood volume response occurs at 1 hr. after estrogen treatment (8), the hormone or solvent was always administered 55 min prior to the injection of radioiodine-labelled human serum albumin (RIHSA) for blood volume determination.

RIHSA was used for the measurement of uterine blood volume. The application of the method and its validation are described in detail elsewhere (8). A solution of RIHSA (1 μCi) was injected during 10 sec into the tail vein in a volume of 0.1 ml (0.3–0.5 mg albumin per ml in 0.9% saline). It has previously been shown that radioactivity in the blood reaches a stable level after 5 min

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following administration of the tracer and remains stable for periods up to 60 min (9). In order to insure that radioactivity in the tissue at any moment was a reflection of that in blood, the animals were killed instantaneously by immersion into liquid nitrogen, 5 min following the administration of RIHSA. Care was taken to limit immersion time (10–20 sec) to only that required to stop the blood circulation but not to freeze the internal organs. Blood samples (25 μl) to be used as internal reference standard were removed from the left ventricle of the heart after the animal was opened. The uterus was exposed by a midline abdominal incision, was dissected free and removed. Both uterine horns were cut at the vaginal and tubal junctions and all loose parametrial tissue was stripped away from the organ. The uteri were subsequently weighed on tared wax paper and placed at the bottom of a plastic counting tube containing 5 ml of perchloric acid (see below). The blood samples were placed in counting tubes containing 2 ml of distilled water for rinsing of the transferring pipette. Radioactivity was determined with an autogamma spectrometer (Packard). The uterine blood volumes were calculated by dividing cpm/g uterine tissue by cpm/ml heart blood, and the data are given as mean (±SEM) values of μl blood per g tissue wet weight.

In those tubes containing perchloric acid the uterine tissues were analyzed for their content of histamine (10) and norepinephrine (11). In some animals small pieces from the uterine horns were removed prior to immersion in perchloric acid. These were frozen immediately in a propane–propylene mixture and prepared for examination of formaldehyde-induced fluorescence using the Falck-Hillarp method (12). The uterine tissues were examined in a Zeiss fluorescence microscope for the presence of adrenergic nerves and serotonin-containing mast cells. Mast cells were also evaluated by light microscopy after staining sections with toluidine blue.

Results. Fluorescence histochemical examination of the mouse uterus revealed that the untreated uterus had fluorescent adrenergic nerve terminals associated primarily with uterine blood vessels. Serotonin-containing mast cells were also observed in the uterine tissue. Following treatment with compound 48/80, adrenergic nerve terminals were still visualized while no fluorescent mast cells could be found. In uteri from animals treated with 6-hydroxydopamine, the mast cells were still present but no adrenergic nerves could be visualized. When the sections were stained with toluidine blue no mast cells were seen in samples from 48/80 treated mice, while they were routinely found in uteri from 6-hydroxydopamine treated animals.

Compound 48/80 caused a significant reduction in the histamine content of the uterus from a value of 5.26 ± 0.81 μg/g wet wt (6 experiments) to 3.92 ± 0.50 μg/g wet wt (7 experiments) ($P < 0.05$). Six-hydroxydopamine depleted the tissue of its norepinephrine content from 1.43 ± 0.16 μg/g (6 experiments) in untreated animals to unmeasurable amounts ($P < 0.001$; 5 experiments) in 6-hydroxydopamine treated animals.

The effects of estrogen on uterine blood volume in untreated ovariectomized mice, ovariectomized mice treated with compound 48/80 and ovariectomized mice treated with 6-hydroxydopamine are summarized in Fig. 1. The blood volume was slightly reduced in ovariectomized animals receiving compound 48/80 and 6-hydroxy-

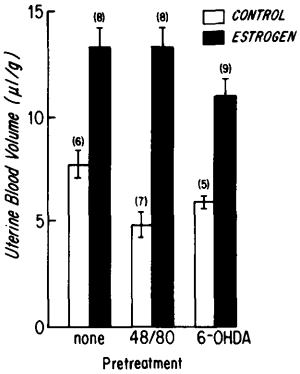

FIG. 1. The effect of estrogen on uterine blood volume in untreated ovariectomized mice, ovariectomized mice treated with compound 48/80 and ovariectomized mice with 6-hydroxydopamine (6-OH-DA). The blood volumes are given as mean (± SEM) of μl blood per g wet wt. Number of animals within parentheses. Each increase produced by estrogen was statistically significant ($P < 0.001$).

dopamine as compared to untreated ovariectomized animals. Estrogen significantly ($P < 0.001$) increased blood volume in all groups receiving the hormone. The uterine blood volume increased by 74% in the untreated animals, 171% in 48/80 treated animals, and 83% in 6-hydroxydopamine treated mice

Discussion. In an earlier report it was demonstrated that a dramatic increase in uterine blood volume is produced by the administration of estrogen to ovariectomized mice and rats (8). Since this change coincides in time with increased blood flow it was suggested that uterine blood volume provides a convenient index of the changes in uterine hemodynamics (8). In the present study the uterine blood volume response to estrogen is not significantly affected by pharmacologic treatments, which alter the uterine content of mast cell histamine and norepinephrine.

Compound 48/80 was demonstrated to destroy mast cells (none were seen with either fluorescence or light microscopy) and reduce the content of histamine in the mouse uterus. The large fraction of histamine remaining in the uterus after treatment with compound 48/80 probably represents therefore a nonmast cell pool of histamine. Since the uterine hyperemic response to estrogen was, if anything, exaggerated by compound 48/80, as appeared to be the case by both visual observation (the uteri appeared much redder) and by measurement of uterine blood volume, the conclusion which must be drawn is that mast cell histamine *per se* does not play a primary role in mediating the response to estrogen in mice. These data do not rule out the possibility, however, that the response to estrogen is mediated in part or *in toto* by histamine derived from a nonmast cell source. In a recent study McKercher *et al.* (7) showed that the reduction in histamine content of rat uterus produced by estrogen was associated with an apparent decrease in the fluorescence intensity of mast cells visualized with *O*-phthaldialdeyhde for their histamine content. However, the hyperemic response to estrogen as estimated visually, was unaltered following compound 48/80 in doses sufficient to destroy mast cells and deplete uterine histamine. Thus, studies performed in two species of rodents both indicate that mast cell histamine does not appear to be a requirement for a normal hyperemic response to estrogen although McKercher *et al.* (7) suggest that estrogen may influence the binding of histamine by mast cells.

The observation that local intraluminal administration of compound 48/80 produces uterine hyperemia (5) is not necessarily inconsistent with present results since massive acute liberation of mast cell histamine would be anticipated to increase local blood flow. The effects of estrogen, although qualitatively similar in terms of hyperemia are probably not equatable with those of 48/80.

Adrenergic nerves innervate uterine blood vessels (13, 14) and play a functional vasoconstrictor role in the uterine vascular bed (15). Estrogen administration to ovariectomized rats reduced uterine norepinephrine content by almost 40% (7). Inhibition of release or functional depletion of adrenergic transmitter by estrogen could therefore promote vasodilatation. In an effort to determine whether uterine adrenergic innervation is involved in the estrogen-induced hyperemia, experiments were performed in animals in which uterine norepinephrine was depleted by pretreatment with 6-hydroxydopamine. This treatment resulted in a total disappearance of norepinephrine from the uterine tissue as shown both histochemically and chemically. Despite this, estrogen produced a normal increase in uterine blood volume. It appears therefore that the uterine response to estrogen does not depend in any primary way upon the presence of normal amounts of adrenergic transmitter.

We demonstrated recently that prostaglandins are involved as mediators of estrogen-induced uterine hyperemia since the blood volume response was reduced by two different inhibitors of prostaglandin synthesis (16). It remains to be determined whether there is any relationship between uterine prostaglandins and uterine nonmast cell histamine which could help promote uterine hyperemia following estrogen treatment.

Summary. Uterine blood volume increases after administration of estrogen to ovariectomized mice. The effect on uterine blood volume was not altered by depletion of mast cell histamine with compound 48/80 or by depletion of norepinephrine from uterine nerves by 6-hydroxydopamine. Thus, neither reduction in the adrenergic transmitter content nor release of mast cell histamine appears to play any primary role in mediating the changes in uterine hemodynamics produced by estrogen, at least as they are reflected by changes in uterine blood volume.

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