

OXYTOCINASE AND ITS POSSIBLE SIGNIFICANCE IN THE DEGRADATION OF OXYTOCIN DURING PREGNANCY*

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1. Introduction

Although, during the past four decades, a great deal of work has been focused on factors known to regulate human pregnancy and parturition, the complex mechanisms involved in these physiological events are still not completely known. Since 1930, when Fekete [1] observed that the plasma from pregnant women inactivated the oxytocic principle from the posterior pituitary gland, the enzyme oxytocinase causing this inactivation has been assigned an important role, in regulating the blood oxytocin during pregnancy. The present review will provide a basis for discussion of the possible physiological significance of this oxytocin-oxytocinase equilibrium for the initiation of labour at the end of pregnancy.

In fig. 1, the inactivation of oxytocin caused by oxytocinase is included as one of the processes, which regulate the free circulating amount of oxytocin in blood, besides the uptake and metabolism of the hormone in the liver, kidneys and possibly also in other tissues. Indicated as an uncertain mechanism is the possible complex formation between oxytocin and blood proteins. It is apparent from this scheme, that the relative physiological importance of oxytocinase will, of course, depend on the concentration of the enzyme, the presence of enzyme inhibitors or activators in the blood, the inactivation rate, i.e. the properties of oxytocinase, as well as the time needed to transport the oxytocin from the pituitary

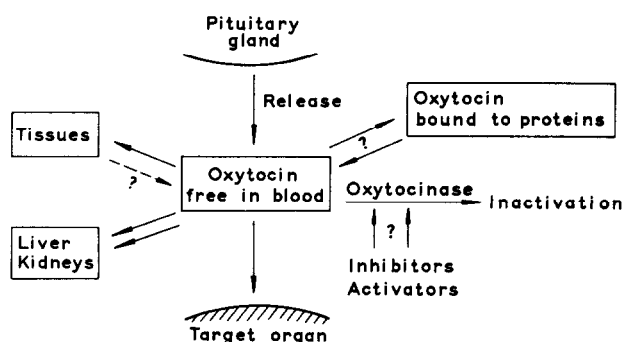


Fig. 1. Summary of some mechanisms regulating the free concentration of oxytocin in blood of pregnant women.

gland to the target organ, i.e. the half-life ($t_{1/2}$) of oxytocin in the blood, and the extent of the uptake and inactivation of oxytocin, which takes place in the liver, kidneys and other tissues.

2. Oxytocinase

Oxytocinase reaches its highest concentration in the blood at term [2–5]. No decline in the enzyme activity has been observed immediately before delivery, thus giving no support to the theory that a diminished synthesis of oxytocinase initiates labour. The individual variations are, however, large. Even if certain pathological conditions are known to increase or decrease the blood oxytocinase [3,4,6,7,8], the differences from the normal values will be too small to yield statistically significant discrepancies. This

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means, unfortunately, that the value of oxytocinase estimations as a diagnostic tool has, hitherto, been limited. However, in cases of multiple pregnancy, the oxytocinase concentration has been proved to be higher than that in single pregnancies [3,9].

In view of its high oxytocinase content, retroplacental blood serves as a convenient starting material for preparation of the enzyme and essentially homogeneous preparations have been obtained after ethanol fractionation and chromatography on hydroxylapatite, Sephadex G-200 or ion exchangers [10–14]. Purified oxytocinase gives rise to two bands in both polyacrylamide-gel and vertical starch-gel electrophoresis [15–21]. The two bands have the same ability to degrade oxytocin, lysine-vasopressin and L-cystine-di- β -naphthylamide [18] and form a reversible pH-dependent equilibrium with each other in the pH range 8.2 to 9.6 [19]. The two bands represent two different conformations of the enzyme, most probably obtained by different spatial arrangement of the peptide chains around the sialic acid residues, as treatment of the enzyme with neuraminidase yields a preparation, which gives only *one* band in starch-gel and polyacrylamide-gel electrophoresis [13, 19, 22]. In some cases, sera from pregnant women have been shown to contain also a third new peptidase band [23–25], the properties of which, however, are not yet known.

Tuppy et al. initially showed that oxytocinase is a glycoprotein, containing sialic acid [22]. Additionally, we have found in preliminary experiments neutral hexoses and amino sugars, but no 2-deoxy sugars. The total carbohydrate content in oxytocinase has been calculated to be around 40%. Its molecular weight, estimated on the basis of the sedimentation constant and the Stokes' radius obtained from gel-filtration data, is 325,000 [26].

The enzyme specificity of oxytocinase is presently well documented, as far as synthetic amino acid derivatives [22, 27], cysteine peptides [11, 28–30], other dipeptides and oligopeptides [2, 31–34] are concerned. The enzyme has a broad specificity, hydrolyzing all natural peptide bonds, except imido-peptide bonds, from the *N*-terminal end, when the *N*-terminal amino acid has a free amino group and is of L form. This property gives oxytocinase the position as the only single enzyme known to hydrolyze *N*-terminally situated half-cystinyl bonds, which ex-

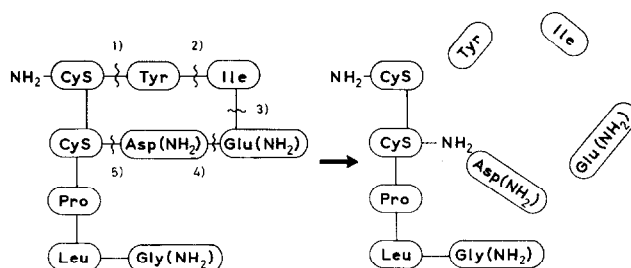


Fig. 2. Degradation of oxytocin with oxytocinase.

plains the synonymous use of the term "cystine aminopeptidase"(CAP) for oxytocinase.

Because of its broad specificity, oxytocinase causes complete degradation of oligopeptides into amino acids and, if proline is present, peptides with proline next to the *N*-terminus [32–34]. This is illustrated in fig. 2, showing degradation of oxytocin with a pure oxytocinase preparation [33]. However, large polypeptides such as insulin, or proteins such as human serum albumin, are only inappreciably degraded [34]. The size of a peptide showing the highest affinity between peptide and enzyme and the highest reaction velocity remain to be investigated.

3. Oxytocin

To allow any conclusions to be drawn about the physiological significance of oxytocinase in blood, the distribution rate of oxytocin from blood, i.e. the half-life ($t_{1/2}$), and the relative uptake in the target organ(s) compared with the uptake and storage in other tissues, have to be known. Ginsburg has earlier compiled in a review [35] the facts known up to 1963 in this field.

The clearance from blood of oxytocin, vasopressin and some of their synthetic analogues has been shown to proceed rapidly, the $t_{1/2}$ being estimated as about 100 sec in the rat [35, 36], 4–8 min for arginine-vasopressin in males [37, 38], and 1.2–4 min for oxytocin in pregnant women [39]. In the great majority of cases, the estimations were based on the biological activity remaining after intravenous single doses, or after interruption of steady-state infusions. In these situations, however, large "pharmacological" doses

were given, making the physiological interpretation uncertain. Since radioactively labeled hormones with high specific radioactivity are now available [40], it has been possible to decrease the doses used substantially (unfortunately not yet to the "physiological" level), and to make more extensive studies under different hormonal conditions in animals and in human beings.

The half-life of oxytocin in blood seems to be influenced by other hormones. This has been found to be so in the rat, since pregnancy causes a decrease in $t_{1/2}$ of both oxytocin and lysine-vasopressin (from 97 and 113 sec to 79 and 99 sec, respectively [36]). The values from the non-pregnant rats are in good accord with those obtained with higher doses of non-radioactive hormones, which may imply that the dose is of secondary importance. Moreover, it is to be noted that oophorectomy causes an increased clearance from the blood, and that oestrogen treatment of these rats partly restores the $t_{1/2}$ to non-pregnant values [36].

Clearance studies of oxytocin in pregnant and non-pregnant women are scarce, owing to the difficulties of estimating small amounts of oxytocin. The values of Gonzales-Panizza et al. ($t_{1/2} = 1.2-4$ min) were obtained from 4 patients with dead fetuses near the calculated term, or *post partum* after macro-

infusions of 8-18 IU per min [39]. Our own studies [42] were performed on about 50 non-pregnant and pregnant volunteers. The non-pregnant women consisted both of those menstruating normally, who were classified according to the day of their menstruating cycle, and of those taking contraceptive tablets with gestagenic and oestrogenic hormones. The pregnant women were hospitalized for legal abortion, either by abrasio (pregnancy ≤ 12 weeks) or by intra-amniotic instillation of hypertonic glucose (pregnancy 14-20 weeks). In the latter group, $t_{1/2}$ was also determined within 24 hr of the abortion, in addition to the estimation made in all patients just before the instillation or operation. The results are summarized in table 1.

The values in the non-pregnant group taking contraceptive tablets are significantly lower than those in women in the oestrogen-dominated part of the cycle, which is in accord with the results in the rats. Among the pregnant women, a decrease in $t_{1/2}$ was seen in all cases after abortion, and, generally, the change was very evident. The decrease in $t_{1/2}$ was not followed by a similar marked increase in the blood oxytocinase concentration (CAP). Although a slight increase was seen in many patients, this increase cannot be considered as significant, as tissue injuries are often followed by an increase of the peptidase activity in blood.

Table 1
Half-life of oxytocin in blood of non-pregnant and pregnant women.

Group of women	Hormonal stage	$t_{1/2}^*$ (sec)
Non-pregnant	Proliferative phase	272 ± 14.2 (14)
	Secretory phase (18th-22nd day)	221 ± 14.9 (5)
	On oral contraceptives (gestagens + oestrogens)	199 ± 9.8 (10)
Pregnant	≤ 12 weeks pregnancy	178 ± 13.9 (6)
	14-17 weeks pregnancy	
	prior to abortion	295 ± 47.2 (6)
	after abortion	229 ± 21.5 (6)
	18-20 weeks pregnancy	
	prior to abortion	282 ± 21.3 (6)
	after abortion	181 ± 14.0 (6)

* Expressed as arithmetic mean \pm standard deviation followed by the number of observations in parentheses.

The increased CAP activity might then be due to damage to the placenta, which contains large amounts of oxytocinase [8]. The markedly decreased $t_{1/2}$ after abortion might instead have been due to a change in the ratio between gestagenic and oestrogenic hormones, but it is more probable that more complex unknown regulatory mechanisms are involved in the distribution of oxytocin. This is further illustrated early in pregnancy (before the 12th week), when oxytocin is distributed more rapidly than later (in the 14th–20th weeks), despite a smaller myometrium and placenta. Nevertheless, the studies on the half-life of oxytocin have revealed that gestagens increase the distribution rate, and oestrogens decrease it. It remains to be shown whether these results are due to effects on unspecific organs such as the liver and kidneys or on the specific target organs.

Slight effects on the liver and kidneys can be expected to result in marked changes in the $t_{1/2}$ in blood. Both vasopressin and oxytocin are mainly taken up in these organs [35,41,43,44]. By direct measurement of the radioactivity content in different tissues in the rat after intravenous injection of tritiated oxytocin, previous evidence has been quantitatively confirmed [45,46]. Twenty to thirty percent of the total amount of oxytocin injected was taken up in the liver and kidneys 60 sec after injection, which corresponds to more than 50% of the amount which has left the circulation [46], as $t_{1/2}$ is around 100 sec. In women, 40–50% of the injected radioactivity was recovered in the urine within 3 hr [42].

4. Conclusions

Oxytocin is of fundamental importance in regulating uterine muscle activity during delivery. Oxytocinase has also been ascribed a role as the specific oxytocin-inactivating component of the blood. Convincing proof exists that oxytocinase has the ability to degrade the hormone enzymatically, but, at present, the physiological significance of the inactivation mechanism is doubtful. Some facts about oxytocin and oxytocinase are known from late stages of the pregnancy, or from women in labour, but most knowledge has been gained from studies in animals, in non-pregnant women or in women in early pregnancy. Certainly, hormonal and other regulatory conditions

are changed up to delivery, but if the results are assumed to be valid during delivery as well, the following short summary can be made of the apparently most important factors arguing for and against oxytocinase as an important regulating enzyme, at the end of pregnancy.

Against the importance of oxytocinase

(1) The concentration of oxytocin in blood is very low ($< 1 \mu\text{unit/ml}$), and even if the release of oxytocin from the pituitary is increased during labour [47–49], the low concentration decreases the chances of oxytocin being degraded because of competitive inhibition of other peptides.

(2) The half-life of oxytocin in blood is short.

(3) Oxytocin is taken up extensively in the liver and kidneys, and probably almost completely inactivated there. This means that the relative importance of inactivation in the blood is small.

(4) In view of the broad specificity of oxytocinase, other peptides are also attacked by the enzyme. However, the relative affinity of oxytocin to the enzyme and the inactivation rate in comparison to that of other peptides have not hitherto been established.

(5) The transport form of oxytocin is not yet known. There still exists the possibility, which is not, however, probable as judged by the short $t_{1/2}$, that oxytocin is bound to a protein in blood. This can take place in such a way that the inactivation is either excluded (as for instance, when the α -amino group is bound to neurophysin in the pituitary gland) or decreased.

For the importance of oxytocinase

(1) Oxytocinase reaches its highest concentration at term. However, the blood level is still low, about 4 mg/l [13].

(2) In pure incubation mixtures, oxytocin is readily degraded by oxytocinase.

Uncertain factors are, among others, the oestrogens and gestagens, which reach high concentrations near term, and which have been shown to considerably affect the clearance rate of oxytocin from blood. The mechanism of their action, however, still remains unclear. Another very important factor, which still is unknown, is the threshold dose of oxytocin for response in the target organ and the effect of small variations around this dose. Knowledge in these matters will simplify the estimation of the importance of oxytocinase during late pregnancy.

To sum up, the physiological importance of oxytocinase in blood does not at present appear convincing. It therefore seems reasonable to speculate that the presence of the enzyme in blood has no functional explanation, but merely reflects the increased metabolic activity of the placenta, from which the enzyme leaks out into the circulation. The latter hypothesis is also supported by the results of Riad [50] and Branda et al. [51], who found that oxytocinase was released into the blood during labour.

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