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THE ANTAGONISM OF DISULPHIDE POLYPEPTIDES BY THIOLS

BY

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In 1957, Guttman, Jaquenoud & Boissonas reported the synthesis of an oxytocin antagonist which was related in structure to oxytocin. Since then several oxytocin analogues with antagonistic activity have been described. These compounds are all disulphide polypeptides, like oxytocin itself, and presumably act as competitive antagonists (Rudinger & Krejci, 1962). This paper deals with a different class of antagonists, simple aliphatic compounds containing a free sulphydryl group which produce a specific pharmacological antagonism of disulphide polypeptides.

It has been known that thiols such as thioglycollate can inactivate oxytocin and vasopressin chemically by breaking their disulphide bonds (Dyke, Chow, Greep & Rothen, 1942). This reaction has been used to detect the presence of posterior pituitary disulphide polypeptides in tissue extracts, by incubating them with thioglycollate and testing whether their pharmacological activities are subsequently abolished. We have confirmed that thioglycollate inactivates oxytocin chemically, but have found that the rate of inactivation is too slow to account for the abolition of the pharmacological activity of oxytocin. Thioglycollate appears to antagonize oxytocin at the tissue receptor level, and another thiol, α-thioglycerol, also exhibits this antagonism although its ability to destroy posterior pituitary polypeptides is negligible. Some of this work has already been briefly reported (Martin & Schild, 1962, 1964).

METHODS

Rat isolated uterus preparation. Uterus horns from virgin rats preferably in oestrus were suspended in de Jalon solution of the following composition (%): NaCl 0.9, KCl 0.042, $CaCl_2$ 0.006, $NaHCO_3$ 0.05, and glucose 0.05. Recordings were by isotonic lever on a smoked drum, except in a few experiments in which isometric recording with a Statham strain gauge transducer connected to a Grass polygraph ink-writer was used. The muscle was suspended in a jacketed isolated organ-bath usually controlled at 30° C. Agonists were added to the bath through a side-arm by means of a syringe; antagonists were added to the stock solutions. Before carrying out assays preparations were stabilized by eliciting maximal contractions with oxytocin at intervals of 4 min over a period of 1 to 3 hr by an automatic syringe. Assays were performed by a four-point randomized block procedure as described by Schild (1942).

Isolated mammary gland preparation. White lactating rats of about 300 to 400 g in the 2nd to 8th day of lactation were anaesthetized with pentobarbitone sodium. Radial strips including the nipple were cut out as described by Smith (1961) and kept in the refrigerator at 2° C. The preparations were suspended in Tyrode solution at room temperature and the bath was strongly oxygenated through a disc of sintered glass. Recordings were made isometrically by a strain gauge transducer with an initial load of 500 mg.

Perfused hind-limb preparation. The rat preparation of Fastier & Smirk (1942) in which the toes are cut off was perfused with Locke solution (composition (%): NaCl 0.92, KCl 0.042, $CaCl_2$ 0.012, $NaHCO_3$ 0.015, and glucose 0.1) at room temperature. The outflow was recorded by a Thorp drop-counter.

Uterus in vivo. Rats of 200 g received 0.2 mg of stilboestrol 1 day before the experiment. The animals were anaesthetized with urethane (0.6 ml. of a 25% solution per 100 g) and their body temperatures were kept at 30° C by means of a rectal thermometer which controlled the temperature of the operating table. A cannula was inserted through a cut into the lumen of the uterus from which pressures were recorded by a Grass strain-gauge transducer.

Thiol concentration in plasma. This was measured by amperometric titration with mercuric chloride with a rotating platinum electrode as described by Kolthoff & Harris (1946). When tested in this way heparinized plasma from a rat anaesthetized with urethane contained the equivalent of 1.2±0.1 mM (standard error) of thiol; after intravenous injections of thioglycollate the thiol equivalents of the plasma rose to 20 to 40 mM as discussed in the text.

Drugs. The following were used: oxytocin (Syntocinon, Sandoz); vasopressin (lysine-vasopressin, Sandoz); bradykinin (Parke Davies); desamino-oxytocin (Professor Du Vigneaud); thioglycollic acid (B.D.H.) neutralized before experiment to *p*H 7.4 as all other organic acids; dimercaprol, cyanoacetic acid, dithioglycollic acid, thiodiglycol, glycollic acid, 2-mercaptoethanol, α- and β-mercaptopropionic acids, methionine, methyl thioglycollate, *N*-ethylmaleimide, thiolacetic acid, thioethane, α-thioglycerol and thiomalic acid (mercaptosuccinic acid) (all Light & Co.); ascorbic acid, L-cysteine (B.D.H.); and angiotensin (val_5-hypertensin II-asp-β-amide, Ciba).

RESULTS

The antagonism between thioglycollate and oxytocin on the isolated uterus

Thioglycollate produces a selective antagonism of the contractile effect of oxytocin on the rat isolated uterus. Fig. 1 shows an experiment in which the activity ratios of oxytocin and bradykinin were determined at 20° C before and after addition of 20 mM-thioglycollate to the bath fluid; each assay consisted of four 2+2 blocks. The oxytocin curve is displaced to the right by thioglycollate giving a dose-ratio of 10, whereas the bradykinin curve is only marginally displaced.

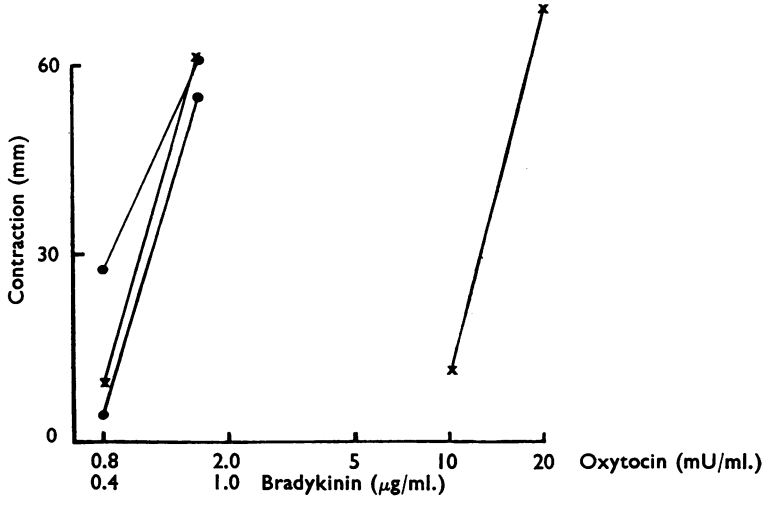

Fig. 1. Log dose/response curves of oxytocin (×) and bradykinin (●) with and without 20 mM-thioglycollate. Rat uterus preparation in de Jalon solution at 20° C; isotonic recording. Each point is the mean of four responses.

TABLE 1

DOSE-RATIOS FOR OXYTOCIN, ACETYLCHOLINE AND BRADYKININ WITH THIOGLYCOLLATE ON THE RAT UTERUS PREPARATION

Values are geometric means, with numbers of experiments in parentheses

Temperature (°C)	Thioglycollate (mM)	Dose-ratio for		
		Oxytocin	Acetylcholine	Bradykinin
30	5	1·6 (2)	1·0 (1)	
	10	2·9 (4)	1·7 (4)	
	20	25·0 (1)	1·3 (1)	
	40	150·0 (1)	6·0 (1)	
20	10	7·0 (4)	2·5 (4)	
	20	26·0 (2)		1·4 (2)

Table 1 shows the results of a series of comparative assays of oxytocin and acetylcholine or bradykinin on rat uterus with and without thioglycollate, each experiment involving eight assay blocks. The dose-ratios of oxytocin increase regularly with the concentration of thioglycollate and rather more than in proportion to the concentration of the antagonist (that is, more than in simple competitive antagonism). The other two stimulants show a small and irregular antagonism by thioglycollate which is considerably less than with oxytocin. The log dose/response curves of oxytocin in the presence of thioglycollate were generally parallel (Fig. 1); only with 40 mM-thioglycollate was there a significant decrease of the slope. The effects of oxytocin on the guinea-pig and rabbit isolated uterus preparations were similarly antagonized by thioglycollate.

Rate of inactivation of oxytocin by thioglycollate

The possibility was considered that thioglycollate inactivates oxytocin chemically during its brief sojourn in the isolated organ-bath. In order to evaluate the relevance of this factor for the antagonism by thioglycollate, experiments were carried out in which oxytocin was incubated with thioglycollate *in vitro* and the activity remaining after various incubation periods was measured by biological assay on the rat uterus. To avoid interference with the bioassay by any thioglycollate in the incubation mixtures it was necessary to dilute these so as to contain 1 mM or less at the stage of assay, and this in turn necessitated the use of relatively high initial concentrations of oxytocin. The initial concentration chosen was 500 mU/ml. It seemed justifiable to use this procedure in view of the finding, reported below, that thioglycollate inactivates oxytocin at an exponential rate which is independent of concentration of substrate.

The time course of inactivation is illustrated in Fig. 2 which shows the destruction rate of oxytocin incubated with various concentrations of thioglycollate for various times at room temperature. Each point is derived from a separate assay with a separate incubation mixture in a composite experiment. The time course of destruction is approximately exponential. The relationship between concentration of thioglycollate and destruction rate is approximately linear. It can be seen by a comparison of Figs. 1 and 2 that chemical inactivation cannot by itself account for the antagonistic effect of thioglycollate. Thus 20 mM-thioglycollate, 20° C, produced a dose-ratio of 10, but in order to achieve this dose-ratio 90% of oxytocin would need to be inactivated in the bath. This degree of inactivation would require 20 min, which is far in excess of the interval between injection of oxytocin into the bath and maximum response, which is from 1 to 4 min.

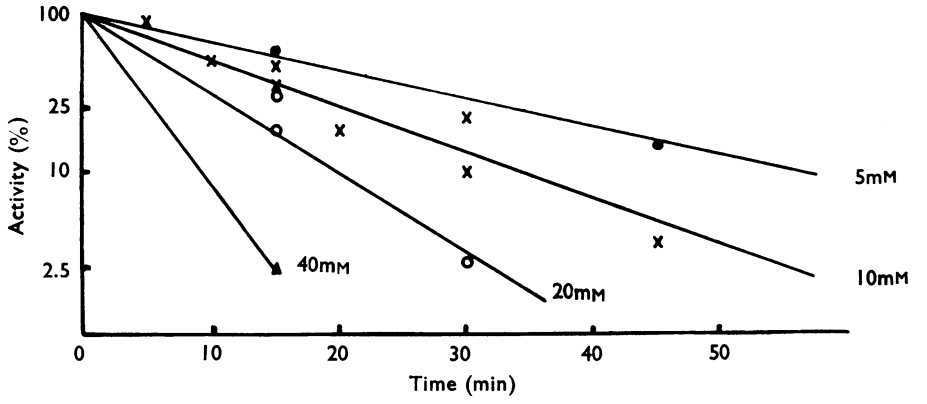

Fig. 2. Rate of inactivation of oxytocin by four concentrations (in mM) of thioglycollate at room temperature. All incubations started with 500 mU/ml. of oxytocin.

Fig. 3 shows the effect of temperature on the inactivation rate. The rate is approximately doubled by raising the temperature from 20 to 30° C. As shown in Table 1, the same temperature rise produced no increase in the dose-ratio of oxytocin but, if anything, a decrease. This is a further argument against chemical antagonism. Nevertheless, our findings do not exclude that chemical inactivation in the bath may contribute to the total antagonistic effect of thioglycollate and it was therefore of interest to find a compound which would antagonize oxytocin without destroying it chemically.

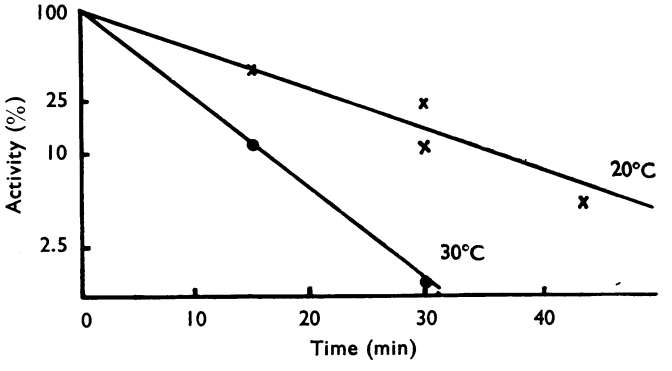

Fig. 3. Effect of temperature on the rate of inactivation of oxytocin by 10-mM thioglycollate.

Antagonism of thioglycerol and oxytocin

α-Thioglycerol antagonizes oxytocin without destroying it appreciably *in vitro*. The specificity of the antagonism is illustrated in Fig. 4. Addition of 10 mM-thioglycerol to the bath fluid completely abolished the oxytocin response leaving the bradykinin response unchanged. The antagonism was fully reversible by washing. A series of experiments with 10 and 20 mM-thioglycerol in which dose-ratios of oxytocin and bradykinin were determined in parallel is summarized in Table 2. Bradykinin gave small dose-ratios which did not increase with concentration of thioglycerol. Oxytocin gave dose-ratio 4.8 with

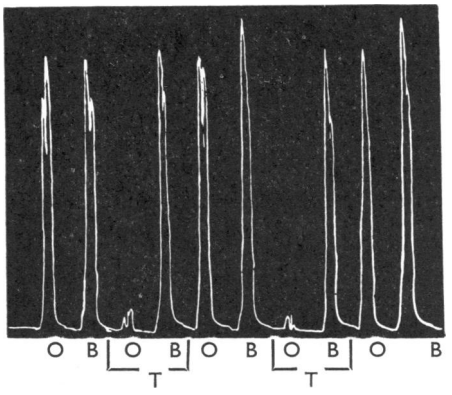

Fig. 4. Effect of 10 mM-thioglycerol (T) added to de Jalon solution on the responses of the rat uterus preparation to oxytocin (O, 3 mU) and bradykinin (B, 2 μg), 30° C; 4-ml. bath; time cycle, 4 min; isotonic record.

TABLE 2

DOSE-RATIOS WITH α-THIOGLYCEROL ON THE RAT UTERUS PREPARATION AT 30° C

	With 10 mM-α-thioglycerol		With 20 mM-α-thioglycerol	
	Oxytocin	Bradykinin	Oxytocin	Bradykinin
	3·0		13·2	1·1
	4·6	1·0	19·5	1·4
	3·9	1·5		
	5·8	1·7		
Means	4·8	1·4	16·3	1·3

10 mM- and 16.3 with 20 mM-thioglycerol. As with thioglycollate, the dose-ratio increased rather more than twofold (3.4-fold) between 10 and 20 mM although the factor of chemical inactivation is absent in this case. The log dose/response curves of oxytocin were parallel as with thioglycollate.

Compared to thioglycollate, thioglycerol had only negligible oxytocin-destroying ability. When 5 mM-thioglycollate and 50 mM-α-thioglycerol were incubated with oxytocin for 30 min at room temperature the former produced 50% inactivation whereas the latter produced 10% or less inactivation.

Chemical requirements for oxytocin antagonism

Various other thiols, as well as disulphides, reducing agents and thiol blocking agents were investigated for antagonism of oxytocin. The standard procedure was to carry out a comparative assay of oxytocin and bradykinin before and after addition of the potential antagonist to the bath. The assays were performed on the rat isolated uterus preparation at 30° C as a rule using two groups of four (2+2) assay blocks. The substances tested fell into two distinct groups, one antagonized oxytocin at 10 and 20 mM more or less specifically, the other antagonized unspecifically or not at all.

Table 3 lists the specific antagonists. Chemically they are thiols characterized by a free sulphydryl group attached to a terminal or intermediate carbon atom, and a second

TABLE 3

DOSE-RATIOS WITH VARIOUS THIOLS ON RAT UTERUS PREPARATION

Values are geometric means with numbers of experiments in parentheses. * 5 mM antagonized oxytocin but not bradykinin

	With 10 mM concentration		With 20 mM concentration	
	Oxytocin	Bradykinin	Oxytocin	Bradykinin
α-Thioglycerol [SH.CH_2.CHOH.CH_2OH]	4·7 (4)	1·4 (3)	16 (2)	1·3 (2)
β-Mercaptoethanol [SH.CH_2.CH_2OH]	5·3 (1)	1·1 (1)	12·9 (1)	1·6 (1)
β-Mercaptopropionate [SH.CH_2.CH_2.COO^-]	8·1 (1)	1·4 (1)		
α-Mercaptopropionate [CH_3.CHSH.COO^-]	5·2 (3)	1·2 (3)	12 (1)	4·3 (1)
L-Cysteine [SH.CH_2.CH(NH).COO^-]	4·8 (3)	1·6 (1)		
Thiomalate [$^-$OOC.CHSH.CHOH.COO^-]	4·1 (1)	2·1 (1)	16·6 (1)	5·6 (1)
Methyl thioglycollate* [SH.CH_2.$COOCH_3$]				

substituent attached to another carbon which is either carboxyl or hydroxyl or (in one instance) methyl ester. The latter (methyl thioglycollate) was relatively insoluble and could be tested only at 5 mM, when it antagonized oxytocin selectively. The remaining compounds were tested at 10 and 20 mM. Table 3 shows the dose-ratios obtained. It is seen that these compounds antagonized oxytocin to approximately the same extent as equimolar concentrations of thioglycerol. The antagonism of bradykinin was always less than of oxytocin, but two of the compounds (thiomalate and α-mercaptopropionate) produced appreciable antagonism of bradykinin at 20 mM.

Substances which failed to exhibit any specific antagonism of oxytocin are listed in Table 4. Compounds 1 to 3 contain at least one free sulphydryl group. Of these, dimercaprol, a dimercaptoalcohol, might have been expected to exhibit specific antagonism, but it produced unspecific depression of contractility at less than 1 mM which could have

TABLE 4

INACTIVE OR NONSPECIFIC COMPOUNDS

Ach = Acetylcholine, Brady = bradykinin, Oxy = oxytocin

Compound	Concentration (mM)	Result
1. Dimercaprol	0·5	Nonspecific depression (Oxy, Ach)
	10	Prolonged nonspecific depression
2. Thioethane	20	No antagonism (Oxy, Brady)
3. Thiolacetic acid [CH_2.COSH]	20	No antagonism (Oxy, Brady)
4. Methionine	10	No antagonism (Oxy, Brady)
	50, 100	Small nonspecific depression
5. Dithioglycollate [$^-$OOC.CH_2.SS.CH_2.COO^-]	20	No antagonism (Oxy)
6. Thiodiglycol [CH_2OH.CH_2.S.CH_2.CH_2OH]	20	Small nonspecific depression (Oxy, Brady)
7. Glycollate	100	Small nonspecific depression (Oxy, Brady)
8. Cyanoacetate	100	Nonspecific depression (Oxy, Brady)
9. Ascorbic acid	100	Small nonspecific depression (Oxy, Brady)
10. Sodium sulphite	20	Nonspecific depression (Oxy, Ach)
11. Ethylmaleimide	0·1	Nonspecific depression (Oxy, Ach)

masked a specific action. Thioethane at 20 mM, on the other hand, was quite inactive against oxytocin (Fig. 5); it may be relevant that thioethane, although it is a thiol, differs from the specific antagonistic thiols in lacking a second polar substituent group. Thiolacetic acid, a thioacid, was inactive.

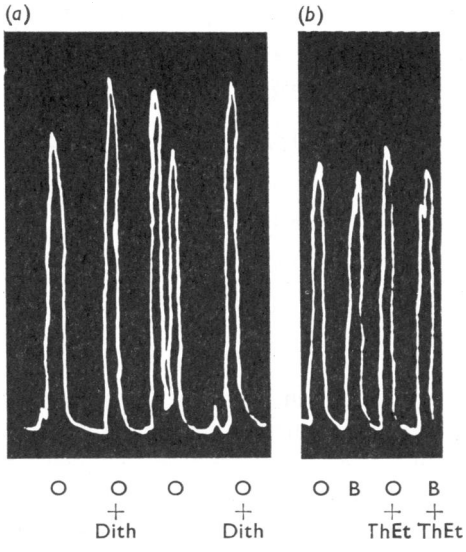

Fig. 5. Lack of antagonism of oxytocin by (*a*) 20 mM-dithioglycollate (Dith); (*b*) 20 mM-thioethane (ThEt). Rat uterus preparation in de Jalon solution at 30° C. Oxytocin, 2 and 1 mU/ml.

Compounds 4 to 6 are disulphides or thioethers lacking a free sulphydryl group; they were all inactive at 20 mM (Fig. 5). Glycollate and cyanoacetate are analogues of thioglycollate in which the sulphydryl group is replaced by a hydroxyl or a cyanide group; their ineffectiveness demonstrates the essential nature of the sulphydryl group for anti-oxytocin activity. Two non-thiol reducing agents, ascorbic acid and sodium sulphite, were tested. Ascorbic acid (100 mM) produced only a slight depression of contractility which affected bradykinin as well as oxytocin; 20 mM-sodium sulphite produced an unspecific depression of both stimulants. Finally a powerful blocker of sulphydryl groups, *N*-ethylmaleimide, was tested because sulphydryl-blockers had previously been shown to antagonize the stimulant effect of oxytocin on the sodium permeability of frog skin (Schwartz, Rasmussen, Schoessler, Silver & Fong, 1960). In the rat isolated uterus it acted powerfully but unspecifically, causing a progressive decrease of contractility at 0.1 mM with equal depression of the responses to oxytocin and acetylcholine.

Effects of thioglycerol on the depolarized uterus

The potassium-depolarized rat uterus responds to oxytocin by a slow sustained contraction which differs from the rapid and frequently intermittent response to oxytocin in sodium-Ringer solution; this difference reflects the fact that in the depolarized preparation oxytocin acts solely on individual smooth muscle cells, whilst propagated impulses in the smooth muscle syncytium are abolished (Evans, Schild & Thesleff, 1958).

Thioglycerol antagonized the effect of oxytocin in the depolarized uterus. Fig. 6 shows the effect of 20 mM-thioglycerol on the oxytocin response of a uterus immersed in isotonic potassium sulphate-Ringer (Edman & Schild, 1962). Compared with the effect of oxytocin alone the response in the presence of thioglycerol is not only reduced but less maintained and altered in shape. The finding of a change in the shape of the response curve in the presence of thioglycerol in the depolarized preparation was made repeatedly; it provides an argument against competitive antagonism in which the quality of the response would be expected to be unaltered by the antagonist.

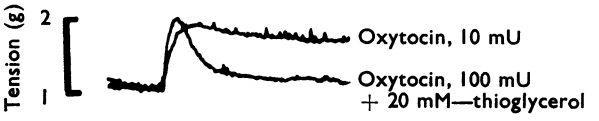

Fig. 6. The effect of 20 mM-thioglycerol on the responses to oxytocin of a depolarized rat uterus. Isometric record; potassium sulphate-Ringer in a 2-ml. organ-bath at 30° C.

Effect of thioglycollate on the uterus in situ

In preliminary experiments the concentrations of thioglycollate in plasma resulting from single intravenous injections into anaesthetized rats were determined. 1 ml. of 1 M-thioglycollate was injected into rats weighing about 200 g; after a measured time the animals were bled and the concentration of thiol in plasma determined by a rotating platinum electrode. The following results (means and standard errors) were obtained:

No. of rats	Time after injection (min)	Concentration in plasma (mM)
6	2	40·2±6·5
6	30	22·4±2·8

In a further series of experiments in which intrauterine pressures were recorded the effects of oxytocin and angiotensin were measured before and after intravenous injections of thioglycollate. Doses of 1 μg of angiotensin and 100 mU of oxytocin produced approximately equal contractile responses of the uterus. After intravenous injection of 2 to 3 ml. of 1 M-thioglycollate the responses to both drugs declined and two to four times larger doses were required to reproduce the original effects. No differential antagonism of oxytocin was observed, as is illustrated in the experiment shown in Fig. 7. A series of similar experiments is summarized in Table 5, which shows that injections of doses of thioglycollate, theoretically sufficient to produce concentrations from 40 to 60 mM in plasma, that is well above those effective *in vitro*, produced no appreciable differential antagonism of the contractile action of oxytocin on the uterus *in vivo*.

The isolated mammary strip

Isolated strips of rat mammary gland suspended in Tyrode solution at 20° C responded to oxytocin and acetylcholine. Both produced sustained contractions, but the responses to oxytocin were slower to reach a maximum than those to acetylcholine. Both drug effects were reversible and could be reproduced several times.

Thioglycollate antagonized the effect of oxytocin but not of acetylcholine. Fig. 8,*a* shows graded responses to 2 and 4 mU/ml. of oxytocin. After the addition of 20 mM-

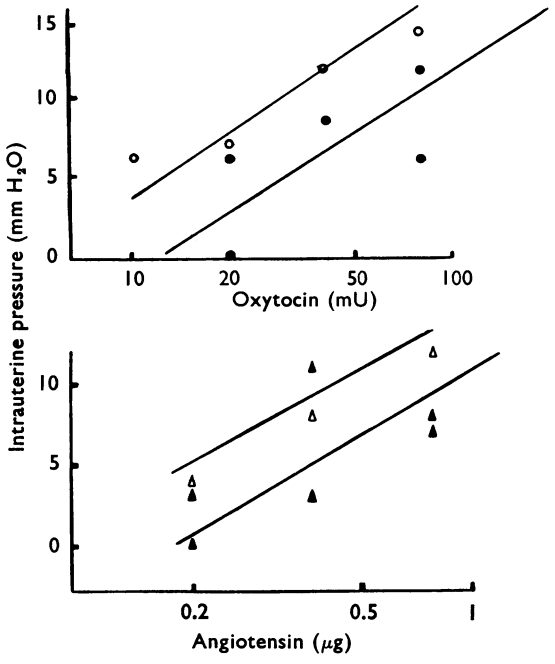

Fig. 7. Intrauterine pressures in response to intravenous doses of oxytocin and angiotensin before (○, △) and after (●, ▲) an intravenous injection of 2 ml. of 1 M-thioglycollate. Urethane anaesthesia. Isometric recording. Note equal displacement of hypertensin and oxytocin curves after thioglycollate.

TABLE 5

DOSE-RATIOS OF OXYTOCIN AND ANGIOTENSIN AFTER INTRAVENOUS THIOGLYCOLLATE ON RAT UTERUS *IN VIVO*

Dose of 1 M-thioglycollate (ml.)	Dose-ratios for	
	Oxytocin	Hypertensin
2	3·3	4·7
2	2·6	2·0
2	3·5	4·3
2	2·4	3·0
3	6·0	2·1

thioglycollate to the bath a fifty times greater dose of oxytocin produced rather less than the original effect and the dose/response curve was flattened; after removal of thioglycollate the original sensitivity and slope were practically restored. Fig. 8,*b* shows lack of antagonism of acetylcholine by 20 mM-thioglycollate. In a series of similar experiments the following dose-ratios were obtained with oxytocin: 10 mM-thioglycollate, no appreciable antagonism (three experiments); 15 mM, dose-ratio 15 in one experiment; 20 mM, dose-ratio 40 to 50 in two experiments and an unsurmountable antagonism with dose-ratio >100 in two experiments. Thioglycerol in concentrations up to 100 mM produced no antagonism in this preparation. In two experiments on rabbit isolated mammary strips, 20 mM-thioglycollate produced an antagonism of oxytocin with dose-ratios 4 and >10.

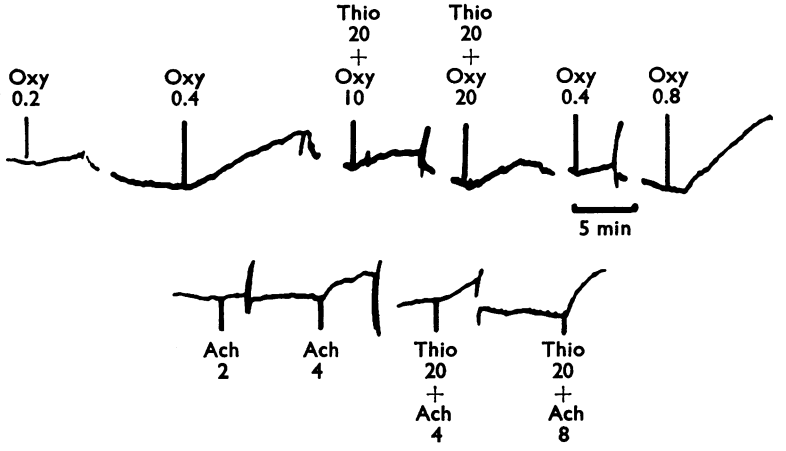

Fig. 8. Rat isolated mammary strip preparation, in 100-ml. organ-bath, isometric record. (*a*) Reversible antagonism and decrease in oxytocin slope in presence of 20 mM-thioglycollate. (*b*) Absence of antagonism of response to acetylcholine by thioglycollate. Tyrode solution was used throughout. Doses of oxytocin (Oxy) in U, of thioglycollate (Thio) in mM, and of acetylcholine (Ach) in μg.

In summary, thioglycollate, but not thioglycerol, antagonized the contractile effects of oxytocin in the myoepithelial preparation without antagonizing acetylcholine. Calculation showed that this effect could not be accounted for by destruction of oxytocin in the bath.

The increase of dose-ratio with concentration of antagonist was very marked in this preparation. 10 mM-Thioglycollate was ineffective whilst 20 mM produced high dose-ratios or unsurmountable antagonism.

Interactions of thiols and vasopressin

Lysine-vasopressin was found to be approximately thirty times less active on the rat isolated uterus than was oxytocin. Thioglycerol antagonized the effects of equiactive doses of oxytocin and vasopressin approximately equally as shown in Fig. 9. Another disulphide polypeptide, desamino-oxytocin, which was obtained through the kindness of Professor du Vigneaud, was also antagonized by thioglycerol to the same extent as oxytocin. It would appear thus that the three disulphide polypeptides acted on the same receptors on the uterus.

By contrast, the vasoconstrictor effect of vasopressin in the Ringer-perfused rat hind-limb preparation was not appreciably antagonized by either thioglycerol or thioglycollate. The procedure was to establish dose/response curves for vasopressin with Locke solution, followed by Locke solution containing a thiol compound and then again Locke solution alone. Fig. 10 shows an experiment of this kind in which 20 mM-thioglycollate failed to produce an appreciable antagonism of vasopressin. In four experiments with 20 mM-thioglycollate and two experiments with 20 mM-thioglycerol dose-ratios varying between 1 and 2 were obtained for vasopressin.

Chemical destruction of vasopressin by thiols. The destruction rate of vasopressin by thiols was similar to that of oxytocin. Both were 50% inactivated by 5 mM-thioglycollate in 30 min, at 30° C, and failed to be inactivated by 50 mM-thioglycerol under similar

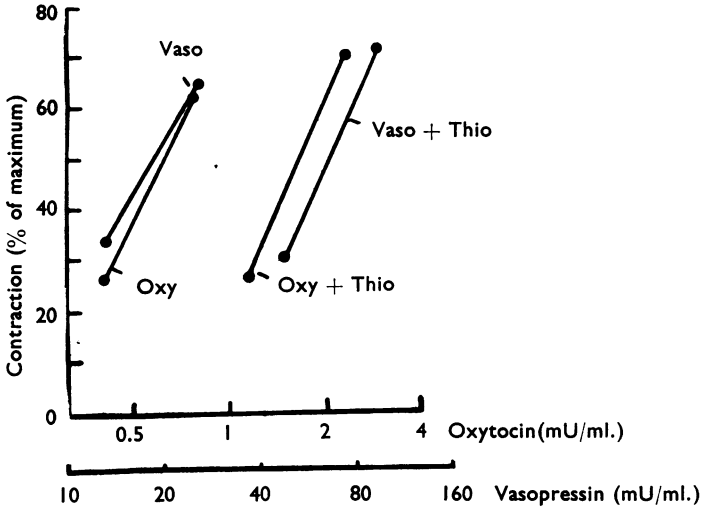

Fig. 9. Antagonism of oxytocin (Oxy) and lysine-vasopressin (Vaso) by 10 mM-α-thioglycerol (Thio) in rat isolated uterus preparation at 30° C.

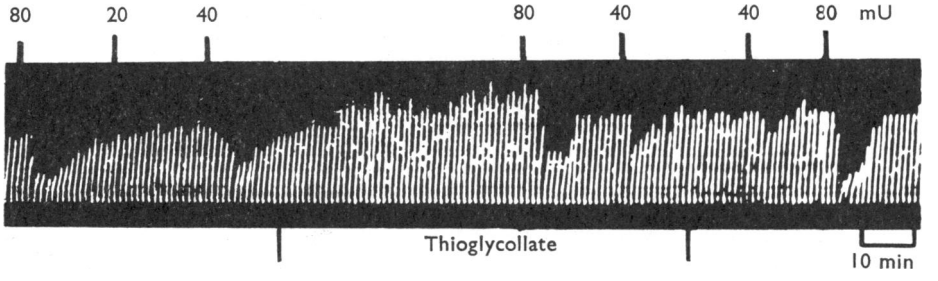

Fig. 10. Lack of antagonism of vasopressin (doses in mU, above) by 20 mM-thioglycollate added (between lines) to Locke perfusate of rat hind-limb at 20° C.

conditions. Pressor and oxytocic activities of vasopressin were destroyed at the same rate. In a series of experiments 500 mU/ml. of vasopressin was incubated with 10 mM-thioglycollate for 15 min at 20° C. The following activities were recovered at the end of the incubation period: as measured on the rat blood pressure, 32% (mean of thirteen experiments); as measured on the rat uterus, 52% (mean of five experiments). The difference was not statistically significant ($0.3<P<0.4$).

Rate of destruction of vasopressin in the organ-bath. It seemed possible that chemical destruction of disulphide polypeptides, although normally slow *in vitro*, might be accelerated in the presence of a tissue. In order to test this point experiments were carried out in which the isolated uterus suspended in an organ-bath was stimulated by vasopressin in the presence of 20 mM-thioglycollate at 20° C; after 3 min an aliquot was removed from the bath and immediately assayed on the rat blood pressure for vasopressin activity. (Thioglycollate produces no appreciable antagonism of the pressor effect of vasopressin on the blood pressure preparation.) The assays were carried out in the form of 2+2 assays. An

average activity of 64% of the original was recovered in five experiments which indicates no acceleration of destruction since it corresponds to the rate of loss to be expected from a 3-min incubation with 20 mM-thioglycollate at 20° C.

DISCUSSION

It has been shown that certain thiols act as specific pharmacological antagonists of disulphide polypeptides when tested on isolated preparations of uterus and mammary gland. This antagonism cannot be attributed to chemical destruction since it is also exhibited by a thiol such as thioglycerol which does not inactivate disulphide polypeptides. Nor can it be due to an unspecific depression of contractility since the response to other stimulants is little affected, or to an interference with membrane potential since the antagonism occurs also in depolarized preparations. It may be concluded, employing a functional definition of receptors, that the effect is exerted at the receptor level.

Thiols are chemically highly reactive compounds which are capable of forming covalent links with a variety of functional groups in tissues (Boyer, 1958); this distinguishes them from typical competitive antagonists which are as a rule chemically unreactive and form electrovalent rather than covalent links with receptors. There were indications that the antagonism between thiols and disulphide polypeptides is not typically competitive: the log dose/response curves although parallel at first became flat with high concentrations, especially in the myoepithelial preparation; the displacement of the curves was greater than would be expected from competition for a single receptor; and in the depolarized preparation the shape of the response curve became altered in the presence of antagonist. On the other hand, the pharmacological effects of the thiols were readily reversible in contrast to other antagonists forming covalent bonds with receptors such as the haloalkylamines. A plausible explanation is that, although thiols react chemically with the oxytocin receptor, this reaction is readily reversible as would be expected particularly from a reaction with a disulphide group. The antagonism might thus be due to a reversible receptor inactivation.

The sulphydryl-disulphide system is characterized by great power of breaking disulphide bonds in spite of its low oxidation-reduction potential (Foss, 1961). There is evidence that the reaction between thiols and disulphides is not normally a true oxidation-reduction reaction but a two-stage ionic displacement:

(1) $XS\text{–}SX + YS^- = XS\text{–}SY + XS^-$
(2) $XS\text{–}SY + YS^- = YS\text{–}SY + XS^-$

leading to the formation of mixed disulphides as intermediates. Eldjarn & Pihl (1957) have demonstrated by means of isotope experiments that mixed disulphides occur in the body after the injection of thiols and that these interchanges lead rapidly to the establishment of new reversible equilibria. It is conceivable that a similar reversible interaction occurs between a thiol and an essential disulphide group, such as cystine in the receptor. Alternatively, thiols may react with the disulphide group of a lipid such as lipoic acid. That thiols do not act simply as reducing agents is suggested by the ineffectiveness of ascorbic acid in antagonizing oxytocin.

Assuming that an intact disulphide group is required for the functioning of the oxytocin receptor, it does not necessarily follow that the oxytocin molecule reacts directly with it.

The first possibility is that the disulphide group plays a structural stabilizing role maintaining the configuration of the receptor. Alternatively, it may be functional and react with the disulphide group of polypeptides by some process akin to a disulphide-disulphide interchange (Sanger, 1953). Although this reaction is normally potentiated rather than inhibited by trace amounts of thiols, it is possible that thiol in excess will disrupt a delicately balanced process. Schwartz *et al.* (1960) have suggested that the oxytocin receptor in frog skin contains a free sulphydryl (not disulphide) group and that the disulphide group of the polypeptides reacts with a sulphydryl group in the receptor with formation of a polypeptide-receptor disulphide bond. Such a process would also be expected to be antagonized by excess thiol. Although it is tempting to assume that the disulphide group in oxytocin reacts with a disulphide or sulphydryl group in the receptor, these ideas may have to be revised in view of the recently reported finding of Rudinger & Jost (1964) of an active oxytocin analogue lacking a disulphide bridge. It would be interesting to know whether the new compound is antagonized by thiols, since a positive outcome would favour the first possibility discussed above.

Our findings indicate that specific oxytocin antagonism is confined to a limited range of chemical structures; the active compounds were all thiols containing an additional polar group. Thioethane which lacks the additional group was inactive. The hydrophilic group may aid the orientation of the molecule in relation to the receptor surface; alternatively, the electron withdrawing properties of a substituent in the chain may increase the reactivity of the sulphydryl group. A thioacid, thiolacetate, was inactive, thioethers and disulphides were also inactive.

A puzzling feature of our experiments was the lack of activity of thioglycollate on the uterus *in vivo*. It is conceivable that reactions such as hydrogen bonding (Boyer, 1958) occur *in vivo* which reduce the effectiveness of the sulphydryl group but so far the discrepancy is unexplained. On the other hand, it seems possible that under suitable conditions naturally occurring thiols, such as glutathione, might modify the sensitivity of oxytocin receptors also *in vivo*.

The lack of activity of thiols on the Ringer perfused hind-limb preparation could be due to a genuine difference in receptors. Receptors are most conveniently classified by antagonists and it may be that thiol-sensitive receptors such as those in the uterus and myoepithelial cell can be distinguished from thiol-insensitive ones in the blood vessels. In order to investigate this matter further it would be desirable to carry out experiments on isolated vascular strips suspended in Ringer solution under conditions more nearly comparable to those in other isolated preparations.

SUMMARY

1. Thioglycollate (10 to 20 mM) produces a reversible pharmacological antagonism of oxytocin on the rat isolated uterus preparation. The antagonism occurs at the receptor level.

2. The rate of chemical inactivation of oxytocin by thioglycollate was measured; inactivation proceeded exponentially and was too slow to account for the observed antagonism. Another thiol, thioglycerol, also acted as antagonist although it did not inactivate oxytocin chemically.

3. Thiol antagonism appears to be specific for disulphide polypeptides; the actions of vasopressin and desamino-oxytocin were similarly antagonized but not those of bradykinin and acetylcholine.

4. Several other thiols acted as antagonists but not disulphides, thioethers and non-thiol reducing agents.

5. Thioglycollate also antagonized oxytocin on the isolated uterus of rabbit and guinea-pig, on the isolated potassium-depolarized rat uterus and on the isolated mammary gland of rat and rabbit. No antagonism was obtained in the uterus *in vivo*. The vasoconstrictor effect of vasopressin in the perfused hind-limb preparation was not antagonized.

6. There is evidence that the antagonism between thiols and disulphide polypeptides is not typically competitive; it may be caused by a reversible inactivation of essential disulphide groups in the receptor.

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