ELIMINATION OF DEAMINO-DICARBA-OXYTOCIN FROM BLOOD STREAM AND UTERINE COMPARTMENT OF RAT

J.VANĚČKOVÁ[a], T.BARTH[a], K.JOŠT[a], I.RYCHLÍK[a], P.FROMAGEOT[b] and J.L.MORGAT[b]

[a] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6 and*
[b] *Service de Biochimie, Centre d'Études Nucleaire de Saclay 91, Gif-sur-Yvette, France*

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The half-life of the uterotonic effect of deamino-dicarba-oxytocin and of oxytocin infused to rats and the levels of both tritium-labelled compounds in blood during and after the infusion were investigated. The half-life of the uterotonic effect and the half-life of deamino-dicarba-oxytocin in blood are higher than the corresponding parameters of oxytocin. Deamino-dicarba-oxytocin is not inactivated in rat blood plasma.

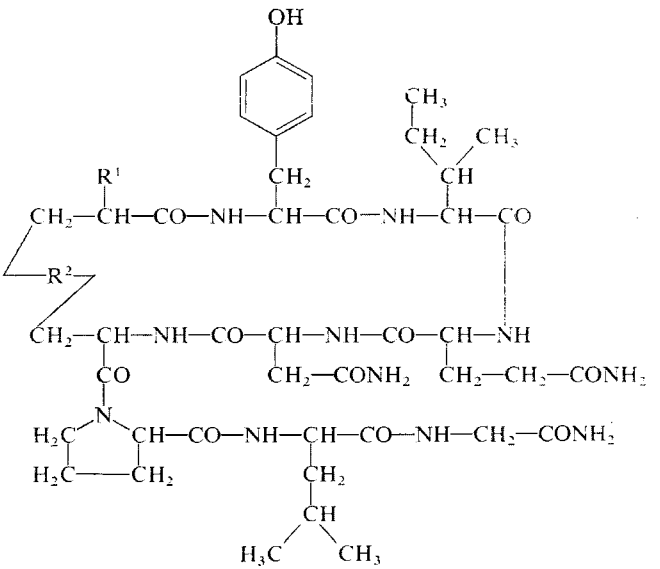
The degree of metabolic stability of the analogs of neurohypophyseal hormones is one of the factors limiting the duration of their biological effect. We have observed with oxytocin earlier[1] that especially the changes in the amino-terminal portion of its molecule lead to a protraction of the uterotonic effect while its high specific activities are retained. These changes involve the replacement of the α-amino group of cysteine at position 1 by a hydrogen atom and the replacement of the disulfide bond by a thioether or ethylene group; the replacement of the sulfur atom of cysteine at position 6 of the polypeptide chain by a methylene group shows a markedly profound effect. This type of analogs (the so-called carba analogs) is inactivated at a far slower rate by homogenates of certain target tissues[2,3]. We considered interesting to study the relation between the stability of the carba analogs in the uterus, the half-life of their uterotonic effect, their half-life in the blood stream and their stability towards the enzymic degradation systems of the blood. To cast light on these relations we chose deamino-dicarba-oxytocin (*Ib*) and oxytocin (*Ia*) as a reference substance.

EXPERIMENTAL

Materials

Deamino-dicarba-oxytocin was synthetized in the Institute of Organic Chemistry and Biochemistry and its characteristics were identical to those reported elsewhere[4]. Tritiated oxytocin[5] of specific activity 41 Ci/mmol and tritiated deamino-dicarba-oxytocin[6] of specific activity

60 Ci/mmol were prepared in the Centre d'Études Nucleaire de Saclay. Oxytocin used in the biological experiments was a commercial product of Spofa, Prague. Hyamine hydroxide was from Koch-Light (England).



Oxytocin *Ia*, $R^1 = NH_2$, $R^2 = S—S$
Deamino-dicarba-oxytocin *Ib*, $R^1 = H$, $R^2 = CH_2—CH_2$

Methods

Uterine activity *in vivo* was determined as described earlier[1,7]. Female Wistar rats (farm Konárovice) weighing 180—220 g were used in the experiments. Uterine activity was examined by the isometric method. Oxytocin or deamino-dicarba-oxytocin were infused at a rate of 0·6 ml/h into the femoral vein. The infusion was terminated after attaining steady state approximately after 25—30 min. The labelled hormone or analog were added to the solution infused approximately 10—15 min before the end of the infusion. Blood samples (three to four, 100—150 μl each) were withdrawn from the other femoral vein during the infusion for radioactivity measurement. (The rats were administered 500 units of heparin into the femoral vein before the removal of the blood samples). The remaining samples were withdrawn after the end of the infusion: at 30-s intervals during the period of 3—4 min after the infusion and at 2—5 min intervals later. The uterine activity of the experimental animals was continuously registered during the infusion and thereafter. The samples were centrifuged and plasma was removed. Aliquots of plasma (50 μl) were mixed with 0·3 ml of hyamine hydroxide and the mixture was heated 30 min at 60°C in a water bath. A mixture (10 ml) of the Bray solution and a toluene solution (1 : 2) was added to the sample dissolved and radioactivity was measured in Model 3375 Packard Tri Carb Scintillation Spectrometer. The half-life of the uterine response was calculated from the decrease in the number of uterotonic contractions by the method of Pliška[7]. The half-life of the tritiated com-

pounds in blood was calculated from the rate constants of elimination of the hormones from plasma; the elimination was regarded as a first order reaction.

Incubation of Oxytocin and Deamino-dicarba-oxytocin with Rat Plasma

The incubation mixture contained $2{\cdot}2 \cdot 10^{-4}$ μmol of oxytocin or $2{\cdot}5 \cdot 10^{-4}$ μmol of deamino--dicarba-oxytocin and 100 μl of blood plasma (approximately 5—6 mg of proteins); the volume of the mixture was made up to 1 ml with water. The samples were incubated at 37°C and the reaction was discontinued by 3 min boiling at time intervals of 0—90 min. The precipitate was centrifuged off and the concentration of the active substance remaining in the supernatant was determined on isolated rat uterus according to Holton[8].

RESULTS AND DISCUSSION

The method described permitted us to simultaneously examine the level of the hormone in blood and in the receptor compartment of the rat uterus. The record of the change in radioactivity during the infusion of the labeled hormone and after the

TABLE I

Elimination of Deamino-Dicarba-Oxytocin and Oxytocin from Uterine Compartment and Blood
The number of experiments is given in brackets.

Compound	Half-life of response	Half-life of labelled compound in blood
Oxytocin *Ia*	2·98 ± 1·12 (5)	4·42 ± 2·08 (5)
Deamino-dicarba-oxytocin *Ib*	11·7 ± 1·24 (3)	7·7 ± 3·35 (4)

infusion, as well as the changes in uterotonic activity are indicated of Fig. 1. The values of half-lives are given in Table I (after processing by the Student test for confidence interval 0·95). A longer half-life of deamino-dicarba-oxytocin in the receptor compartment of rat uterus, demonstrated by us earlier[1], was observed when equimolar solutions of compounds *Ia* and *Ib* were infused and equal doses of radioactive compounds were applied. A comparison of the half-lives of tritiated compounds *Ia* and *Ib* points to a lower rate of disappearance of deamino-dicarba-oxytocin from the blood stream.

In this study the metabolic stability of deamino-dicarba-oxytocin *Ib*, observed with the homogenates of liver, kidney, and uterus, was investigated in blood plasma. The difference in the metabolic stability of the two compounds is clearly shown

in Fig. 2, demonstrating the time profile of the change in concentration of oxytocin and deamino-dicarba-oxytocin during their incubation with blood plasma. Deamino--dicarba-oxytocin is not inactivated under the experimental conditions chosen. This leads us to postulate that the removal of radioactivity of blood plasma observed can be accounted for by the decrease of labeled radioactive deamino-dicarba-oxytocin by tissues. In view of the lower stability of oxytocin in some tissues, even its degradation products can contribute to the radioactivity of plasma samples. We may therefore expect that the difference in the half-lives of intact compounds *Ia* and *Ib* in the blood stream will be in fact higher than the difference experimentally determined in this study.

Since deamino-dicarba-oxytocin is not degraded by plasma alone and since its half-life in blood *in vivo* is increased its protracted effect can be accounted for by higher stability in tissues and especially in the uterine compartment. This conclusion is in agreement with the observations made with tissues homegenates.

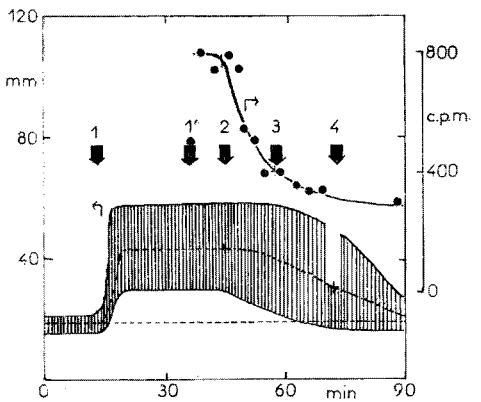


FIG. 1

Level of Tritiated Deamino-dicarba-oxytocin in Blood and Uterine Activity (Schematic) of Rat during and after Infusion of Analog *Ib*

1 Beginning of infusion of analog *Ib*, 1' beginning of infusion of mixture of *Ib* and tritiated deamino-dicarba-oxytocin, 2 end of infusion, 3 half-life of tritiated deamino--dicarba-oxytocin in blood, 4 half-life of biologic effect. Both records are synchronous, abscissa-time (min), ordinate-uterine contraction in mm, radioactivity, c.p.m. (●)

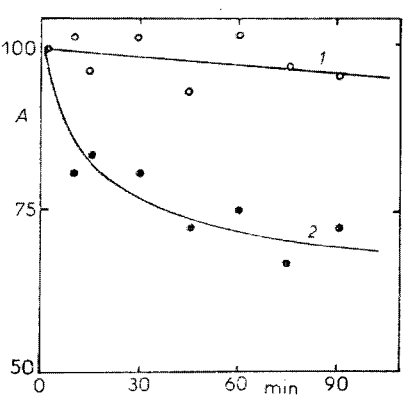


FIG. 2

Inactivation of Deamino-dicarba-oxytocin and Oxytocin

See Experimental for the detailed arrangement of the experiment. Abscissa-time (min), ordinate-per cent of remaining biological activity. *1* Deamino-dicarba-oxytocin, *2* oxytocin.

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