UP TAKE OF OXYTOCIN IN TISSUES
AFTER INTRAVENOUS ADMINISTRATION OF
TRITIATED OXYTOCIN IN RATS

By
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ABSTRACT

The distribution of oxytocin in the kidneys, liver, uterus and skeletal muscle of the rat was followed during 10 min after intravenous injection of tritium labelled oxytocin. Oxytocin was found to be taken up and degraded mainly in the kidneys and the liver. After 150 seconds no intact oxytocin could be detected in these organs.

The time course of the distribution of the radioactivity in the liver and the skeletal muscle showed no noteworthy characteristics, whereas a different course was found in the kidneys and in the uterus. In the kidneys, the radioactivity increased continuously from 60 to 200 seconds after the injection, indicating an accumulation of oxytocin or its metabolites in the kidneys. In the uterus a high initial uptake was observed, followed by a decrease of the radioactivity from 60 to 100 seconds after the injection. This distribution pattern was specific to oxytocin, since the uptake of tritiated tyrosine and tritiated water was almost constant during the same time period. These findings may indicate a preferential distribution of oxytocin to the uterus.

Oxytocin has been shown to be readily distributed from the blood (for a review, see Ginsburg 1964). Using tritium-labelled oxytocin, the half-life in the blood of rats at different hormonal stages was estimated to be 73–97 s (Sjöholm & Rydén 1967). Pregnancy caused an almost significant decrease in half-life, indicating that the larger pregnant uterus might play an important role in the
distribution of oxytocin. The shorter half-life in pregnancy could, however, be
due to some other physiological changes, e.g., an altered blood distribution.
Ginsburg & Smith (1959) found, by exclusion of different organs, that the liver
and the splanchnic vascular area in rats are highly effective in taking up oxy-
tocin from the circulation. This was shown by Chaudhury & Walker (1959) to
be also true for the rabbit. However, in view of the rapid degradation of pep-
tides in tissues, it has not been possible to make any quantitative study of the
distribution by means of biological methods. The present quantitative study
with labelled oxytocin was therefore performed to investigate the factors reg-
gulating the half-life of oxytocin in blood, and to ascertain whether oxytocin is
specifically taken up in the uterus.

MATERIALS

Tritiated oxytocin was synthetized according to Carlsson & Sjöholm (1966) and
Sjöholm & Carlsson (1967). L-3-3H-tyrosine was used as starting material for the
synthesis. The specific activity was 4.2 μCi/IU oxytocin.

Female non-pregnant rats of the Sprague-Dawley strain, weighing 210–300 g, were
used.

METHODS

Preparation of tissue samples

The rats were anaesthetized with 25% urethane (ethyl carbamate) given intra-
peritoneally (0.5 ml/100 g body weight). A tracheal cannula was introduced, and thin
polyethylene tubes were inserted into the jugular vein on one side, and into the com-
mon carotid artery on the opposite side. The rats were heparinized with 400 IU of
heparin, and were given about 120 milliunits of tritiated oxytocin (1 × 10⁶ dpm) intra-
venously in 0.25 ml of physiological saline. In control experiments, the rats were given
tritiated tyrosine and tritiated water in the same way. The polyethylene tube was
rinsed with 0.10 ml of saline. Injection of the sample took about 10 s. A stopwatch
was started when half the amount had been injected. A blood sample of about 200 μl
was collected from the arterial cannula 30 s after the injection, and at a fixed time,
the abdominal cavity was opened. Within as short an interval as possible, the liver,
kidneys, uterus and a piece of the rectus muscle were cut out and put into small pre-
weighed glass flasks. When the radioactive metabolites had to be identified, the flasks
were cooled in dry ice and the flasks with the tissue slices stored in the deep freeze
until the analysis of the radioactive metabolites was performed.

Determination of tritium in tissues

Suitable amounts of the different tissues were freed from connective tissue and
blood, cut into pieces and dissolved in 1 ml of NCS solution (Nuclear Chicago Corp.)
at 40°C for 2–4 h in a counting vessel. Since blood-rich tissues, e.g. liver and kidneys,
cause severe colour quenching when the radioactivity is measured in the scintillation
spectrometer, it was found preferable to use not more than 60–80 mg of these tissues.
From the uterus and muscle, about 100–200 mg were taken. 10 ml of a solution of 0.3 g
dimethyl POPOP and 5 g PPO in 1000 ml toluene was added, and the tritium counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3875. The counting efficiency was calculated from the automatic external standardization (AES) built into the apparatus. The AES was calibrated with internal standards.

Thin-layer chromatography was performed as described previously (Sjöholm & Ryrfeldt 1967).

RESULTS

Distribution of oxytocin in tissues

The amount of tritiated oxytocin which reached the circulation was generally about 160 000 DPM/ml of serum, but varied down to 55 000 DPM/ml. To allow comparisons between experiments in different rats, the blood value (DPM/500 µl of serum) obtained in a blood sample taken 30 s after the injection was used as reference value. In these cases the radioactivity in the tissues was expressed as DPM/g wet tissue divided by the blood value at 30 s.

The distribution of oxytocin expressed as the total amount of radioactivity is shown in Table 1. Only values from early samples and from experiments with a high blood concentration of oxytocin are listed in Table 1. The kidneys and the liver showed the highest initial concentration of oxytocin, and even after 60 s, about 30% of the radioactivity injected was already recovered in these tissues. Although the amount of oxytocin taken up in the uterus was small, the concentration was 2–3 times higher than that in skeletal muscle.

The results of 15 experiments at different times are collected in Figs. 1–3. The values obtained are summarized in 4–5 groups. The first group consists of values from organs removed as soon as possible, i.e., about 60 s after the injection. Values after a half-life in blood of oxytocin (about 90 s) constitute the second group. The remaining groups are results from 3, 5 and 10 min, respectively, after the injection.

In all figures, the oxytocin distribution can be compared with that of tritiated tyrosine and water. These compounds were chosen as unspecific control substances, to investigate whether oxytocin is distributed specifically. The controls are mean values from a total of 21 separate experiments.

As expected, the distribution of tyrosine and water (the control) was more rapid than that of oxytocin, which reached lower relative concentrations in the liver, uterus and muscle. In the kidneys, however, a relatively greater concentration of oxytocin was found, and the radioactivity increased continuously from 60–200 s (Fig. 3). In pregnant women Rydén & Sjöholm (1969) found that after an intravenous injection of tritiated oxytocin between 25–50 per cent of the radioactivity was recovered in the urine within 3 h. Thin-layer chromatography did not reveal any traces of intact oxytocin in the urine. These results indicate that the kidneys are of great significance in the degradation of oxy-
Table 1.
Tritium distribution after intravenous injection of tritiated oxytocin (0.45 μCi, 0.12 IU) in the rat.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Blood serum After 30 s dpm/500 μl</th>
<th>Liver After 55–65 s</th>
<th>Kidneys After 50–60 s</th>
<th>Kidneys After 75–90 s</th>
<th>Uterus After 60–75 s</th>
<th>Uterus After 80–90 s</th>
<th>Skeletal muscle After 80–100 s dpm/g</th>
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<tr>
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Radioactivity content of rat liver and abdominal muscle at different times after intravenous injection of tritiated oxytocin. The tritium content at different times (+) is calculated as dpm/g wet weight divided by the radioactivity content of serum (dpm/500 µl) taken 30 s after the injection. ○ denotes the mean values at different time intervals, □ the mean values of the tritium content after injection of tritiated tyrosine and tritiated water as controls.

Radioactivity content of rat uterus at different times after intravenous injection of tritiated oxytocin. The calculations and symbols used are the same as in Fig. 1.
Radioactivity content of rat kidneys at different times after intravenous injection of tritiated oxytocin. The calculations and symbols used are the same as in Fig. 1.

tocin. The time course of the distribution of oxytocin showed no noteworthy characteristics in the liver and skeletal muscle (Fig. 1), whereas in the uterus a somewhat different pattern was found. Thus a high initial uptake was seen, followed by a decrease of the radioactivity (Fig. 2). The distribution pattern was specific for oxytocin, since the uptake of tyrosine and water was almost constant during the relevant time interval.

Thin-layer chromatography

Thin-layer chromatography disclosed that tritiated oxytocin was rapidly degraded in the liver and kidneys. A chromatogram was previously presented showing that practically no intact oxytocin remains in kidneys removed 150 s after injection of oxytocin (Sjöholm & Ryrfeldt 1967). Moreover, Fig. 4 shows that only about 10 % of the radioactivity in a liver removed as early as 60 s after injection, could be ascribed to intact oxytocin. In the blood, however, the main amount of the radioactivity for a relatively long time after the injection originated from oxytocin. This can be seen in Fig. 5, which is a chromatogram of a serum sample taken 120–180 s after the injection of tritiated oxytocin.

DISCUSSION

Aroskar et al. (1964) studied the distribution of radioactivity in male rats after the injection of high doses of labelled oxytocin with tritium in the leucyl residue. They found a rapid urinary excretion of the radioactivity, and estimated the distribution in body tissues after 20 and 60 min. After such a long time, however, the oxytocin is completely degraded, which implies that their figures
Thin-layer chromatography in n-butanol-dioxane - 2 n ammonia on cellulose-silica gel G of an extract of a rat liver taken 60 s after intravenous injection of tritiated oxytocin. The upper part shows the distribution of the radioactivity, and the lower part the migration of oxytocin (Oxyt), the opened oxytocin nonapeptide (Nona) and tyrosine (TYR).

Fig. 4.

do not show the distribution of oxytocin. This was also evident from their biological and chromatographical investigations of the urine. Only 9 units of the total dose of 75 units of oxytocin were excreted, and two oxytocin metabolites were identified chromatographically. In the present study, about 50 mU of oxytocin per 100 g body weight were administered, and the distribution was studied very soon after the injection. Our chromatograms of samples from liver and kidney tissues also indicate a very rapid degradation of oxytocin in these organs, since only small amounts of intact oxytocin could be detected 60–150 s after injection. As it can be expected that equilibrium with the circulating blood will be reached within a few minutes, the values obtained from the last 3 groups of rats (180–600 s after injection) cannot reflect the tissue distribution of oxytocin. However, as the main part of the radioactivity in the blood is intact oxytocin as long as 120–180 s after injection, the values obtained in the first two groups will, in fact, reflect the distribution of oxytocin, even if the oxytocin, when taken up in the organs, is thereafter rapidly degraded. After intravenous injection of tritiated oxytocin, the main part of the radioactivity is rapidly taken up in the liver and the kidneys. The concentration in the kid-
Thin-layer chromatography in n-butanol-dioxane – 2 N ammonia on cellulose-silica gel G of a serum sample from a rat taken 120–180 s after intravenous injection of tritiated oxytocin. The upper part shows the distribution of the radioactivity, and the lower part that of oxytocin (Oxyt) and tyrosine (Tyr).

neys after 100 s is about 3 times that in the liver, 10 times that in the uterus and 15 times that in skeletal muscle. As the kidneys weigh about 1.5 g together, the uterus 0.25 g and the liver 6–9 g, this implies that the clearance and degradation of oxytocin in the kidneys and the liver play the most important role in the metabolism of oxytocin. Thus, it can be seen from Table 1 that about 30% of the injected oxytocin is taken up in the liver and kidneys 60 s after injection, or altogether much more than 50% of the amount which has left the circulation, since the half-life of oxytocin in blood is about 90 s. At the same time, only about 0.5% of the oxytocin is bound in the uterus. Even if more oxytocin is bound to the pregnant uterus, the figures clearly demonstrate that non-targent organs, such as the liver and kidneys, play a fundamental role in determining the half-life of oxytocin in the blood. This is most probably also true for other physiologically active peptides, which have been shown to have a short half-life in blood. The tritium content of the liver and skeletal muscle showed no drastic changes between 60–600 s. However, the uterus curve (Fig. 2) had a different course, with a high initial concentration of radioactivity, which decreased from 60 to 100 s after the injection. That this distribution pattern is
specific for oxytocin was further demonstrated with the help of tritiated tyrosine and tritiated water. Their distribution was studied in the same manner as that of oxytocin. In contrast to oxytocin, these probably inert substances exhibited no high initial uptake in the uterus. Our results may indicate that oxytocin has a relatively high affinity for the uterus in non-pregnant rats.

Doubts have previously been expressed about the physiological significance of the oxytocin-inactivating enzyme, plasma-oxytocinase, on the inactivation of oxytocin in the blood of pregnant women (Rydén 1966; Sjöholm 1968). The rapid degradation of oxytocin in the liver and the kidneys, as well as the high affinity of oxytocin for the uterus, cast further doubts on this hypothesis. Certainly, the physiological conditions in women can differ considerably from the experimental conditions used in this study. However, a short half-life of oxytocin in the blood and a rapid excretion of the degradation products of oxytocin in the kidneys was recently demonstrated in both non-pregnant and pregnant women (Rydén & Sjöholm 1969). Thus, the liver and kidneys are probably equally important for the metabolism of oxytocin in man, thus decreasing the relative significance of the inactivation which occurs in the blood of pregnant women.

ACKNOWLEDGMENTS

The financial support by the Swedish Medical Research Council (Project No. B68-14X-2079-02) and the technical assistance of Miss Ulla Skogh are gratefully acknowledged.

REFERENCES


Received on October 2nd, 1968.