THE EFFECT OF SOME STRUCTURAL MODIFICATIONS OF THE OXYTOCIN MOLECULE ON THE DURATION OF THE GALACTOGOGIC RESPONSE

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The duration of the galactogogic response to analogues of oxytocin, modified mainly in the aminoterminal part of the molecule, was determined in experiments with lactating rats. The influence of the individual structural modifications was investigated by determining the dependence of both the intensity of the effect and the total effect of the compound on the log dose.

The biological response to neurohypophysial hormones is distinctively short-lasting. Synthetic modifications of the hormone molecule resulted in a number of analogues with pronouncedly prolonged action[1]. Apart from the intensity of the effect, other parameters were introduced[2] in order to evaluate the duration of the response (the total effect) and the rate of elimination of the compound (the elimination constant and persistence index). The antidiuretic and uterotonic effects of oxytocin and vasopressin analogues with protracted action were characterized by these parameters not only when protracted action was due to higher metabolic stability (or to different distribution), but also when the compounds were of hormonogen nature and the duration of the response was influenced by the formation of more active hormones or analogues from the hormonogens[2,3].

We have already described and partially characterized the protracted action of some analogues in the galactogogic assay[4,5]. When studying compounds of hormonogen nature, it appears more suitable to compare their potencies by calculating the threshold doses not only of the relationship between the intensity of the effect and the log dose (the way in which the galactogogic potencies of the analogues of neurohypophysial hormones have been expressed so far), but also of the dependence of the total effect on the log dose. These considerations served as a basis for the evaluation of the potencies of some analogues in the present paper.

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EXPERIMENTAL

*Material.** The analogues used had the same properties as described earlier: N^{α}-glycyl-oxytocin[7], N^{α}-glycyl-1-carba-oxytocin[8], [2-O-methyltyrosine]oxytocin[9], N^{α}-glycyl[2-O-methyltyrosine]oxytocin[10], [2-O-methyltyrosine]deamino-1-carba-oxytocin[11], [2-O-methyltyrosine]-deamino-6-carba-oxytocin[12], [4-glutamic acid methyl ester]oxytocin[13], [4-glutamic acid methyl ester]deamino-oxytocin[13], [4-glutamic acid methyl ester]deamino-1-carba-oxytocin[14], [2-O-methyltyrosine, 4-glutamic acid methyl ester]deamino-1-carba-oxytocin[14], [2-O-methyltyrosine, 4-glutamic acid]deamino-1-carba-oxytocin[14], [4-glutamic acid]deamino-1-carba--oxytocin[15].

Methods. The galactogogic potency was assayed[4,16] using anaesthetized female rats (8—14 days postpartum). The potency of the analogues was evaluated by comparing the threshold doses obtained by plotting the intensity of the response against the log dose, and also the threshold doses obtained by plotting the total galactogogic response against the log dose, with those of oxytocin. The intensity of the response is given by the amplitude of the initial response. The value of the total effect is obtained by integrating the whole response.

RESULTS AND DISCUSSION

As analogues of neurohypophysial hormones with prolonged action appeared, the question of expressing their potency correctly presented itself. In the case of analogues, the prolonged action of which is due to their metabolic stability, it is advantageous to calculate the index of persistence from the ratio of the elimination constants of the standard and the compound studied. This index is also used to characterize compounds of hormonogen nature; in this case one must take into account that the rate of elimination is influenced by the rate at which more active compounds are formed from the hormonogens and that they often elicit a two-phase response[2,3,17].

We determined and compared the total effects of analogues of neurohypophysial hormones with prolonged galactogogic action that had been characterized so far mainly by the intensity of their effect and only in some cases by their elimination constants. Table I gives the potencies of selected analogues, determined on the basis of the intensity of their action and on the basis of the total effect. The compounds presented in Table I have several types of structural modification: stabilization of the hormone structure (the substitution of the disulphide bond by a thioether group), aminoacylation of the primary amino group of cysteine, methylation of the hydroxyl group of tyrosine in position 2 of the peptide chain and combinations of these modifications. We have already shown that derivatives containing O-methyltyrosine have protracted action in the uterotonic and galactogogic assays[4,5]. The carba-modification (substitution of the disulphide bond by a thioether group) enhances the prolongation of the effect. A similar relationship was observed in the case of aminoacyl derivatives of oxytocin and 1-carba-oxytocin. N^{α}-Glycyl[2-O-methyltyrosine]oxytocin can serve as an example of a multiple hormonogen, the direct

* The nomenclature of oxytocin analogues follows the published recommendation[6].

action of which is significantly inhibitory in some target organs[10]; its protracted action *in vivo* is apparently due to the gradual formation of more active compounds. It is better to characterize the potency of this type of compound by the total effect than by its intensity; the intensity of the effect reflects the affinity of the initial com-

TABLE I

Galactogogic potency of oxytocin analogues (I.U./mg)

Compound	Intensity of effect	Total effect
Oxytocin	450	450
[2-O-Methyltyrosine]deamino-1-carba-oxytocin	35[a]	178
[2-O-Methyltyrosine]deamino-6-carba-oxytocin	18[b]	142
[2-O-Methyltyrosine]oxytocin	11·8[a]	71
N^{α}-Glycyl-oxytocin	1·0	11·3
N^{α}-Glycyl-1-carba-oxytocin	2·35	45·0
N^{α}-Glycyl[2-O-methyltyrosine]-oxytocin	0·64[c]	8·0

[a] Ref.[4]; [b] ref.[12]; [c] ref.[10].

TABLE II

Biological activities of oxytocin analogues (I.U./mg)

Compound	Intensity of galactogogic activity	Uterotonic activity *in vitro*
Oxytocin	450	450
Deamino-oxytocin	266[a]	795[b]
Deamino-1-carba-oxytocin	604[c]	1 899[d]
[4-Glutamic acid methyl ester]oxytocin	101	18[e]
[4-Glutamic acid methyl ester]deamino-oxytocin	271	43[f]
[4-Glutamic acid methyl ester]deamino-1-carba-oxytocin	1 307[g]	26[g]
[4-Glutamic acid]deamino-1-carba-oxytocin	38[g]	1[g]
[2-O-Methyltyrosine, 4-glutamic acid methyl ester]deamino--1-carba-oxytocin	2·5[h]	0·2[g]
[2-O-Methyltyrosine, 4-glutamic acid]deamino-1-carba-oxytocin	2·5[i]	0·1[g]

[a] Ref.[4]; [b] ref.[19]; [c] ref.[5]; [d] ref.[20]; [e] ref.[18] states 10·2 I.U./mg; [f] ref.[18] states 21·4 I.U./mg; [g] ref.[14]; [h] ref.[14] states 2 I.U./mg; [i] ref.[14] states 0·5 I.U./mg.

pound to the receptor which can be rather low. The total activity is directly related to metabolic and elimination processes in the living organism that determine the duration of the response.

The substitution of the amide group of glutamine in position 4 of the peptide chain by a methyl ester group led to a significant dissociation of the uterotonic and galactogogic activities in favour of the galactogogic action[14]. The values of the galactogogic potencies of these analogues, determined from the intensity of the effect and the total effect, did not differ. The response to these compounds was of short duration. The protracted response, described earlier[14], is noticeable only when the doses are higher than those resulting in the half-maximal intensity of the effect. The introduction of the methyl group into the side chain of tyrosine led to a pronounced decrease of the intensity of the effect (Table II). However, this substitution did not influence the duration of the galactogogic response. The fact that the action of this analogue is not protracted and does not differ from that of the analogue with glutamic acid in position 4 can be explained by the metabolic instability of the ester bond (*cf.*[18]). It can be seen from Table II that the deamination of the oxytocin molecule decreases the specificity of the galactogogic action, whereas the deamination of the analogue with glutamic acid methyl ester in position 4 does not have this effect. Deamino-1-carba-oxytocin has approximately the same specificity as deamino--oxytocin; the substitution of the disulphide bond by a thioether group cannot, therefore, increase by itself the specificity of the galactogogic action. Nevertheless, the combination of this substitution with the replacement of glutamine by glutamic acid methyl ester results in an analogue with significantly higher specificity than that of the analogue containing a disulphide bridge. No structural modification has been found so far that would result in the prolongation of the galactogogic action and at the same time ensure sufficient dissociation of the effects in favour of the galactogogic action.

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