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The role of the adrenal glands and of α- and β-adrenergic receptors in bronchodilatation of guinea-pig lungs *in vivo*

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Adrenaline, aminophylline, isoprenaline, noradrenaline, papaverine and phenylephrine exerted non-specific bronchodilator effects in guinea-pig lungs *in vivo*. The descending order of bronchodilator potency was isoprenaline > adrenaline > noradrenaline > phenylephrine. These agents also exerted a bronchoconstrictor effect, the descending order of bronchoconstrictor potency being phenylephrine > noradrenaline > adrenaline > isoprenaline. Bronchodilator activity could be antagonized by either α- or β-adrenergic receptor antagonists. The bronchodilator action of aminophylline, papaverine, phenylephrine and noradrenaline was partly mediated via the adrenal gland.

BRONCHODILATATION induced by sympathomimetic substances can be antagonized by β-adrenergic receptor antagonists (Nagasaka, De Schaepdryver & Heymans, 1964; Römer & Weidmann, 1964; Farmer & Lehrer, 1966). Such bronchodilatation, however, is not antagonized by α-antagonists (Römer & Weidmann, 1964; Bianchi & De Vleeschouwer, 1960). Although no evidence for the presence of α-constrictor receptors could be found in guinea-pig isolated trachea (Foster, 1966), the presence of such receptors has been reported in guinea-pig isolated lung (Nagasaka, Bouckaert & others, 1964), in dog lung *in vivo* (Castro de la Mata, Penna & Aviado, 1962) and possibly in cat isolated tracheal muscle (Türker & & Kiran, 1965). These findings imply that the adrenergic system has a dual effect on bronchial muscle, the dilator effect being dominant.

The present experiments were undertaken to determine the mode of action on guinea-pig lungs *in vivo* of some well known bronchodilator drugs. By the use of selective α- and β-adrenergic receptor antagonists and by removal of the adrenal glands, the bronchodilator action of these drugs was investigated.

Experimental

MATERIALS AND METHODS

The bronchodilator drugs used were adrenaline tartrate, aminophylline, isoprenaline sulphate, noradrenaline bitartrate, papaverine sulphate and phenylephrine hydrochloride. To block α- and β-adrenergic receptors, tolazoline hydrochloride and pronethalol hydrochloride respectively were used. As bronchoconstricting agents, acetylcholine bromide, bradykinin, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate (5-HT) were used. Bradykinin was synthesized according to Nicolaides & De Wald (1961). Vasopressin was used in experiments on blood pressure. All drugs were administered intravenously in solution in 0·9% w/v saline.

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Guinea-pigs of either sex weighing 200–400 g were anaesthetized with urethane (1·25–2·5 g/kg) intraperitoneally, the dose being adjusted to suppress spontaneous respiration. Animals were prepared for intravenous administration of substances and for recording air overflow volume by the method of Konzett & Rössler (1940). Bronchodilator activity was assessed from the reduction of the increased air overflow volume after administration of acetylcholine (5–20 μg/pig), bradykinin (0·5–2 μg/pig), histamine (1–4 μg/pig) or 5-HT (1–4 μg/pig). Acetylcholine or histamine was administered at 5 min and bradykinin or 5-HT at 10 min intervals. Acetylcholine was used in experiments to determine the antagonism of bronchodilatation by tolazoline (5 mg/kg) or by pronethalol (5 mg/kg) or by bilateral adrenalectomy. Acetylcholine was also used in all dose-ratio experiments. Doses of bronchodilating drug were adjusted to give submaximal antagonism of the acetylcholine response. This antagonism was measured at its peak effect, usually 30 sec.

Bilateral adrenalectomy was performed after midline incision 15 min before test. Dose ratios were obtained from the ratio of the dose of agonist (bronchodilator) given after antagonist to that given before antagonist that gave a similar quantitative response. Blood pressure was measured from an indwelling cannula in the carotid artery connected to a Statham pressure transducer. In these experiments, bronchoconstriction was assessed from the increase in tracheal pressure measured by a "Greer" differential micromanometer connected to the side arm of the tracheal cannula. The results were recorded on an electronic multichannel recorder.

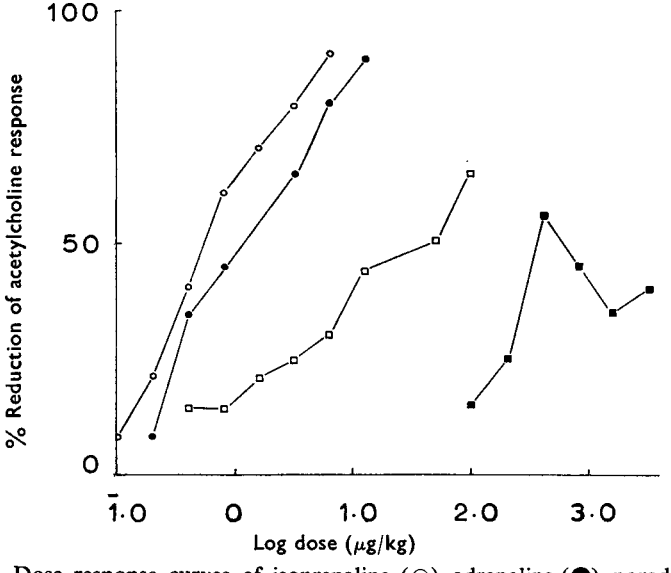

FIG. 1. Dose response curves of isoprenaline (○), adrenaline (●), noradrenaline (□) and phenylephrine (■), all given intravenously, assessed on guinea-pig lung *in vivo*. The response was assessed as the % reduction of the bronchoconstrictor effect of acetylcholine given 30 sec after the bronchodilating agent. All curves are the mean of at least 5 experiments.

Results

All the bronchodilator drugs tested antagonized bronchoconstriction induced by acetylcholine, bradykinin, histamine or 5-HT, indicating a non-specific effect. Fig. 1 gives the mean dose-response curves for four bronchodilator compounds, selected for their ability to stimulate predominantly β-(isoprenaline) or α-(phenylephrine) or both β- and α-(adrenaline and noradrenaline) adrenergic receptors. Each point is the mean of at least five observations. With the exception of isoprenaline, these compounds also induced bronchoconstriction.

Table 1 gives the percentage antagonism by tolazoline, by pronethalol or by adrenalectomy of the bronchodilator activity of adrenaline, aminophylline, isoprenaline, noradrenaline, papaverine and phenylephrine. For reasons discussed below, the percentage antagonism by pronethalol or by tolazoline of bronchodilatation induced by noradrenaline was determined in animals in which both adrenal glands had been removed. After adrenalectomy, pronethalol or tolazoline still exerted an approximately equal and almost complete antagonism of noradrenaline (Table 1).

TABLE 1. ANTAGONISM BY α- AND β-ADRENERGIC BLOCKING AGENTS AND BY BILATERAL ADRENALECTOMY OF BRONCHODILATOR ACTIVITY OF SOME DRUGS ON GUINEA-PIG LUNGS *in vivo*. All substances were given intravenously. Acetylcholine (5–20 μg/pig) was used as bronchoconstricting agent. Tolazoline and pronethalol were given at 5 mg/kg. An interval of 15 min was allowed after bilateral adrenalectomy; N.O., not obtainable.

Bronchodilator		Tolazoline		Pronethalol		Bilateral adrenalectomy % antagonism (90% limits)
Name	Dose (mg/kg i.v.)	% antagonism (90% limits)	Dose-ratio (range)	% antagonism (90% limits)	Dose-ratio (range)	
Adrenaline	0·002	22 (12–31)	18 (16–64)	70 (64–76)	108 (64–128)	3 (−23 to 29)
Aminophylline	5	10 (−1 to 21)	2·6 (2–4)	60 (32–89)	5·6 (4–16)	66 (41–116)
Isoprenaline	0·001	6 (0–12)	4 (nil)	99 (83–115)	56 (32–128)	28 (11–44)
Noradrenaline	0·02 0·02 0·008	91 (76–105) 84 (67–102) 58 (43–73)	6 (4–8)	92 (81–103) 81 (65–96) 59 (35–84)	11 (4–16)	44 (8–80) 58 (30–86)
Papaverine	5	−6 (−14 to 3)	2·6 (1–8)	33 (25–44)	9 (4–32)	52 (31–73)
Phenylephrine	0·5	77 (41–113)	N.O.	−14 (−44 to 15)	N.O.	62 (30–93)
			After bilateral adrenalectomy			
Noradrenaline	0·02	73 (55–92)	N.O.	81 (64–98)	N.O.	

The effect of tolazoline and pronethalol on the potency of the bronchodilator compounds in Table 1 has also been estimated as a dose ratio. The results obtained by the dose ratio method were similar to those obtained as a percentage antagonism. The values given in the Table are the mean of at least 5 observations. Dose ratios for noradrenaline after adrenalectomy and for phenylephrine could not be obtained, since the high doses needed to obtain the ratio caused intense bronchoconstriction.

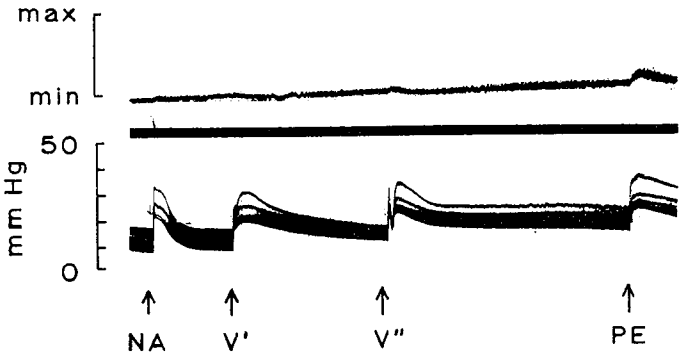

FIG. 2. Effect of noradrenaline, vasopressin and phenylephrine on the tracheal pressure (upper trace) and carotid arterial blood pressure (lower trace) of the guinea-pig. NA, 1 μg/kg of noradrenaline; V′, 1 unit/kg and V″, 5 units/kg of vasopressin; PE, 1 mg/kg of phenylephrine, all given intravenously; min, 10 cm H_2O; max, 23·5 cm H_2O.

Fig. 2 shows that phenylephrine induced rises in both blood pressure and tracheal pressure, whereas doses of noradrenaline and vasopressin induced a comparable rise in blood pressure but did not appreciably raise tracheal pressure.

Discussion

The relative bronchodilator potencies of the compounds illustrated in Fig. 1 was in descending order, isoprenaline > adrenaline > noradrenaline > phenylephrine. This confirms previous results obtained in guinea-pig lung (Carminati & Cattorini, 1966; Foster, 1966). This order is similar to that of these compounds in stimulating β-receptors (Ahlquist & Levy, 1959; Furchgott, 1960). The bronchoconstrictor potencies of the compounds of Fig. 1 were in reverse order of their bronchodilator potencies, i.e. phenylephrine > noradrenaline > adrenaline > isoprenaline. This, in turn, is similar to their relative potencies in stimulating α-receptors (Ahlquist & Levy, 1959; Furchgott, 1960). These correlations suggest that bronchodilator activity is due to the stimulation of β-receptors and bronchoconstrictor activity to stimulation of α-receptors. This presupposes that α-receptors are present in guinea-pig lung, a view held by Nagasaka & others (1964). The bronchoconstriction obtained, particularly with higher doses of phenylephrine, is unlikely to be due to an increase in arterial blood pressure, since equi-vasopressor doses of vasopressin did not cause bronchoconstriction (Fig. 2). The presence of α-constrictor receptors in guinea-pig lung would explain why some of the dose-response curves in Fig. 1 did not approach 100% irrespective of the dose, and why the curve for phenylephrine was "bell shaped."

In the case of adrenaline, aminophylline, isoprenaline and papaverine, but not of noradrenaline and phenylephrine, pronethalol was a more potent antagonist of bronchodilatation than was tolazoline (Table 1). Isoprenaline, a substance known to act predominantly on β-receptors, was

antagonized completely by a β- but not by an α-receptor blocking agent. This result agrees with those of other authors (Nagasaka & others, 1964; Römer & Weidman, 1964; Farmer & Lehrer, 1966). Adrenaline, a substance acting on both β- and α-receptors, was antagonized by both β- and α-receptor antagonists, whereas phenylephrine, an α-receptor stimulant, was antagonized only by an α-receptor antagonist. The effect of α- and β-receptor antagonists on bronchodilatation due to sympathomimetic substances was therefore more dependent on whether the bronchodilating agent was an α- or β-receptor stimulant than on the relative number of α- or β-receptors in the lung (Nagasaka & others, 1964). That an α-receptor blocking drug antagonized bronchodilatation is contrary to the results of Bianchi & De Vleeschouwer (1960). At least three explanations are possible: (1) that tolazoline acts on β- as well as α-receptors; (2) that tolazoline has sympathomimetic effects; (3) that α-dilator receptors are also present in guinea-pig lung. The first two possibilities seem unlikely since, if tolazoline acted on β-receptors or had sympathomimetic effects, its efficacy against all bronchodilators would be similar. The present results therefore support the third possibility.

Noradrenaline was antagonized equally by pronethalol or tolazoline. At high doses of noradrenaline (20 μg/kg), the antagonism was almost complete, although at lower doses, an approximate 50% reduction of response was obtained with either antagonist. This result could be explained if high doses of noradrenaline mediated its bronchodilator effect on either β- or α-receptors indirectly, i.e. via the adrenal glands. The result obtained (Table 1) showed that pronethalol and tolazoline still exerted an approximately equal and almost complete antagonism in adrenalectomized animals, indicating that noradrenaline did not mediate an indirect effect on either β- or α-receptors, through the adrenal glands. The result that two different types of receptor antagonist almost completely antagonized the noradrenaline response is paralleled by the antagonism by morphine and phenoxybenzamine of the contraction of guinea-pig isolated ileum induced by 5-HT (Day & Vane, 1963). The authors concluded that either the antagonism by morphine or by phenoxybenzamine was not specific. Since pronethalol has weak α-receptor blocking activity (Gulati, Gokhale & Udwadia, 1965), this may explain the present results.

The bronchodilator actions of aminophylline, papaverine, phenylephrine and noradrenaline are partly mediated via the adrenal glands, since removal of the adrenal glands much reduced the activity of these compounds (Table 1). This result is consistent with the view that some sympathomimetic drugs act via the adrenal medulla (Rubin & Jaanus, 1966; Schümann & Philippu, 1962). The findings of Farmer & Chick (1967) confirm that the bronchodilator activity of papaverine is partly mediated via the adrenal glands. The sum of the percentage antagonism of the bronchodilator activity of papaverine and aminophylline due to α- and β-receptor blockade was approximately 50%, indicating a dual action, i.e. sympathetic and probably a direct action. The activity of the remaining compounds appears to be mainly sympathetically mediated.

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