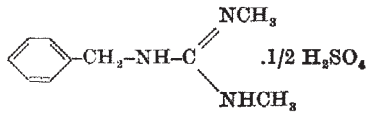
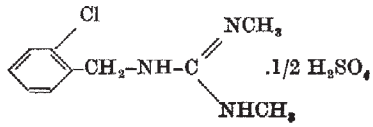
pyrimidines (*N*-2-guanidino-pyrimidine sulphate and *N*-4-guanidino-2,6-pyrimidine sulphate) maintain a high guanidine-like anti-polio activity. Indeed, not only is their antiviral spectrum identical to that of guanidine, but they are also ineffective against guanidine-resistant poliovirus and can replace guanidine in supporting the growth of guanidine-dependent polioviruses[4,5].

During our investigations on antiviral activity of guanidine-derivatives in order to find some structure-activity relationships, we were able to find that *BW* 467*C*60 (Bethanidine) and its derivative *BW* 392*C*60 were endowed with anti-polio and anti-vaccinia activity *in vitro*.




Bethanidine




*O*-chlorobethanidine

The viral strains and the procedures used for the culture of human amnion cells (Mascoli's line) were as described previously[6]. The assays of poliovirus cytopathic effect (CPE) were performed by the plaque technique of Dulbecco[7]; those of vaccinia virus CPE were described previously[8]. The inhibition of polio- and vaccinia-virus replication was evaluated by counting the cytopathic units (CPU) present at various intervals in the cell cultures. Since *BW* 467*C*60 and *BW* 392*C*60 lower the *p*H of the medium, this was kept at 7·3 by adding a few drops of 1 per cent $NaHCO_3$ solution.

The data in Tables 1 and 2 demonstrate that the two compounds exert a clear inhibition of the CPE and multiplication of polio and vaccinia viruses.

Table 1. Inhibition by Bethanidine (467*C*60) and its *O*-Chloro-derivative (392*C*60) of Polio 1 and Vaccinia Virus Cytopathic Effect

| Viral strain | Compound tested (μg/ml.)* | | Percentage inhibition (mean and range) | |
|---|---|---|---|---|
| Polio 1 | Bethanidine | 125·0 (5) | 92·2 | (75–100) |
| Polio 1 | Bethanidine | 62·5 (4) | 81·0 | (57–96) |
| Polio 1 | Bethanidine | 41·0 (2) | 3·5 | (0–7) |
| Polio 1 | *O*-Chl. Beth. | 125·0 (2) | 81·0 | (62–100) |
| Polio 1 | *O*-Chl. Beth. | 62·5 (2) | 50·0 | (24–76) |
| Polio 1 | *O*-Chl. Beth. | 41·0 (2) | 0·0 | — |
| Vaccinia | Bethanidine | 125·0 (3) | 89·0 | (72–98) |
| Vaccinia | Bethanidine | 62·5 (3) | 64·0 | (50–72) |
| Vaccinia | Bethanidine | 41·0 (2) | 13·0 | (6–20) |
| Vaccinia | *O*-Chl. Beth. | 125·0 (2) | 90·0 | (85–95) |
| Vaccinia | *O*-Chl. Beth. | 62·5 (2) | 48·0 | (40–56) |
| Vaccinia | *O*-Chl. Beth. | 41·0 (2) | 2·0 | (0–4) |

* No. trials in parentheses.

Table 2. Inhibition by Bethanidine (467*C*60) of Polio 1 and Vaccinia Virus Replication

| Viral strain | Inoculum (CPU) | Bethanidine (μg/ml.)* | CPU detected after 36 h (mean and range) |
|---|---|---|---|
| Polio 1 | $10^4$ | — (4) | $5{\cdot}8 \times 10^7$ ($6{\cdot}8 \times 10^6$–$9{\cdot}7 \times 10^7$) |
| Polio 1 | $10^4$ | 125 (4) | $6{\cdot}1 \times 10^5$ ($4 \times 10^5$–$6{\cdot}8 \times 10^5$) |
| Vaccinia | $5 \times 10^3$ | — (4) | $1{\cdot}5 \times 10^6$ ($10^6$–$3 \times 10^6$) |
| Vaccinia | $5 \times 10^3$ | 125 (4) | $1{\cdot}9 \times 10^4$ ($6 \times 10^3$–$3 \times 10^4$) |

* No. trials in parentheses.

*BW* 392*C*60 and bethanidine seem to act through a different mechanism from that of guanidine.

In fact, (*a*) the spectra of their virus-inhibiting action differ from that of guanidine, (*b*) they are fully effective in inhibiting guanidine-resistant poliovirus strains, and (*c*) they cannot replace guanidine in supporting the growth of guanidine-dependent strains: on the contrary, they inhibit their guanidine-conditioned multiplication.

It appears that antiviral activity is present in several guanidine derivatives, but they seem to exert their inhibiting properties by different mechanisms.

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[1] Loddo, B., *Boll. Soc. Ital. Biol. Sperim.*, **37**, 395 (1961).
[2] Loddo, B., Ferrari, W., Brotzu, G., and Spanedda, A., *Nature*, **193**, 97 (1962).
[3] Crowther, D., and Melnick, J. L., *Virology*, **15**, 65 (1961).
[4] Loddo, B., Garzia, A., and Ferrari, W., *Experientia*, **20**, 12 (1964).
[5] Loddo, B., Garzia, A., and Ferrari, W., *Boll. Soc. Ital. Biol. Sperim.*, **39**, 1542 (1963).
[6] Loddo, B., Ferrari, W., Brotzu, G., and Spanedda, A., *Boll. Ist. Sieroter. Milanese*, **41**, 111 (1962).
[7] Dulbecco, R., *Proc. U.S. Nat. Acad. Sci.*, **38**, 747 (1952).
[8] Loddo, B., Schivo, M. L., and Ferrari, W., *Lancet*, ii, 914 (1963).

## Bronchoconstrictor Action of Bradykinin, Kallidin and Eledoisin

Collier *et al.*[1] reported in 1959 that partially purified natural bradykinin, made by incubating trypsin with ox globulin, causes bronchoconstriction when injected into guinea-pigs. This action was confirmed for the synthetic nonapeptide[2,3] as well as for the decapeptide kallidin[4] and the endecapeptide eledoisin[5]. In contrast to the bronchoconstrictor action of serotonin and histamine, the effect of bradykinin and kallidin was completely abolished by pretreatment with acetylsalicylic acid and phenylbutazone[1,4].

These experiments were carried out in artificially respired guinea-pigs using the sensitive overflow method of Konzett and Rössler[6]. In spontaneously respiring guinea-pigs, however, we found that doses of bradykinin and kallidin up to 16 μg/kg (intravenous) did not decrease but rather increased the respiratory minute volume by more than 100 per cent[7,8], while higher doses (20–60 μg/kg; intravenous) produced apnoea which is probably of central origin[9].

In the experiments to be described the actions of synthetic bradykinin and kallidin on tidal volume and on inflation volume were examined in guinea-pigs under urethane anaesthesia by means of body-plethysmography according to the method of Koller[10]; the effects were compared with those of eledoisin and serotonin. All injections were made through a cannula in the external jugular vein.

In spontaneously breathing guinea-pigs graded doses of serotonin and eledoisin produced a dose-dependent reduction of the tidal volume (Fig. 1). Furthermore, a tachypnoic response of short duration was regularly observed. Following the injection of high doses of serotonin (10 μg/kg) and of eledoisin (5 μg/kg), the tidal volume became virtually zero although the movements of the respiratory muscles (Fig. 1, bottom trace) had increased. Bradykinin, however, reduced the tidal volume only slightly in doses up to 20 μg/kg and caused a long-lasting tachypnoea, while higher doses (25 μg/kg) produced an arrest of respiration in expiratory position. Similar results were obtained with kallidin.

In artificially respired guinea-pigs serotonin and eledoisin reduced the inflation volume by an amount depending on the given dose (Fig. 2). To reduce the inflation volume by 50 per cent ($ED_{50}$) approximately 1 μg/kg of eledoisin and 9 μg/kg of serotonin were required. The dose-response curve of bradykinin differed from those of eledoisin and serotonin by its slope. 1 μg/kg of bradykinin had nearly

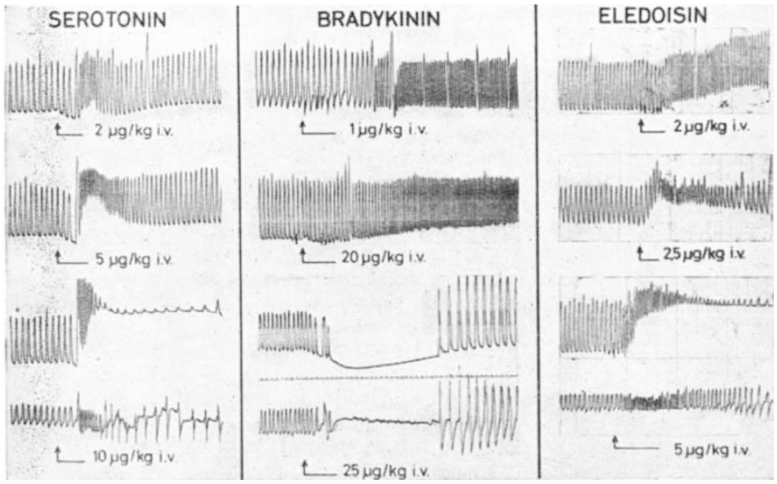


Fig. 1. Effect of bradykinin, eledoisin and serotonin on the tidal volume in the guinea-pig. Guinea-pig (420 g) under urethane anaesthesia (1·5 g/kg; subcutaneous). Body-plethysmography; for details of method see Gjuriš *et al.*[8]. Bottom trace: movements of respiratory active muscle groups

maximal effect, and even extremely high doses (up to 200 µg/kg) were unable to reduce the inflation volume by more than 50 per cent. The dose response curve of the less-active kallidin closely resembled that of bradykinin.

The reduction of inflation volume by small doses of bradykinin and kallidin (5 µg/kg) was completely prevented by the broncholytic agents isoprenaline (1–10 µg/kg) or epinephrine (0·5–10 µg/kg). High doses of the plasma kinins (50 µg/kg), although not significantly more effective, remained unaffected or were only slightly reduced by the broncholytics which, on the other hand, readily abolished even the maximal effects of eledoisin or serotonin.

Acetylsalicylic acid (5–10 mg/kg) and phenylbutazone (5–10 mg/kg), which did not prevent the reduction of inflation volume caused by eledoisin or serotonin, abolished only the action of small doses of bradykinin and kallidin (5 µg/kg), but did not antagonize the action of higher doses of the plasma kinins (50 µg/kg). Even pretreatment with 100 mg/kg of acetylsalicylic acid failed to prevent the action of 50 µg/kg of bradykinin or kallidin.

The different slopes of the dose-response curves of bradykinin and kallidin as compared with those of eledoisin and serotonin suggest that the action of the plasma kinins cannot be explained simply by bronchoconstriction. Since the pleura of the guinea-pig's lung contains an exceptionally large number of smooth muscle fibres[11], it seems possible that the reduction of inflation volume caused by the plasma kinins is at least partly due to the contraction of pleural muscle fibres. This view is supported by the finding that bradykinin is much more effective in reducing the inflation volume when applied locally to the pleural surface instead of being administered by inhalation or by intravenous injection, while the reverse holds true for histamine[4,12].

Recently, it has been reported[13,14] that in guinea-pigs small doses of the plasma kinins produce constriction of pulmonary vessels (possibly of the pulmonary veins) which was also prevented by salicylates. Thus, an increased blood volume of the lungs as caused by the constriction of pulmonary veins might be an additional factor in the action of the plasma kinins.

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[1] Collier, H. O. J., Holgate, J. A., Schachter, M., and Shorley, P. G., *J. Physiol.* (Lond.), **149**, 54 P (1959); *Brit. J. Pharmacol.*, **15**, 290 (1960).
[2] Boissonas, R. A., Gottmann, St., Jaquenoud, P. A., Konzett, H., and Stürmer, E., *Experientia* (Basle), **16**, 326 (1960).
[3] Konzett, H., and Stürmer, E., *Brit. J. Pharmacol.*, **15**, 544 (1960).
[4] Bhoola, K. D., Collier, H. O. J., Schachter, M., and Shorley, P. G., *Brit. J. Pharmacol.*, **19**, 190 (1962).
[5] Erspamer, V., and Falconieri Erspamer, G., *Brit. J. Pharmacol.*, **19**, 337 (1962).
[6] Konzett, H., and Rössler, R., *Arch. exp. Path. und Pharmakol.*, **195**, 71 (1940).
[7] Gjuriš, V., Heicke, B., Holtz, P., and Westermann, E., *Experientia* (Basle), **18**, 385 (1962).
[8] Gjuriš, V., Heicke, B., and Westermann, E., *Arch. exp. Path. und Pharmakol.*, **247**, 429 (1964).
[9] Gjuriš, V., Heicke, B., and Westermann, E., *Arch. exp. Path. und Pharmakol.*, **248**, 540 (1964).
[10] Koller, E., *Helv. physiol. pharmacol. Acta*, **20**, 97 (1962).
[11] Miller, W. S., *Amer. Rev. Tuberc.*, **5**, 689 (1921).
[12] Herxheimer, H., and Stresemann, E., *J. Physiol.* (Lond.), **158**, 38 P (1961).
[13] Moog, E., and Greeff, K., *Arch. exp. Path. und Pharmakol.*, **246**, 16 (1963).
[14] Klupp, H., and Konzett, H., *Arch. exp. Path. und Pharmakol.*, **246**, 19. (1963).

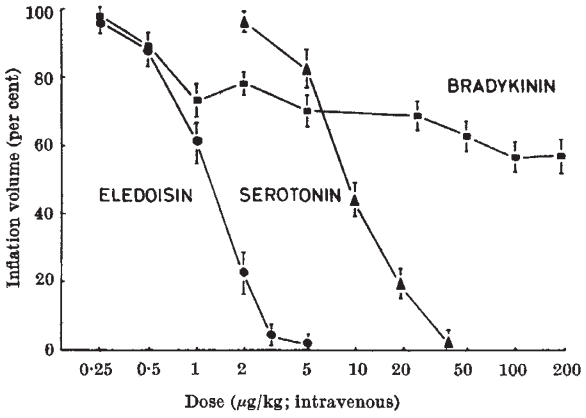


Fig. 2. Effect of bradykinin, eledoisin and serotonin on the inflation volume in guinea-pigs. Body-plethysmography in guinea-pigs (350–520 g) under urethane anaesthesia (1·5 g/kg; subcutaneous). Artificial respiration with a Starling pump (40 strokes per min) at constant pressure (100 mm $H_2O$). Paralysis of the respiratory muscles with succinyl-*bis*-choline (0·2 mg/kg; intravenous) 1 min before the intravenous injection of bradykinin, eledoisin and serotonin resp. Inflation volume before the injection of the bronchoconstrictor agents was assumed to be 100 per cent. Each point represents the mean ± *S.E.* of 6–13 experiments

The interesting observation of Gjuriš and Westermann that bradykinin differs from 5-hydroxytryptamine in its action on the tracheobronchial muscle of the guinea-pig accords with previous findings. For example, Jänkälä and Virtama[1] concluded from radiography that 5-hydroxytryptamine "causes vigorous constriction of all the constrictable bronchial tree of the guinea-pig", whereas "bradykinin apparently only affects the respiratory bronchioles". I am glad that Gjuriš and Westermann support the suggestion we made some time ago[2], that bradykinin may also affect the smooth muscles of the pleural surface of the lung.

Since bradykinin probably acts on a smaller part of the bronchial tree than does 5-hydroxytryptamine, it cannot be expected to reduce to the same extent the volume of air entering the lungs. In our hands, intravenous doses of 8 or more µg/kg of bradykinin usually reduce by > 50 per cent the inflation volume of a guinea-pig, which is artificially ventilated after suppression of spontaneous respiration with urethane. The difference from the 40 per cent reduction obtained with 50–200 µg/kg of bradykinin by Gjuriš and Westermann (Fig. 2) may perhaps arise from the fact that repeated doses of bradykinin readily cause refractoriness of the bronchoconstrictor response[3]. Repeated doses of 5-hydroxytryptamine, on the contrary, often elicit successively larger responses.