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ACTIONS OF SOME PEPTIDES ON BRONCHIAL MUSCLE

BY

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The decapeptide kallidin-10, substance P and angiotensin increased the resistance of guinea-pig lungs to inflation; lysine- or arginine-vasopressin and oxytocin were inactive. Acetylsalicylate antagonized this action of kallidin-10, as it does that of bradykinin, but it failed to antagonize substance P or angiotensin. Bradykinin also increased resistance to inflation of rabbit lungs and, to a lesser extent, rat lungs. It caused a relatively slow contraction of guinea-pig tracheal and bronchial muscle *in vitro*, but it did not contract isolated rabbit, dog or human bronchus. The relative potencies of different substances on different bronchial test preparations, and also in different species, were not parallel.

Recent experiments have demonstrated that the peptide released from plasma by salivary or urinary kallikrein differs from bradykinin only in having an N-terminal lysine added to the molecule (Pierce & Webster, 1961a; Webster & Pierce, 1962; Werle, Trautschold & Leysath, 1961). It has therefore been called kallidin-10, in distinction to kallidin-9 (bradykinin), which is derived from it by the action of a plasma peptidase. We have studied the bronchoconstrictor action of this decapeptide and also that of other endogenous peptides, such as substance P and angiotensin, and compared their actions with those of bradykinin, histamine and acetylcholine.

In the present experiments, earlier observations on the action of bradykinin on guinea-pig lung and its antagonism by acetylsalicylate (Collier, Holgate, Schachter & Shorley, 1959, 1960) have been extended to other species.

METHODS

Materials. The following substances were used: synthetic bradykinin (Nicolaides & DeWald, 1961); synthetic kallidin-10 (Nicolaides, DeWald & McCarthy, 1961), purified chromatographically by Pierce & Webster (1961b); substance P, prepared by the method of Pernow (1953), containing 75 and 13.8 units/mg; angiotensin (val^5-hypertensin II Asp-β-amide) (Ciba); lys^8-vasopressin, arg^8-vasopressin and oxytocin (Syntocinon, "Sandoz"); and other substances as previously described (Collier & Shorley, 1960).

Whole animal preparations. Guinea-pigs, rabbits and rats were prepared for recording resistance of lungs to inflation by the method of Konzett & Rössler (1940), as modified by Collier *et al.* (1960), and acetylsalicylate antagonism was determined by the procedure of Collier & Shorley (1960). For rabbits (1 to 3 kg), the stroke volume of the pump ranged from 18 to 24 ml., the frequency from 72 to 92 strokes/min and the resistance of the escape valve from 7.5 to 10 cm water. Rabbits were anaesthetized by slowly infusing 25% (w/v)

urethane intravenously. For rats (150 to 250 g), the stroke volume of the pump was lowered to 4 to 6 ml., its frequency increased to 92 strokes/min and the resistance of the escape valve reduced to 5 cm water. Intravenous injections were made by the femoral vein in the rabbit and rat, and by the jugular vein in the guinea-pig. Substances were dissolved in Tyrode solution (0.05 to 0.2 ml.) and applied as drops to the exposed pleural surface of the lung.

Isolated bronchus or trachea. The excised trachea or bronchus of guinea-pig, rabbit or dog was opened with a longitudinal cut along the mid-dorsal surface and a series of transverse cuts made successively from alternate sides, so that they overlapped one another but did not transect the preparation. This was suspended in an organ bath at 37° C. For guinea-pig and rabbit, we used Krebs-Henseleit solution aerated with oxygen containing 5% carbon dioxide; for dog, magnesium-free Tyrode solution. Drugs were added to the bath and movements of the trachea recorded on smoked paper by a lever with a frontal writing point. Bradykinin was left in the bath for 1.5 min and histamine and acetylcholine for 1 min. Pieces of human bronchus, recovered from lungs excised for carcinoma, were prepared as described by Hawkins & Schild (1951), except that the bronchus was cut as above.

Isolated guinea-pig lungs. The trachea was cannulated and perfused with Tyrode solution from a reservoir, fluid escaping from the surface of the lungs. The pulmonary artery was also cannulated and perfused with Tyrode solution, drugs being injected into the rubber tubing above either cannula. The rate of flow in the tracheal channel was obtained by recording the bubbles entering the reservoir (Sollmann & von Oettingen, 1928).

RESULTS

Responses of guinea-pig to kallidin-10, substance P and angiotensin

Kallidin-10 was about one-third as active as bradykinin in increasing resistance of the lungs to inflation *in vivo*. The time-courses of responses to both peptides were similar (Fig. 1). Fig. 1 also shows that acetylsalicylate antagonized the response

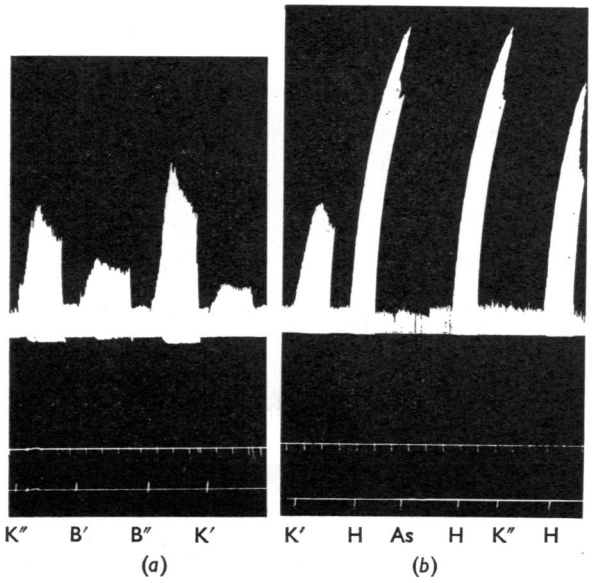

Fig. 1. Resistance to inflation of guinea-pig lungs *in vivo*. Konzett-Rössler preparation. All doses per kg intravenously. (*a*) Guinea-pig, 500 g.; B′, 1 μg, and B″, 2 μg bradykinin; K′, 3 μg, and K″, 6 μg kallidin-10. (*b*) Guinea-pig, 610 g.; K′, 3 μg, and K″, 6 μg kallidin-10; H, 2 μg histamine; As, 4 mg calcium acetylsalicylate. Time, 30 sec.

to kallidin-10. The minimal effective dose of calcium acetylsalicylate (2 mg/kg intravenously) was the same as that required to antagonize an equipotent dose of bradykinin.

Substance P and angiotensin also increased resistance to inflation (Fig. 2). Angiotensin was considerably less active than the other compounds and did not show a pronounced dose-response effect. Acetylsalicylate, atropine, mepyramine and lysergic acid diethylamide failed to antagonize substance P or angiotensin (Figs. 2 and 3).

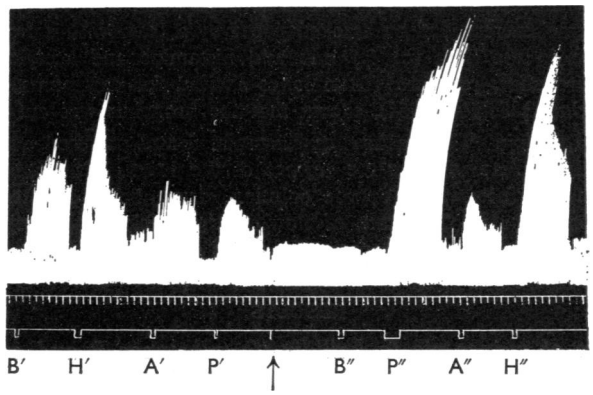

Fig. 2. Resistance to inflation of guinea-pig lungs *in vivo*. The response to substance P and angiotensin is not antagonized by Ca acetylsalicylate. At arrow, Ca acetylsalicylate (5 mg/kg) injected i.v.; B′, 2.5 μg, and B″, 3.75 μg bradykinin; H′, 2.5 μg, and H″, 3.75 μg histamine; A′, 20 μg, and A″, 30 μg angiotensin; P′, 50 u., and P″, 75 u. substance P (13.8 u./mg). Guinea-pig 800 g. Time, 10 sec.

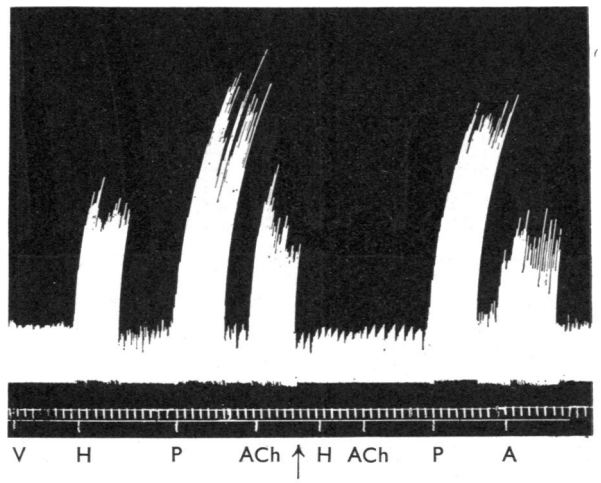

Fig. 3. Resistance to inflation of guinea-pig lungs *in vivo*. The response to substance P and angiotensin is unaffected by atropine, mepyramine and lysergic acid diethylamide. At arrow, atropine (20 μg/kg), mepyramine (10 μg/kg) and lysergic acid diethylamide (10 μg/kg) injected intravenously. V, 100 m-u. arg^8-vasopressin; H, 2 μg histamine; P, 60 u. substance P (13.8 u./mg); ACh, 2 μg acetylcholine; A, 40 μg angiotensin. Guinea-pig 600 g. Time, 10 sec.

Lys^8-vasopressin (100 m-u.), arg^8-vasopressin (100 m-u.) and oxytocin (2,000 m-u.) had little or no effect.

Responses of guinea-pig to bradykinin

Bradykinin contracted the isolated guinea-pig trachea. The contraction, however, was slower than that caused by histamine, and the dose-response slope was much less steep (Fig. 4). Phenylbutazone (Fig. 5) and amidopyrine relaxed the tracheal strip and depressed the response to bradykinin more effectively than that to histamine or acetylcholine.

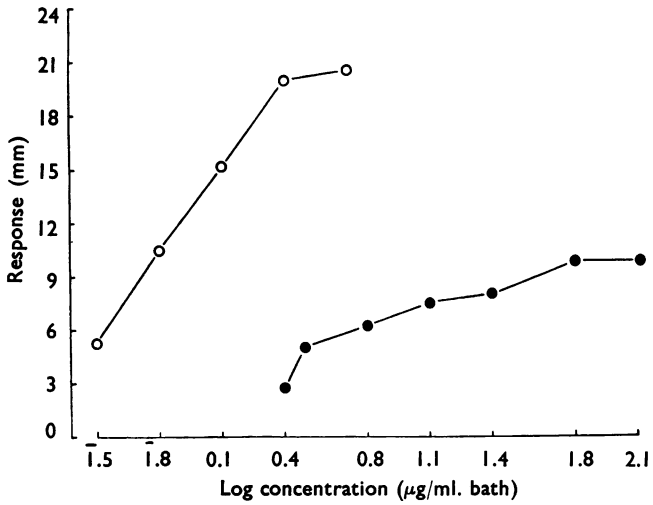

Fig. 4. Isolated guinea-pig trachea. Dose-response curves of bradykinin (● —— ●) and histamine (○—— ○).

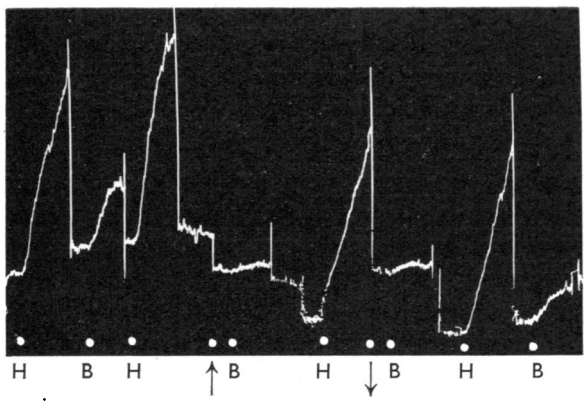

Fig. 5. Isolated guinea-pig trachea in 15 ml. bath. Responses to bradykinin and histamine and effects of phenylbutazone. B, 50 μg bradykinin; H, 20 μg histamine; between arrows 1.5 mg phenylbutazone.

In isolated guinea-pig lungs, bradykinin, histamine and acetylcholine, injected into either the tracheal or arterial perfusion channel, decreased the intratracheal perfusion rate, indicating increased resistance to flow. Both bradykinin (1 to 10 μg/ml.) and acetylcholine (10 to 100 μg/ml.) caused a definite though small increase in resistance to inflation when applied to the pleural surface of the lung *in vivo* (Fig. 6).

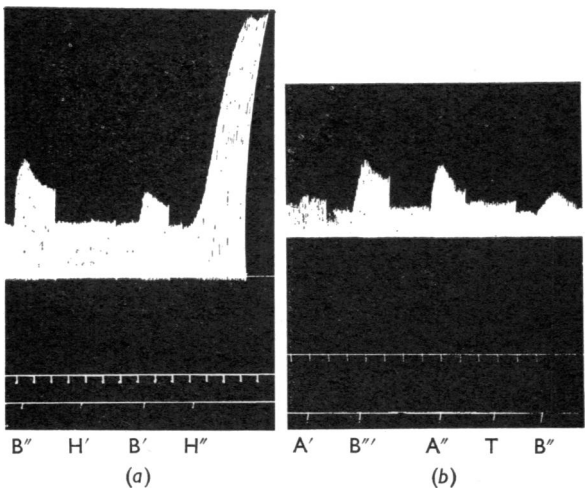

Fig. 6. Resistance to inflation of guinea-pig lungs *in vivo*. Responses to bradykinin, histamine and acetylcholine dropped on to the pleural surface of the lung in 0.05 ml. Tyrode solution. (*a*) Guinea-pig, 540 g.; B′, 0.05 μg, and B″, 0.5 μg bradykinin; H′, 5.0 μg, and H″, 50.0 μg histamine. (*b*) Guinea-pig, 580 g.; A′, 5.0 μg, and A″, 50.0 μg acetylcholine; B″, 0.5 μg, and B‴, 5.0 μg bradykinin; T, Tyrode solution. Time, 30 sec.

Histamine was also effective by this route, but a solution containing 0.1 to 1 mg/ml. was needed to evoke a response, which then developed more slowly (Fig. 6). This response, however, reached a higher maximum and lasted longer that that produced by the same dose of bradykinin or acetylcholine. Intravenous acetylsalicylate (100 mg/kg) or intravenous atropine (500 μg/kg) did not abolish the responses to topically applied bradykinin or acetylcholine respectively. However, high concentrations of atropine (0.2 ml. containing 1 mg/ml.) applied topically did abolish the response to topical acetylcholine.

The lowest doses of bradykinin, histamine and acetylcholine effective in the different test preparations are summarized in Table 1. This shows that the relative effectiveness of the different substances differs according to the test preparation and the route of administration.

Responses of other species to bradykinin

Bradykinin, acetylcholine and histamine, given intravenously, also increased resistance of rabbit lungs to inflation (Fig. 7). The rat, however, responded only to very large doses of bradykinin and acetylcholine (Fig. 7), although 5-hydroxytryptamine was effective in doses of 10 to 100 μg/kg, being about 5 to 10 times as

TABLE 1

EFFECTIVE DOSES OF BRADYKININ, HISTAMINE AND ACETYLCHOLINE IN VARIOUS PREPARATIONS OF GUINEA-PIG

Preparation	Route	Units	Minimal effective dose: Brady-kinin	Hist-amine	Acetyl-choline
Trachea *in vitro*	Into organ bath	μg/ml.	2·5–10	0·5–2	0·25–1
Perfused lung *in vitro*	Intratracheal	μg	0·5–5	0·1–1	0·05–0·2
	Intra-arterial	μg	0·5–2·5	0·1–0·5	0·05–0·2
Konsett-Rössler *in vivo*	Intravenous	μg/kg	0·5–1	0·5–1	2·5–10
	Topical	μg in 0·05 ml.	0·05–0·5	5–50	0·5–5

potent as bradykinin or acetylcholine. Acetylsalicylate, in contrast to its action in the guinea-pig, showed little or no antagonism to bradykinin in the rabbit or rat (Fig. 7).

Isolated rabbit trachea or bronchus contracted to acetylcholine (0.06 to 0.5 μg/ml.), the response being related to dose. Neither histamine nor bradykinin (up to 625 μg/ml.) was effective.

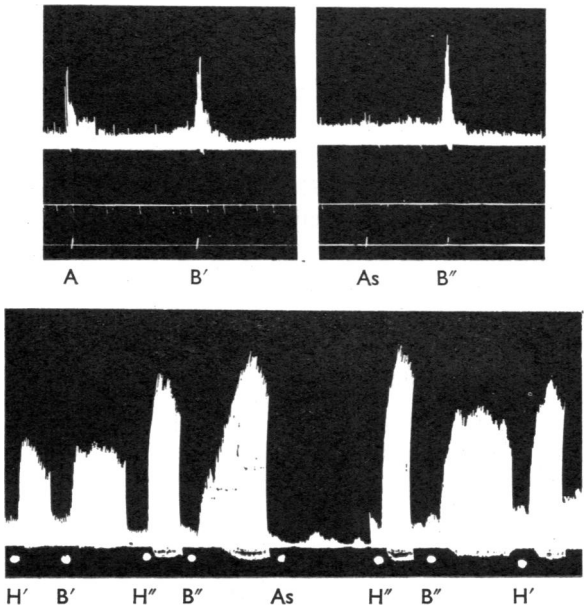

Fig. 7. Resistance to inflation of rat and rabbit lungs *in vivo*. Action of bradykinin and failure of antagonism by acetylsalicylate. Upper panels: rat, 185 g.; A, 450 μg ACh; B′, 450 μg bradykinin; As, 100 mg calcium acetylsalicylate; B″, 900 μg bradykinin. Lower panel: rabbit, 2.7 kg.; H′, 1.8 μg, and H″, 3.6 μg histamine; B′, 1.8 μg., and B″, 3.6 μg bradykinin; As, 10 mg calcium acetylsalicylate. All doses per kg intravenously. Time, 30 sec.

Bradykinin did not contract isolated dog bronchus (15 μg/ml.), nor 4 preparations of human bronchus (up to 667 μg/ml.) that responded to histamine (0.067 to 0.67 μg/ml.) and acetylcholine (0.2 to 0.67 μg/ml.).

DISCUSSION

If 1 μg pure substance P contains 30 to 35 u. (Franz, Boissonnas & Stürmer, 1961), this peptide is of the same order of potency as bradykinin in increasing resistance of the lungs to inflation in the guinea-pig *in vivo*. Angiotensin was considerably less potent than kallidin-10 and substance P and the dose-response curve was flatter. However, it contracted guinea-pig trachea *in vitro* and therefore probably acts on the bronchial muscle *in vivo*. The failure of acetylsalicylate, mepyramine, atropine and lysergic acid diethylamide to antagonize substance P shows that the activity of this preparation was not due to contamination by, or release of, bradykinin, kallidin-10, histamine, acetylcholine or 5-hydroxytryptamine.

The fact that acetylsalicylate antagonized kallidin-10 adds another peptide to those (bradykinin and wasp kinin) known to be antagonized by antipyretic drugs. Substance P or angiotensin, which share many properties with this group, may therefore be differentiated from it by the failure of this antagonism. The failure of acetylsalicylate to antagonize bradykinin in the rabbit lung *in vivo* emphasizes the narrow limits of this antagonism.

The findings that bradykinin contracted isolated guinea-pig trachea and reduced the rate of perfusion through isolated lung support the conclusion (Collier *et al.*, 1960) that bradykinin acts on the smooth muscle of the bronchial tree in the guinea-pig *in vivo*. When applied to the pleural surface of the lungs, bradykinin was more potent than histamine. This may be because different smooth muscles are not equally sensitive to different agents. The smooth muscles nearest the pleural surface include interstitial fibres (Baltisberger, 1921) and fibres around the mouths of alveoli and atria (Miller, 1921). These muscle fibres may differ from those in bronchioles in being relatively more sensitive to bradykinin than to histamine. The fact that atropine, administered intravenously, failed to block the bronchoconstrictor action of acetylcholine applied topically is probably due to the relatively low concentration of atropine at the site of action of acetylcholine. This explanation is in accord with the fact that a high concentration of atropine applied topically antagonizes effectively.

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REFERENCES

BALTISBERGER, W. (1921). Über die glatte Muskulature der menschlichen Lunge. *Zeit. Anat. Entwickl.*, **61**, 249–282.

COLLIER, H. O. J., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1959). An apparent bronchoconstrictor action of bradykinin and its suppression by some anti-inflammatory agents. *J. Physiol.*, **149**, 54*P*.

COLLIER, H. O. J., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1960). The bronchoconstrictor action of bradykinin in the guinea-pig. *Brit. J. Pharmacol.*, **15**, 290–297.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Brit. J. Pharmacol.*, **15**, 601–610.

FRANZ, J., BOISSONNAS, R. A. & STÜRMER, E. (1961). Isolierung von Substanz P aus Pferdedarm und ihre biologische und chemische Abgrenzung gegenüber Bradykinin. *Helv. Chim. Acta*, **44**, 881–883.

HAWKINS, D. F. & SCHILD, H. O. (1951). The action of drugs on isolated human bronchial chains. *Brit. J. Pharmacol.*, **6**, 682–690.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.*, **195**, 71–74.

MILLER, W. S. (1921). The musculature of the finer divisions of the bronchial tree and its relation to certain pathological conditions. *Amer. Rev. Tuberc.*, **5**, 689–704.

NICOLAIDES, E. D. & DEWALD, H. A. (1961). Studies on the synthesis of polypeptides. Bradykinin. *J. org. Chem.*, **26**, 3872–3876.

NICOLAIDES, E. D., DEWALD, H. A. & MCCARTHY, D. A. (1961). The synthesis of a biologically active decapeptide having the structure proposed for kallidin II. *Biochem. Biophys. Res. Comm.*, **6**, 210–212.

PERNOW, B. (1953). Studies on substance P purification, occurrence and biological properties. *Acta Physiol. Scand.*, 29, Suppl.

PIERCE, J. V. & WEBSTER, M. E. (1961a). Human plasma kallidins; isolation and chemical studies. *Biochem. Biophys. Res. Comm.*, **5**, 353–357.

PIERCE, J. V. & WEBSTER, M. E. (1961b). Personal communication.

SOLLMANN, T. & VON OETTINGEN, W. F. (1928). Bronchial perfusion of isolated lung as a method of studying pharmacologic reactions of bronchial muscle. *Proc. Soc. exp. Biol. N.Y.*, **25**, 692–695.

WEBSTER, M. E. & PIERCE, J. V. (1962). Role of kallikreins in release of kallidins. *Proc. N.Y. Acad. Sci.*, in press.

WERLE, R., TRAUTSCHOLD, I. & LEYSATH, G. (1961). Isolierung und Struktur des Kallidins. *Zeit. physiol. Chem.*, **326**, 174–176.