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Antibronchoconstrictor activity of two new phosphodiesterase inhibitors, a triazolopyrazine (ICI 58 301) and a triazolopyrimidine (ICI 63 197)

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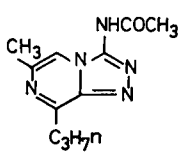
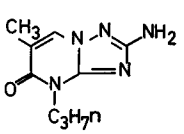
A triazolopyrazine (ICI 58 301) and a triazolopyrimidine (ICI 63 197), both potent inhibitors of 3′5′-cyclic adenosine monophosphate phosphodiesterase, protected conscious guinea-pigs against an otherwise lethal bronchospasm caused by inhalation of histamine. Both compounds were active when given by mouth and the activity was both rapid in onset and persistent. Bronchospasm caused by histamine or acetylcholine in guinea-pig isolated lungs was reduced when the lungs were perfused with dilute solutions of the compounds and the bronchodilator activity of catecholamines was potentiated.

In anaesthetized animals the compounds were relatively more active against severe bronchospasm than against milder stimuli. The antibronchoconstrictor effects on conscious guinea-pigs were blocked by propranolol but not by practolol even in much larger doses. Propranolol did not, however, block the effect on the isolated lung. It appears possible that the antibronchoconstrictor activity is a manifestation of the inhibition of phosphodiesterase.

A study of the pharmacological properties of purine isosteres, made in these laboratories during the past 15 years has revealed that two series of compounds, triazolopyrazines and triazolo-pyrimidines are particularly potent inhibitors of bronchospasm in guinea-pigs. The representatives of these series now discussed are 3-acetamido-6-methyl-8-n-propyl-s-triazolo [4,3-a] pyrazine (ICI 58 301) (Maguire & Rose, 1969; Maguire, Paton & Rose, 1969) and 2-amino-6-methyl-5-oxo-4-n-propyl-4,5-dihydro-s-triazolo [1,5-a] pyrimidine (ICI 63 197) (Dukes, 1971). ICI 58 301 will be referred to as A and ICI 63 197 as B.



A = ICI 58 301



B = ICI 63 197

Both compounds are potent inhibitors of 3′5′-cyclic adenosine monophosphate (cyclic AMP) phosphodiesterase (Somerville, Rabouhans & Smith, 1970; Somerville, personal communication). A brief account of the chemical, biological and biochemical properties of A has been given by Davies, Rose & Somerville (1971) and the present paper describes in detail the activities of both compounds as inhibitors of bronchospasm in guinea-pigs.

MATERIALS AND METHODS

Animals

The guinea-pigs were specific pathogen free animals (Alderley Park Strain 1) of either sex and weighing between 250 and 350 g.

Histamine-induced bronchospasm in conscious guinea-pigs

The method was essentially that described by Loew, Kaiser & Moore (1945). The apparatus consisted of a cylindrical Perspex chamber, capable of holding up to 4 animals, into which a 1:80 aqueous solution of histamine acid phosphate (equivalent to 0·45% histamine base) was sprayed for 45 s. Ten min from the start air was drawn through the chamber for 2 min. The times at which animals died were recorded and the number surviving at 15 min was counted.

Isolated perfused lung of guinea-pig

Animals were killed by a blow on the head, the thorax opened and the heart and lungs taken out. The pericardium was removed and a cannula tied into the pulmonary artery. Most of the blood was flushed from the lung by injecting 10–20 ml of warmed Tyrode solution into the cannula, the effluent being allowed to escape through an incision in the left auricle. A second cannula was tied into the trachea and the preparation mounted in the apparatus described by Bhattacharya & Delaunois (1955). A bellows pump, delivering 7 ml of air 25 times min^{-1} was attached to the chamber in which the lungs were suspended, the lungs being inflated as air was withdrawn from the chamber. Changes in pressure during respiration were recorded by means of a pressure transducer attached to the side-arm of the tube leading to the tracheal cannula and a Devices recorder, or in the early experiments, with a tambour-activated lever writing on a smoked drum. An aspirator containing Tyrode solution with 2% dextran (pH 7·3) was attached, via a warming coil, to the arterial cannula and the vascular system perfused at a rate of 2–3 ml min^{-1}.

Bronchoconstrictor agents, in 0·1 ml volumes, were injected close to the arterial cannula and other compounds either in the same manner or by perfusion through the vascular system of the lung from a second aspirator. Since with this apparatus bronchospasm caused smaller pressure changes in the trachea the degree of spasm (S) could be calculated from the expression $S = (X - Y)/X \times 100$ where X and Y were respectively the excursion (in mm) of the recording 1 min before and 1 min after injection of spasmogen.

At the start of an experiment, various doses of bronchoconstrictor agents were given at 4 min intervals until the response to the dose given had stabilized at about 80–95% spasm. When other drugs were injected the first dose was given 4 min after the preceding dose of bronchoconstrictor. Four min later the injections of bronchoconstrictor were continued at 4 min intervals, until any effect of the drug had worn off.

Percent inhibition of bronchospasm was calculated from the expression

$$\% \text{ inhibition} = \frac{\text{S control} - \text{S treated}}{\text{S control}} \times 100$$

Bronchospasm in anaesthetized guinea-pigs

Guinea-pigs were anaesthetized with pentobarbitone sodium (70 mg kg^{-1} intraperitoneally) and cannulae tied in the trachea and jugular vein. The apparatus was that described by Konzett & Rössler (1940), but whereas the original method measures an increase in overflow volume caused by increased resistance of the lungs to inflation, the present technique measured the increase in pressure due to the overflow air by substitution of a pressure transducer for the more usual volume recorder. Air was delivered from a pump at a rate of 68 strokes min^{-1} and a constant stroke volume between 6 and 8 ml which was adjusted at the beginning of the experiment to give a minimal overflow volume and hence a minimal increase in pressure. To assist recovery of the lungs from severe spasm the side vent of the recording arm of the intratracheal cannula was clamped automatically for 10 s during each 30 s to inflate the lungs more forcibly as described by Collier & James (1967). Recordings of increased pressure were taken to indicate bronchospasm. Maximal spasm was measured at the start of an experiment by momentarily clamping the tube between the side vent and the trachea. The degree of bronchospasm was measured at intervals and calculated from the expression

$$\% \text{ bronchospasm} = \frac{X - B}{M - B} \times 100$$

where M, B and X respectively were the heights (in mm) of the maximal recording, the recording before spasmogen and the recording after spasmogen.

Drugs

Sources of the various compounds used were: histamine acid phosphate (BDH); acetylcholine chloride (Sigma); dextran (Dextraven, Fisons); propranolol (Inderal, ICI); practolol (Eraldin, ICI); adrenaline bitartrate (Evans); isoprenaline sulphate (Burroughs Wellcome); salbutamol (Ventolin, Allen and Hanbury).

The samples of A and B used were both synthesized in these laboratories, the former by Dr. F. L. Rose and the latter by Dr. M. Dukes. For all experiments solutions were prepared as follows. Compound A was dissolved with gentle warming in 0·1 N NaOH at 40 mg ml^{-1} and the solutions diluted with the required amount of warm saline, B was dissolved in warm saline at 1 mg ml^{-1} and diluted as required. All solutions were prepared and used on the same day.

RESULTS

Histamine bronchospasm in conscious guinea-pigs

Under the experimental conditions used, more than 90% of the undosed animals died 5 to 9 min after exposure to the aerosol of histamine, further deaths after this time being rare. Oral administration of A or B had marked protective effects which were rapid in onset and persistent. When given 1 h before histamine a dose of 0·5 mg kg^{-1} of A or 0·05 mg kg^{-1} of B protected about half the animals. At rather higher doses (5 mg kg^{-1} of A or 0·1 mg kg^{-1} of B) protective effects were evident from 5 min to 5 h after dosing. Both compounds were clearly more effective than aminophylline (Table 1).

The behaviour of animals dosed with any of the above compounds and exposed to this very severe challenge is worthy of comment. All, including those which are

Table 1. *Effects of ICI 58 301 (A) and ICI 63 197 (B) on histamine bronchospasm in conscious guinea-pigs.*

Compound	Oral dose (mg kg^{-1})	Proportion of animals surviving when the compound was given at the indicated time before exposure to histamine						
		5 min	15 min	30 min	1 h	2 h	3 h	5 h
A	0·1				0/8			
	0·5			1/8	14/27	3/8		
	1·0	0/4	8/16	18/40	32/64	5/20	0/8	
	5·0	12/28	12/24	31/48	49/64	10/16	20/24	8/16
	10·0	14/23	11/16	15/20	47/60	14/16	14/16	13/16
B	0·01				0/4			
	0·025				1/4			
	0·05	0/4	0/4	4/7	13/24	6/8	5/8	1/4
	0·1	3/8	3/8	4/8	19/24	4/8	6/8	2/4
	0·25	1/8	5/8	7/8	10/12	8/8	8/8	1/4
	0·5	5/8	7/8		14/16	6/8	5/8	4/4
Aminophylline	100	0/4	1/12	5/12	6/12	3/8		

Table 2. *Effects of ICI 58 301 (A) on bronchospasm induced by acetylcholine (0·2 to 2 μg) in guinea-pig isolated lungs.*

Period of perfusion	Concentration of A in perfusion fluid (μg ml^{-1}) Mean % inhibition of spasm (with range)		
With compound	5·0 (n = 3)	2·5 (n = 2)	1·0 (n = 1)
4 min	55(31–77)	47(42–51)	10
8 min	43(27–58)	49(46–51)	6
Subsequently with Tyrode			0
4 min	34(4–58)	17(0–33)	

Dose of acetylcholine chosen to give 80–95% spasm during preliminary perfusion with Tyrode solution.

n = number of lungs.

Table 3. *Effect of ICI 63 197 (B) on bronchospasm induced by acetylcholine (0·2 to 2 μg) in guinea-pig isolated perfused lungs.*

Period of perfusion	Concentrations of B in perfusion fluid (μg ml^{-1}) Mean % inhibition of spasm (+s.e.)			
	0·5	0·25	0·125	0·0625
With compound				
4 min	69·6 ± 6·2	47 ± 5·7	43·8 ± 11·9	30·4 ± 14·4
8 min	68·8 ± 4·2	47·7 ± 5·8	53·9 ± 11·9	33·3 ± 10·3
12 min	69 ± 6·5	48·5 ± 7·0	41·3 ± 9·8	37·5 ± 12·6
Subsequently with Tyrode				
4 min	56·7 ± 8·3	24·7 ± 10	29·9 ± 8·5	16 ± 6·7
8 min	29·4 ± 17·0	23·6 ± 10	29·9 ± 8·5	0·8 ± 2·5

Dose of acetylcholine chosen to give 80–95% spasm during preliminary perfusion with Tyrode solution. 5 lungs used for each concentration of B.

eventually going to survive, are indistinguishable from undosed animals during the first 3 min or so after they have inhaled histamine but the eventual survivors gradually recover and may be breathing normally at the end of the 15 min period. The effect of isoprenaline or a similar compound is different; animals treated with doses which protect half (for example 0·006 mg kg^{-1} of isoprenaline given subcutaneously 15 min before exposure) appear normal for 5 min or so, but some then exhibit bronchospasm of gradually increasing severity.

Inhibition of bronchospasm in the guinea-pig isolated perfused lung

When the isolated lungs were perfused with Tyrode solution containing either A or B the effects of bronchoconstrictor agents were inhibited. In early experiments lungs were perfused first with Tyrode solution and then with gradually decreasing concentrations of A. Every 4 min, bronchospasm was produced by injection of acetylcholine (0·5 μg in 0·1 ml). Perfusion with A at 10 μg ml^{-1} caused an immediate increase in the height of the tracing and virtual abolition of the bronchospasm. Inhibitory effects were also evident at concentrations down to 1 μg ml^{-1} but no dose-response relation was apparent possibly because of the carry-over of effects from the preceding higher concentrations.

In subsequent experiments a single lung was used for each concentration. When histamine in doses of 0·5 or 1 μg was used as spasmogen, A was inhibitory at 10–20 μg ml^{-1} and there was some continued inhibition when the lungs were subsequently perfused with plain Tyrode solution. However these experiments were not entirely satisfactory because the formation of oedema restricted the number of doses of histamine that could be given. This problem did not occur when acetylcholine in doses from 0·2 to 5 μg was used as spasmogen. Compound A was inhibitory at 2·5 or 5 μg ml^{-1} but not at 1 μg ml^{-1} (Table 2). More complete experiments were done with B. Table 3 shows the mean results when 5 lungs were used for each concentration. Inhibitory effects were detectable at concentrations as low as 0·06 μg ml^{-1}.

Potentiation of the bronchodilator activity of sympathomimetic amines

The doses of 3 sympathomimetic amines minimally effective against acetylcholine-induced bronchospasm in isolated lungs were found to be: salbutamol and adrenaline 0·05 μg and isoprenaline 0·01 μg when injected 4 min before acetylcholine. Compound A, although active when perfused at a concentration of 5 μg ml^{-1}, was ineffective when 5 μg was given as a single injection 4 min before acetylcholine and the same was true for B when perfused at 1 μg ml^{-1} or given as a single injection of 1 μg. However, when these amounts of the two compounds were given as single injections with minimally active doses of the amines an intensification and prolongation of the bronchodilator activity was seen (Table 4 and Fig. 1).

In these experiments one lung was used for one concentration of each compound or combination of compound and amine. When either compound was used with a sympathomimetic amine the compound was always given immediately before the amine.

Effect on histamine bronchospasm in anaesthetized guinea-pigs

Groups of 4 animals were given either saline or A (2·5 mg kg^{-1}) or B (250 μg kg^{-1}) intravenously and 1 min later histamine at either 10 μg kg^{-1} or 150 μg kg^{-1} by the same route. The degree of bronchospasm was recorded for 8 min and then further

Table 4. *Potentiation in guinea-pig isolated lungs of the bronchodilator activities of catecholamines by ICI 58 301 (A) and ICI 63 197 (B).*

Treatment				Mean % inhibition of acetylcholine bronchospasm		
Catecholamines	μg ml^{-1}	Compound	μg ml^{-1}	Time after treatment		
				4′	8′	12′
Adrenaline	0·05			28 ± 4·3 (6)	7 ± 6·5 (5)	12·5 ± 0·5 (2)
,,	0·05	A	5	57 ± 8·2 (6)	38 ± 11·5 (5)	30 ± 4·4 (4)
,,	0·05	B	1	60 ± 9·1 (5)	31 ± 10·6 (5)	18 ± 7·5 (3)
Salbutamol	0·025			6 ± 3·1 (4)		
,,	0·05			25 ± 3·5 (15)	7 ± 3·8 (4)	6 ± 4·4 (2)
,,	0·05	A	5	53 ± 4·3 (10)	31 ± 4·4 (10)	18 ± 9·9 (3)
,,	0·025	B	1	60 ± 9·1 (5)	31 ± 10·6 (5)	18 ± 7·5 (3)
Isoprenaline	0·01			19 ± 3·7 (5)	8 ± 1·4 (5)	7 ± 2·8 (5)
,,	0·01	B	1	45 ± 7·4 (5)	25 ± 0·7 (5)	2 ± 0·5 (5)
None		A	5	1·3 ± 1·8 (4)		
,,		B	1	0 (4)		

Figures in brackets are the number of lungs used.

injections of histamine given at intervals of 8 min. Both compounds showed similar effects. When the higher dose of histamine was used the initial peak spasm was the same in both treated and control groups but the treated animals showed a more rapid recovery. Subsequent injections of histamine produced progressively less severe effects in animals treated with either A or B, whereas increasingly severe effects occurred in the control animals, 3 of the 4 dying after the third injection (Fig. 2b). When the lower dose of histamine was used the only effect seen was a slight inhibition of the first spasm when B was used (Fig. 2a).

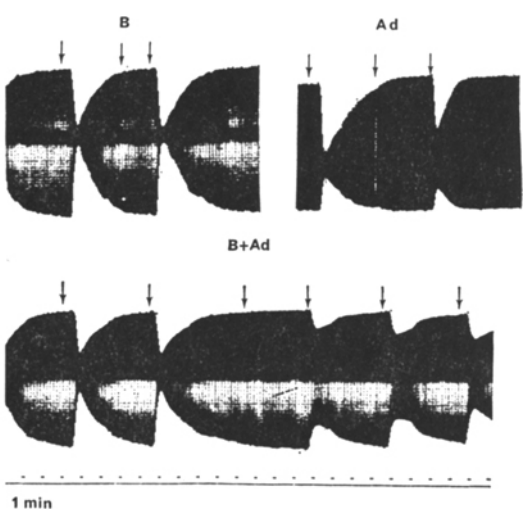


FIG. 1. Potentiation of antibronchoconstrictor activity of adrenaline by ICI 63 197 (B). Each unlabelled arrow represents the injection of acetylcholine (1 μg); B = compound B (1 μg); Ad = adrenaline (0·05 μg); B + Ad = compound B (1 μg) with adrenaline (0·05 μg). Each experiment was carried out on separate tissue.

Table 5. *Effect of β-adrenoceptor blocking drugs on the activities of ICI 58 301 (A) and ICI 63 197 (B) against histamine bronchospasm in conscious guinea-pigs.*

Compound	Dose (mg kg^{-1})	β-adrenoceptor blocker	Dose (mg kg^{-1})	Number of animals	Number Surviving
A	5·0	—	—	8	8
,,	,,	Propranolol	0·1	8	0
,,	,,	,,	0·05	8	1
,,	,,	Practolol	4·0	8	8
B	1·0	—	—	8	8
,,	,,	Propranolol	0·5	8	0
,,	,,	,,	0·1	8	2
,,	,,	,,	0·05	8	6
,,	,,	Practolol	4·0	8	7
None	—	Propranolol	1·0	8	0
,,	—	Propranolol	0·5	16	0
,,	—	Practolol	4·0	16	0
,,	—	,,	—	24	0

Compounds A and B administered orally 1 h before histamine β-adrenoceptor blocking drugs administered subcutaneously 30 min before histamine.

Effect of β-adrenoceptor blockade in conscious guinea-pigs

Propranolol (0·05 — 0·1 mg kg^{-1}) completely prevented the protective action of both compounds against histamine bronchospasm whereas the cardio-selective drug practolol (4 mg kg^{-1}) did not (Table 5). Both propranolol and practolol were given subcutaneously 30 min before histamine exposure.

Propranolol potentiates histamine bronchospasm by blockade of the reflex β-adrenoceptor activation following bronchoconstrictor stimuli (McCulloch, Proctor & Rand, 1967). The interference by propranolol with the activity of the compounds was at first thought to be due to the increased bronchoconstrictor action of a given dose of histamine making the test too severe for the compounds to show activity. This explanation, however, appeared unlikely when it was found that both maintained

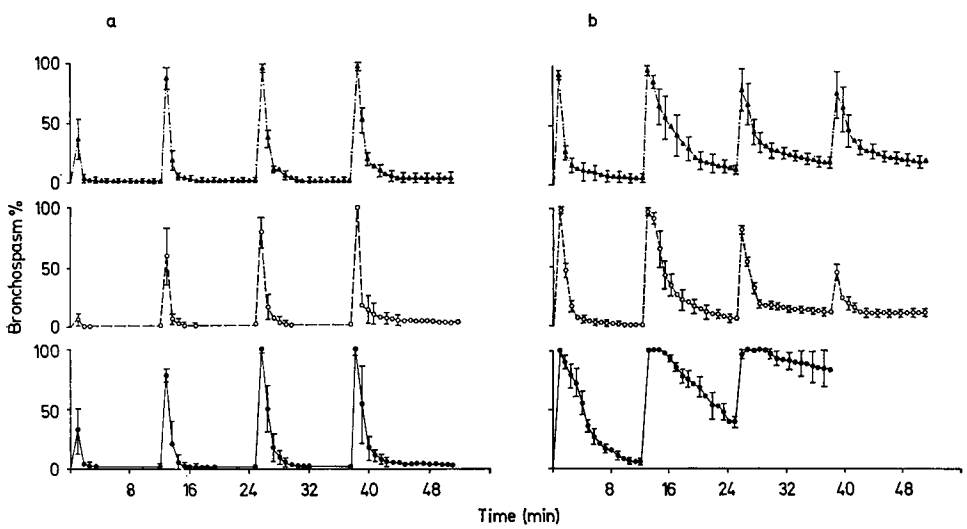


FIG. 2. Inhibition of histamine bronchospasm in anaesthetized guinea-pigs. (a) Histamine 10 μg kg^{-1}, (b) histamine 150 μg kg^{-1}. ▲, compound A (2·5 mg kg^{-1}); ○, compound B (250 μg kg^{-1}); ●, controls. Histamine and drugs administered intravenously.

their activities even when, without propranolol, eight times the normal concentration of histamine was used.

In contrast to these effects in the whole animals, propranolol, at concentrations up to 1 μg ml^{-1}, did not block the activity of either ICI compound against bronchoconstrictor challenge in the isolated lung.

DISCUSSION

Both compounds A and B at low doses, protect guinea-pigs from death due to histamine bronchospasm and they also show effects in isolated lung preparations consistent with activity as bronchodilators. Four lines of evidence, however, point to a conclusion that the effects seen in the whole animal are not those of direct-acting bronchodilators. These are: (i) in conscious animals the compounds did not delay the onset of bronchospasm following inhalation of histamine but did allow the animals to survive; (ii) the amounts of compound required for effects on isolated lungs were relatively large (1–10 μg ml^{-1} for A and 0·06–1 μg ml^{-1} for B) in view of the low blood levels achieved by doses active *in vivo* (0·1 μg ml^{-1} for A and less than 0·05 μg ml^{-1} for B) (Case, personal communication); (iii) in anaesthetized guinea-pigs, activity was seen mainly against severe challenge; and (iv) although activity in conscious animals was blocked by propranolol, there was no blockade of activity in isolated lungs. A possible explanation of all these observations became apparent when it was shown that both compounds are potent inhibitors of 3,5-cyclic AMP phosphodiesterase (Somerville & others, 1970; Somerville, personal communication).

The inhibitor constants against guinea-pig lung phosphodiesterase are $4{\cdot}98 \times 10^{-4}$M for compound A and $4{\cdot}6 \times 10^{-6}$M for compound B, i.e. compound B is 108 times as potent as compound A (Skidmore & Somerville, personal communication).

It may be argued that relief of bronchospasm by sympathetic stimulation is proportional to the amount of available cyclic AMP (Szentivanyi, 1968). In the absence of further treatment, the amount of cyclic AMP formed as a result of reflex stimulation may well be insufficient to confer significant protection since it will be removed by the action of phosphodiesterase. Compounds A and B, by inhibiting the activity of phosphodiesterase, might be expected to delay this destruction and allow the cyclic AMP to confer protection.

The amount of histamine used in the present experiments in conscious guinea-pigs is lethal to undosed animals; it may be assumed that any reflex sympathetic stimulation leading to increased levels of cyclic AMP is ineffective because the action of phosphodiesterase is unhindered. In isolated preparations there is no sympathetic stimulation and hence higher levels of the two compounds are necessary to maintain the smaller amounts of cyclic AMP formed by some other mechanism. The experiment in anaesthetized animals also became explicable since mild challenge may not produce sufficient cyclic AMP to be effective even though it is protected from destruction by inhibition of phosphodiesterase and anaesthesia will have the additional effect of reducing the degree of sympathetic stimulation. In the presence of larger amounts of cyclic AMP resulting from the more severe challenge the effect of inhibition of phosphodiesterase becomes apparent as reduction in the duration of bronchospasm. Blockade by propranolol of β-adrenoceptor stimulation will prevent the accumulation of cyclic AMP in response to challenge. It also follows that increased production of cyclic AMP by administration of β-adrenoceptor stimulants may be more effective

in the presence of a phosphodiesterase inhibitor as is shown by the potentiation of the bronchodilator activity of sympathomimetic amines.

These speculations on the mode of action are presented as a working hypothesis which can be given weight only if it can be demonstrated that the predicted changes in levels of cyclic AMP in the respiratory tract of guinea-pigs do in fact occur.

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