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Lack of antagonism between thioglycerol and an oxytocin analogue not containing a disulphide bond

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Summary

1. The antagonistic effect of thioglycerol against oxytocin and an analogue of oxytocin not containing a disulphide bridge (desamino-1-carba-oxytocin) has been compared in the rat isolated depolarized uterus.

2. Thioglycerol clearly differentiated between the two compounds, 40 mM producing a dose ratio of 12 with oxytocin but only 1·5 with the carba compound.

3. It was confirmed that thioglycerol produces no appreciable destruction of oxytocin *in vitro*.

4. The mode of action of thiols in antagonizing specifically S–S polypeptides is discussed. It is concluded that they probably do not produce their effect either by inactivating receptors or by inactivating S–S polypeptides in solution. A possible mechanism is that thiols potentiate the destruction of S–S polypeptides at the receptor site.

Introduction

It was shown by Martin & Schild (1965) that thioglycerol and other straight chain aliphatic thiols antagonize the contractile effect of oxytocin and other S–S polypeptides on the isolated uterus. This antagonistic effect occurred under conditions in which there was little or no destruction of oxytocin in solution, suggesting an antagonism at the receptor level rather than chemical destruction of oxytocin. Two types of receptor interaction were considered.

First, the receptor might contain an S–S or an –SH group (Rasmussen & Schwartz, 1964) in its active site, with either of which the S–S group of the polypeptide could interact by disulphide interchange, thus initiating a pharmacological response. Thiols would interfere with the disulphide interchange and thus antagonize oxytocin. This theory lost force after the discovery (Jost & Rudinger, 1967) that polypeptides containing CH_2–S or CH_2–CH_2 in place of the S–S bridge and of a structure that would not permit a disulphide interchange reaction, nevertheless had oxytocin-like effects.

Second, the receptor might contain a structural S–S group required for maintaining receptor configuration but not participating directly in the drug-receptor interaction. If a thiol cleaved this S–S bridge, it could be expected to inactivate the receptor and therefore antagonize not only S–S polypeptides but other isosteric polypeptides acting on the same receptor. It thus became of interest to test the effect of a thiol against an oxytocin analogue not containing an S–S bridge.

I was able to obtain the CH_2–S analogue of desamino-oxytocin ("da-carba" analogue) through the kindness of Dr. Rudinger and Dr. Jost and have tested whether its effect on the uterus is antagonized by thioglycerol.

Methods

The experiments were carried out on the rat isolated depolarized uterus. The use of this preparation for receptor studies has been discussed previously (Schild, 1969). Responses were recorded isometrically; the general methodology was as in the previous paper. The potassium Ringer solution used had the following composition (mM): 104 K_2SO_4, 11 K_2HPO_4, 1 KH_2PO_4, 6 glucose, 0·1 $CaCl_2$, 0·5 $MgCl_2$; it was bubbled with oxygen. When thioglycerol or sucrose were added to Ringer solution, the concentrations of the other constituents were adjusted so as to maintain isotonicity. The rate of destruction of oxytocin by thioglycerol was measured as follows. Oxytocin (10 mu./ml) was incubated with 40 mM thioglycerol in potassium Ringer solution at 30° C. After incubation for a measured time, the entire solution was added to a bath containing a uterus strip immersed in an equal volume of thioglycerol-free potassium Ringer solution. By thus diluting thioglycerol the sensitivity of the uterus to oxytocin was increased. The ensuing responses were compared with those of similarly prepared unincubated solutions.

Drugs used were: alpha thioglycerol (Koch-Light); oxytocin (Syntocinon, Sandoz); 1-desamino-6 1-cystathionine-oxytocin (desamino-1-carba-oxytocin, da-carba-oxytocin) in ampoules of 0·05 mg/ml obtained from the Research Institute of the Czechoslovak Academy of Sciences. The sample of da-carba compound supplied had activity of approximately 150 International Units/mg when assayed against oxytocin (approximately 450 i.u./mg) in the rat depolarized uterus preparation.

Results

Table 1 shows the results of five comparative assays of oxytocin and the da-carba compound in potassium Ringer solution in the presence and absence of 40 mM thioglycerol. The experiments were carried out either as successive 2+2 assays in sucrose-containing Ringer solution and afterwards in thioglycerol-containing Ringer solution, or as randomized 2+2+2+2 assays of the two agonists in either sucrose- or thioglycerol-Ringer solution. The respective Ringer solutions were admitted to the bath 2 min before addition of the polypeptide. The data in Table 1 are in terms of shift of log dose response curves by 40 mM thioglycerol. Thioglycerol produced a differential effect with a mean dose ratio of 12 for oxytocin and 1·5 for the da-carba compound. The difference in the dose ratios is statistically highly

TABLE 1. *Antagonistic effect of* 40 *mM thioglycerol against oxytocin and da-carba analogue*

Expt.	Log dose ratio after thioglycerol		Number of blocks of 8	Design
	Oxytocin	Da-carba		
1	1·08	0·14	4	Random
2	0·93	0·3	2	Random
3	1·0	0·12	2	Consecutive
4	1·08	0·08	2	Consecutive
5	1·37	0·34	4	Random
Mean	1·09	0·19		

significant. Thioglycerol (40 mM) also produced some antagonism of acetylcholine, comparable to that of the da-carba compound.

Figure 1 shows tracings of responses with 40 mM sucrose and 40 mM thioglycerol. The oxytocin dose had to be considerably increased in the presence of 40 mM thioglycerol and the shape of the isometric response curve was altered ; the response was not maintained and was followed by a rapid fade, an observation which is in agreement with previous findings (Martin & Schild, 1965). By contrast, the shape of the isometric response curve of the da-carba compound was unaltered by thioglycerol. Measurements were made on a fast drum of the time interval between injection of oxytocin into a thioglycerol containing bath and the maximal response as this should provide an upper limit of time available for the destruction of oxytocin in the bath. The mean of nine such measurements was $21 \pm 1{\cdot}6$ s (S.E.).

It was shown by Martin & Schild (1965) that the inactivation of oxytocin by thioglycerol is a slow process. In these earlier experiments the destruction rate was calculated by extrapolation from measurements made in concentrated solution.

In the present experiments a more direct procedure was used ; the inactivation rate was measured at concentrations of oxytocin of an order similar to those used for the 2+2 assays. In four separate experiments, 40 mM thioglycerol in potassium Ringer solution at 30° C produced some inactivation of oxytocin but the time required for half inactivation was more than 10 min in each case, which contrasted with the time of 21 s during which oxytocin was in contact with thioglycerol in the bath before its effect was completed. Clearly at this rate chemical inactivation of oxytocin in the bath is far too slow to account for the observed dose ratios.

Discussion

It has been shown that 40 mM thioglycerol differentiates between oxytocin and its da-carba analogue, causing a pronounced dose shift and a characteristic change of shape of the isometric oxytocin curve and a slight, possibly unspecific, dose shift of the da-carba curve without change of shape of the response trace. This raised

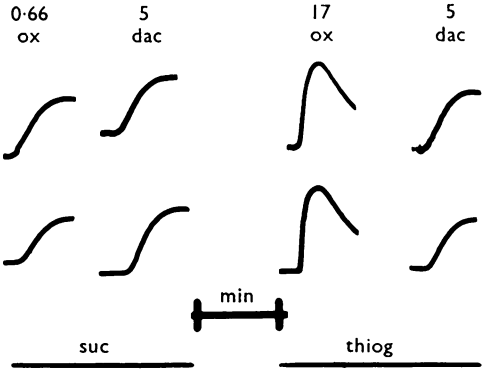

FIG. 1. Isometric responses to oxytocin (ox, mu./ml) and da-carba analogue (dac, ng/ml) in potassium Ringer solution containing either 40 mM sucrose (suc) of 40 mM thioglycerol (thiog). Records from two horns of same rat uterus, 30° C. Note changed character of response and increased dose of oxytocin, but not of da-carba compound, after thioglycerol.

the question of the mode of action of thiols in antagonizing specifically S–S polypeptides. Like oxytocin, desamino-oxytocin, the parent compound of the da-carba analogue, is antagonized by thiols (Martin & Schild, 1965).

The antagonistic effect of thiols could be exerted at one of two sites.

1. An action on receptors. Oxytocin and the da-carba compound might act on different receptors, of which only the oxytocin receptor is affected by thiol, but this possibility seems unlikely in view of the closely similar pharmacological effects of the two compounds. Although their three-dimensional structures have not yet been elucidated, it is probable that they are very nearly isosteric (Jost & Rudinger, 1967) and could therefore be expected to act on the same receptor. However, in that case the differential effect of thioglycerol could not be explained. In summary, the present experiments have provided no support for the hypothesis that thiols inactivate receptors.

2. An action on the S–S polypeptide. Although a simple chemical interaction seems excluded by the slow rate of destruction of oxytocin by thioglycerol *in vitro*, a special situation may conceivably obtain in the receptor region. If S–S polypeptides became inactivated at the receptor site or its vicinity, this local process might be potentiated by thiols, thus accounting for the diminished activity of S–S polypeptides in the presence of thiols. The inactivating process might be sufficiently confined not to affect the bulk concentration of oxytocin in an isolated organ bath appreciably, as was found to be the case by Martin & Schild (1965). The stable da-carba analogue would be expected to be unaffected by thiols. The " fade " phenomenon could perhaps be explained by assuming that oxytocin produces first stimulation as it attaches to the receptor followed by loss of stimulation as it becomes inactivated on the receptor.

Some support for these speculative views is afforded by the work of Audrain & Clauser (1960) who found that rat uterine tissue destroys oxytocin under anaerobic conditions. The destruction is a two-stage process consisting of a reversible cleavage of the disulphide bond followed by the enzymatic inactivation of the reduced moiety. If a two-stage reaction of this kind occurred at the receptor site or in its vicinity, thiols might be expected to potentiate the first stage of cleavage of the disulphide bond.

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