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The bronchoconstrictor action of the tetradecapeptide bombesin in the guinea-pig

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The tetradecapeptide bombesin was found to exert a potent spasmogenic action on the bronchiolar muscle of the anaesthetized guinea-pig. The threshold bronchoconstrictor dose ranged between 50 and 200 ng kg^{-1}. Other spasmogenic substances tested in the same experimental conditions, except physalaemin, were much less effective. Since antagonists of acetylcholine, histamine or 5-hydroxytryptamine did not inhibit the bronchoconstriction it is suggested that bombesin acts either by direct stimulation of the smooth muscle or by the prior release of other, as yet unidentified, spasmogenic substances.

Bombesin is a tetradecapeptide recently isolated from the skin of the European frogs *Bombina bombina* and *Bombina variegata variegata* by Anastasi, Erspamer & Bucci (1971). It has the following amino-acid sequence:

Pyr*–Gln–Arg–Leu–Gly–Asn–Gln–Trp–Ala–Val–Gly–His–Leu–Met–NH_2

This structure has little in common with most of the known natural peptides. Moreover, bombesin shows a peculiar spectrum of activity as described by Erspamer (1971), Anastasi (1971) and Bertaccini (1971). Recently, details of the activity of bombesin on isolated smooth muscle and on the systemic blood pressure were published by Erspamer, Falconieri-Erspamer & others (1972), and by Erspamer, Melchiorri & Sopranzi (1972); according to their data it is evident that some of the actions of bombesin resemble those of bradykinin (e.g. stimulation of guinea-pig ileum and rat uterus, relaxation of rat duodenum). Since bradykinin is one of the most potent bronchoconstrictor agents known at present, we decided to investigate the activity of bombesin on the bronchiolar muscle of the guinea-pig which so far has not been studied.

METHODS

Guinea-pigs of either sex weighing approximately 500 g were kept on a standard laboratory diet with free access to water. They were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.) then treated with decamethonium (syncurine) (4–5 mg kg^{-1}, i.v.) to maintain suppression of spontaneous respiratory movements.

The method of Konzett & Rossler (1940) modified by Impicciatore & Giovati (1973) was used for recording resistance of lungs to inflation. The trachea was cannulated and the lungs inflated with air by means of a miniature Palmer pump operating on a partially closed circuit (approximately 10 ml stroke volume at 70 strokes per min). A sidearm from the cannula permitted some air to enter a

* Pyr = pyroglutamyl.

differential transformer pressure transducer connected with an amplifier and a direct writing polygraph. In some experiments, blood pressure from the carotid artery and a tachogram were recorded. Drugs were injected through a cannula inserted in the external jugular vein.

Drugs. The following compounds were used: synthetic bombesin with analogues represented by partial sequences of bombesin (C-terminal nona-, octa-, hepta- and hexapeptide), eledoisin, physalaemin, caerulein and 5-hydroxytryptamine generously supplied by Farmitalia Laboratories (Milan); synthetic substance P which was a gift from Dr. Susan Lehman (Harvard, U.S.A.); prostaglandin E_2 (PGE_2) was kindly supplied by Dr. J. Pike (Upjohn). Other drugs used were: bradykinin and methysergide (Sandoz), angiotensin II (hypertensin, Ciba), isoprenaline, histamine, atropine and mepyramine (Fluka).

RESULTS

In all the experiments, bombesin increased resistance of the lungs to inflation. The threshold doses were always very low and ranged in the different animals between 50 and 200 ng kg^{-1}. At the low doses bombesin could be easily distinguished from other common bronchoconstrictor agents, like 5-hydroxytryptamine (5-HT) and histamine, because of its slower onset which resembled that of bradykinin. Recovery usually occurred within 10 min. However, with doses 3 to 5 times threshold, and higher, bombesin caused a more rapidly developing bronchoconstriction which persisted for a much longer time (Fig. 1a).

Tachyphylaxis was seen in every experiment using these higher doses, though the degree was variable, and for this reason no complete dose/response curve was

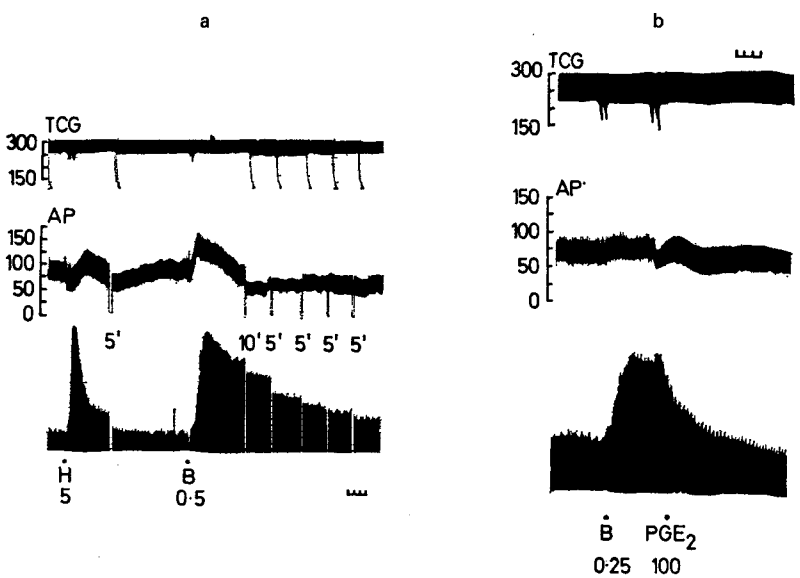

FIG. 1a. Effects of histamine (H) and bombesin (B) in the anaesthetized guinea-pig. From top to bottom: TCG = tachogram. AP = arterial pressure in mm Hg. Resistance of lungs to inflation. Doses in $\mu g\ kg^{-1}$. Recordings stopped for the times indicated. Time marks 10 s.

b. Reversal of bombesin bronchoconstriction by PGE_2 in the anaesthetized guinea-pig. From top to bottom: TCG = tachogram. AP= arterial pressure in mm Hg. Resistance of lungs to inflation. Time marks 10 s.

obtained. The only drug which behaved as bombesin was bradykinin which, however, was always much less potent than bombesin.

The activity of bombesin was compared with that of peptide-like and non peptide-like drugs which stimulate the bronchiolar muscle. The ratios of activity, considering the peak effect at doses exceeding by 2–3 times the threshold doses, is shown in Table 1.

It is clear from Table 1 that bombesin was the most active drug in increasing resistance to inflation of guinea-pig lungs. Other compounds, except physalaemin (which showed a short-lasting, histamine-like effect), were far less effective. The experiments performed with partial sequences of the bombesin molecule revealed that the crucial part for the maintenance of the potency of bombesin is the C-terminal nonapeptide which, in our experimental conditions, was approximately as active as bombesin. The C-terminal octapeptide and heptapeptide retained respectively 10 and 1% of the activity of the parent substance while the hexapeptide was inactive in doses 500 times greater than those of bombesin. These synthetic analogues behaved like bombesin as regards the persistence of the effect and tachyphylaxis.

Table 1. *Bronchoconstrictor effect elicited in the guinea-pig by intravenous injection of several drugs in comparison with bombesin.* Figures represent the number of moles required to give the same effect as 1 mol of bombesin. Equivalence obtained with doses 2–3 times higher than the threshold dose.

Drug	Mean	Range	Number of experiments
Bombesin	1		
Physalaemin	1	(0·9– 1·3)	4
Eledoisin	3	(2– 5)	4
Substance P	10	(5– 20)	4
Bradykinin	30	(22– 35)	5
Angiotensin	40	(15– 50)	4
Caerulein	*		3
Histamine	100	(50–200)	7
5-Hydroxytryptamine	75	(50–125)	6

* The compound was virtually inactive (300 mol were less effective than 1 mol of bombesin).

In an attempt to investigate the mode of action of the tetradecapeptide, several compounds were tested for their ability to interfere with the activity of bombesin: atropine (2 mg kg^{-1}), methysergide (0·15 mg kg^{-1}), and mepyramine (5 mg kg^{-1}) administered either alone or together failed to modify the spasmogenic activity of bombesin. Conversely, isoprenaline (10 μg kg^{-1}) completely abolished the effect of the peptide, or prevented it if given before bombesin. The same was observed after administration of relatively high doses (30–100 μg kg^{-1}) of prostaglandin E_2 (Fig. 1b).

DISCUSSION

The findings show that bombesin exerts a marked spasmogenic activity on the bronchiolar muscle of the guinea-pig. All other compounds tested were much less effective with the exception of physalaemin which had approximately the same potency as bombesin though its action was much less persistent. Both the persistence of action and the tachyphylaxis seen with bombesin in the present experiments have been reported by other workers investigating different actions of the peptide, e.g.

investigations on blood pressure (Erspamer & others, 1972), stimulation of motility in isolated organs (Erspamer & others, 1972) and stimulation of gastric secretion (Bertaccini, Impicciatore & Erspamer, 1973).

As to the structure/activity relation, the minimum fragment which retained virtually the whole activity of the parent substance seemed to be the C-terminal nonapeptide. The loss of the asparagine residue in position 9 (from the C-terminus) caused a pronounced fall in the activity and the further elimination of glutamine from position 8 produced an even more dramatic fall. No effect was found with the hexapeptide even in doses 500 times higher than that of bombesin. These results are in good agreement with those obtained by De Castiglione and co-workers (personal communication) who investigated other preparations (guinea-pig large intestine, kitten small intestine, rat uterus, rat urinary bladder).

The mechanism of action of bombesin is at present obscure. The lack of inhibitory effect of antagonists of acetylcholine, histamine and 5-hydroxytryptamine seems to suggest a direct activity of bombesin on the bronchiolar muscle. However, an indirect effect mediated through the possible release of other spasmogenic substances (e.g. kinins, prostaglandins) cannot be excluded. In this connection it must be noted that bombesin was found to be an effective releasing factor; it was shown to release renin (Melchiorri, Sopranzi & Erspamer, 1971) gastrin (Bertaccini & others, 1973) and probably cholecystokinin (Erspamer, Melchiorri & others, 1973).

It was not unexpected that isoprenaline would antagonize bombesin because of its bronchodilator activity, and the same is true of PGE_2 which has also been shown to cause bronchodilation in several animal species and under various experimental conditions (for review see Cuthbert, 1973).

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