THE UPTAKE OF NOREPINEPHRINE BY RAT UTERINE SUBEPITHELIAL CELLS UNDER NORMAL AND EXPERIMENTAL CONDITIONS

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During the oestrous cycle of the rat, there is a variation in the catecholamine content of the uterus (Wurtman, Chu & Axelrod, 1963). Numerous studies, recently reviewed by Marshall (1970), have shown that the uterine response to nerve stimulation and the quantity of neurotransmitter present vary with the hormonal status of the animal. In the present study, the uptake of norepinephrine in the rat uterus was studied during the various stages of the oestrous cycle and during pregnancy and pseudopregnancy. In addition, the effects of ovarian hormones on the uptake of norepinephrine in the uterus of ovariectomized animals was evaluated.

Female nulliparous Sprague-Dawley rats weighing 175 to 225 g were housed in a room with a 14-hr light (05.00 to 19.00 hours) and 10-hr dark regimen. Oestrous cycles were monitored daily between 08.00 and 10.00 hours by microscopic evaluation of aqueous vaginal lavages. For pregnancy studies, females in pro-oestrus were housed with a male of proven fertility. The presence of spermatozoa or a vaginal plug the following morning established Day 1 of pregnancy. On Days 1 through 11 and Days 16 and 18, uterine tissue was removed from pregnant rats and processed for the demonstration of norepinephrine. Pseudopregnancy was induced in oestrous rats by tapping the cervix rapidly with a polished glass rod 2 to 3 mm in diameter (De Feo, 1963). The first day of predominant leucocytic lavage following cervical stimulation was taken to indicate Day 1 of pseudopregnancy. Uterine tissue was studied on Days 4 to 11 of pseudopregnancy for the demonstration of norepinephrine. For hormonal studies, some rats were anaesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and bilaterally ovariectomized. Rats were allowed at least 10 days to recover from surgery and subsequently one uterine horn was removed as a control and processed for the presence of norepinephrine. These ovariectomized hemi-hysterectomized rats were injected daily subcutaneously with 1 µg oestradiol-17β, 5 mg progesterone, or oestradiol-17β plus progesterone. All hormones were dissolved in 0.5 ml sesame oil for injection. Control rats received only the oil vehicle. After 1 week on hormone therapy, rats were killed and the remaining uterine horn was processed.

At specified days of the oestrous cycle, pregnancy, pseudopregnancy, or
following hormonal injections in ovariectomized animals, rats were assigned to one of three treatment groups. Rats in Group I were injected with nialamide, a monamine oxidase inhibitor (100 mg/kg, intraperitoneally), 5 hr before killing and norepinephrine (1 mg/kg, saphenous vein route) 15 min before killing. Rats in Group II received nialamide alone 5 hr before killing. Rats in Group III were injected with L-Dopa (100 mg/kg, intraperitoneally), the precursor of norepinephrine, 2 hr before killing. At autopsy, the uterus was removed and trimmed of the mesentery and excess fat. Two segments, each approximately 1 cm long, were cut from the centre of a uterine horn. One segment was quenched rapidly, freeze-dried, treated with formaldehyde gas for 1 hr at 80°C, infiltrated with paraffin wax under vacuum, sectioned at 13 µm, and analysed with fluorescence microscopy, all according to the procedure of Falck & Owman (1965). The other segment was placed in 10% neutral buffered formalin, sectioned at 7 µm and stained with haematoxylin and eosin.

### Table 1. Presence of extraneuronal norepinephrine indicated by uterine subepithelial cell fluorescence during the oestrous cycle, pregnancy, pseudopregnancy and following hormonal treatment in ovariectomized rats

<table>
<thead>
<tr>
<th>Hormonal status</th>
<th>Subepithelial cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrous cycle:</td>
<td></td>
</tr>
<tr>
<td>Pro-oestrus</td>
<td>–</td>
</tr>
<tr>
<td>Oestrus</td>
<td>–</td>
</tr>
<tr>
<td>Dioestrus</td>
<td>–</td>
</tr>
<tr>
<td>Metoestrus</td>
<td>–</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>+ 4 to 9</td>
</tr>
<tr>
<td>Pseudopregnancy</td>
<td>+ 4 to 9</td>
</tr>
<tr>
<td>Ovariectomized:</td>
<td></td>
</tr>
<tr>
<td>Sham treatment†</td>
<td>–</td>
</tr>
<tr>
<td>Oestrogen treatment†</td>
<td>–</td>
</tr>
<tr>
<td>Progestosterone treatment†</td>
<td>+</td>
</tr>
<tr>
<td>Oestrogen and progesterone treatment†</td>
<td>–</td>
</tr>
</tbody>
</table>

All animals injected with nialamide (100 mg/kg intraperitoneally) 5 hr before killing and norepinephrine (1 mg/kg, saphenous vein route) 15 min before killing.

* –, Non-fluorescent subepithelial cells; +, fluorescent subepithelial cells; numbers indicate days of pregnancy or pseudopregnancy.
† For the treatment of ovariectomized rats, see text.

Adrenergic nerves were present in all uteri studied. The pattern of fluorescent nerves was similar to that reported previously (Sjöberg, 1967). Arteries of the mesometrial triangle and myometrium had extensive perivascular plexuses. Mast cells were especially numerous in the mesometrial triangle of all uteri. Pregnant, pseudopregnant and progesterone-treated rats injected with nialamide and norepinephrine (Table 1) showed a greenish-yellow fluorescence in uterine subepithelial cells, identical to the fluorescence of adrenergic nerves (Pl. 1, Fig. 1). Rats injected with nialamide alone or L-Dopa did not show this
Fig. 1. Day 5 of pseudopregnancy in a rat. The antimesometrial side is at the top of the figure. The subepithelial cells show catecholamine fluorescence. E, epithelium; arrowhead, autofluorescent lipofuscin. Injected with nialamide and norepinephrine (see text). × 127.

Fig. 2. Day 8 of pregnancy in a rat. The decidual mass (D) expanding from the antimesometrial side obliterates the uterine lumen. Catecholamine fluorescence is restricted to mesometrial subepithelial stromal cells. E, epithelium; arrow, autofluorescent artifact. Injected with nialamide and norepinephrine (see text). × 139.
Subepithelial fluorescence. Specificity tests for norepinephrine (Corrodi, Hillarp & Jonsson, 1964; Corrodi & Jonsson, 1967) confirmed that this subepithelial fluorescence was due to extraneuronal norepinephrine. Fluorescence was seen in subepithelial cells of the endometrial stroma of 4- to 9-day pregnant and pseudopregnant rats and in ovariectomized rats treated with progesterone for 7 days. A spatial periodicity was observed in the subepithelial cell fluorescence wherever it occurred. Fluorescent zones alternated with intervals of non-fluorescent zones. This fluorescence was not seen in subepithelial cells during the oestrous cycle or in ovariectomized rats treated with oestrogen or oestrogen plus progesterone. During pregnancy, the fluorescent subepithelial cells were aggregated, enlarged and polygonal, and apparently corresponded to the primary decidual cells of Krehbiel (1937).

Extraneuronal fluorescence was first noted on the antimesometrial side of the lumen (Day 4), later extending laterally (Days 5 to 6), and still later a narrow circumferential band of fluorescence was observed throughout the subepithelial zone. The non-fluorescent subepithelial cells in the interfluorescent zone were loosely arranged, enlarged, polygonal types with abundant cytoplasm. Grossly visible secondary decidual swellings of pregnancy were not fluorescent (Pl. 1, Fig. 2).

In the present study, the pattern of uterine subepithelial fluorescence corresponded both temporally and spatially with the primary decidual zone of Krehbiel (1937). Since ultrastructural studies of rat subepithelial stroma have not shown chromaffin-like cells (Jollie & Bencosme, 1965; Finn, 1971), it is suggested that the fluorescence found in the present study was due to the extraneuronal uptake of norepinephrine. Extraneuronal uptake of catecholamines (uptake₂) has been demonstrated in sympathetically innervated peripheral effector tissues such as vascular smooth muscle, cardiac muscle and glandular tissues (Iversen, 1973). The significance of the extraneuronal amine uptake is not fully understood, but three functions have been proposed: (1) uptake₂ may facilitate the rapid removal and inactivation of circulating norepinephrine (Iversen, 1971); (2) uptake₂ may potentiate the response of effectors to catecholamines (Kaumann, 1972); (3) uptake₂ may function in intracellular regulation unrelated to the immediate response of the cell (Gillespie, 1973).

Various steroids, including corticosterone, progesterone and oestradiol-17β, produced a dose-dependent inhibition of the uptake of [³H]norepinephrine by the uptake₂ mechanism in the isolated perfused rat heart (Iversen & Salt, 1970; Salt, 1972). Corticosterone also inhibits the extraneuronal uptake of norepinephrine in rat salivary glands (Almgren & Jonason, 1973). In the present study, treatment of ovariectomized rats with oestradiol-17β inhibited the uptake of norepinephrine by uterine subepithelial cells. On the other hand, uptake₂ of norepinephrine by subepithelial cells was always demonstrated during pregnancy, pseudopregnancy, or in ovariectomized rats treated with progesterone. Examination of the data suggests that progesterone is necessary for the uptake of norepinephrine by subepithelial cells because: (1) no uterine subepithelial fluorescence was observed at any stage in the rat oestrous cycle. It is well known that the rat ovary does not secrete significant quantities of progesterone unless
it is activated by the process of pregnancy or pseudopregnancy; (2) ovariectomized rats treated with progesterone showed a well-defined subepithelial fluorescence reaction. Ovariectomized rats treated with oestrogen alone, or oestrogen plus progesterone, or oil alone did not show subepithelial fluorescence. The action of progesterone may have been antagonized by the oestrogen in the combined steroid treatment group.

The results of the present study suggest progesterone may facilitate extraneuronal uptake \( (uptake_2) \) of the adrenergic neurotransmitter by the uterus and any of the three functions cited above may be involved. Inactivation of norepinephrine by subepithelial cells may be an important uterine adrenergic regulatory mechanism. Alternatively, the possibility that these subepithelial cells may be effectors or that the catecholamine may exert a trophic or regulatory function on the physiology of subepithelial uterine stromal cells cannot be ruled out.

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REFERENCES


De Feo, V. J. (1963) Temporal aspect of uterine sensitivity in the pseudopregnant or pregnant rat. Endocrinology, 72, 305.


Salt, P. J. (1972) Inhibition of noradrenaline uptake \( (uptake_2) \) in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. Eur. J. Pharmac. 20, 329.
