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Deamino-oxytocin: Inactivation by Plasma of Women in Labor

Abstract. *Incubation of deamino-oxytocin with plasma obtained from women in labor reduced the potency of this analog of oxytocin when assayed on an isolated strip of mammary gland taken from a lactating rat. Plasmas of nonpregnant women had no detectable effect on this activity of deamino-oxytocin, and the effect of plasmas from women just previous to the beginning of labor was not significant. The original activities of the incubated deamino-oxytocin solutions could be restored by treatment with hydrogen peroxide. The inactivation may be caused by reductive cleavage of the disulfide bridge of deamino-oxytocin, this bridge being reformed by oxidation.*

Deamino-oxytocin (*1*) is an analog of the neurohypophysial polypeptide hormone oxytocin and, in contrast to oxytocin, does not possess a free amino group (Fig. 1). It has more powerful uterotonic (in rats), milk-ejecting (in rabbits), and vasodepressor (in fowls) activities (*2*) than the natural hormone does (*1*, *3*).

In humans, oxytocin appears to be the hormone responsible for milk ejection and to be implicated in the processes of labor. Yet deamino-oxytocin, when given to women in labor or puerperium, is even more potent than oxytocin in producing contractions of the uterus (*4*) and mammary gland (*5*).

These results, of great importance in attempts to clarify the mode of action of oxytocin, have furthermore led to an increasing interest in the possible use of deamino-oxytocin in obstetrics.

During human pregnancy an enzyme capable of inactivating oxytocin appears in the blood. It is apparently produced by the placenta (*6*). It has been called "plasma oxytocinase" and is an aminopeptidase of rather broad specificity (*7*), and still undetermined function. The enzyme produces a rupture of the ring of oxytocin by hydrolysis of the hemicystinyl-tyrosyl bond. Deamino-oxytocin, lacking an amino group, would not be expected to be attacked by this aminopeptidase, and indeed incubation with serum from women in labor does not affect the avian vasodepressor activity of deamino-oxytocin under conditions in which this activity of oxytocin is virtually completely destroyed (*8*).

There exists another "oxytocinase," which occurs in highest concentrations in the liver and kidneys and which appears to cleave the disulfide bridge in oxytocin by acting as a thiol oxidoreductase for disulfide bonds in protein. This action is followed by aminopeptidase degradation (*9*). This system, "tissue oxytocinase," is not specific to pregnancy, but there is evidence that it does occur in placental tissue. It is very much less stable than "plasma oxytocinase" and rapidly loses its activity at room temperature (*6*). There are reports of the finding of trophoblastic fragments in the general circulation in postpartum (*10*), which may mean that "tissue oxytocinase" of placental source could also occur in the blood, although this enzyme system is apparently not normally found extracellularly. Deamino-oxytocin, possessing a disulfide bond, should be susceptible to attack by a thiol oxidoreductase. The resultant dithiol-containing peptide would still presumably be resistant to amino peptidase attack. *S,S'*-Dihydrodeamino-oxytocin, deamino-oxytoceine, is much less potent than deamino-oxytocin as an avian vasodepressor agent (*11*). Therefore the formation of deamino-oxytoceine by the action of "tissue oxytocinase" on deamino-oxytocin could be detectable by biological assay.

We have investigated the effect of plasma of women in late pregnancy and labor on deamino-oxytocin, as expressed by changes in its biological activity, with care being taken to avoid the inactivation of labile enzyme systems.

Blood (20 ml) from the brachial vein of women in advanced pregnancy or active labor was collected in a cooled receiver. Immediately after extraction it was centrifuged at approximately 2°C for 10 minutes at 1000*g*, and the plasma was used at once. Plasma (2 ml) at 37°C was added to a mixture of barbital buffer (*p*H 7.9, 1 ml) and either oxytocin (*12*) (1 ml aqueous solution, containing approximately 2×10^{-3} μmole, with about 1 unit of milk-ejecting activity) or deamino-oxytocin (*12*) (1 ml aqueous solution containing approximately 2×10^{-3} μmole, with about 1 unit of milk-ejecting activity) at 37°C. A sample (1 ml) was taken from each mixture immediately after the addition of the plasma (zero time) and was added to a test tube containing water (3 to 4 ml) in a water bath at

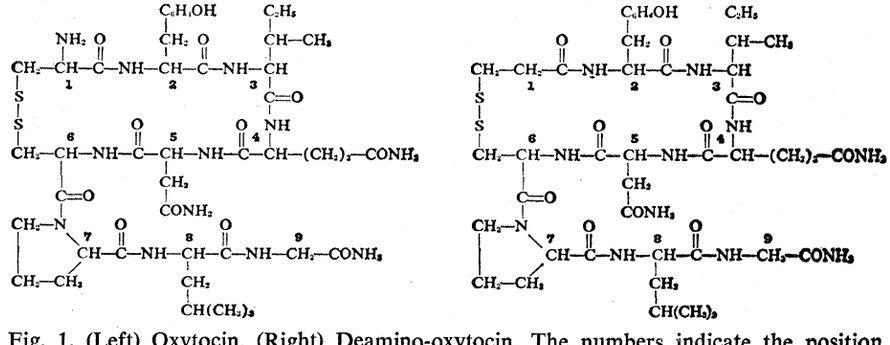

Fig. 1. (Left) Oxytocin. (Right) Deamino-oxytocin. The numbers indicate the position of individual amino acid residues in the structure.

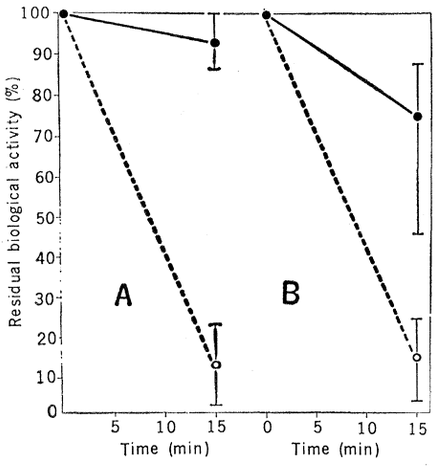

Fig. 2. Average percentage of residual biological activity (contraction of isolated strip of mammary gland of lactating rat) found in solutions of oxytocin (○) and deamino-oxytocin (●) after incubation with plasma from women just before labor (A) and during labor (B). The experimental range is shown for each average value.

90°C. After 10 minutes the tubes were taken from the water bath and cooled to room temperature, when the volumes were adjusted to 5 ml.

The procedures of sampling and heat-inactivation of the enzymes were repeated 15 minutes after the addition of the plasma. All mixtures were kept frozen until they could be assayed. Control samples taken from nonpregnant women were treated similarly.

The method of assay was the measurement of the contraction produced by oxytocin or deamino-oxytocin on an isolated strip of mammary gland taken from a lactating rat (*13*). Tyrode solution was used as the bathing fluid and for the preparation of all necessary dilutions of the solutions to be assayed. In one series of assays, synthetic oxytocin was used as the standard for the assay of the oxytocin solutions and deamino-oxytocin for that of the deamino-oxytocin solutions. In another series, the samples incubated for 15 minutes were assayed against the zero-time samples. Similar results were obtained from both methods.

We have studied the effect on the activity of oxytocin and deamino-oxytocin of incubation with plasma from seven women just before the onset of labor, eight women in active labor, and five nonpregnant women, the last group serving as controls. The biological assays were performed by means of the four-point parallel line assay method (Fig. 2).

The plasmas from the patients just before onset of labor appear to slightly inactivate deamino-oxytocin but this was not statistically significant. However the plasmas from the patients in labor do significantly inactivate deamino-oxytocin. The average of the activity after 15 minutes of incubation was 74.6 percent of the original. The control plasmas had no effect on the activity of deamino-oxytocin under consideration.

One patient was admitted with intermittent, painful uterine contractions and some cervical dilatation and her plasma caused some inactivation. The contractions then stopped, and after 2 days without further progress her plasma caused no inactivation; inactivation was again produced by her plasma when she was in active labor.

If, as we have suggested, this inactivation of deamino-oxytocin is caused by the reductive cleavage of the disulfide bridge to give deamino-oxytoceine, and if no further degradation takes place, it should be possible to restore the original activity by re-formation of the disulfide bridge by mild oxidation. From another viewpoint, if oxidation restores the activity, this would provide some evidence that the loss of activity on incubation had been due to reduction. The most readily reversible reduction in molecules possessing the ring of oxytocin, occurs at the disulfide bridge.

Therefore, in three cases in which inactivation of deamino-oxytocin had been observed in the 15-minute sample, we added a solution of hydrogen peroxide (50 μl of a $2 \times 10^{-3}M$ solution) to both the zero-time and 15-minute samples. After this treatment, the zero-time and 15-minute samples were reassayed and found to be undistinguishable in potency, showing that the original biological activity had been restored.

There are several other obvious factors to be examined. The plasma of women postpartum must be examined to determine how long the effect on deamino-oxytocin, noted during labor, lasts. If, as we have suggested is possible, deamino-oxytocin is inactivated by "tissue oxytocinase," inactivation should be more marked at *p*H's lower than the ones used in our series of experiments (7.9) since the *p*H for optimum activity of "tissue oxytocinase" is about 6.3 (*6*). If the product of the observed inactivation of deamino-oxytocin is deamino-oxytoceine, this product may cause some contraction in the strip of mammary gland, in which case the degree of inactivation measured would be somewhat less than the true extent of molecular cleavage.

We do not feel that these results conflict with those of Golubow *et al.* (*8*), who found that the avian vasodepressor activity of deamino-oxytocin was unaffected after 4 hours of incubation at *p*H 7.9 with serum from women in labor. These authors were interested in determining whether the relatively stable "plasma oxytocinase" could attack deamino-oxytocin, and for that purpose, they used serum for their studies. We think that we may be studying the action of the extremely labile "tissue oxytocinase," the activity of which could be lost in the time taken for serum to separate (*14*).

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12. The milk-ejecting potencies of the synthetic oxytocin and the deamino-oxytocin used were approximately 400 and 500 units per milligram, respectively.
13. On the basis of the observation that the avian vasodepressor potency of deamino-oxytoceine is much lower than that of deamino-oxytocin (*11*), we have made the assumption that deamino-oxytoceine would also have a lower potency than deamino-oxytocin when assayed on an isolated strip of mammary gland from a lactating rat. [M. W. Smith, *Nature* **190**, 541 (1961)].
14. Indeed, in a single experiment, we found that plasma from blood taken from a woman in labor inactivated deamino-oxytocin, whereas serum separated from the same blood had no effect on the biological activity under consideration.
15. We thank Dr. B. Anido of Laboratorio Dispert, S.A., Montevideo, for providing the rats for the biological assays. Supported by PHS grant HD-01587.

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