UP TAKE OF
[14C]5-HYDROXYTRYPTAMINE BY THE UTERUS OF
RATS TREATED WITH OESTROGEN
AND PROGESTERONE

E. KOREN*, Y. PFEIFER AND F. G. SULMAN**

Department of Applied Pharmacology, School of Pharmacy,
Hebrew University, Jerusalem, Israel

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SUMMARY

Radioactive [14C]5-hydroxytryptamine (5-HT, serotonin) creatinine sulphate was injected intrapleurally into female rats treated with oestrogen and/or progesterone which had received, immediately before the injection of 5-HT, homogenates of placenta, foetus, uterus or plasma, taken from pregnant rats on the 19th day of pregnancy. The placental homogenate produced a significant increase in 5-HT uptake by the myometrium, when the rats had been primed with moderate doses of oestrogen plus progesterone. Higher doses prevented the increased uptake, and oestrogen treatment alone did not induce 5-HT uptake. The highest level of 5-HT accumulation in the uterus was produced by placental extract after pretreatment with 0.5 mg. oestradiol plus 10 mg. progesterone/rat/day.

These results suggest that the placenta contains a 'trans-serotonin' system which is dependent on the oestrogen-progesterone balance and serves to accumulate 5-HT in the placenta and myometrium. Shifts of the hormonal balance may contribute to the release of 5-HT and thus promote uterine contractions at any stage of pregnancy.

INTRODUCTION

The stimulant effect of 5-hydroxytryptamine (5-HT) on the uterus and the variable sensitivity of this organ to 5-HT are well known. During oestrus, uterine sensitivity to 5-HT is very high, and a rat uterus, after priming with oestrogen, may react with maximum responses to 5-HT dilutions as low as 10^-8 or 10^-9 (Abrahams & Pickford, 1956). Snyder, Wurtman, Axelrod & Chuw (1964) have shown that the affinity of the uterus for 5-HT is hormone-conditioned. Thus, it appears that the uterus can bind and store 5-HT, depending on the hormonal situation prevailing and this may influence uterine motility under varying physiological conditions. 5-HT administered to pregnant mice and rabbits has been shown to lead to foetal death and

* Present address: Department of Obstetrics and Gynecology, Michigan Medical Center, Ann Arbor, Michigan 48104, U.S.A.
** Requests for reprints should be addressed to F. G. Sulman.
to produce changes in the placenta reminiscent of those seen in toxæmia of pregnancy (Poulson, Botros & Robson, 1960).

We have reported (Sadowsky, Pfeifer, Sadowsky, Tsur & Sulman, 1963) that the urinary levels of the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA) are raised in certain women suffering from habitual abortion not amenable to hormone therapy. Analysis of the urine of such cases showed that emotional stress with increased 5-HT production may result in increased contractility of the pregnant uterus. This observation was strengthened by unusually high peaks of 5-HIAA excretion 1 day before abortion. On the day of abortion, 5-HIAA disappeared entirely from the urine and 5-HT only was excreted. This points to a breakdown in normal 5-HT metabolism due to placent al monoamine oxidase (MAO) deficiency (Sadowsky, Pfeifer, Sadowsky & Sulman, 1964). It seemed desirable, therefore, to determine which factors contribute to the accumulation of 5-HT in the uterus.

MATERIALS AND METHODS

Radioactive 5-HT ([3-14C]5-hydroxytryptamine-creatine sul phate) obtained from the Radiochemical Centre, Amersham (Great Britain) was used for injecting steroid-primed female albino rats, of the Hebrew University ‘Sabra’ strain. The normal virgin rats used weighed 200 ± 10 g. and were kept on a stock diet.

Six groups of ten female rats each were prepared with different doses of oestrogen, progesterone or both in olive oil, as follows. Group 1: 10 mg. oestradiol benzoate/rat as one s.c. depot injection. Group 2: 1 mg. oestradiol benzoate/rat/day by s.c. injections for 9 days. Group 3: 50 mg. medroxyprogesterone acetate/rat as one s.c. depot injection.

Group 4: 50 mg. medroxyprogesterone acetate/rat in one s.c. depot injection and 10 mg. oestradiol benzoate/rat/day s.c. for 9 days. Group 5: 10 mg. progesterone/rat/day s.c. for 9 days. Group 6: 0.5 mg. oestradiol benzoate and 10 mg. progesterone/rat/day s.c. for 9 days. Group 7 received control injections of oil. Vaginal smears were taken daily. They showed permanent oestrus in Groups 1, 2 and 4; permanent anoestrus in Groups 3, 5, and alternating oestrus in Groups 6 and 7. Ten days after the first injections, extracts of placentae, foetuses, uteri or plasma were injected followed by [14C]5-HT. The four organ extracts were prepared from pregnant rats killed on the 19th day of gestation. Their abdomen was opened under light ether anaesthesia and blood was taken from the vena cava, cooled and immediately centrifuged for 10 min. at 2000 rev./min. At the same time placentae, foetuses and uteri were removed and 1 g. of each tissue was homogenized in 2 ml. 0.9% NaCl solution, the test tubes being cooled on ice. Immediately thereafter 2 ml. of each of the four extracts were injected s.c. into the above six groups of steroid-primed rats, as detailed in Table 1. A control group received 2 ml. saline only. Ten minutes later each rat was injected with 3 mg. [14C]5-HT (= 0.3 μCi) in 1 ml. saline into the pleural space and 30 min. later the rats were killed with ether. The intrapleural space was chosen since in working with abdominal organs intraperitoneal injections had to be avoided. Absorption after intrapleural injection was found to be very fast (Koren, Pfeifer & Sulman, 1966a).

During ether anaesthesia 2 ml. blood were drawn from the abdominal vena cava of each rat and plasma prepared as above. Then all the rats were killed by ether.
Table 1. Uptake of 5-hydroxytryptamine (5-HT) (µg/g. tissue) by the uterus in six groups of ten steroid-primed adult female rats each and a control group, after intrapleural injection of [14C]5-HT (3 mg. = 0.3 µc in 1 ml. saline) preceded by subcutaneous injection of homogenate (1 g./2 ml.) of various organs (means ± S.E.)

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oestradiol benzoate, s.c. depot (10 mg./rat)</td>
<td>Oestradiol benzoate, s.c. for 9 days (1 mg./rat/day)</td>
<td>Medroxy-progesterone acetate, s.c. depot (50 mg./rat) + oestradiol benzoate s.c. depot (50 mg./rat)</td>
<td>Progesterone s.c. (0·5 mg./rat/day) + progesterone (10 mg./rat/day for 9 days)</td>
<td>Oestradiol benzoate s.c. (0·5 ml./rat/day)</td>
<td>Controls, oil s.c. (0·5 ml./rat/day for 9 days)</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>22 ± 2</td>
<td>2 ± 0·2</td>
<td>29 ± 2</td>
<td>4 ± 0·2</td>
<td>30 ± 3</td>
<td>19 ± 2</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Foetus</td>
<td>21 ± 2</td>
<td>1 ± 0·1</td>
<td>25 ± 2</td>
<td>2 ± 0·1</td>
<td>31 ± 3</td>
<td>14 ± 1</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Uterus</td>
<td>34 ± 3</td>
<td>2 ± 0·2</td>
<td>12 ± 1·5</td>
<td>3 ± 0·2</td>
<td>32 ± 2·5</td>
<td>18 ± 1</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Plasma</td>
<td>25 ± 2·5</td>
<td>3 ± 0·3</td>
<td>16 ± 1·5</td>
<td>3 ± 0·2</td>
<td>30 ± 2</td>
<td>15 ± 1</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>Saline</td>
<td>31 ± 3</td>
<td>6 ± 0·3</td>
<td>24 ± 2</td>
<td>3 ± 0·2</td>
<td>27 ± 2</td>
<td>16 ± 1</td>
<td>27 ± 2·5</td>
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anaesthesia, their uteri were removed, cut into small sections, homogenized, poured into 2 ml. boiling 46% KOH, immediately cooled on ice and diluted with water to 10 ml. Then 0.1 ml. of each digest was chromatographed on Whatman paper no. 1. The distance between spots was 5 cm. A similarly treated solution of radioactive 5-HT (0.01 μc/ml.) was used as control and standard. It showed that the above treatment did not destroy the indole moiety. The paper strips were scanned with a Packard Radiochromatogram Scanner Model 7200, connected to a Packard recording rate-meter Model 380. The setting was as follows: 0.5 cm. speed/min. and 5 mm. window opening. It was found that under these conditions the size and the form of the spot affected the linearity of the determination only minimally. Radioactivity was calculated by cutting out the area of the recorded peaks, weighing them and comparing the weight with that of the standard. The final calculation took into account dilution, weight of the whole uterus and the standard error of the mean.

RESULTS

Table 1 shows the uptake of [14C]5-HT by blood plasma and uteri of steroid-primed rats which received homogenates of placentae, foetuses, uteri or plasma and of controls. Oestrogen treatment did not promote 5-HT accumulation in the uterus. Progesterone facilitated the accumulation of 5-HT in the uterus.

In columns 1 and 2, which refer to rats injected with oestrogen, 5-HT uptake/g. uterine tissue was small and not significantly ($P > 0.1$) different from the oil controls. None of the injected homogenates influenced 5-HT uptake by the uterus. Columns 3 and 4 show that treatment with large doses of progesterone increased 5-HT uptake by the uterus four- to six-fold, but none of the four injected homogenates produced a significantly higher uptake of 5-HT. When progesterone was given alone (column 5), the uterine uptake of 5-HT was approx. 50% higher than that in the other rats where placental homogenate was also given. In rats which received progesterone together with a low dose of oestrogen (column 6), the highest 5-HT uptake was obtained by adding placental extract: +260% uptake (14 μg./g. uterus in the saline control rats compared with 37 μg. in the rats injected with placental homogenate).

DISCUSSION

The results show that placental extracts played an important role in the accumulation of [14C]5-HT in the uterus. This role was particularly evident when the animals were pretreated with a combination of oestrogen and progesterone. Oestrogen treatment alone did not promote the uptake of 5-HT. This was also shown by Snyder et al. (1964).

It is well known that the 5-HT content of the uterus and of the placenta at different stages of pregnancy shows considerable fluctuations as a result of a decrease in placental content of monoamine oxidase (Koren, Pfeifer & Sulman, 1965). This has recently been confirmed by Contractor, Jones, Lee & Morris (1968).

In mice, Robson & Senior (1964) found an accumulation of 5-HT in placental and foetal tissue and suggested that the foetus is the primary site accounting for the rise in 5-HT in the intra-uterine tissue, though they did not exclude the possibility that the placenta may also be involved. The present study indicates that it is the placenta
**5-HT uptake by rat uterus**

which promotes uptake and accumulation of 5-HT in uterine tissue which may contain a protein system (‘trans-serotonin’) binding free 5-HT. Such a globulin system exists also for steroids (trans-cortin), iron (trans-ferrin) and vitamin B₁₂ (trans-corrin). ‘Trans-serotonin’ availability would seem to depend on an optimal oestrogen–progesterone ratio in the myometrium.

Another aspect of the presence of a placental ‘trans-serotonin’ is its possible role in binding 5-HT in the uterus during pregnancy and releasing it at the end of pregnancy due to a shift in the oestrogen–progesterone balance known to occur at term. It has been shown by us that this may trigger uterine contractions (Koren, Pfeifer & Sulman, 1966b).

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**REFERENCES**