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POTENTIATION OF SOME BRADYKININ EFFECTS BY THIOL COMPOUNDS

BY

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It is well known that bradykinin and kallidin are very rapidly destroyed by plasma and tissue polypeptidases (Werle, 1955; Van Arman, 1955; Habermann, 1963; Lewis, 1960; Werle, Hochstrasser, Trautschold & Leysath, 1964), a fact constituting a serious difficulty in detecting the polypeptides in various physiological and pathophysiological conditions. Van Arman (1955) showed that cysteine, approximately 1 mg/ml., almost entirely prevented loss of bradykinin from the serum within several hours at 37° C. Recently, Werle *et al.* (1964) demonstrated that thioglycollic acid very efficiently prevented the inactivation of bradykinin in plasma and greatly augmented the hypotensive effect elicited by the polypeptide in dogs. Also, the authors mentioned that thioglycollic acid did not modify the contractions elicited by bradykinin on the rat uterus. Consequently, in a recent investigation we used thioglycollic acid and cysteine (1 mg/ml.) in order to prevent loss of bradykinin in the interval between blood sampling and plasma testing on rat uterus or guinea-pig ileum preparations (Cîrstea, Suhaciu & Butculescu, 1965). As the amount of bradykinin in plasma was rather small, 2 ml. of plasma were used for each test, so that approximately 0.002 M-thioglycollic acid or cysteine was reached in the bath. Control experiments, in which these thiol compounds were added to bradykinin solutions immediately before the tests, showed that in the given doses both thioglycollic acid and cysteine greatly potentiated the contractions elicited by bradykinin on guinea-pig gut as well as on rat uterus.

In the present experiments we examine this potentiation and the mechanism of its production.

METHODS

Guinea-pig ileum was suspended in a 9 ml. organ-bath, containing air-bubbled Tyrode solution; composition (g/l.): NaCl 8, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, glucose 1 and $NaHCO_3$ 1, with atropine sulphate (0.1 mg/l.) at 35° C. Rat uterus was suspended in rat uterus-Ringer solution; composition (g/l.): NaCl 9, KCl 0.2, $CaCl_2$ 0.04, glucose 0.5 and $NaHCO_3$ 0.5, at 27° C.

The tests were performed at 5 min intervals with a contact time of 60 sec. Before the start of the assays, the differentiating capacity of the ileum was investigated and only those preparations with a good discriminating capacity were employed for the actual tests.

Synthetic bradykinin (Sandoz) and a sample of bradykinin, prepared by the technique of Diniz, Carvalho, Ryan & Rocha e Silva (1961) modified by Cirstea *et al.* (1965) and standardized against synthetic bradykinin, were used. 0.1 M solutions of thioglycollic acid (Union Chimique Belge) and cysteine (Merck) were prepared every second day in brown flasks with ground stopper, brought to *p*H 7 with 10 N-sodium hydroxide solution and stored in a refrigerator until used. Thioglycollic acid and cysteine were mixed in the syringe with bradykinin or the other smooth muscle stimulating agents immediately before being added to the organ-bath.

RESULTS

Potentiation of the contraction due to bradykinin by thioglycollic acid and cysteine. As seen in Table 1, and illustrated in Figs. 1, 2 and 3, thioglycollic acid and cysteine (0.001 M) induce a 50% potentiation of the contractions elicited by bradykinin both on the guinea-pig ileum and on the rat uterus preparations. In a higher concentration (0.002 M) the two thiol compounds produce a 100% increase of contractions due to bradykinin. The potentiating effect almost completely disappears after washing for 90 sec. In this dose range neither thioglycollic acid nor cysteine alone elicit a contraction.

TABLE 1

POTENTIATION BY THIOGLYCOLLIC ACID AND CYSTEINE OF CONTRACTIONS DUE TO BRADYKININ

Each row represents a single experiment

Test organ	Thiol compound used	Concentration ($\times 10^{-3}$ M)	Dose of bradykinin (μg)	Potentiation (%)
Guinea-pig ileum	Thioglycollic acid	1	0·1	50
Guinea-pig ileum	Cysteine	1	0·1	50
Guinea-pig ileum	Thioglycollic acid	2	0·5	100
Guinea-pig ileum	Cysteine	2	0·5	100
Guinea-pig ileum	Thioglycollic acid	2	0·2	100
Guinea-pig ileum	Cysteine	2	0·2	100
Guinea-pig ileum	Thioglycollic acid	2	0·1	100
Rat uterus	Thioglycollic acid	1	0·005	50
Rat uterus	Thioglycollic acid	2	0·01	75
Rat uterus	Thioglycollic acid	3	0·01	100
Rat uterus	Thioglycollic acid	2	0·001	100
Rat uterus	Cysteine	2	0·001	100

Effect of thioglycollic acid upon the contractions elicited by histamine, potassium chloride and acetylcholine. Results concerning the influence of thioglycollic acid upon the contractions elicited by histamine and potassium chloride on the guinea-pig ileum and by acetylcholine on the rat uterus are given in Table 2 and illustrated in Figs. 1, 2 and 3. As seen in Table 2, the contraction elicited by histamine on guinea-pig ileum is only augmented by 20 to 30% (Fig. 1) and that elicited by potassium chloride (Fig. 2) is not at all influenced by

TABLE 2

EFFECT OF THIOGLYCOLLIC ACID UPON THE CONTRACTIONS ELICITED BY HISTAMINE AND POTASSIUM CHLORIDE ON GUINEA-PIG ILEUM AND BY ACETYLCHOLINE ON RAT UTERUS

Each row represents a single experiment

Test organ	Concentration of thioglycollic acid ($\times 10^{-3}$ M)	Potentiation (%) of the contractions elicited by			
		Histamine	Potassium chloride	Acetylcholine	Bradykinin
Guinea-pig ileum	2	20–30	0		100
Guinea-pig ileum	2	20–30	0		100
Guinea-pig ileum	2	0	0		100
Guinea-pig ileum	2	20			100
Guinea-pig ileum	2	0			100
Rat uterus	2			20–30	100
Rat uterus	2			0	50
Rat uterus	4			20–30	100
Rat uterus	2			20	100

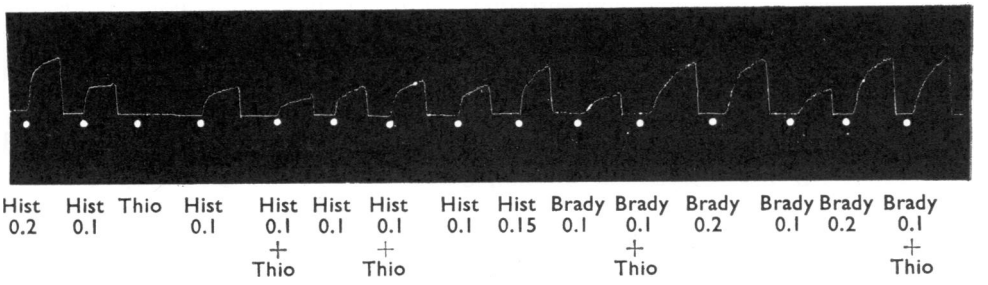

Fig. 1. Guinea-pig ileum in Tyrode solution, at 35° C, containing atropine sulphate, 0.1 mg/l. Hist= histamine, doses in μg. Thio=sodium thioglycollate, 2×10^{-3} M. Brady=bradykinin, doses in μg. Sodium thioglycollate was mixed with histamine and bradykinin immediately before adding to the bath.

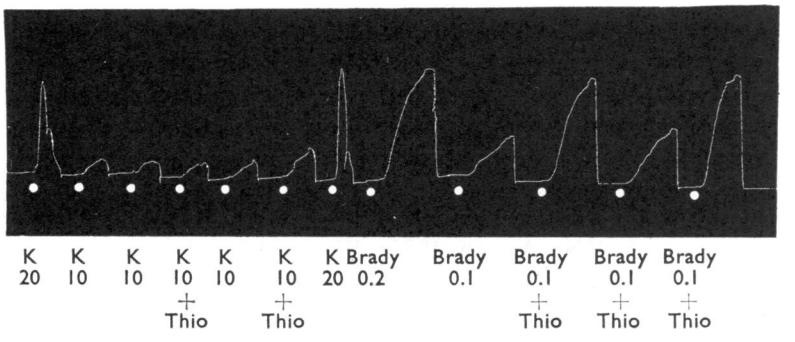

Fig. 2. Guinea-pig ileum in Tyrode solution, at 35° C, containing atropine sulphate, 0.1 mg/l. K= potassium chloride, doses in mg. Thio=sodium thioglycollate, 2×10^{-3} M. Brady=bradykinin, doses in μg. Sodium thioglycollate was mixed with potassium chloride and bradykinin immediately before adding to the bath.

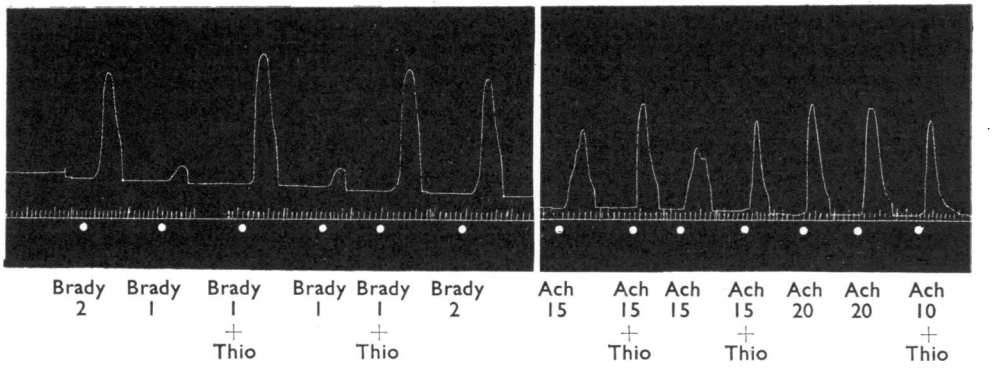

Fig. 3. Rat uterus in rat uterus-Ringer solution at 27° C. Thio=sodium thioglycollate, 2×10^{-3} M. Brady=bradykinin, doses in ng. Ach=acetylcholine, doses in μg. Sodium thioglycollate was mixed with bradykinin and acetylcholine immediately before adding to the bath. Time marks, 10 sec.

thioglycollic acid in the same concentrations at which bradykinin contractions were augmented by 100%. Similarly, contractions elicited by acetylcholine on the rat uterus were only slightly potentiated (20 to 30%) compared with contractions due to bradykinin (Fig. 3).

Bradykinin-inactivating capacity of the guinea-pig ileum. In five experiments the bradykinin inactivating capacity of the guinea-pig ileum was investigated. Fragments of guinea-pig gut, similar in size to those used for biological estimation, were incubated at 35° C for 2, 3, 4 and 5 (two experiments) min with 5 ml. of Tyrode solution, containing bradykinin (0.1 μg/ml.). Control samples similarly treated, but without gut fragments, were included in the test. No detectable bradykinin inactivation took place during incubation with the tissue for 2, 3 or 4 min, but after incubation for 5 min there was a 20% decrease in one experiment and a 20 to 30% decrease in the other. No detectable inactivation was found in the control samples after incubation for the same time interval.

DISCUSSION

Our results show that, when mixed with bradykinin immediately before being added to the organ-bath, thioglycollic acid and cysteine, in concentrations that have no actual stimulating effect, potentiate by 100% the contractions elicited by the polypeptide on guinea-pig ileum and rat uterus preparations. There are at least three mechanisms to which this phenomenon may be ascribed.

(1) Inhibition of tissue polypeptidases which are kinin-destroying enzymes (Lewis, 1960; Habermann, 1963; Van Arman, 1955; Werle, 1955; Werle *et al.*, 1964).

(2) Sensitization of smooth muscle myofilaments following the reduction of disulphide groups to sulphydryl groups, as thiol groups play an important part in the formation of actomyosin and in adenosine triphosphatase activity displayed by this molecular complex (Huxley, 1960). It is relevant here that the analogy between the basic mechanisms of contraction of striated and smooth muscles was recently strengthened (Csapo, 1962; Lowy & Hanson, 1962).

(3) An increase in the amount of bradykinin receptors following the rupture of disulphide bridges and the reversible denaturation of the tissue proteins.

Our results give some indications about the extent to which each of the above-mentioned mechanisms can be involved in the effect. Firstly, it must be emphasized that, under the same conditions, the contractions elicited by histamine on the guinea-pig gut and by acetylcholine on the rat uterus are definitely less augmented and those elicited by potassium chloride are not at all potentiated. These findings show that the potentiation of bradykinin-induced contractions is primarily specific. However, the influence of thiol compounds on the smooth muscle stimulating effects of other peptides has not been investigated in the present experiments. Consequently, this potentiating effect might be specific to peptides in general and not necessarily to bradykinin in particular.

Secondly, our results show that the bradykinin destroying capacity of the guinea-pig gut under the conditions of testing is not large enough to ascribe to a great extent this potentiation to the inhibition of tissue polypeptidases. Thus, the potentiating effect upon the contractions is complete during 1 min, while a detectable destruction of bradykinin is found only after an incubation period of 5 min. However, as the concentration of brady-

kinin in the incubation fluid was several times higher than the concentration reached in the organ-bath during testing, the fact must be cautiously interpreted and the possibility that the inhibition of tissue polypeptidases may play some part in this phenomenon cannot be rejected. The second possible mechanism, sensitization of the actomyosin myofilaments following the rupture of disulphide bridges, cannot play an important role in this phenomenon, because such a mechanism would have a nonspecific character and the contractions elicited by any stimulating agent would be potentiated to a similar extent.

The third possible mechanism, an increase in the amount of bradykinin receptors following the unfolding of the protein complexes by the rupture of disulphide bridges, appears to fit better with the experimental findings. In fact, this hypothesis explains satisfactorily not only the augmentation of the contractions elicited by bradykinin, but also the weaker augmentation of the contractions elicited by histamine and acetylcholine, as well as the lack of potentiation of the contractions elicited by potassium chloride. Indeed, one may assume that the unfolding of the protein complexes also renders available some normally unavailable histamine and acetylcholine receptors. The quantitative difference between the potentiation of the contractions elicited by bradykinin, on the one hand, and of those elicited by histamine and acetylcholine, on the other, may be due to differences in localization of the corresponding receptors. Histamine and acetylcholine receptors could be situated primarily at the outer surface of the protein macromolecules, while bradykinin receptors could be equally distributed at the outer as well as the inner macromolecular surfaces. The lack of potentiation of the contractions elicited by potassium chloride may be due to a different mechanism of action, that may not involve specific receptors. If, however, there were specific potassium receptors, the findings would show that all of them are equally available.

SUMMARY

1. Thioglycollic acid and cysteine (0.001 and 0.002 M) augment by 50% and by 100%, respective to dose, the contractions elicited by bradykinin on guinea-pig ileum and on rat uterus preparations.

2. With these doses, the thiol compounds have no stimulating effect on either guinea-pig gut or rat uterus.

3. Under the same conditions, thioglycollic acid and cysteine augment by only 20 to 30% the contractions elicited by histamine on guinea-pig ileum and by acetylcholine on rat uterus and do not at all potentiate the contractions elicited by potassium chloride.

4. Following incubation of a fragment of guinea-pig gut (similar in size to those used for biological estimation) with bradykinin (0.1 μg/ml.) no detectable inactivation of bradykinin is found after incubation for 2 to 4 min, and a 20% decrease in activity is found only after 5 min incubation.

5. Three possible mechanisms of the potentiation of bradykinin-induced contractions by thiol compounds are discussed, and the hypothesis of an increased amount of bradykinin receptors following the rupture of disulphide bridges with subsequent unfolding of the protein macromolecules is considered the most plausible.

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NOTE ADDED IN THE PROOF

While this paper was in the press, we became acquainted with Picarelli, Henriques & Oliveira's paper "Potentation of bradykinin action on smooth muscle by cysteine" published in *Experientia (Basel)*, 1962, **18**, 77–79. Our results concerning the effect of cysteine largely agree with those of the above authors, who ascribe, however, this effect wholly to the inhibition of tissue kininases.

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