BIOLOGICAL ACTIVITIES AND PROTRACTED ACTION OF CARBA-ANALOGUES OF DEAMINO-OXYTOCIN WITH O-METHYLTYROSINE IN POSITION 2

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The biological potency and the duration of the effect of two analogues of deamino-oxytocin, having the disulfide bond substituted by a thioether group and the tyrosine residue replaced by O-methyltyrosine, were compared in the uterotonic and galactogogic assay *in vivo*. [2-O-Methyltyrosine]deamino-6-carba-oxytocin had a very low formal elimination constant (*i.e.* strongly protracted action) in the uterotonic assay.

The analogues of neurohypophysial hormones with one or both sulfur atoms of the disulfide bridge substituted by methylene groups (so-called carba analogues) were proved to have an affinity to receptors in target tissues[1] and increased metabolic stability[2,3]. These properties are directly responsible for their protracted action on the uterus *in vivo*[4]. Furthermore, it was found that the two sulfur atoms were not equivalent when substituted by methylene groups; this fact was demonstrated by differences in the magnitude of the response to the given analogues and its duration[5–7]. When describing the properties of [2-O-methyltyrosine]deamino-1-carba-oxytocin[8], we mentioned the possibility that the presence of the methyl ether group could give the analogue the properties of a hormonogen, thus enhancing its prolonged action.

The present paper describes the biological activity of [2-O-methyltyrosine]deamino-6-carba-oxytocin and compares it with that of the corresponding analogue in the 1-carba series.

EXPERIMENTAL

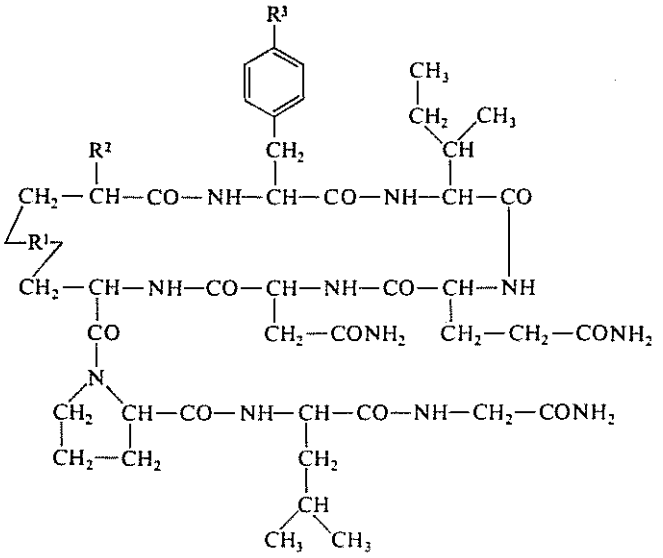
Oxytocin (*Ia*) is a commercial preparation (Léčiva, Prague). The properties of the analogues used were the same as stated in the papers describing their preparation: [2-O-methyltyrosine]-deamino-6-carba-oxytocin[9] (*Ie*), [2-O-methyltyrosine]deamino-1-carba-oxytocin[10] (*Id*), deamino-6-carba-oxytocin[6] (*Ic*) and deamino-1-carba-oxytocin[5] (*Ib*).

Uterotonic activity was determined on an isolated rat uterus[11,12] and *in situ*, using anaesthetized rats[13]; the potency of the analogues was estimated by means of the four-point test or by comparing the threshold doses of the given compound with those of oxytocin. Galactogogic activity

was assayed[14,15] on anaesthetized lactating rats (9—15 days after parturition). The elimination constants were determined in the uterotonic and galactogogic assays by following the rate of decline of the response using the two-dose method[16].

RESULTS

Table I gives the activities and formal elimination constants of analogue *Ie* and some reference compounds, as determined in the uterotonic and galactogogic assays *in vivo* and on the isolated rat uterine strip. In all the tests, the analogues containing a methyl ether group (*Id* and *Ie*) followed a similar trend in the shift of activities as the analogues with a free hydroxyl group of tyrosine (*Ib* and *Ic*). In other words, the analogues in the 6-carba series had higher uterotonic activity *in situ*, and lower uterotonic activity *in vitro* and galactogogic *in situ*. However, methylation of the tyrosine residue decreased the activities of the two analogues by 1 to 2 orders of ten.



Ia, $R^1 = S—S$, $R^2 = NH_2$, $R^3 = OH$; *Ic*, $R^1 = S—CH_2$, $R^2 = H$, $R^3 = OH$
Ib, $R^1 = CH_2—S$, $R^2 = H$, $R^3 = OH$; *Id*, $R^1 = CH_2—S$, $R^2 = H$, $R^3 = OCH_3$
Ie, $R^1 = S—CH_2$, $R^2 = H$, $R^3 = OCH_3$

All four analogues showed protracted action in the two assays *in vivo*. The duration of the uterotonic response was apparently influenced mainly by the position of the methylene group (compound *Ic* had the lowest formal elimination constant). The protracted galactogogic response was probably caused mainly by the presence of the methyl ether group. The time course of the two biological responses *in situ* to analogues *Ic* and *Ie* is shown in Fig. 1.

TABLE I

Biological Activities and Elimination Constants of Oxytocin Analogues

Compound	Biological activity[a]			Formal elimination constant	
	uterotonic *in vitro*	uterotonic *in vivo*	galactogogic *in vivo*	uterus k_1, min^{-1}	mammary gland k_1, min^{-1}
Oxytocin *Ia*	450	450	450	0·237 ± 0·071	1·4—21·2[b]
Deamino-1-carba-oxytocin *Ib*	1 899[c]	1 206[c]	604[d]	0·172 ± 0·050[c]	>0·26[g]
Deamino-6-carba-oxytocin *Ic*	929[c]	2 792[c]	456[d]	0·041 ± 0·011[e]	>0·28[g]
[2-O-Methyltyrosine]deamino-1-carba--oxytocin *Id*	17[f]	45[h]	35[d]	0·079 ± 0·044	0·111 ± 0·038[b]
[2-O-Methyltyrosine]deamino-6-carba--oxytocin *Ie*	3·1	75	18	0·049 ± 0·017	0·180 ± 0·020

[a] The activities are given in I.U./mg; [b] ref.[8]; [c] ref.[7]; [d] ref.[15]; [e] ref.[4]; [f] ref.[10]; [g] the extent to which the response to these compounds was protracted varied greatly (apparently due to the state of the mammary gland of the individual experimental animals); the table gives the lowest values registered.

DISCUSSION

The uterotonic activity of analogue *Ie* is lower than that of the non-methylated compound *Ic*. If we, however, base our calculations on the total effect, it appears to be rather high, in view of its long duration. The formal elimination constants of compounds *Ic* and *Ie* are closely similar (even the carba-analogues *Ib* and *Ic*, however, have significantly protracted action in the uterotonic assay *in vivo*). Notwithstanding, the time course of the uterotonic response to the two compounds differs (Fig. 1); the onset of the action of analogue *Ie* is more gradual and the curve is less steep. We must keep in mind that the formal elimination constants, as formerly defined[17], may not characterize the initial compound investigated. This applies mainly to compounds the chemical structure of which may change in the period after their application and before their binding to the receptor in the target tissue. In these cases (*e.g.* hormonogens[18]), the formal elimination constants may not define the compounds for which they have been calculated; in other words, the value of an elimination constant for a given compound can in fact belong to another chemical individual.

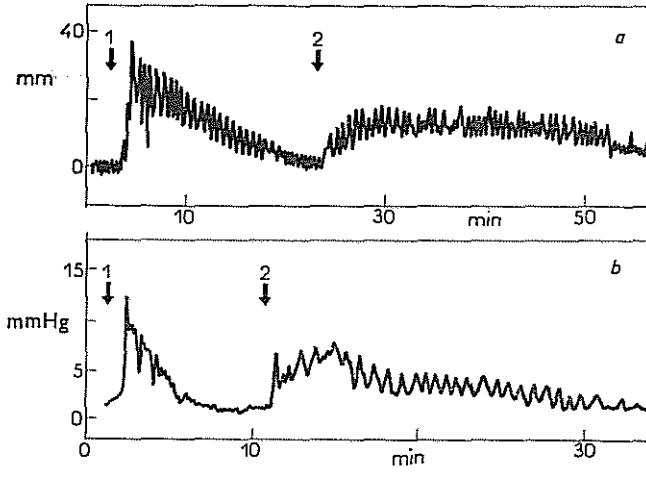


FIG. 1

Response to the Action of Analogues *Ic* and *Ie*

a) Uterotonic; ordinate, contractions in mm; abscissa, time in min, *1* $2 \cdot 10^{-6}$ mg *Ic*, *2* $5 \cdot 10^{-5}$ mg *Ie*. *b*) Galactogogic; ordinate, intramammary pressure in mm Hg; abscissa, time in min, *1* $1 \cdot 10^{-6}$ mg *Ic*, *2* $4 \cdot 10^{-5}$ mg *Ie*.

We have suggested earlier[8] that analogues containing an O-methyltyrosine residue in position 2 instead of tyrosine could act as "hormonogens". This assumption was based on the analysis of the time course of the galactogogic response. The enzymic system responsible for the demethylation reaction has not as yet been isolated. The introduction of a methyl group into the analogue *Ib* decreased the formal elimination constant of the resultant compound *Id* in the uterotonic assay by one half. By contrast, the analogous pair of compounds derived from the 6-carba series (*Ic* and *Ie*) had the same elimination constant. This might be due to the different distribution of compounds *Ic* and *Ie*. It is also possible that the methylated analogues *Ie* and *Id* have different affinities to the enzymic system assumed to be responsible for their demethylation.

The galactogogic potency of analogue *Ie* was considerably lower than that of compound *Ic*. Nevertheless, the introduction of the methyl group into the peptide molecule significantly prolonged the duration of the response, as can be seen from the time course of the response (Fig. 1) and from the values of the elimination constants of compounds *Ic* and *Ie*. A similar difference between the elimination constants of compound *Ic* in different target tissues had been observed[4] by comparing the course of the uterotonic and antidiuretic responses.

Compounds with selectively prolonged or shortened action on a given target tissue could have considerable theoretical and practical importance. The results presented here show that it might be possible to reach this goal. However, the particular steps that would lead us to the solution of this problem are not quite clear as yet.

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