THE PHYSIOLOGICAL DISPOSITION OF C^{14}-SEROTONIN IN THE RAT UTERUS

SOLOMON H. SNYDER, RICHARD J. WURTMAN, JULIUS AXELROD AND ELIZABETH W. CHU

Laboratory of Clinical Science, National Institute of Mental Health, and Department of Pathologic Anatomy, Clinical Center, National Institutes of Health, Bethesda, Maryland

Accepted for publication August 21, 1964

The biogenic amines have been implicated in diverse uterine functions. There is a cyclic variation in uterine epinephrine content during the estrous cycle. The concentration of epinephrine, but not of norepinephrine, doubles during the estrous phase (Rudzik and Miller, 1962). During parturition the uterus releases most of its epinephrine content (Wurtman *et al.*, 1963a). The installation of histamine or serotonin into the lumen of the rat uterus leads to uterine hyperemia, edema, and to an increase in mitotic figures, similar to that produced by estrogen (Spaziani, 1963). Histamine installation also causes an enhancement in the net incorporation of labeled amino acids into uterine protein (Szego and Lawson, 1964). It has been postulated that estrogen administration effects a degranulation of uterine mast cells and a concomitant release of histamine and serotonin (Szego and Sloan, 1961).

Studies on the uptake of circulating H^3-epinephrine have proved useful in characterizing the binding of this amine by the rat uterus. It has been shown that this process is endocrine-sensitive; there is a 4-fold increase in uterine H^3-epinephrine uptake between diestrus and estrus (Wurtman *et al.*, 1963b). The subcellular distribution of H^3-epinephrine and the effects of drugs on the uptake and release of this amine differ in the uterus and heart, suggesting that the locus of epinephrine binding in the uterus may differ from that in other organs studied (Wurtman *et al.*, 1964). Studies described here will show that C^{14}-serotonin is also bound by the rat uterus. The dynamics and locus of binding and its variation with the estrous cycle will also be described.

METHODS AND MATERIALS. Adult female Sprague-Dawley rats were used for studies of the effects of drugs and of the estrous cycle on the uptake of C^{14}-serotonin by the uterus, and for subcellular experiments. For tissue distribution studies, 28-day-old Sprague-Dawley female rats pretreated with 10 micrograms of estradiol-17β 18 hours before receiving the C^{14}-serotonin were used, in order to eliminate differences in amine uptake caused by the estrous cycle. Vaginal smears were examined by the method of Papanicolaou (1954) and classified as described before (Wurtman *et al.*, 1963b). Tissue C^{14}-serotonin concentrations were measured by a procedure previously described (Axelrod and Inscoe, 1963). Serotonin-2-C^{14} hydrogen oxalate (10 mc/mmole) was obtained from Nuclear-Chicago.

RESULTS. *Uptake of C^{14}-serotonin in rat uterus, heart, and spleen.* Immature rats received 5.2 μc (80 μg) of C^{14}-serotonin intravenously and were killed at various time intervals ranging from 1 minute to 24 hours. Hearts, uteri, and spleens were rapidly removed and assayed for C^{14}-serotonin. One minute after its intravenous injection, the heart contained about 6 times as much C^{14}-serotonin as the uterus (fig. 1). The heart released almost 80% of its serotonin in the next 10 minutes, while the uterus lost very little during this time. Subsequently, both organs lost the amine at the same slow rate, so that at 2, 4, and 24 hours, the heart and uterus contained about the same amount of C^{14}-serotonin. The half-life for C^{14}-serotonin during this period was about 48 hours in both heart and uterus. The disposition of C^{14}-serotonin in the spleen differed from that observed in the heart and uterus. Splenic C^{14}-serotonin concentration increased about 40-fold between 1 and 10 minutes after injection, and remained at a constant high level for at least 24 hours.

Subcellular distribution of C^{14}-serotonin in the uterus, heart, and spleen. Adult rats received 5.2 μc of C^{14}-serotonin subcutaneously and were killed 1 hour later. The uterus, heart, and spleen were removed and homogenized with 5 volumes of cold isotonic potassium chloride and centrifuged

Received for publication June 17, 1964.

in a refrigerated centrifuge (Spinco) at 1,000 × *g*, 10,000 × *g*, and 100,000 × *g*. The particulate fractions and the 100,000 × *g* supernatant fluid were assayed for C14-serotonin. In the uterus, about 75% of the C14-serotonin was present in the soluble supernatant fraction (table 1). About 75% of the C14-serotonin in the spleen was present in the 1,000 × *g* fraction. In the heart, the C14-serotonin content was divided between the 1,000 × *g* fraction and the soluble supernatant fraction. Considerable amounts of C14-serotonin were also present in the cardiac 100,000 × *g* fraction; this fraction has previously been shown to contain the norepinephrine storage granules (Potter and Axelrod, 1963).

The 1,000 × *g* fractions of these three organs were examined by phase contrast microscopy, and then treated with Wright's stain and re-examined. In the spleen, most of the particulate matter appeared to consist of platelets, white blood cells, and erythrocytes, while in the heart about half of the pellet resembled blood elements and the remainder appeared to contain nuclei and unbroken cells not derived from the circulation. Blood elements were largely absent in the 1,000 × *g* fraction prepared from the uterus.

Effects of drugs on the release of C14-serotonin in the uterus and spleen. Rats received 6.5 μc of C14-serotonin subcutaneously and were killed 18 hours later. Reserpine (5 mg/kg), lysergic acid diethylamide (1 mg/kg) or compound 48-80 (0.5 mg/kg) was administered intraperitoneally 4 hours before the animals were killed. Uteri and spleens were examined for C14-serotonin (table 2). Reserpine liberated almost all (96%) of the C14-serotonin from the spleen and considerable amounts (67%) from the uterus. Lysergic acid diethylamide, a serotonin antagonist, and compound 48-80, a drug which liberates serotonin from mast cells, had no effect on C14-serotonin in the uterus or spleen.

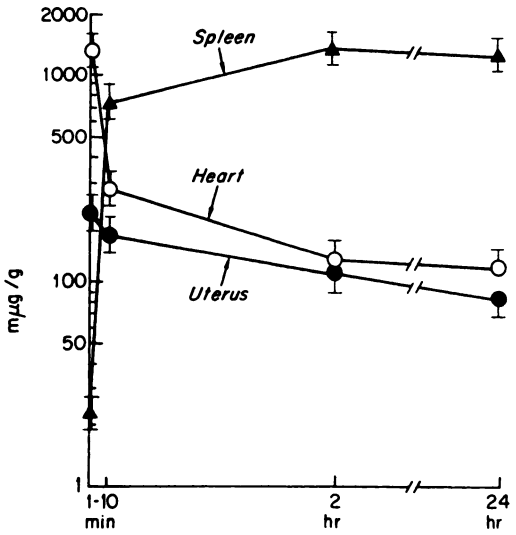

FIG. 1. Immature rats pretreated with estrogen received C14-serotonin intravenously and were killed at time intervals ranging from 1 minute to 24 hours.

Hearts, uteri, and spleens were assayed for C14-serotonin.

TABLE 1

Subcellular distribution of C14-serotonin in rat uterus, heart and spleen

Subcellular Fraction	% Total C14-Serotonin		
	Uterus	Heart	Spleen
1,000 × *g*	14	40	77
10,000 × *g*	6	10	13
100,000 × *g*	6	12	2
Supernatant fluid	74	38	18

Adult rats received C14-serotonin subcutaneously and were killed 1 hour later. Subcellular fractions obtained by differential centrifugation were assayed for C14-serotonin.

It has been shown that in certain organs, such as the heart, repeated injections of compound 48-80 are required to deplete mast cells of their histamine content (Feldberg and Talesnik, 1953). Accordingly, experiments were performed utilizing a more rigorous dosage schedule of compound 48-80 to examine for the possibility that C14-serotonin in the uterus is stored in mast cells which are relatively resistant to the actions of compound 48-80. Rats were given 15 μc of C14-serotonin subcutaneously. Beginning two hours after C14-serotonin injection, compound 48-80 (1 mg/kg) was administered intraperitoneally at 8-hour intervals for 3 days. Rats were killed 18 hours after the last dose along with saline injected controls, and uteri were assayed for C14-serotonin (table 2). This dosage schedule of compound 48-80 had no effect on C14-serotonin content of uterus or spleen. In the experiment involving repeated doses of compound 48-80, C14-serotonin in the heart, an organ whose histamine content can be depleted by repeated

treatment with compound 48-80 (Feldberg and Talesnik, 1953), was also assayed and was unaffected by the drug treatment. Uptake of C^{14}-serotonin by rat skin was negligible, making studies of the effect of drugs in this tissue not feasible.

Changes in C^{14}-serotonin binding in the uterus during the estrous cycle. Vaginal smears were taken from 70 adult rats immediately before the subcutaneous administration of 5.2 μc of C^{14}-serotonin. One hour later, the rats were killed and the C^{14}-serotonin contents of the uterus and spleen were measured. There were marked variations in the C^{14}-serotonin concentration in the uterus which were related to the state of estrus (table 3). During proestrus, estrus, and metaestrus, the C^{14}-serotonin concentration was significantly lower than during diestrus, when the weight of the uterus was least. The C^{14}-serotonin content of the whole uterus did not vary during the estrous cycle. The splenic C^{14}-serotonin concentration was unaffected by the estrous cycle. Endogenous serotonin, estimated by a microfluorometric method (Kuntzman *et al.*, 1961), could not be detected in pooled uteri from rats in different stages of the estrous cycle.

DISCUSSION. Our studies show that circulating serotonin is taken up by the rat uterus and retained for long periods of time. There are considerable differences in the uptake and retention of serotonin in the uterus, heart, and spleen. One minute after the intravenous injection of C^{14}-serotonin, the heart took up more than 6 times as much of the amine as an equal weight of the uterus. The higher initial uptake by the heart may represent differences in the fractions of the cardiac output which perfuse this organ and the uterus. Each unit weight of rat heart receives about three times as much of the circulating blood as an equal amount of uterine tissue (Sapirstein, 1958; Wurtman *et al.*, 1963a). Hence, a considerably greater amount of circulating serotonin is delivered to the heart in the first minute after injection. In the subsequent 10 minutes, the heart loses almost 80% of its initial serotonin content, while the uterus loses only 15%. Then, both organs retain the remaining serotonin for long periods of time. The spleen, on the other hand, has a low initial uptake of the amine. Its C^{14}-serotonin content then rises rapidly, so that after 2 hours this organ contains about 10 times the cardiac or uterine concentration. The splenic concentration of serotonin, like that of heart and uterus, then falls very slowly. The high uptake and retention of C^{14}-serotonin by the spleen may be related to the number of platelets present in the organ.

Studies on the subcellular distribution of C^{14}-serotonin showed marked differences in the way the compound was stored in the different organs. In the spleen, most of the serotonin was present in that fraction associated with the blood elements. This would indicate that its actual concentration in the splenic pulp is much lower. The major fraction of uterine C^{14}-serotonin was found in the cell sap. This would suggest that, like uterine epinephrine, serotonin is firmly

TABLE 2

Effects of drugs on uterine C^{14}-serotonin

Treatment	Uterine C^{14}-Serotonin mμg/g ± S.E.	Splenic C^{14}-Serotonin mμg/g ± S.E.
Saline (control)	41.4 ± 4.2	1305 ± 168
Reserpine, 5 mg/kg	13.7 ± 3.3*	54 ± 22*
LSD, 1 mg/kg	37.8 ± 7.0	1354 ± 310
Compound 48-80, 0.5 mg/kg	32.3 ± 4.3	1329 ± 84
Compound 48-80, 1 mg/kg × 9[a]		
Control	6.4 ± 1.0	426 ± 61
Drug treated	6.2 ± 0.8	435 ± 53

* $P < .001$.

Adult rats received C^{14}-serotonin subcutaneously and were killed 18 hours later. Drugs were given intraperitoneally 14 hours after C^{14}-serotonin.

[a] Details of dosage are given in the text.

TABLE 3

Uterine C^{14}-serotonin in different stages of the estrous cycle

Phase of Estrous Cycle	N	Uterine Wt. (mg)	C^{14}-Serotonin		
			mμg per whole uterus	Uterus mμg/g	Spleen mμg/g
Proestrus	8	279 ± 14*	8.5 ± 0.8	31.0 ± 3.5**	1306 ± 248
Estrus	13	248 ± 16*	7.0 ± 1.0	29.2 ± 4.2**	1397 ± 147
Metaestrus	16	230 ± 14*	7.6 ± 1.0	33.6 ± 4.0**	1344 ± 316
Diestrus	33	168 ± 8	8.7 ± 0.8	53.0 ± 5.4	1333 ± 201

Differs from diestrus value * $P < .001$, ** $P < .01$.

Vaginal smears were taken immediately before the subcutaneous administration of C^{14}-serotonin. Rats were killed 1 hour later and uteri and spleens were assayed for C^{14}-serotonin. Data are presented as means ± standard error of the mean.

bound to a soluble cell constituent in such a way as to protect it from enzymatic destruction by the large amounts of monoamine oxidase which have been shown to be present in this organ (Wurtman *et al.*, 1964). In the heart, C^{14}-serotonin appears to be bound in three separate fractions: in the 1,000 × *g* fraction, as in the spleen; in the soluble supernatant fraction, as in the uterus; and in the 100,000 × *g* fraction which has been shown also to contain catecholamine storage granules (Potter and Axelrod, 1963). It has been suggested that endogenous uterine serotonin is stored in mast cells (Szego and Sloan, 1961). The failure of compound 48-80, a drug which disrupts mast cells, to release C^{14}-serotonin from the uterus would indicate that serotonin taken up from the circulation is not stored in these cells.

The concentration of C^{14}-serotonin by the uterus has been found to vary with the estrous cycle, the retention being greatest per unit weight of tissue during diestrus. The enhanced uterine concentration of C^{14}-serotonin in diestrus is in contrast to uterine binding of epinephrine, which is greatest during the estrus phase (Wurtman *et al.*, 1963b). Epinephrine and serotonin are known to exert opposite effects on the motility of the rat uterus. The catecholamine relaxes the rat uterus, while serotonin has contractile effects. Thus, changes in uterine binding of these biogenic amines may influence uterine motility under varying physiologic conditions.

Our inability to detect endogenous uterine serotonin may be due to low levels of this amine in the tissue or to the lack of sufficient sensitivity of the fluorometric method employed. Bogdanski *et al.* (1958) were unable to detect measurable levels of endogenous uterine serotonin by a fluorometric method, but were able to obtain uterine serotonin levels of 2 to 4 micrograms per gram after an infusion of 5-hydroxytryptophan. Studies in our laboratory (Snyder and Axelrod, 1964) have shown that rat uterine tissue has considerable 5-hydroxytryptophan decarboxylase activity, and that there are marked changes in the activity of this enzyme with the estrous cycle (Snyder *et al.*, unpublished observations). There is a 3-fold increase of enzyme activity in the uterus between estrus and diestrus. Moreover, estrogen administration to oophorectomized rats causes a marked decrease in uterine 5-hydroxytryptophan decarboxylase.

SUMMARY

C^{14}-serotonin is taken up and retained for long periods of time by uterus, heart, and spleen. The C^{14}-amine is firmly bound by constituents in the cell sap of the uterus, by the blood elements in the spleen, and by a combination of these as well as by a small granule fraction in the heart.

Reserpine, but not lysergic acid diethylamide, nor compound 48-80, can release bound C^{14}-serotonin from uterus and spleen.

There is a greater concentration of C^{14}-serotonin in the uterus during the diestrus phase of the estrous cycle than during estrus.

REFERENCES

Axelrod, J. and Inscoe, J. K.: J. Pharmacol. **141:** 161, 1963.

Bogdanski, D. F., Weissbach, H. and Udenfriend, S.: J. Pharmacol. **122:** 182, 1958.

Feldberg, W. and Talesnik, J.: J. Physiol. **120:** 550, 1953.

Kuntzman, R., Shore, P. A., Bogdanski, D. and Brodie, B. B.: J. Neurochem. **6:** 226, 1961.

Papanicolaou, G. N.: Atlas of Exfoliative Cytology, Harvard University Press, Cambridge, Massachusetts, 1954.

Potter, L. T. and Axelrod, J.: J. Pharmacol. **142:** 291, 1963.

Rudzik, A. D. and Miller, J. W.: J. Pharmacol. **138:** 88, 1962.

Sapirstein, L. A.: Amer. J. Physiol. **193:** 161, 1958.

Snyder, S. H. and Axelrod, J.: Biochem. Pharmacol. **13:** 805, 1964.

Spaziani, E.: Endocrinology **72:** 180, 1963.

Szego, C. M. and Lawson, D. A.: Endocrinology **74:** 372, 1964.

Szego, C. M. and Sloan, S. H.: Gen. comp. Endocrin. **1:** 295, 1961.

Wurtman, R. J., Axelrod, J. and Kopin, I. J.: Endocrinology **73:** 501, 1963a.

Wurtman, R. J., Chu, E. W. and Axelrod, J.: Nature, Lond. **198:** 547, 1963b.

Wurtman, R. J., Axelrod, J. and Potter, L. T.: J. Pharmacol. **143:** 314, 1964.