THE EFFECT OF MAGNESIUM IONS ON THE UTEROTONIC ACTIVITY OF CARBA ANALOGUES OF OXYTOCIN MODIFIED IN POSITION 4

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Five analogues of deamino-1-carba-oxytocin, in which the γ-carboxyl group of glutamine in position 4 had been modified (free acid, monomethylamide, dimethylamide, hydrazide, *p*-tolylamide), were investigated regarding their uterotonic activity *in vitro*. The slope of the dose-response curve and the magnitude of the maximum response were compared with those of oxytocin in magnesium-free and 0·5 mM Mg-containing media. In the case of [4-glutamic acid] deamino-1-carba-oxytocin, the $R_{Mg^{2+}}$ value equalled 22. The other compounds had $R_{Mg^{2+}}$ values of 2—3. All the analogues evoked the same maximum response as oxytocin in both the media tested.

The fact that the ionic composition of the medium influences the activity of oxytocin and its analogues in the uterotonic assay *in vitro* is well known[1]. The major role among the various ions can be ascribed to magnesium. The optimum concentration (0·5 mM) of this ion in the medium, at which the biological activity of most analogues is potentiated, was established and the $R_{Mg^{2+}}$ index – which denotes the ratio of the analogue's activity in a medium containing 0·5 mM magnesium to the analogue's activity in a Mg-free medium, was introduced[2]. Determination of the activities of compounds in media with different ionic composition can help to solve the relationship between chemical structure (including spatial arrangement) and biological activity. At present, two steps are distinguished in the action of a hormone, namely the binding of the hormone to the receptor (its affinity to the receptor) and the action of the hormone after its binding to the receptor (intrinsic activity). It follows that it is necessary to know not only the activity of the analogue, *i.e.* the formal value that enables its comparison with oxytocin, but also the maximal attainable response and the slope of the so-called dose-response curve.

The present paper deals with the effect of magnesium ions on the uterotonic activity *in vitro* of several oxytocin analogues modified in position 1 and 4. We investigated the ability of the analogues to evoke the maximum response achieved by oxytocin and compared the slopes of the dose-response curves.

EXPERIMENTAL

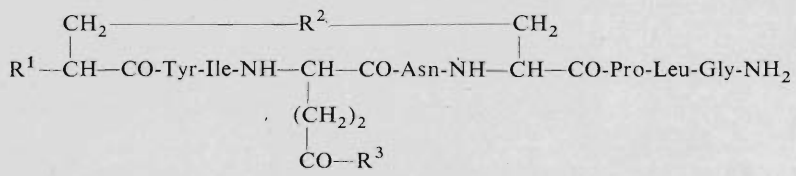
The uterotonic activity of the oxytocin (*I*) preparation used was 450 IU/mg. The properties of the analogues[3] studied (Fig. 1) have already been described[4,5]. The uterotonic assay[2,6] was

performed using magnesium-free medium and a medium containing 0·5 mM magnesium. In the case of compound *III* a medium containing 1 mM magnesium was also used. In our experiments, doses were applied cumulatively and the activity of the analogues was calculated from the threshold dose[7,8]. The compounds were applied until the maximum response was reached. The standard curve for oxytocin was assayed at the beginning and at the end of the experiment or more often, if necessary.

RESULTS AND DISCUSSION

Most oxytocin analogues[1,9,10] with lower activity than that of oxytocin are potentiated in the presence of magnesium ions, some 2–3 times, others reach the activity of oxytocin. Compounds with activities equal to, or higher than, the activity of oxytocin were found to have lower activity in the presence of magnesium. Our results are in agreement with these findings. Table I summarizes the activities of the analogues assayed in magnesium-free medium and in the medium containing 0·5 mM magnesium; the maximum response achieved is given in percentages of the maximum response to oxytocin. Table I also states the values concerning the analogue deamino--1-carba-oxytocin[9], for reference. It is apparent that the analogues studied were potentiated in the presence of magnesium. Compounds *IV*–*VII* were potentiated 2–3 times.

Strong potentiation was observed in the case of [4-glutamic acid] deamino-1-carba oxytocin; in the presence of 0·5 mM-Mg^{2+}, the potentiation was 22fold, in the presence of 1 mM-Mg^{2+} 66fold. A formally similar result was obtained in the case of [5-aspartic acid]oxytocin[11]; the activity, assayed on isolated uterine strips, was 20 I.U./mg in Mg-free medium and equalled the activity of oxytocin in the presence



I, $R^1 = NH_2$, $R^2 = S—S$, $R^3 = NH_2$
II, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = NH_2$
III, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = OH$
IV, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = NHCH_3$
V, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = N(CH_3)_2$
VI, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = NH—C_6H_4—CH_3(p)$
VII, $R^1 = H$, $R^2 = CH_2—S$, $R^3 = NHNH_2$

FIG. 1
Structural modifications of the oxytocin (*I*) molecule

of 1 mM-Mg^{2+}. It does not seem probable that the increase in activity of these two analogues is caused only by the formation of a magnesium salt. Some possible ways, in which the activity of oxytocin analogues could be influenced by magnesium, have been discussed[12]. Unfortunately, the effect of magnesium ions on uterotonic activity has been estimated only in a few of the analogues of neurohypophysial hormones prepared so far. Apart from the two above-mentioned analogues, strong potentiation of the activity in the presence of magnesium was observed only in the case of [7-glycine]oxytocin[13] (from 93 to 965 I.U./mg) and [3-norvaline]oxytocin[14] (from 4 to 44 I.U./mg). The case of tocinamide is controversial; on the one hand, a ten-fold potentiation has been observed[15] and, on the other, a four-fold decrease[16].

The influence of magnesium ions is apparently complex, and can be of either static or dynamic nature[12]. In the first case, the spatial arrangement of the analogue or the receptor would be affected (biologically active conformation) or a ternary complex would be formed (analogue–Mg^{2+}–receptor). In the second case, magnesium could influence the dissociation of the hormone–receptor complex or affect the enzymatic inactivation of the effector when it is bound to the receptor[17,18]. According to ref.[14], the ion must act at the stage of the specific interaction of the neurohypophysial peptides with oxytocin receptors or at a stage closely following it.

The analogues studied were capable of evoking the same maximum response as oxytocin in both media and the slopes of their dose-response curves in magnesium-free medium were parallel (within limits of experimental error); this indicates that the intrinsic activity of the analogues is identical with that of oxytocin[19]. The lower

TABLE I

Uterotonic activity of oxytocin analogues. The maximum response achieved is given as % of the maximum response of oxytocin; the values in brackets give the lowest maximum response and the ratio of the number of assays in which the analogue evoked a 100% response as compared with oxytocin, to the number of assays in which the maximum response was lower

Analogue	Mg^{2+} free medium		0·5 mM-Mg^{2+}		$R_{Mg^{2+}}$
	I.U./mg ± S.E.	%	I.U./mg ± S.E.	%	
II[a]	1 899 ± 98	—	921 ± 70		0·48
III	1 ± 0·25	99·3 (96·3; 8 : 2)	22·7 ± 5·9	97·1 (82·5; 8 : 2)	22·7
IV	40·0 ± 4·5	91·3 (67·8; 4 : 4)	80·1 ± 16·7	93·6 (83·3; 4 : 4)	2·0
V	4·3 ± 0·3	91·6 (63·4; 4 : 4)	13·5 ± 2·4	98·0 (86·3; 6 : 2)	3·1
VI	21·2 ± 7·6	93·4 (59·2; 5 : 3)	58·6 ± 20·2	98·6 (92·0; 6 : 2)	2·8
VII	19·4 ± 5·1	97·7 (91·0; 6 : 2)	43·7 ± 3·8	98·0 (89·0; 5 : 3)	2·3

[a] Ref.[9].

maximum response, observed in some experiments, can be explained by self-inhibitory action of the analogue concerned, which has been sometimes observed when using the cumulative method of dose application[20]. The maximum response to oxytocin analogues was achieved regardless of the position in which the structural modification was performed[8,11,21–24]. In the medium containing 0·5 mM magnesium, the slope of the dose-response curve was slightly more vertical. It is difficult, however, to decide what change in the action mechanism this phenomenon reflects.

REFERENCES

1. Munsick R. A. in the book: *Handbook of Experimental Pharmacology* (B. Berde, Ed.), Vol. 23, p. 443. Springer, Berlin 1968.
2. Munsick R. A.: Endocrinology *66*, 451 (1960).
3. Tentative Rules on Biochemical Nomenclature. Biochemistry *6*, 362 (1967); Biochem. J. *126*, 773 (1972).
4. Lebl M., Jošt K.: This Journal *43*, 523 (1978).
5. Lebl M., Dimeli A., Bojanovska V., Slaninová J., Barth T., Jošt K.: This Journal *44*, 2556 (1979).
6. Holton P.: Brit. J. Pharmacol. *3*, 328 (1948).
7. Ariens E. J., de Groot W. M.: Arch. Int. Pharmacodyn. Ther. *99*, 193 (1954).
8. Rudinger J., Krejčí I.: Experientia *18*, 585 (1962).
9. Barth T., Krejčí I., Kupková B., Jošt K.: Eur. J. Pharmacol. *24*, 183 (1973).
10. Turan A., Manning M., Haldar J., Sawyer W. H.: J. Med. Chem. *20*, 1169 (1977).
11. Walter R., Skala G., Smith C. W.: J. Amer. Chem. Soc. *100*, 972 (1978).
12. Bentley P. J.: J. Endocrinol. *32*, 215 (1965).
13. Lowbridge J.: J. Med. Chem. *20*, 120 (1977).
14. Krejčí I., Poláček I.: Eur. J. Pharmacol. *2*, 393 (1968).
15. Ressler C.: Proc. Soc. Exp. Biol. Med. *92*, 725 (1956).
16. Zaoral M., Flegel M.: This Journal *37*, 1539 (1972).
17. Schild H. O.: Brit. J. Pharmacol. *39*, 69 (1970).
18. Jošt K., Procházka Z., Cort J. H., Barth T., Škopková J., Prusík Z., Šorm F.: This Journal *39*, 2835 (1974).
19. Walter R., Wahrenburg M.: Pharmacol. Res. Commun. *8*, 81 (1976).
20. Walter R.: Fed. Proc., Fed. Amer. Soc. Exp. Biol. *36*, 1872 (1977).
21. Krejčí I., Poláček I., Rudinger J. in the book: *Endogenous Substances Affecting the Myometrium* (V. R. Pickles, R. J. Fitzpatrick, Eds). Memoirs Soc. Endocrinol. Vol., *14*, p. 171. Cambridge Univ. Press, London 1966.
22. Krejčí I., Poláček I., Rudinger J.: Brit. J. Pharmacol. Chemother. *30*, 506 (1967).
23. Chan W. Y., Kelley N.: J. Pharmacol. Exp. Ther. *156*, 150 (1967).
24. Smith C. W., Chan S., Walter R.: J. Pharm. Exp. Ther. *203*, 120 (1977).

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