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Estrogen-Induced Uterine Hyperemia and Edema Persist during Histamine Receptor Blockade[1] (39947)

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Introduction. Estrogen exerts a profound effect on the uterine vasculature, initially producing uterine vasodilation, then increased vascular permeability and uterine edema. Uterine vasodilation does not occur immediately following administration of exogenous estrogen, but rather after a time lag (1, 2). The presence of a time lag in the estrogen response suggests that a vasodilator mediator is released and acts to produce relaxation of uterine blood vessels. Also, it has not been clarified whether the estrogen-induced increases in vascular permeability observed following the initial vasodilation are mediated by the same vasodilator or by a secondary mediator.

Three endogenous vasodilator substances, acetylcholine (3), histamine (4-7), and prostaglandins (8) have been implicated as possible mediators of estrogen-induced uterine hyperemia and increased vascular permeability. The present study concerns an evaluation of the possible role of histamine in both estrogen-induced uterine vasodilation and increased vascular permeability.

Histamine was implicated as a mediator of estrogen-induced hyperemia as early as 1924 (9), when it was observed that administration of histamine could increase uterine size and produce hyperemia in rats. Westin (10) reported that exogenously administered estradiol significantly reduced the number of histamine-laden mast cells found in the uteri of castrated mice. In addition, the number of mast cells fluctuated during the estrous cycle, being lowest at estrus (10). Spaziani and Szego (4) and McKercher *et al.* (5) reported that uterine histamine content is lowest at estrus (4) and that uterine histamine content decreases by 25% following the administration of exogenous estradiol in physiological doses to rats. Based on these observations, Spaziani and Szego (4, 6) suggested that estrogen liberates histamine which then produces uterine hyperemia and increased capillary permeability by a direct action on the uterine vasculature. The source of the histamine does not appear to be mast cells since their destruction did not alter the uterine response to estrogen (11). Additional evidence which suggests that histamine might mediate the estrogen response has been summarized by Szego (6) and more recently by Spaziani (12).

Several attempts have been made to block these estrogen-induced changes by the use of antihistamines. Kaiser (13) found that the antihistamine tripelennamine reduced estrogen-induced hyperemia in rabbit endometrium autotransplanted to the eye. Spaziani and Szego (7) reported that water imbibition occurs following intraluminal injections of estradiol-17β, histamine, or the histamine liberator compound 48/80. In addition, the antihistamines chlorpheniramine and diphenhydramine, when injected intraluminally, greatly attenuated water imbibition. However, Cecil *et al.* (14) were unable to duplicate these findings and reported that both histamine and diphenhydramine caused severe damage to the tissue of the uteri when injected intraluminally. Thus, some question remains as to whether estrogen-induced hyperemia and increased permeability can be altered by antihistamines.

Recently, Black and co-workers (15) identified a second distinct type of histamine receptor involved in vasodilation (H_2 recep-

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tors) which is not blocked by the classical H_1 receptor antihistamines (diphenhydramine, tripelennamine, pyrilamine, etc.) used in previous uterine studies. The present experiments were designed to determine if either estrogen-induced vasodilation or increased uterine vascular permeability were mediated by H_1 and/or H_2 histamine receptors.

Methods and materials. Estrogen surge model. Female Sprague–Dawley rats weighing between 180 and 200 g (90–100 days of age) were housed under cycling light (14-hr light, 10-hr dark) and received Tech-lab mouse and rat diet and water *ad libitum*. Rats were bilaterally ovariectomized under ether anesthesia using a dorsal approach without regard to the stage of the estrus cycle, and the incision was closed using wound clips. On the seventh day following ovariectomy each rat received an estrogen supplement in the form of a subcutaneous injection of estradiol benzoate dissolved in corn oil (estradiol-3β benzoate, Sigma Chemical Co.) at a dose of 1 μg/kg body weight.

On the 14th day after ovariectomy the effects of the antihistamines pyrilamine and burimamide on estrogen-induced increases in uterine blood volume and vascular permeability were determined. In these experiments, animals were lightly anesthetized with ether and the femoral vein was exposed. Rats received antihistamines iv just prior to the administration of either saline or estrogen (0.5 or 5.0 μg/kg iv). The femoral incision was closed with wound clips and the animal was allowed to regain consciousness.

Measurement of uterine blood volume. Uterine blood volume was determined using RISA-^{131}I (radioiodinated ^{131}I-labeled serum albumin). In this series of experiments, 10 μCi of RISA-^{131}I (0.1 ml of saline containing 3–4 mg of albumin/ml) was injected into the contralateral femoral vein 2 hr after the injection of either estrogen or the vehicle, and the incision was closed with a wound clip. An equilibration time of 5 min was allowed, after which the animals were sacrificed by cervical dislocation followed by immersion in liquid nitrogen. The uterus and vena cava were exposed via a midline incision. Vena caval blood was withdrawn and a 100-μl aliquot was placed in a counting tube containing 2 ml of distilled water. The uterus was removed by sectioning at the cervical–vaginal junction. Excess mesometrium and fat were removed and the uterus was placed in a tared counting vial for uterine blood volume determination. All tissues were immediately weighed and the radioactivity in each tissue sample was determined with a Beckman Autogamma Counter. After counting, uterine samples were dried at 100° for 3 to 4 days and reweighed. Uterine blood volume was determined on both a wet and dry weight basis by using the following equation:

$$\text{ml of blood/g of tissue} = \frac{\text{cpm/g of tissue}}{\text{cpm/1000 } \mu\text{l of blood}}$$

On the basis of previous work (2) the increase in uterine blood volume was found to be maximal at 2 hr following estrogen and returned toward baseline at 4 hr. At 2 hr this change was due to a true increase in vascular space and not to enhanced leakage of the tracer protein produced by a change in permeability (2). Therefore in experiments designed to determine the role of histamine in estrogen-induced uterine hyperemia, uterine blood volume was determined 2 hr after estradiol administration.

Measurement of edema. Estrogen-induced increases in vascular permeability as reflected by augmented water content were determined by measuring uterine tissue weights at the time of removal from the animal and after drying for 3–4 days at 100°. Preliminary results (see Results section) indicated that water content was maximum 4 hr after administration of 5 μg/kg of estradiol-17β. This time and dose of estradiol were used to assess the effect of antihistamines on estrogen-induced uterine edema.

Drug preparation. The estrogen solution used on the day of the experiment was prepared by dissolving estradiol-17β (Sigma Chemical Co.) in 95% ethanol and then diluting it with physiological saline to obtain a final concentration of 0.5 or 5.0 μg/ml in a 1% ethanolic solution. Antihistamines used were dissolved in saline and administered intravenously.

Determination of dose of antihistamine to

be used. The dose of the classical H_1 receptor antagonist used in the experiments was determined from pilot studies in rats in which H_1 histamine receptor blocking agents attenuated the systemic vasodepressor responses to histamine for at least a 4-hr time period. The response to histamine was not completely abolished by H_1 histamine receptor antagonists, but was totally abolished when the combination of the H_1 and H_2 (burimamide) histamine receptor blocking agents was utilized. Burimamide alone had little effect on the histamine-induced vasodepressor response. The dose of H_2 receptor blocking agents was determined from the following information: (i) In preliminary experiments histamine-induced relaxation of isolated rat uteri was completely blocked by burimamide (1 μg/ml), suggesting that H_2 receptors exist in the rat uterus and that they can be blocked by burimamide; (ii) the concentration used in the isolated rat uterus study has also been shown to be the ED_{50} dose for inhibition of histamine-stimulated gastric secretion in rats (15); (iii) the half-life of burimamide in the rat is approximately 2 hr (15); and (iv) burimamide (1 mg/kg), given after an H_1 histamine receptor antagonist, completely blocked systemic vasodepressor responses for 2 hr. The length of blockade was increased to 4 hr by increasing the dose of burimamide to 5 mg/kg intravenously.

Results. Effect of histamine receptor blockade on uterine hyperemia. The effects of the H_1 histamine receptor antagonist pyrilamine, the H_2 histamine receptor antagonist burimamide, and the combination of H_1 and H_2 histamine receptor antagonists are shown in Fig. 1. Two hours following the administration of estradiol-17β (0.5 μg/kg) uterine blood volume was significantly increased in control animals. None of the antihistamine pretreatments altered either the baseline uterine blood volume or the normal estrogen-induced increase in uterine blood volume.

In order to determine if the failure to block estrogen-induced uterine hyperemia with the antihistamines pyrilamine and burimamide was due to the dose of the agents used, doses of both agents were increased to 10 mg/kg. Responses to estrogen were not significantly altered in the presence of these higher doses. In addition, pretreatment with the H_1 blockers tripelennamine and diphenhydramine (10 mg/kg) failed to alter the estrogen response.

Effect of histamine receptor blockade on uterine edema. Estrogen-induced uterine edema was related to both the dose of estradiol used and the time elapsed between estrogen administration and sacrifice. Two hours following the administration of estrogen neither uterine tissue weight (Fig. 2) nor tissue water content (Fig. 3) was significantly altered at the lowest dose of estradiol (0.5 μg/kg) tested. This is in contrast to the results observed at the higher dose of estradiol (5.0 μg/kg) which produced a significant increase in uterine tissue weight (Fig. 2) and increased the uterine water content (Fig. 3). Four hours after the administration

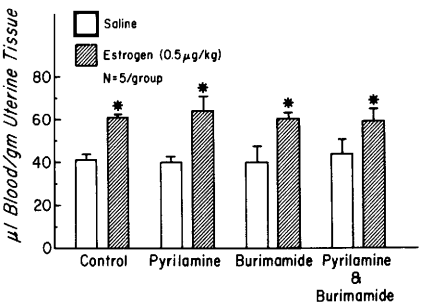

FIG. 1. The effect of the H_1 receptor antagonist pyrilamine, the H_2 histamine receptor antagonist burimamide, and the combination of both H_1 and H_2 histamine receptor blockade on estrogen-induced increases in uterine blood volume. Values represent means ± standard errors of the means. $^*P < 0.05$. In this and remaining figures, groups were compared by group-comparison Student's t test.

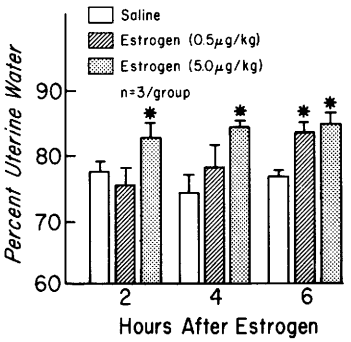

FIG. 2. The effect of estradiol on uterine tissue weight. Each value represents the mean ± standard error of the mean. $^*P < 0.05$.

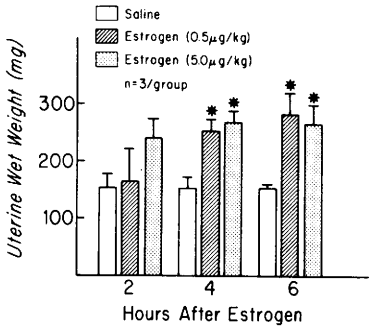

FIG. 3. The effect of estradiol on uterine water content. Each value represents the mean ± standard error of the mean. $^*P < 0.05$.

of estrogen, uterine tissue weight was significantly increased at both 0.5 and 5.0 μg/kg of estradiol, while the uterine water content was significantly augmented only at the highest dose of estradiol examined (Fig. 3). By 6 hr, the uterine tissue weight (Fig. 3) was significantly increased at both doses of estradiol. Although wet weights of uterine tissues increased following estrogen, tissue dry weights were not significantly modified, suggesting that edema rather than tissue growth had occurred. Based on these observations, estradiol was administered at a dose of 5 μg/kg and the effect of antihistamines was determined at 4 hr.

The effects of the antihistamines pyrilamine and burimamide on uterine edema are shown in Fig. 4. Estrogen produced a significant increase in uterine water content in control uteri at 4 hr. Pretreatment with pyrilamine alone did not increase uterine water content of saline-treated animals and prevented a statistically significant increase in the uterine water content following estrogen. Pretreatment of animals with the H_2 histamine receptor antagonist burimamide did not significantly alter either the saline-control uterine water content, nor did it attenuate the normal estrogen-induced increase in uterine water imbibition. Similarly, the combination of pyrilamine and burimamide did not alter the estrogen response.

Discussion. It could be anticipated that uterine hyperemia can be attenuated by pharmacologic intervention. In earlier studies, inhibition of prostaglandin synthesis (8) and α-adrenergic receptor blockade (16) both reduced the response to estrogen. Thus selected interference with potential mediators will alter the capacity of estrogen to increase uterine blood volume.

In the present experiments attempts to attenuate the estrogen-induced increase in uterine blood volume in rats with H_1 histamine receptor antagonists (pyrilamine, diphenhydramine, tripelennamine), the H_2 receptor antagonist burimamide, or the combination of H_1 and H_2 receptor antagonists failed. The doses of antihistamine were sufficient to block systemic depressor responses to intravenous histamine. In addition, increasing the dose of pyrilamine and burimamide did not significantly alter the normal estrogen response. It is possible that the histamine receptors in uterine vascular smooth muscle are insensitive to the antihistamines which were tested. This possibility seems unlikely, however, since the H_2 receptor-mediated relaxation of rat uterine smooth muscle was inhibited by burimamide at low concentrations *in vitro*. If it is assumed that uterine vascular receptors, like other vascular histamine receptors, respond to antihistamines at concentrations similar to those which block myometrial receptors (13), the doses of antihistamines used appear to be sufficient. These results suggest therefore that histamine does not play a major role in mediating estrogen-induced increases in uterine blood volume.

Ideally, the role of histamine as the vasodilator mediator of the estrogen response

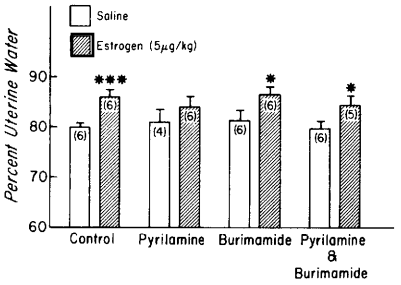

FIG. 4. The effect of the H_1 histamine receptor antagonist pyrilamine, the H_2 histamine receptor antagonist burimamide, and the combination of both H_1 and H_2 histamine receptor blockade on estrogen-induced uterine accumulation of water. Values represent means ± standard errors of the means. $^*P < 0.05$; $^{***}P < 0.001$. Numbers in parentheses indicate group size.

would best be tested by determining if antihistamines abolish the increase in uterine blood flow produced by local intraarterial injection of histamine or estrogen. For technical reasons this has not been possible in the rat, however, Resnik *et al.* reported recently that diphenhydramine totally blocked uterine vasodilator responses to histamine in unanesthetized sheep, while it did not alter estrogen-induced increases in uterine blood flow (17).

In contrast to the effects of estrogen on uterine hemodynamics, the time of onset of estrogen-induced water imbibition and edema was dose dependent. This observation was originally reported by Astwood (18) in 1938 and was suggested as a bioassay for estrogen. In the present study estrogen-induced edema was related both to the dose of estradiol used and the interval of time following estrogen administration. Attempts to block estrogen-induced water imbibition with antihistamines failed, with the exception of pyrilamine which when used by itself slightly reduced the magnitude of estrogen-induced edema. Since other H_1 blockers and the combination of pyrilamine and burimamide were without effect, the pyrilamine result does not appear to have any biological importance.

Overall these results, which demonstrate that certain antihistamines fail to affect either the hyperemia or edema produced by estrogen, are at variance with a large body of evidence which has been reviewed by Szego (6) and Spaziani (12). There may be a number of reasons why the present findings differ from others in which antihistamines have been shown to be effective in attenuating uterine responses to estrogen. Most previous studies have used qualitative estimates of the change in uterine circulation such as the degree of redness. In the present studies the uterine response was quantified using measurement of blood volume. The antihistamines were administered by the systemic route in doses which were shown to prevent the depressor response to histamine and which by themselves had little influence on arterial pressure. In earlier studies the antihistamines have often been administered directly to the uterus via the intraluminal route, a route which Spaziani suggests may well be unphysiological (12). Perhaps the major difference between this and other studies is the application of new H_2 receptor blocking agents to this problem. It has never been possible to completely block the vascular effects of histamine with H_1 receptor blocking agents alone (19). The combined use of H_1 and H_2 receptor blockers provides a powerful tool for studying possible histamine mediation of vascular events because the combination abolishes the vasodilator effects of histamine (19, 20). In the present study this combination, even at doses larger than needed to prevent the depressor effect of histamine, did not alter the uterine response to estrogen.

Summary. The effect of H_1 and H_2 histamine receptor antagonists on estrogen-induced increases in uterine blood volume and uterine water content (edema) was determined. Neither the H_1 or H_2 histamine receptor antagonists nor the combination of both significantly altered either estrogen-induced increases in uterine blood volume or uterine water content. These results suggest that histamine does not play a major role in these physiological responses of the uterus to estrogen.

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