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# POTENTIATION OF BRADYKININ BY DIMERCAPTOPROPANOL (BAL) AND OTHER INHIBTORS OF ITS DESTROYING ENZYME IN PLASMA*

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**Abstract**—Metal-binding agents such as BAL, versene, thioglycolic acid, cysteine and 8-hydroxyquinoline were found to inhibit the bradykinolytic activity of rat plasma and with the exception of 8-hydroxyquinoline, to potentiate the effect of synthetic bradykinin upon the guinea pig ileum. Our results agree with the suggestion that carboxy-peptidase-B might be the enzyme responsible for the inactivation of bradykinin in plasma. A general similarity seems to exist between inhibition of the bradykininolytic activity and potentiation of the action of bradykinin upon isolated preparations except in the case of 8-hydroxyquinoline. This suggests that the potentiation of bradykinin may be due to inhibition of the enzyme which inactivates it, presumably present in the biological preparations.

ELLIOTT *et al.*[1, 2] described the destructive effect of carboxypeptidase upon purified preparations of bradykinin and Erdös[3] indicated that the enzyme in plasma responsible for the destruction of bradykinin has the characteristics of carboxypeptidase-B. Following this observation we have tried several inhibitors, *in vitro* and *in vivo*, for their capacity to increase the physiological effects of bradykinin upon different biological structures. The rationale for this approach to the problem was the indication that carboxypeptidases contain an atom of zinc which constitutes a functional part of the enzyme.[4–7] Metal-chelating agents, such as 1:10-phenanthroline, 8-hydroxyquinoline-5-sulphonic acid, and 2:2′-dipyridyl, were found to inhibit a purified preparation of carboxypeptidase-B, from autolysed porcine pancreas tissue.[7] In the present paper, we have utilized other agents, such as BAL, EDTA, cysteine, 8-hydroxyquinoline and thioglycolic acid, as possible inhibitors of the bradykininolytic enzyme in plasma and also as potentiators of the biological effect of bradykinin.

## MATERIAL AND METHODS

### *Enzyme inhibitors*

Two classes of compounds were utilized in the experiments on *in vitro* potentiation and protection of bradykinin when added to fresh plasma: (a) sulphydryl compounds, such as cysteine HCl, thioglycolic acid (TGA), and 2:3-dimercaptopropanol (BAL), and (b) metal chelating agents such as versene (EDTA) and 8-hydroxyquinoline (8-HQ). Cysteine-HCl was neutralized with an equivalent of NaOH before use. The

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other compounds were added to plasma and found not to change the pH of the incubating mixture, in the concentrations utilized. The origin of the compounds is indicated at the end of this section.

In all experiments synthetic bradykinin (SBR 640) kindly supplied by Sandoz Ltd. (Basel) was used. The material was found to contain 2 units of our standard per $\mu$g.[14]

*Biological preparation*

The ileum of the guinea pig was suspended in a 4-ml flask at 35 °C using Tyrode solution (NaCl 8 g; KCl 0·2 g; $CaCl_2$ 0·2 g; $MgCl_2$ 0·1 g; $NaHCO_3$ 1 g; $NaH_2PO_4$ 0·5 g, and dextrose 1 g per l. of solution) as bathing fluid. The volume of the assaying mixture added to the bathing fluid varied from 0·05 to 0·1 ml.

*Collection of rats' plasma*

The blood samples were collected into heparinized syringes from the abdominal aorta, with the animals under light ether anaesthesia. The blood was centrifuged and the separated plasma was kept at room temperature for 1 hr before assaying for its action upon the guinea pig ileum. As a rule, after this interval of time, the plasma was inactive upon this preparation. When a contraction persisted after this time (possibly due to histamine contamination) the plasma was discarded in order to avoid the use of anti-histaminics which might complicate the course of the experiments.

*Bradykininolytic assay*

All enzymatic assays were done at 10 °C in a Dewar flask, in glass test tubes, stoppered with rubber coverings. The use of polyethylene tubes was found unnecessary on account of the long time the plasma had been standing at room temperature. The small amounts of bradykinin spontaneously released by contact with glass were destroyed during this interval.

To each tube, 0·5 ml of plasma and 0·5 of saline (with or without the inhibitors to be tested) were added. After 2 or 3 min allowed for the temperature equilibration, a control sample (0·1 ml) from each tube was tested upon the guinea pig ileum. To the remaining volume (0·9 ml) 0·1 ml of synthetic bradykinin (1 $\mu$g/0·1 ml) was added giving a final concentration of bradykinin of 1 $\mu$g/ml in the incubating mixture. The final concentration of inhibitors in the incubating mixtures were the following: BAL, $2{\cdot}02 \times 10^{-3}$ M; 8-HQ, $3{\cdot}41 \times 10^{-3}$ M; TGA, $5{\cdot}45 \times 10^{-3}$ M; EDTA, $2{\cdot}62 \times 10^{-3}$ M, and cysteine, $8{\cdot}25 \times 10^{-3}$ M.

Aliquots (0·1 ml) of the incubating mixtures were taken at different intervals of time and assayed directly upon the biological preparation. Each sample of plasma was used only once or twice for the assay of a given inhibitor. As a rule, the controls with fresh plasma diluted to 1 : 1 with saline would destroy 1 $\mu$g/ml of SBR in less than 15 min at 10 °C.

As bradykinin standard, a mixture containing 1 $\mu$g/ml of SBR, plus the proper concentration of the different inhibitors, was utilized.

*Computation of the data from the biological assay*

Owing to the limited volumes at our disposal and the short time interval for the assays, we had to develop a method to deduce the amounts of active material (bradykinin) remaining in the solution after the indicated time intervals; for this purpose a double reciprocal plot (reciprocals of the doses and of the heights of the contractions)

was utilized in the course of an assay upon the same preparation. Two doses of the standard (0·1 and 0·05 $\mu$g of BRS) were assayed repeatedly during the experiment, and the reciprocals of the averages of the responses were plotted against the reciprocal of each dose.

The coincidence of the reciprocal of the response of the unknown with that of the standard, was checked graphically upon the standard lines. Among other possible methods to rectify the dose–response curve, the double reciprocal plot has been found to fulfil the desideratum over a large range of doses and responses.[8]

*Drugs and inhibitors*

The following drugs were used: dimercaptopropanol (BAL) (California Corporation for Biochemical Research); cysteine hydrochloride (Eastman Organic Chemicals); EDTA, di-sodium salt of ethylene diaminetetra-acetic acid dihydrate (Bersworth Chemical Co.); histamine diphosphate (Abbott); acetylcholine (Roche); thioglycolic acid and 8-hydroxyquinoline were kindly supplied by the Department of Biochemistry, F.M.R.P.

## RESULTS

*Potentiation of bradykinin effects upon the guinea pig ileum*

Small concentrations of BAL and TGA, of the order of $10^{-5}$ to $10^{-6}$ M, strongly potentiate the stimulating effect of bradykinin upon the isolated guinea pig gut.

As shown in Fig. 1, a concentration of $5{\cdot}24 \times 10^{-6}$ M of BAL was already enough to produce an enormous increase in the effect of 0·05 $\mu$g of synthetic bradykinin added to the 4·0 ml bath.

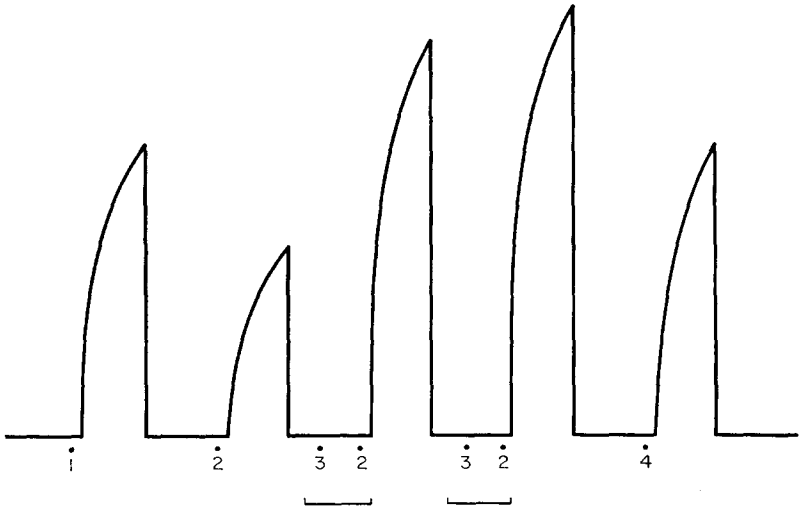


FIG. 1. Potentiation by BAL of the action of bradykinin upon the guinea pig ileum: (1) 0·1 $\mu$g synthetic bradykinin (BRS); (2) 0·05 $\mu$g BRS; (3) BAL, final molar concentration $5{\cdot}24 \times 10^{-6}$; (4) 0·05 $\mu$g BRS, 3 min after washing out the previous additions. Note a residual potentiation after the treatment with BAL. Volume of the bath: 4·0 ml.

Histamine, and less so acetylcholine, were also potentiated by large concentrations of BAL, as shown in Table 1. However, under similar conditions, the potentiation of bradykinin was almost invariably more pronounced than that of histamine (except in one experiment with a concentration of $4{\cdot}03 \times 10^{-4}$ M of BAL). The potentiation of acetylcholine was considerably less or doubtful. The absence of correlation between

TABLE 1. POTENTIATION OF BRADYKININ, HISTAMINE AND ACETYLCHOLINE ON GUINEA PIG ILEUM BY BAL CYSTEINE, THIOGLYCOLIC ACID, EDTA*

| Enzyme inhibitor | Molar concentration (final) | Acetylcholine (0·0125 μg)† | Histamine (0·05 μg)† | Bradykinin (0·05 μg)† |
|---|---|---|---|---|
| BAL‡ | $4{\cdot}02 \times 10^{-4}$ | 17 | 26 | 77 |
| | $4{\cdot}03 \times 10^{-4}$ | 2 | 23 | 112 |
| | $4{\cdot}03 \times 10^{-4}$ | 10 | 70 | 51 |
| BAL | $2{\cdot}01 \times 10^{-5}$ | 3 | 0 | 90 |
| | $2{\cdot}01 \times 10^{-5}$ | 34 | 21 | 68 |
| | $2{\cdot}01 \times 10^{-5}$ | 0 | 7 | 102 |
| Cysteine | $8{\cdot}25 \times 10^{-3}$ | | —41 | 97 |
| | $8{\cdot}25 \times 10^{-3}$ | | —36 | 59 |
| | $8{\cdot}25 \times 10^{-3}$ | | —22 | 50 |
| Thioglycolic acid | $1{\cdot}36 \times 10^{-4}$ | | 0 | 54 |
| | $1{\cdot}36 \times 10^{-4}$ | | —10 | 47 |
| | $1{\cdot}36 \times 10^{-4}$ | | 1 | 101 |
| EDTA | $6{\cdot}5 \times 10^{-4}$ | | −27 | 26 |
| | $6{\cdot}5 \times 10^{-4}$ | | −19 | 11 |
| | $6{\cdot}5 \times 10^{-4}$ | | −26 | 40 |

* The results presented in each horizontal line were obtained upon the same ileum, and are expressed in percentage increase of response. Atropine was previously added to the bathing fluid in the assays with cysteine, thioglycolic acid and EDTA.

† These doses are added to a bath of 4 ml.

‡ In a few assays, in which atropine has not been added to the bathing fluid, BAL has been capable of eliciting a small contraction. In such instances, the experiments were discarded. The doses of BAL utilized were much higher than those which already elicited a potentiation of bradykinin; the small doses did not influence histamine or acetylcholine in any consistent way.

potentiation of bradykinin and that of histamine was better shown with cysteine which exerted an inhibitory effect upon histamine, under conditions in which bradykinin was strongly potentiated. Potentiation of histamine by thyoglycolic acid was doubtful in one case, small in another, and negative (inhibition) in the third.

Cysteine in concentrations of $8{\cdot}25 \times 10^{-3}$ M potentiates the action of bradykinin and inhibits the contraction elicited by histamine.

Versene (EDTA) exerted a small potentiating effect upon bradykinin when added in concentrations of $6{\cdot}5 \times 10^{-4}$ M. However, 8-hydroxyquinoline, in concentrations of from less than $6{\cdot}8 \times 10^{-5}$ up to $6{\cdot}8 \times 10^{-3}$ M, did not potentiate the effect of bradykinin upon the guinea pig ileum.

*Inhibition of the bradykininolytic activity of fresh plasma*

As indicated in Fig. 2, BAL and 8-hydroxyquinoline are very efficient in protecting bradykinin when added to samples of rat's plasma. Though the control samples were completely inactivated in less than 13 min, the samples containing these inhibitors

had the full activity after 29 min incubation at 10 °C. Under these conditions cysteine and thioglycolic acid had a partial protecting effect; versene (EDTA) was less effective, than BAL, although 43 per cent of the original bradykinin activity was still present after the standard time of 13 min, while almost all the activity of the control had disappeared.

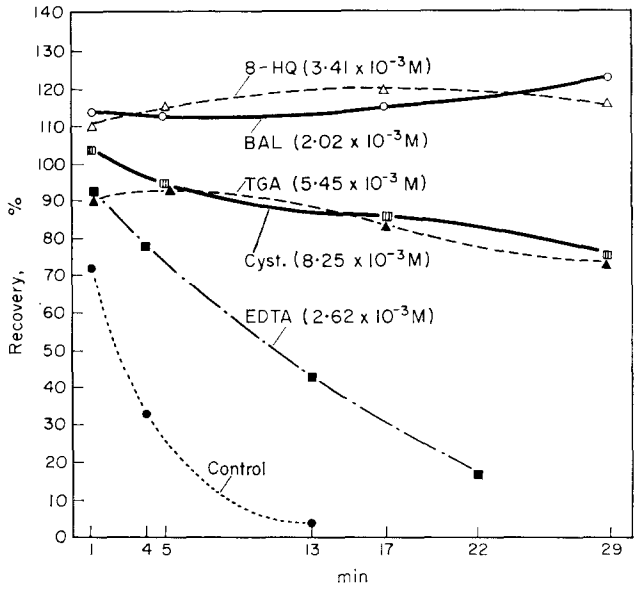


FIG. 2. Recovery of bradykinin activity after incubation of 1 $\mu$g of synthetic bradykinin with rat's plasma diluted 1:1 with saline (control); in the experiments, the diluting saline contained the adequate amounts of inhibitors to give a final concentration in the incubating mixtures:

BAL (dimercaptopropanol), $2{\cdot}02 \times 10^{-3}$ M;
8-HQ (8-hydroxyquinoline), $3{\cdot}41 \times 10^{-3}$ M;
Cyst (cysteine), $8{\cdot}25 \times 10^{-3}$ M;
TGA (thioglycolic acid), $5{\cdot}45 \times 10^{-3}$ M;
EDTA (versene), $2{\cdot}62 \times 10^{-3}$ M.

These results are in conformity with the possibility suggested by Erdös[3] that the enzyme in plasma responsible for the destruction of bradykinin is carboxypeptidase-B. To our knowledge, 8-hydroxyquinoline has not been utilized as an inhibitor of carboxypeptidase-B, though it has been shown, in this paper, to be an excellent inhibitor of the destruction of bradykinin in rat's plasma. However, 8-hydroxyquinoline 5-sulphonic acid is a known inhibitor of carboxypeptidase-B.

## DISCUSSION

Though a general similarity between the potentiation and protection of bradykinin against its destruction in fresh plasma has been reported, there is still a discrepancy as regards the destruction of bradykinin and the potentiation of its action upon the isolated guinea pig ileum. While 8-hydroxyquinoline fully protects bradykinin when

added to the rat's plasma, it had no effect when tried as a potentiator of the biological effect of bradykinin upon the guinea pig ileum. One reason for this discrepancy might be the low solubility of the compound and its inability to reach the active sites of the enzyme in the biological structure. On the other hand, sulphydryl compounds, and especially BAL, would be more suitable to reach such active centres and therefore prevent the destruction of bradykinin in the biological structures.

As shown above, BAL, and to a lesser extent thioglycolic acid, strongly potentiate the effects of bradykinin *in vitro* upon the guinea pig ileum. Bradykinin is also potentiated *in vivo* by these compounds. The results with other structures and in the intact animal (upon the systemic blood pressure or the capillary permeability) will be presented elsewhere.[15]

The potentiation of histamine by BAL deserves special consideration in as much as cysteine has a definite inhibitory effect upon this amine, as known for long time[9, 10] and thioglycolic acid had no effect or a slightly inhibitory one. It might well be that histaminase is also inhibited by BAL, but we have no indications that it might be so.

We have confirmed previous works[11–13] showing that cysteine potentiates the effects of bradykinin upon the guinea pig ileum. In our experiments cysteine and thioglycolic acid were effective to protect bradykinin against its destruction by fresh rat's plasma, although both reagents were much less potent than BAL and 8-hydroxyquinoline.

With versene the potentiation of the biological effects of bradykinin was much smaller than with the sulphydryl compounds, but in conformity with the weak protection exerted by this agent upon bradykinin when incubated with rat's plasma. Folk *et al.*[7] found versene (EDTA) rather inert as an inhibitor of carboxypeptidase-B upon synthetic substrates. It might well be that there occurs in plasma another bradykininolytic enzyme blocked by versene that might explain the small protection obtained in our experiments. Versene, in the concentrations utilized, already had some antagonistic effect on histamine, which might be due to damage to the contractile mechanism of the ileum. If this were so, the potentiation upon the contraction elicited by bradykinin would acquire more significance and would be more in line with its definite, though discrete, protective action on the destruction of bradykinin by rat's plasma.

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