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The effect of directly and indirectly acting sympathomimetic amines on bronchospasm in the guinea-pig during CO_2 inhalation

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The bronchodilator effects of nine sympathomimetic amines were tested by reduction of histamine-induced bronchospasm in guinea-pigs. Their order of potency was: isoprenaline>adrenaline>noradrenaline > orciprenaline > ethylnoradrenaline > phenylephrine > amphetamine=tyramine>ephedrine. In guinea-pigs ventilated with 10% CO_2 in air, there was a marked decrease in the bronchoconstrictor activity of histamine. Under these conditions, isoprenaline, adrenaline, noradrenaline and orciprenaline were as effective as bronchodilators as they had been in guinea-pigs ventilated with air, ethylnoradrenaline was less effective, phenylephrine, amphetamine and tyramine were completely inactive, and ephedrine potentiated the bronchoconstrictor action of histamine. After returning the guinea-pigs to ventilation with air, the bronchoconstrictor activity of histamine and the bronchodilator effects of the sympathomimetic amines were restored. The cardiovascular effects of histamine and of all the sympathomimetic amines used were diminished during ventilation with 10% CO_2 in air and were restored after ventilation with air was resumed.

The sympathomimetic amines commonly employed as bronchodilators in asthma have been investigated by Atkinson & Rand (1968) for their alleged acute cardiac toxicity. These authors found that, in cats, during infusions of either adrenaline, isoprenaline or orciprenaline, there was suppression of the cardiovascular effects involving β-receptors of a single injection of any of these drugs.

In further experiments it was found that in cats ventilated with 10% CO_2 in air, the cardiovascular effects of adrenaline, isoprenaline or orciprenaline involving β-receptors were reduced or abolished (Atkinson and Rand, unpublished). It was therefore decided to examine the effects of sympathomimetic amines during CO_2 inhalation on histamine-induced bronchospasm, to determine whether suppression of β-receptor activity also occurred in bronchial smooth muscle.

EXPERIMENTAL

Methods

Guinea-pigs of either sex, 220–580 g, were anaesthetized by intraperitoneal injection of 4 ml/kg of a solution containing 25% urethane and 1% chloralose in saline. Blood pressure was monitored from the left common carotid with a Statham transducer (type P23AA) coupled with a strain gauge coupler (Offner No. 9872). Needle electrodes were inserted under the skin and connected to a cardiotachometer coupler (Offner No. 9857). The trachea was cannulated and artificial ventilation was applied with a constant volume Palmer respirator. A side-arm on the tracheal cannula was

connected with a Statham transducer to measure changes in intratracheal pressure via a strain gauge coupler. The recordings of heart rate, blood pressure and intratracheal pressure were displayed on an Offner Dynograph (Type R).

Arterial blood samples (0·15 ml) were withdrawn from the blood pressure cannula into a length of nylon tubing and blood pH, pCO_2 and pO_2 were measured at 37° with an IL ultramicro pH-gas Analyser (Model 113-S1). Heparin was routinely administered when the blood pressure cannula was inserted, and a small volume of dilute heparin was introduced into the arterial cannula after each blood sample was taken.

During the initial period of each experiment, blood pH was measured and the stroke volume of the respiratory pump was adjusted to stabilize the blood pH as close to 7·4 as possible. Control responses to histamine injections were then elicited at 6 min intervals until the transient increases in intratracheal pressure produced by histamine became constant. The effect of histamine in increasing intratracheal pressure was taken to represent bronchoconstriction. The bronchodilator drugs were injected 20 s before the histamine injection and the effect on bronchoconstriction was observed during the control period, during a period of ventilation with 10% CO_2 in air, and for a period after returning to ventilation with air. Blood pH, pCO_2 and pO_2 were measured during all three periods.

The dose of histamine was adjusted during the period of ventilation with 10% CO_2 in air, and after resuming ventilation with air, so that the bronchoconstrictor response matched that obtained in the control period.

Histamine bronchoconstriction and its modification by the various sympathomimetics was expressed as follows. The basal intratracheal pressure produced by artificial ventilation immediately before an injection was taken as unity, and the increase above this pressure produced by the injection of histamine was expressed as a multiple of the basal pressure. The effect of a bronchodilator sympathomimetic drug was expressed as the percentage change in this index of the bronchoconstrictor effect of histamine.

Drugs used were: histamine acid phosphate (British Drug Houses Ltd.), (±)-isoprenaline hydrochloride (Isuprel, Winthrop Laboratories), (−)-adrenaline tartrate (Adrenate, Burroughs Wellcome and Co. Ltd.), (−)-noradrenaline bitartrate (Levophed, Winthrop Laboratories), orciprenaline sulphate (Alupent, Boehringer Ingleheim Pty. Ltd.), (±)-ethylnoradrenaline hydrochloride (Bronkephrine, Winthrop Laboratories), (−)-phenylephrine hydrochloride (Neosynephrine, Winthrop Laboratories), tyramine hydrochloride (Koch-Light Laboratories Ltd.), (±)-amphetamine sulphate (L. Light and Co. Ltd.), ephedrine hydrochloride (D.H.A. Laboratories Pty. Ltd.).

RESULTS

Effect of ventilation with 10% CO_2 on histamine bronchospasm

The mean dose of histamine required to produce a bronchospasm approximately 1·5 times the basal intratracheal pressure was 4·8 μg/kg in 56 guinea-pigs. When the guinea-pigs were ventilated with 10% CO_2 in air the response to histamine started to decrease immediately. The sensitivity to histamine usually stabilized within 30 min, at which time almost five times the dose of histamine was required to produce an

approximately equivalent bronchoconstrictor effect to that obtained during ventilation with air; the mean dose of histamine required to match approximately the degree of bronchospasm was increased to 19·7 μg/kg. The sensitivity to histamine usually returned to the initial control level within an hour of restoring the animals to ventilation with air. The data on the mean doses of histamine and mean degrees of bronchospasm produced during the three phases of the experiments are summarized in Table 1.

Effect of ventilation with 10% CO_2 in air on blood pH and pCO_2

In the control period during ventilation with air, a mean blood pH of 7·41 was obtained by adjusting the stroke of the respiration pump and the mean pCO_2 figure was 28·3 mm Hg. The pCO_2 is relatively low compared with figures for human arterial blood: the reason for this is not known. Ventilation with 10% CO_2 in air for 30 min resulted in an increase of the mean arterial blood pCO_2 to more than 95 mm Hg; and the mean pH was reduced to 6·81. When the guinea-pigs were returned to ventilation with air, these values tended to return to control levels. The data are summarized in Table 1.

Table 1. *Effects of ventilation with 10% CO_2 in air on histamine bronchospasm and pH and pCO_2 in guinea-pigs*

Parameter mean ± s.e.	Air	10% CO_2 in air for 30 min	Resumption of air for 60 min
Histamine dose, μg/kg	4·8 ± 0·4	19·7 ± 2·3	4·5 ± 0·7
Degree of bronchospasm (increase/basal tracheal pressure)	1·53 ± 0·07	1·30 ± 0·07	1·47 ± 0·13
pH	7·41 ± 0·11	6·81 ± 0·16	7·28 ± 0·09
pCO_2..	28·31 ± 1·41	96·85 ± 3·33	32·79 ± 2·49

Effects of sympathomimetic drugs on histamine bronchospasm

Nine sympathomimetic amines were examined for their ability to reduce histamine-induced bronchoconstriction. The range of doses of each which reduced the bronchoconstrictor effect of histamine by about one-third was determined in preliminary experiments. They are arranged in rank order of potency, as indicated by the mean doses producing mean reductions of 23 to 55% in histamine bronchospasm, in Table 2.

Table 2. *Effects on histamine-induced bronchoconstriction of sympathomimetics, before, during and after ventilation with 10% CO_2 in air*

Sympathomimetic	Dose (μg/kg) mean ± s.e. (No. of experiments)	Percentage reduction of histamine-induced bronchospasm mean ± s.e. (No. of experiments)		
		Before CO_2 ventilation	During CO_2 ventilation	After CO_2 ventilation
Isoprenaline ..	0·079 ± 0·016 (8)	36·5 ± 5·4 (8)	33·2 ± 8·5 (8)	38·2 (2)
Adrenaline ..	0·104 ± 0·019 (5)	54·3 ± 2·5 (5)	46·2 ± 3·2 (5)	67·0 (2)
Noradrenaline ..	0·700 ± 0·100 (3)	44·7 ± 7·7 (3)	42·3 ± 6·7 (3)	48·5 (2)
Orciprenaline ..	1·63 ± 0·28 (6)	31·3 ± 6·3 (6)	25·4 ± 7·3 (6)	35·5 ± 7·9 (3)
Ethylnoradrenaline	4·63 ± 1·74 (3)	52·0 ± 8·5 (3)	24·8 ± 8·5 (3)	53·5 (2)
Phenylephrine ..	8·70 ± 3·84 (4)	52·2 ± 8·1 (4)	−4·3 ± 1·2 (4)	20·5 ± 0·5 (3)
Amphetamine ..	2000 ± 400 (7)	28·8 ± 3·1 (7)	−0·6 ± 2·3 (7)	34·7 ± 14·4 (3)
Tyramine ..	2100 ± 400 (6)	23·3 ± 3·9 (6)	0·6 ± 0·6 (3)	32·0 ± 5·0 (3)
Ephedrine ..	4000 ± 0 (7)	55·0 ± 9·0 (7)	−44·8 ± 13·7 (6)	46·0 ± 15·0 (3)

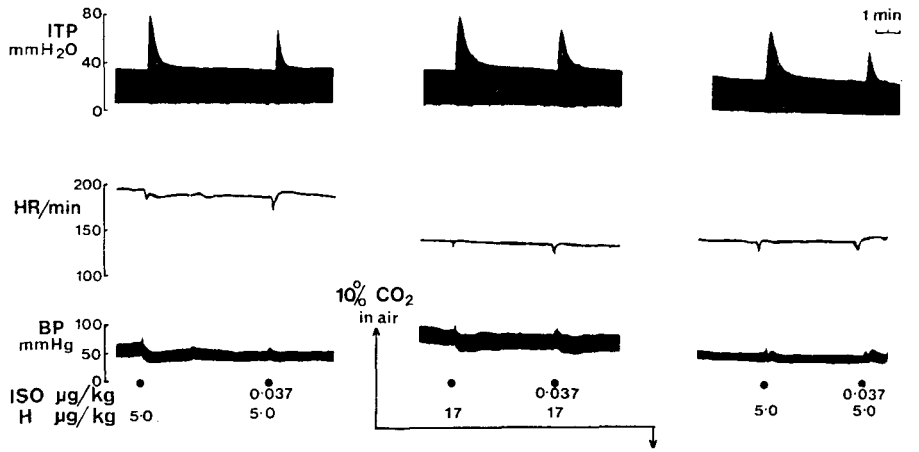

FIG. 1. Records of intratracheal pressure (ITP), heart rate (HR/min) and blood pressure (BP) in anaesthetized guinea-pig. The reduction in histamine-induced bronchospasm produced by isoprenaline (0·087 µg/kg) was unaffected by ventilation with 10% CO_2 in air.

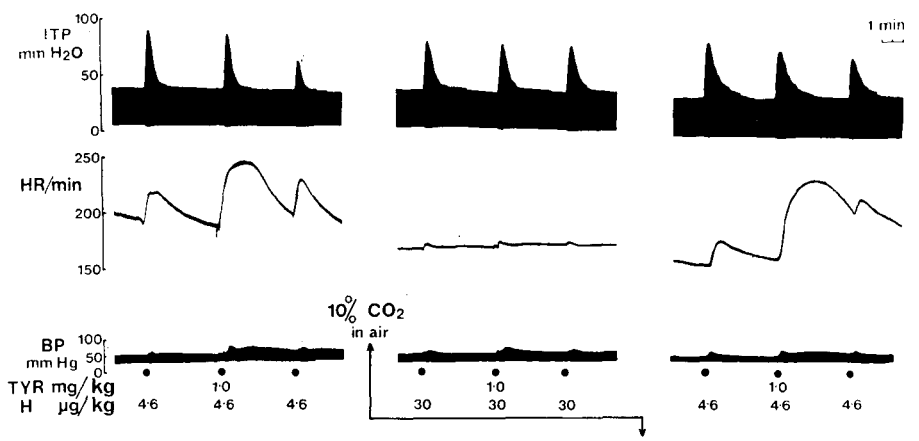

FIG. 2. Records as in Fig. 1. The reduction in histamine bronchospasm produced by tyramine (1 mg/kg) was abolished when the guinea-pig was ventilated with 10% CO_2 in air, but returned after ventilation with air was resumed.

Representative records of experiments with isoprenaline, tyramine and ephedrine are shown in Figs 1–3 (upper tracing, left-hand panel of each figure).

The rates of onset and durations of action of the various sympathomimetic amines differed. For the most potent, from isoprenaline to phenylephrine (see Table 2), the maximal action was observed in the reduction of the responses to the injection of histamine given 20 s later. Their effects were transient and generally did not last the 6 min to the next injection of histamine, although in some experiments a slight bronchodilator effect was seen with ethylnoradrenaline and phenylephrine 6 min after their injection. With amphetamine, tyramine and ephedrine, the maximal bronchodilator effect was observed 6 min after they were injected. Thus, Figs 2 and 3 show that injections of tyramine and ephedrine respectively had greater effects in

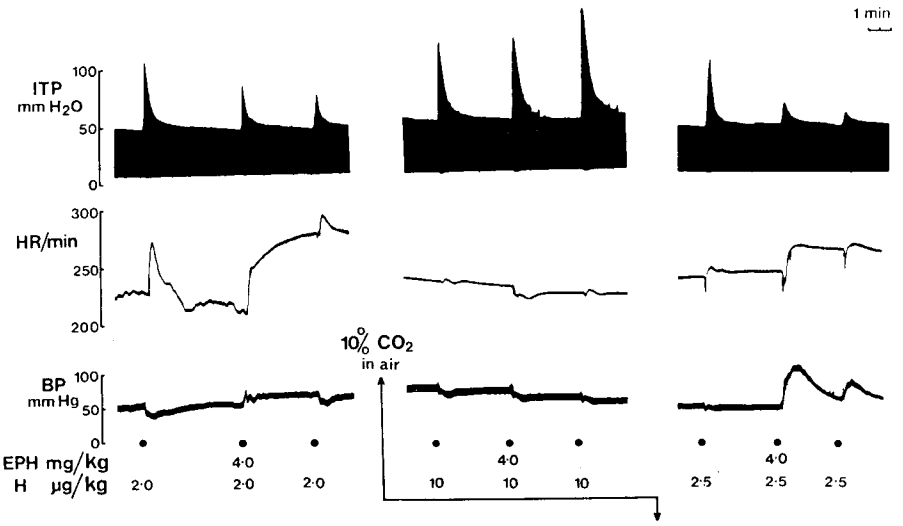

FIG. 3. Records as in Fig. 1. Ephedrine (4 mg/kg) reduced the bronchoconstrictor action of histamine during the control period, but potentiated it when the animal was ventilated with 10% CO_2 in air. The potentiation was sustained during this period. When ventilation with air was resumed, ephedrine again reduced the histamine response.

reducing the bronchoconstrictor responses to histamine given 6 min after their injection than it had on the responses to histamine given 20 s after injection of the sympathomimetic amines. The bronchodilator effects of tyramine and ephedrine lasted from 15 to 60 min. The bronchodilator effect of amphetamine was even more long lasting and never wore off entirely during the course of an experiment. Tachyphylaxis developed to the bronchodilator effect of amphetamine, a second injection being entirely without action. With the other sympathomimetic amines, reproducible responses were obtained with subsequent injections once the effect of the previous injection had passed off.

Effect of ventilation with 10% CO_2 on the bronchodilator effects of sympathomimetic amines

The reduction in histamine-induced bronchospasm produced by the four most potent sympathomimetic amines, isoprenaline, adrenaline, noradrenaline and orciprenaline was slightly but not significantly reduced when the guinea-pigs were ventilated with 10% CO_2 in air. If these sympathomimetics are considered as antagonists of the bronchoconstrictor action of histamine, then they are in fact more potent during ventilation with 10% CO_2 in air since the dose of histamine causing the effect is about five times greater than during ventilation with air. An experiment with isoprenaline is illustrated in Fig. 1 from which it can be seen that the reduction of the bronchoconstrictor effect of histamine in the second panel, during ventilation with 10% CO_2 in air, is as great as on the comparable response to a smaller dose of histamine in the first panel, during ventilation with air.

With the next most potent bronchodilator, ethylnoradrenaline, there was a significant reduction in bronchodilator effect during ventilation with 10% CO_2 in air. The mean reduction in histamine bronchospasm during ventilation with air was 52%

and the mean reduction during ventilation with 10% CO_2 in air was 24·8%, the difference between these means being significant by *t*-test with $P = 0{\cdot}05$.

The less potent sympathomimetic bronchodilators, phenylephrine, amphetamine and tyramine, lost their bronchodilator efficacy in guinea-pigs ventilated with 10% CO_2 in air, as illustrated with tyramine in Fig. 2. Because of the tachyphylaxis which developed after the first injection of amphetamine, a separate series of experiments was performed in which the effect of amphetamine was observed in guinea-pigs ventilated with 10% CO_2 in air without previous administration of a sympathomimetic amine: amphetamine did not reduce the bronchoconstrictor effect of histamine in these experiments.

Ephedrine, the least potent bronchodilator of the sympathomimetic amines tested in guinea-pigs ventilated with air during the control period, potentiated the bronchoconstrictor action of histamine when it was given to guinea-pigs ventilated with 10% CO_2 in air. An experiment illustrating this finding is shown in Fig. 3. The effect of ephedrine in increasing the bronchoconstrictor response to histamine persisted for more than an hour after the injection of ephedrine.

The bronchodilator activity of all those sympathomimetic amines which were less active or inactive during ventilation with 10% CO_2 in air was restored within 1 h of resuming ventilation with air; and occurred at approximately the same time that the sensitivity to the bronchoconstrictor effect of histamine returned to the initial control level. The bronchodilator action of amphetamine was observed after resumption of ventilation with air; the injection given during the period of ventilation with 10% CO_2 was not only ineffective in producing bronchodilatation but also did not produce tachyphylaxis. Restoration of the bronchodilator action of ephedrine occurred after ventilation with air was resumed; this required about 1 h, as with phenylephrine and tyramine.

When the bronchodilator effects of ethylnoradrenaline, phenylephrine, tyramine, amphetamine and ephedrine had been reduced, abolished or reversed by ventilation with 10% CO_2 in air, the guinea-pigs still responded to isoprenaline in the usual manner. The altered response to these amines is therefore apparently not a result of β-receptor blockade.

Blood pressure and heart rate changes

The doses of histamine used generally produced a very slight fall in blood pressure and sometimes an increase in pulse pressure. The effect on the heart rate varied: occasionally, there was a slight decrease, but usually it increased by 10 to 50 beats per min. In the doses used, adrenaline, isoprenaline, orciprenaline and noradrenaline had very little or no effect on blood pressure and heart rate. Ethylnoradrenaline and phenylephrine produced a variable small rise in blood pressure and had no effect on heart rate. With tyramine, amphetamine and ephedrine there was a prolonged increase in blood pressure or pulse pressure. Tyramine, amphetamine and ephedrine occasionally produced a slight fall in heart rate preceding a sustained rise, but more usually there was only a sustained rise.

When the guinea-pigs were ventilated with 10% CO_2 in air, the blood pressure usually increased. The immediate effect on heart rate varied from a slight fall to a slight rise, and there was a gradual fall thereafter. During the period of ventilation with 10% CO_2 in air, the decreased sensitivity to the bronchoconstrictor effects of

histamine was accompanied by a decreased sensitivity to the cardiovascular effects of histamine and all of the sympathomimetic amines used. Sensitivity to the cardiovascular effects of the sympathomimetics started to return at about the same time as bronchial sensitivity after ventilation with air was resumed.

DISCUSSION

The order of potency of a series of sympathomimetics in counteracting histamine bronchospasm in the guinea-pig was found to be isoprenaline>adrenaline>noradrenaline > orciprenaline > ethylnoradrenaline > phenylephrine > tyramine= amphetamine > ephedrine. Phenylephrine was a fairly potent antagonist of histamine-induced bronchospasm although it is regarded primarily as an α-receptor agonist (Levy & Ahlquist, 1961). The dose of phenylephrine required to reduce the histamine response by approximately 50% was only twice that of ethylnoradrenaline, which has both α- and β-agonist activity (Levy, 1959).

It has been shown in this laboratory (Atkinson & Rand, unpublished work) that the cardiovascular effects of isoprenaline, adrenaline and orciprenaline involving β-receptors are suppressed during ventilation with 10% CO_2 in air in cats. The observations on heart rate and blood pressure effects of these amines in guinea-pigs in the present experiments are in agreement with these findings. However, the effects of these three amines in counteracting bronchospasm during ventilation with 10% CO_2 in air were unaffected. This supports the proposal by other workers (Lands & Brown, 1964) that β-receptors in the cardiovascular system differ from those in respiratory smooth muscle. With ethylnoradrenaline, phenylephrine, tyramine, amphetamine and ephedrine, effects involving β-receptors on both the cardiovascular system and bronchial smooth muscle were suppressed during ventilation with 10% CO_2 in air.

An explanation for the difference between isoprenaline, adrenaline, noradrenaline and orciprenaline on the one hand, and of tyramine and amphetamine on the other may lie in their different mechanisms of action, in that the former are directly acting whereas the latter are indirectly acting (Burn & Rand, 1958). However, the mechanism by which a fall in blood pH, or a rise in blood pCO_2 results in loss of action of indirectly acting sympathomimetic amines is a matter of speculation: it may be that the process of uptake of these amines into adrenergic neurons, or the process of release of noradrenaline by them, is inhibited. The main difficulty in the way of suggesting that the difference is due to different types of sympathomimetic action is that Burn & Rand (1958) found phenylephrine to be directly acting and ethylnoradrenaline is also generally regarded as directly acting. Since there are differences between species and even between tissues in the one species in the relative extents to which a given sympathomimetic may act directly and indirectly, it would be desirable to have evidence on the particular mechanism that operates in the guinea-pig bronchial smooth muscle with these two drugs.

The pK_a values of the amines tested are all above 9, so that differences in ionization at the pH produced by ventilating the guinea-pigs with 10% CO_2 in air are unlikely to account for the different effects seen.

The enhanced bronchoconstrictor response to histamine produced by ephedrine during ventilation with 10% CO_2 in air will be subjected to further examination. It

may be due to inhibition of monoamine oxidase by ephedrine (Gaddum & Kwaitkowski, 1938) with protection of histamine from degradation (Zeller, Stern & Blacksma, 1956). Allen & Rand (1969) showed that monoamine oxidase inhibitors potentiated the bronchoconstrictor action of histamine in the guinea-pig.

The findings have implications for the treatment of asthmatic bronchospasm with commonly used sympathomimetic bronchodilators. In an attack which is so prolonged and severe that blood pCO_2 rises and pH falls, then ethylnoradrenaline may be less effective and phenylephrine ineffective, even though they may previously have been effective prophylactically or in less severe attacks; it is possible that ephedrine may even worsen the bronchospasm. In such severe asthma, isoprenaline, adrenaline and orciprenaline may still be fully effective as bronchodilators. Furthermore, in such a condition, there is no evidence to suggest increased cardiac toxicity (Atkinson & Rand, unpublished observations). However, Collins, McDevitt & others (1969) found that the cardiac toxicity of isoprenaline was increased when the blood pO_2 was decreased.

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