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THE PHARMACOLOGY OF ISAMOXOLE [2-METHYL-*N*-BUTYL-*N*(4-METHYLOXAZOL-2-YL) PROPANAMIDE] LRCL 3950, A NEW ANTI-ALLERGIC COMPOUND

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1 Isamoxole [2-methyl-*N*-butyl-*N*(4-methyloxazol-2-yl) propanamide] is an effective orally active anti-allergic compound in animals.

2 Isamoxole inhibits the immunological release of mediators, notably slow-reacting substance of anaphylaxis (SRS-A) from sensitized human and guinea-pig chopped lung *in vitro*.

3 *In vivo*, allergic responses were inhibited in guinea-pigs and rats by doses as low as 25 mg/kg given orally 180 and 30 min before challenge. The effect of Isamoxole was still present 4 h after a single dose of 100 mg/kg orally in the guinea-pig and 3 h in the rat.

4 Isamoxole is a moderately potent, selective inhibitor of SRS-A activity on the guinea-pig ileum *in vitro*, at concentrations that do not antagonize histamine, 5-hydroxytryptamine, or bradykinin.

5 Isamoxole causes human bronchial muscle to relax and antagonizes the bronchoconstriction induced by SRS-A.

Introduction

Bronchial asthma due to an immediate Type 1 allergic reaction may be treated in three ways: by bronchodilators acting directly on bronchial smooth muscle, by antagonists that reduce the effect of pharmacological mediators released due to the antigen-antibody reaction, and by inhibitors which prevent the release of these mediators. In the latter group, disodium cromoglycate (DSCG) is effective in some patients with asthma (Altounyan, 1967). DSCG is ineffective when given orally, since it is poorly absorbed from the gastrointestinal tract; it appears not to inhibit the responses to anaphylactic mediators and has no bronchodilator properties.

Isamoxole [2-methyl-*N*-butyl-*N*(4-methyloxazol-2-yl) propanamide] is an anti-allergic compound with oral activity demonstrable over 4 h in animal models (Figure 1). It is a member of the 2-acylamino oxazole series, which reduces mediator release in immediate hypersensitivity reactions (Ross, Harrison, Jolley, Neville, Todd, Verge, Dawson & Sweatman, 1979).

Methods

Efficacy testing for potential anti-allergic compounds

Guinea-pig chopped lung Guinea-pigs (male, 250 to 350 g body wt) were sensitized with ovalbumin (Sigma, Grade II, 100 mg i.p. and s.c.). Their lungs were removed three weeks later (Mongar & Schild, 1956; Brocklehurst, 1960), perfused with Tyrode solution to remove blood and chopped into 0.5 mm cubes: 100 mg aliquots were suspended in Tyrode (4.5 ml) in each tube, incubated for 5 min (37°C) before addition of Isamoxole or Tyrode and the challenging solution of ovalbumin (5 mg in 0.1 ml, Sigma Grade III). Unchallenged incubates were the controls. Further tubes received semipurified slow reacting substance of anaphylaxis (SRS-A) to determine any direct SRS-A antagonism. All tubes were incubated for 15 min (37°C), the supernatant was removed by straining (200 mesh nylon) and was bioassayed immediately or stored at −20°C. Total histamine was determined by boiling (10 min) each lung aliquot (5 ml Tyrode) and assaying the supernatant. In each experiment, quadruplicate tubes were used.

SRS-A was assayed on segments of guinea-pig ileum treated with mepyramine (0.4 μg/ml) against partially purified SRS-A from guinea-pig lung ($SRS\text{-}A_{gp}$). Results are expressed as percentage inhibition of mediator release after subtraction of basal release levels. Significance of differences was calculated from the absolute values by Student's *t* test. Histamine was assayed similarly using normal segments of guinea-pig ileum.

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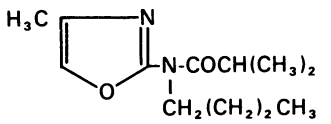

Figure 1 Isamoxole [2-methyl-*N*-butyl-*N*(4-methyl-oxazol-2-yl)propanamide].

Human chopped lung Samples of healthy human lung were placed in ice-cold Tyrode solution within 45 min of removal during operations for carcinoma of the lungs. Within 2 h, the lung samples were washed in Tyrode, chopped with a McIlwain chopper into 0.5 mm cubes, and then incubated for 18 h at 18°C with atopic serum derived from patients allergic to cocksfoot (*Dactylis glomerara*) pollen.

The chopped tissue was treated and assayed in a similar manner to guinea-pig chopped lung, except that the tissue was challenged, after pre-incubation with Isamoxole for 5 min, with specific antigen (pollen extract 0.1 ml, 1000 noon units) or rabbit anti-human IgE serum (0.1 ml diluted 1:200).

Allergic bronchospasm or histamine provocation in conscious guinea-pigs

Allergic bronchospasm was produced as described by Herxheimer (1952). Male guinea-pigs (250 to 350 g body wt) were sensitized as described for the chopped lung test. Three weeks later they were placed in a perspex cylinder, 25 cm i.d. with a depth of 25 cm, and exposed to ovalbumin aerosol (1% w/v solution) generated by a Wright nebuliser using air at a pressure of 1.05 bars. The challenge produced respiratory distress, characterized by increased rate and depth of respiration, followed by a characteristic convulsive cough. After longer exposure, convulsions and death with post mortem findings of severe bronchospasm were observed.

The time of onset of the characteristic cough immediately preceding convulsions was known as the collapse time. The Protection Ratio of a compound was defined as:—

$$\frac{\text{Collapse time after pretreatment with compound}}{\text{Mean collapse times on weeks preceding and following drug treatment}}$$

When an animal is 'protected' it is possible to expose it to antigen aerosol for long periods of time and so an arbitrary maximum protection ratio of 20 was used, equivalent to an exposure of 20 times the untreated collapse time.

Histamine bronchospasm was induced in non-sensitized guinea-pigs with an aerosol of histamine (4 mg/kg, base equivalent), generated as described.

Allergic bronchospasm in anaesthetized guinea-pigs

Guinea-pigs, actively sensitized as described in the preceding section were anaesthetized with pentobarbitone (40 to 50 mg/kg i.p.). The trachea, carotid artery and jugular vein were cannulated. Artificial respiration (Palmer's Ideal pump) was provided at a rate of 12 strokes per min with a volume suitable for the individual guinea-pig and sufficient to maintain a stable arterial blood pressure.

A Y-cannula in the tube from pump to trachea allowed recording of intratracheal pressure (Consolidated Electronics Transducer) according to the method of Dixon & Brodie (1903).

Isamoxole was injected intravenously at various doses before subsequent challenges with antigen, and the responses to histamine and 5-hydroxytryptamine (5-HT) were examined throughout each experiment. In control experiments using animals of the same group, at least four challenges were possible in the same animal. These were made at intervals of one hour, and the doses of antigen increased in steps of 0.1, 0.25, 0.5, 1.0 mg/kg. After four challenges the animals were not totally desensitized, but the severity of the resulting broncho-constriction was less predictable.

Passive cutaneous anaphylaxis (PCA) in the rat

Female Lilly Lodge Moor rats (150 to 160 g) were shaved ventrally under light methohexitone anaesthesia and injected intradermally with 0.05 ml rat serum containing IgE directed towards ovalbumin (anti OA-IgE) at a dilution previously shown to give a positive response; 24 h later, the rats were lightly anaesthetized with methohexitone and challenged intravenously with ovalbumin (20 mg/kg), mixed with Pontamine Sky Blue dye (100 mg/kg) in saline (total volume 4 ml/kg). Forty five min after challenge, the rats were killed by cervical fracture and the skins removed.

Passive peritoneal anaphylaxis in the rat

Female Lilly Lodge Moor rats (190 to 210 g body wt), in groups of 5 animals were injected intraperitoneally with 1 ml of anti OA-IgE (diluted in 0.9% saline to give a PCA titre of 1:100). Two hours after the injection, the test rats were challenged by intraperitoneal injection of ovalbumin (2 mg/rat) in 5 ml Tyrode containing heparin (1 unit/ml) and control rats received 5 ml heparinised Tyrode; 5 min later, the rats were killed by CO_2, the abdomen opened and the peritoneal fluid centrifuged at 14°C (300 *g*, 3 min). Each supernatant was transferred into two sterile capped vials, shell frozen and stored at −20°C. All glassware was siliconized.

Bioassays were carried out on normal and mepyramine-treated guinea-pig ileum segments as detailed in the first section of Methods.

Antagonism in vitro *of pharmacological mediators*

Short segments of guinea-pig terminal ileum were used as described for SRS-A assay in a Cassella automatic biological assay apparatus. Agonists were usually in contact with the tissues for 20 to 30 s every 2 min. The agonists, acetylcholine, histamine, 5-HT, bradykinin, prostaglandins E_2 and $F_{2\alpha}$ and SRS-A_{gp} were administered at two submaximal doses alternately until constant responses were obtained. Isamoxole was added to the bathing solution in increasing concentrations until a constant response of the agonist was obtained. Isamoxole was then removed from the bathing solution and the tissue allowed to recover from its effects. A pA_2 value (Schild, 1947) was determined for Isamoxole against SRS-A. This value was obtained after equilibrium was reached between the tissue and Isamoxole and was measured at a contraction of 50% of maximum.

Phosphodiesterase inhibition

Cyclic nucleotide phosphodiesterase in the 50 × *g* supernatant of guinea-pig lung homogenate (2 g in 2.5 ml Tris 50 mM/sucrose 0.25 M buffer at pH 7.4) was used. The assay was conducted in liquid scintillation vials containing a mixture of 0.025 μCi[^{3}H]-cyclic adenosine 3',5'-monophosphate (cyclic AMP), 0.25 μM cyclic AMP, 0.05 units of nucleotidase, 0.025 μM 5' AMP and 0.25 μM $MgCl_2$ in 50 mM Tris buffer (pH 8.0) (Brookes, Thomas & Appleman, 1968). To this incubation mixture was added 50 μl of Isamoxole to give a final concentration of 1, 0.1 or 0.01 mM and finally, 50 μl of enzyme preparation. The total volume of the incubation mixture was 200 μl. After a 10 min incubation in a counting vial at 37°C, 1 ml resin suspension (AGI-X2, 200 to 400 mesh, chloride form, suspended in equal volume of triple distilled water) was added and incubated at 37°C for a further 10 min; 10 ml of dioxane based scintillator was added, shaken and cooled for 1 h before counting. Zero time and drug controls were included in each experiment. Each group was run in triplicate.

Bronchodilator activity on respiratory tissue

Guinea-pig trachea Spirally cut tracheal strips (Constantine, 1965) were suspended in Krebs solution at 37°C and gassed with 95% O_2 and 5% CO_2 (30 ml bath). Isotonic recordings of contraction and relaxation of the tissue were made with a transducer (Harvard) coupled to a pen recorder (Rikadenki).

Human bronchial muscle strips Spirally cut strips of histologically healthy human bronchial muscle (obtained during operations for lung carcinoma) were suspended in Krebs solution at 37°C and gassed with 95% oxygen/5% carbon dioxide (Collier & Sweatman, 1968). The mean diameter of the airways used was 2 mm. Contractions were recorded as with guinea-pig trachea.

Effect on prostaglandin synthetase

Blade-homogenized frozen ram seminal vesicles or guinea-pig lungs were centrifuged at 10,000 *g* in phosphate buffer (pH 8, 0.1 M) for 10 min, and the supernatant stored on ice. The pellet was resuspended in buffer and again centrifuged at 10,000 *g*. The supernatants from the two centrifugations were centrifuged at 75,000 *g* for 2 h at 0 to 5°C. The supernatant contained prostaglandin metabolizing enzymes and was discarded whilst the final pellet (microsomal fraction) was resuspended in a minimal amount of 0.01 M phosphate buffer at pH 8 and freeze-dried. The incubation mixture consisted of 6 mg particulate (microsomal) enzyme preparation, 200 mg glutathione and 0.2 M Tris buffer (pH 8) to a final volume of 2 ml; after gentle shaking at 37°C Isamoxole was added. Twenty min later [^{14}C]-arachidonic acid (68,000 d/min, Radiochemical Centre, Amersham) at 0.1 to 10 μg/ml was added. After a further 20 min incubation, the reaction was stopped by adjusting to pH 3 with 0.2 M citric acid. The reaction products were extracted with 2 × 5 ml volumes of ethyl acetate, evaporated almost to dryness and separated by t.l.c. in the AI system of Greén & Samuelsson (1964). Standard prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$ and PGD_2 were run at the same time. The plate was scanned and then scraped in 1 cm bands, which were counted in a toluene based scintillation fluid using a scintillation spectrometer (Packard Tricarb). The biological activity eluted from duplicate plates of both test and standard material was assayed on guinea-pig ileum and rat stomach strip preparations.

Materials

Isamoxole was administered orally as an homogenized suspension in 0.5% sodium carboxymethylcellulose with 0.05% Tween 80, or intravenously after being diluted from a concentrated solution of equal parts of Isamoxole, ethanol, water and ethoxylated castor oil (Cremophor EL, BASF). The drugs used were acetylcholine chloride (Sigma), adrenaline hydrogen tartrate (BDH), histamine acid phosphate (BDH), (−)-noradrenaline (Sigma), salbutamol (Allen and Hanburys), bradykinin (Sandoz), 5-hydroxytryptamine creatine sulphate (BDH), PGE_2 and $F_{2\alpha}$ (Lilly Research Centre and Cambrian Chemicals). Lymph

node permeability factor was prepared according to Meacock & Willoughby (1968). SRS-A was prepared from guinea-pig lungs and partially purified according to Brocklehurst (1963).

Results

The release of mediators from guinea-pig chopped lung

In Table 1, experiments 1–7 demonstrate that at 10 μg/ml Isamoxole inhibited the release of SRS-A by 43 to 74% with less effect on histamine release (25 to 53%). Experiments 5 and 7 suggested that the threshold concentration of Isamoxole in this system was between 0.1 and 0.5 μg/ml, and in some experiments, even lower. Experiment 6 showed that Isamoxole maintained its effect when added 15 min before challenge, and there was still a significant response with 30 min pre-incubation. The response is thus not transient, and suggests that the compound is not rapidly inactivated by the tissue, or that a trigger mechanism has been interrupted. No significant antagonism was shown by Isamoxole when incubated with SRS-A standard although inhibitory effects were demonstrated later. This was probably due to the short contact time on the guinea-pig ileum in these studies.

The release of mediators from human chopped lung

In Table 2, experiments 1–6 show that a concentration of 10 μg/ml Isamoxole inhibited the release of SRS-A by 60 to 100%, and 1 μg/ml by 25 to 56%. Its effects on histamine release were slight and variable. Isamoxole inhibited, although inconsistently, SRS-A release at concentrations down to 0.025 μg/ml (Table 2, experiments 7 and 8). The inhibitory effect of 10 μg/ml was maintained if added 30 min before challenge (Table 3, experiment 2) in contrast to the transient effect of 0.1 μg/ml (Table 3, experiment 1). The data in Tables 2 and 3 were obtained using passively sensitized lung tissue challenged with specific antigen and were supported by results with anti-IgE serum challenge (Table 4). The effects here were smaller and more variable, perhaps reflecting the variable amount of IgE antibody normally bound to human tissue.

Allergic bronchospasm in conscious guinea-pigs

Isamoxole, at doses of 2 × 100, 2 × 50 and 2 × 25 mg/kg orally (given 180 and 30 min before challenge) protected guinea-pigs against aerosol antigen challenge (Table 5). In a time course study (Table 6) an

Table 1 Effect of Isamoxole on SRS-A_{gp} and histamine release from chopped sensitized guinea-pig lung

		SRS-A			*Histamine*		
		Total units/g released†			*Total μg/g released*†		
Expt. no.	*Isamoxole (μg/ml)*	*Control* ± s.e.	*Test* ± s.e.	% *Inhib.*	*Control* ± s.e.	*Test* ± s.e.	% *inhib.*
1	10 (0)	325 ± 21	150 ± 12	54***	4.1 ± 0.4	2.6 ± 0.2	38***
2	10 (0)	115 ± 12	50 ± 5	57***	NT	NT	—
3	10 (0)	730 ± 84	415 ± 43	43***	2.8 ± 0.3	1.9 ± 0.2	33*
	1 (0)		570 ± 72	22		2.2 ± 0.2	24
4	10 (0)	30 ± 4	15 ± 3	61***	3.0 ± 0.5	2.2 ± 0.5	25
5	10 (5)	155 ± 16	67 ± 5	57**	3.8 ± 0.2	2.4 ± 0.4	38*
	1 (5)		126 ± 14	17		3.5 ± 0.3	8
	0.1 (5)		150 ± 12	3		3.6 ± 0.3	7
	0.05 (5)		155 ± 12	0		3.5 ± 0.2	8
	0.025 (5)		155 ± 11	0		3.3 ± 0.2	15
6	10 (0)	110 ± 7	50 ± 4	57***	2.2 ± 0.2	1.0 ± 0.1	53***
	10 (5)		53 ± 4	55**		0.8 ± 0.1	62***
	10 (15)		52 ± 4	58***		1.0 ± 0.1	54**
	10 (30)		75 ± 8	31*		1.3 ± 0.1	40*
7	10 (0)	103 ± 15	29 ± 3	74***	2.0 ± 0.1	1.2 ± 0.1	36**
	1 (0)		45 ± 5	57***		1.5 ± 0.1	23
	0.1 (0)		48 ± 7	54***		1.4 ± 0.1	28*
	0.05 (0)		75 ± 5	27		1.4 ± 0.1	26*

Figures in parentheses indicate the time (min) when Isamoxole was added before challenge. †Release expressed per g wet lung tissue. NT = not tested.
Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

apparent biphasic response was seen after a single oral dose of 100 mg/kg, with early and late peaks at 15 and 180 min. In the presence of mepyramine, increased protection ratios were seen, suggesting that Isamoxole was able to control the SRS-A induced component of the bronchospasm. In numerous control experiments, it has not been possible to define clearly the proportion of the response due to histamine and SRS-A; indeed, these proportions seem to vary as judged by the protection ratios achieved by mepyramine alone. However, in most experiments, the protection achieved by mepyramine was increased by concomitant administration of Isamoxole, although this was not statistically significant (Table 5).

Table 2 Effect of Isamoxole on SRS-A and histamine release from passively sensitized chopped human lung

		SRS-A			*Histamine*		
		Total units/g⁺ released			*Total μg/g⁺ released*		
Expt. no.	*Isamoxole (μg/ml)*	*Control* ± s.e.	*Test* ± s.e.	*% Inhib.*	*Control* ± s.e.	*Test* ± s.e.	*% inhib.*
1	10	40 ± 8	3.0 ± 0.5	93***	1.5 ± 0.3	1.5 ± 0.2	0
	1		21.5 ± 0.5	46*		1.5 ± 0.3	0
2	10	25.5 ± 5	5.0 ± 0.7	80***	8.5 ± 0.6	6.5 ± 0.4	23
	1		11.0 ± 1.1	56*		8.5 ± 0.3	0
3	10	119 ± 14	47.5 ± 0.5	60***	7.9 ± 0.7	6.6 ± 0.8	16
	1		89.5 ± 8.0	25		4.9 ± 0.2	37*
4	10	25 ± 5	0	100***	11.9 ± 1.2	11.9 ± 1.1	0
	1		17.0 ± 2.0	33*		11.9 ± 1.5	0
5	10	62.5 ± 10	0	100***	7.3 ± 1.2	7.3 ± 1.3	0
6	10	25 ± 7	6.5 ± 0.7	73	0.6 ± 0.2	0.5 ± 0.2	23
7	10	25 ± 6	0	100***	0.4 ± 0.2	0.2 ± 0.1	37
	1		13 ± 2	53*		0.3 ± 0.2	11
	0.1		22 ± 3	13		0.4 ± 0.1	0
	0.05		22 ± 2	13		0.4 ± 0.1	0
	0.025		25 ± 4	0		0.4 ± 0.2	0
8	10	45 ± 11	3 ± 1	93***	1.7 ± 0.3	1.5 ± 0.5	12
	1		23 ± 2	49*		1.7 ± 0.8	0
	0.1		26 ± 3	51*		1.7 ± 0.8	0
	0.05		26 ± 4	44*		1.7 ± 0.6	0
	0.025		19 ± 1	58*		1.6 ± 0.3	0

⁺Release expressed per g wet lung tissue.
Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Effect of Isamoxole on SRS-A and histamine release from passively sensitized chopped human lung: time course studies

			SRS-A			*Histamine*		
			Total units/g released⁺			*Total μg/g released⁺*		
Expt. no.	*Isamoxole (μg/ml)*	*Time before challenge (min)*	*Control* ± s.e.	*Test* ± s.e.	*% inhib.*	*Control* ± s.e.	*Test* ± s.e.	*% inhib.*
1	0.1	5	191 ± 18	0	100***	NT	NT	NT
		30	191 ± 15	191 ± 20	0			
2	10	15	204 ± 32	0	100***	5.0 ± 0.4	5.0 ± 0.5	0
		30	198 ± 15	0	100***	5.0 ± 0.9	5.0 ± 0.7	0

NT = not tested; ⁺ = release expressed per g wet lung tissue.
Significance of differences: ***$P < 0.001$.

Table 4 Effect of Isamoxole on SRS-A and histamine release from human chopped lung challenged with anti-human IgE serum

		SRS-A			*Histamine*		
		Total units/g⁺ released			*Total μg/g⁺ released*		
Expt. no.	*Isamoxole (μg/ml)*	*Control* ± s.e.	*Test* ± s.e.	*% inhib.*	*Control* ± s.e.	*Test* ± s.e.	*% inhib.*
1	10	37 ± 3	15 ± 2	59***	1.6 ± 0.1	1.6 ± 0.1	0
	1		0	100***		1.3 ± 0.2	19
2	10	24 ± 2	9 ± 1	59***	12.0 ± 1.2	12 ± 1.1	0
	1		14 ± 1	47**		9 ± 1.8	27
3	10	105 ± 3	16 ± 3	81***	19.5 ± 3.2	19.5 ± 2.4	0
	1		105 ± 14	0		19.5 ± 3.0	0
	10	32 ± 3	4 ± 0.5	87***	0.5 ± 0.1	0.5 ± 0.1	0
	1		30 ± 4	7		0.5 ± 0.1	0

⁺Release expressed per g wet lung tissue.
Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5 Effect of oral Isamoxole on allergic bronchospasm induced by aerosol antigen challenge in sensitized guinea-pigs

	Isamoxole (n = 4)			*Mepyramine (Mep)* (n = 5)			*Isamoxole + Mep* (n = 4)		
Isamoxole (mg/kg)	*PR*	*% group totally protected*	†*MCT* ± s.e. *(s)*	*PR*	*% group totally protected*	*MCT* ± s.e. *(s)*	*PR*	*% group totally protected*	*MCT* ± s.e. *s*
2 × 100	2.9	0	463 ± 196**						
2 × 100	5.5	50	810 ± 250**	5.2	40	342 ± 53	6.5	75	265
2 × 100	2.8	0	540 ± 74***	4.9	60	930 ± 74	5.7	100	—
2 × 50	3.8	0	685 ± 299***				4.5	50	816 ± 262
2 × 25	3.6	25	582 ± 281***				5.3	75	1175

MCT = mean collapse time of remainder of group of treated animals; PR = protection ratio (total group); Mep = Mepyramine, 0.5 mg/kg s.c. 30 min before challenge. †The mean collapse time for untreated animals was 162 ± 34.
Significance of differences: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 6 Effect of Isamoxole (100 mg/kg orally) on allergic bronchospasm induced by aerosol antigen challenge in sensitized guinea-pigs (Herxheimer technique)—time course study

		Isamoxole			*Isamoxole + Mep* (n = 4)			
Time before challenge (min)	n	*PR*	*% totally protected*	*MCT*	*Control MCT*	*PR*[1]	*% totally protected*	*MCT*
5	4	1.3	0	178 ± 68	139 ± 33	12.6	50	570 ± 130
15	4	5.0	50	700 ± 35	139 ± 33	9.2	25	458 ± 225
30	8	2.6	0	650 ± 199*	256 ± 30	7.4	25	290 ± 68
60	8	2.7	0	592 ± 163**	221 ± 29	8.2	25	365 ± 102
120	8	1.4	12.5	246 ± 104	209 ± 29	14.6	75	1175
180	8	1.8	0	250 ± 61	142 ± 22	8.8	25	425 ± 150
240	4	0.8	0	104 ± 44	139 ± 33	17.2	100	>1200

[1]Untreated control MCT = 70 ± 11 s. Mepyramine alone = 14.3% totally protected. MCT of remainder ($n = 6$) 604 ± 97 s. Other abbreviations as Table 5.
Significance of differences: *$P < 0.05$; **$P < 0.01$.

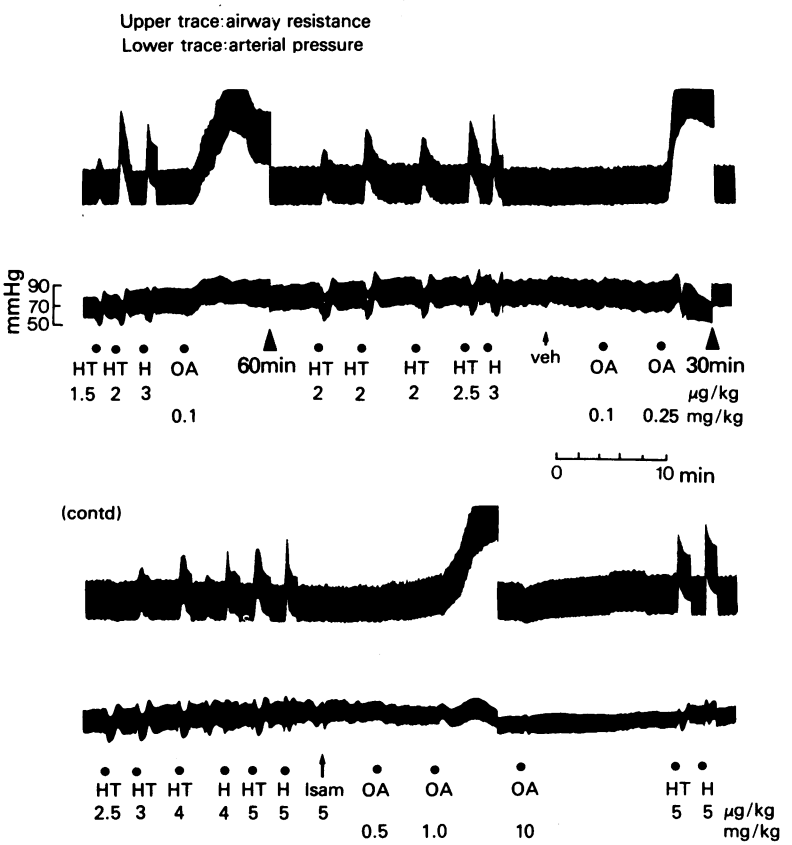

Figure 2 The effect of Isamoxole on the allergic bronchospasm induced in anaesthetized sensitized guinea-pig *in vivo*. The animal was given 5-hydroxytryptamine (HT) and histamine (H) before an initial challenge with 0.1 mg/kg ovalbumin (OA). Isamoxole (Isam) 5 μg/kg i.v. gave good protection against 0.5 mg/kg ovalbumin. Subsequent challenges at increased levels of ovalbumin overcame the protection (1.0 mg/kg OA) and desensitized the animal to high doses of OA (10 mg/kg) when given 40 min after the previous challenge.

Histamine provocation test in guinea-pigs in vivo

Isamoxole had no anti-histamine effect. The mean collapse time was 114 ± 14 s (control time 101 ± 12 s).

Allergic bronchospasm in anaesthetized guinea-pigs

Isamoxole, 50 μg/kg intravenously completely prevented the bronchoconstriction induced by antigen at twice and four times the original concentration over a period of 2 h. Doses of 10 μg/kg and 5 μg/kg Isamoxole (Figure 2) demonstrated the dose-related nature of this effect. Isamoxole did not modify the responses to histamine and 5-HT at any concentration used.

Passive cutaneous anaphylaxis (PCA) in the rat

Isamoxole given orally (2 × 100 mg/kg) or intravenously (10 mg/kg) did not inhibit the PCA reaction.

Passive peritoneal anaphylaxis in the rat

Isamoxole, in oral doses ranging from 2 × 25 mg/kg to 2 × 100 mg/kg, significantly inhibited the release of SRS-A into the peritoneal cavity (Table 7). The effect on histamine release was inconsistent and small. In a time course study, significant activity was noted 3 h after a single oral dose of 100 mg/kg. There was some indication that the effect may be biphasic.

Isamoxole was also effective by the intravenous route, a single intravenous dose of 1 mg/kg being approximately equivalent to 100 mg/kg orally.

Antagonism in vitro *of pharmacological mediators*

SRS-A The pA_2 value for Isamoxole against SRS-A was in four experiments 4.9, 5.2, 5.5 and 5.6. No attempt was made to differentiate between competitive and non-competitive inhibition. Figure 3 shows

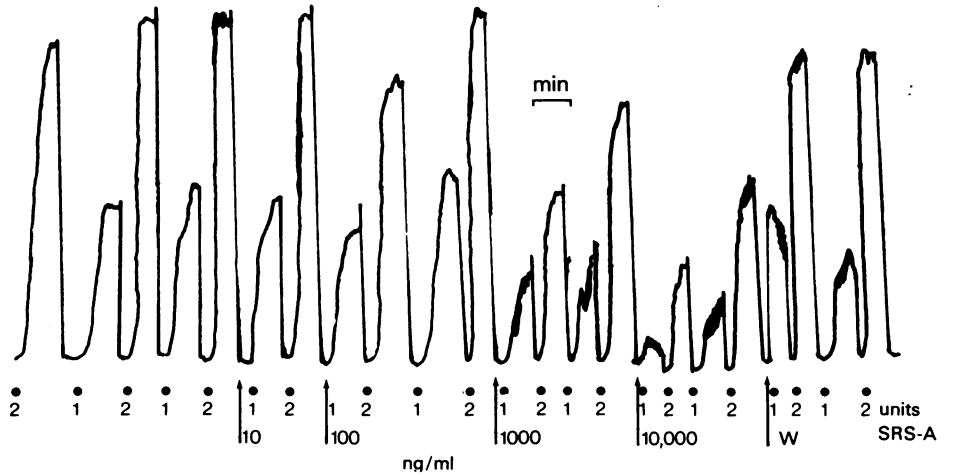

Figure 3 The inhibition of SRS-A_{gp} responses by Isamoxole on the guinea-pig ileum *in vitro*. The guinea-pig terminal ileum was suspended in a 5 ml bath of Tyrode solution containing mepyramine 10^{-6} M and gassed with 95% O_2 and 5% CO_2; 1 or 2 units/ml of SRS-A_{gp} were applied every 4 min and Isamoxole left in continuous contact at 10, 100 and 1000 ng/ml in Tyrode solution until W when the compound was removed and replaced with normal Tyrode solution.

that 100 ng/ml of Isamoxole rapidly inhibited SRS-A responses by 20% and 1 μg/ml by 50%.

In the tachyphylaxis experiments, constant responses to SRS-A were first obtained, and then Isamoxole at a concentration of 100 ng/ml was added to the bathing Tyrode solution. The responses were reduced and this reduction was maintained for 150 min. When Isamoxole was removed from the Tyrode, the response began to return to the pre-drug control values. Thus, the tissue did not exhibit tachyphylaxis to Isamoxole and it was easily washed out. The tissue remained in good condition.

Table 7 Effect of oral or intravenous Isamoxole on the release of histamine and SRS-A from the peritoneal cavity of the rat after passive sensitization and challenge

Dose (mg/kg)	*Time before challenge (min)*	*% inhibition of SRS-A release*		*% inhibition of histamine release†*	
		oral	*i.v.*	*oral*	*i.v.*
2 × 100	180, 30	33**		0	
2 × 50	180, 30	32*		11	
2 × 25	180, 30	21†		14	
1 × 100 oral or 1 × 10 i.v.	5	44**	42*	0	11
	15	31*	46**	20	0
	30	30*	26†	0	0
	60	50**	39*	16	0
	90	31*	33*	20	0
	120	39*	19†	6	6
	180	56**	0†	3	13
1 × 10	60		50**		7
1 × 10	120		12†		14
1 × 1	15		36*		0
1 × 1	60		38*		0
1 × 1	120		30*		0

Significance of differences: *$P < 0.05$; **$P < 0.01$. †Not significant.

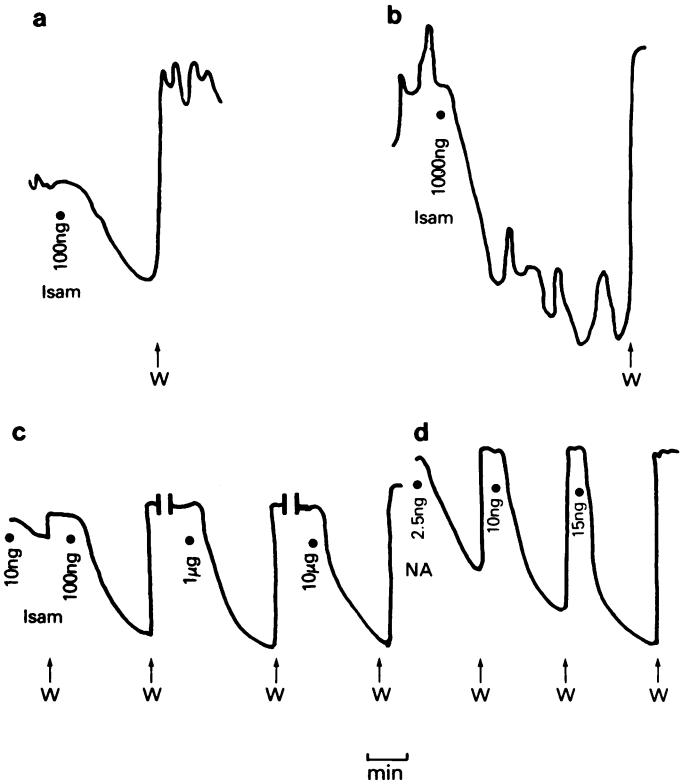

Figure 4 The effect of Isamoxole on human bronchial muscle strips. Spirally cut strips of healthy human bronchial muscle were suspended in Krebs solution at 37 C and gassed with 95% O_2 and 5% CO_2: (a) and (b) show the effect of Isamoxole (Isam) at 100 and 1000 ng/ml upon a spontaneously rhythmic tissue (2 mm original diameter); compare the responses to 10, 100, 1000 and 10,000 ng/ml Isamoxole (c) with those of the same tissue (3 mm original diameter) to 2.5 ng, 10 ng and 15 ng/ml of noradrenaline (NA) (d). W = wash out.

Other agonists Table 8 shows that pA_2 values against other agonists were lower than the mean value against SRS-A by at least 1 log unit, indicating that Isamoxole has a fairly selective action against SRS-A on the guinea-pig ileum.

Table 8 The effect of Isamoxole on the responses of the guinea-pig ileum to various agonists

Agonist	pA_2* *(at equilibrium time)*
$SRS\text{-}A_{gp}$	5.3
PGE_2	4.35
$PGF_{2\alpha}$	4.1
Histamine	3.85
Acetylcholine	3.05
Bradykinin	3.55
5-Hydroxytryptamine	<2

*(Schild, 1947).

Phosphodiesterase inhibition

Isamoxole had a very slight and variable effect on cyclic nucleotide phosphodiesterase at 1 mM. It suggests that phosphodiesterase inhibition is not the mode of action by which this drug inhibits the release of mediators in the allergic reaction.

Bronchodilator action on respiratory tissue

Isamoxole (100 ng/ml to 50 μg/ml) caused a dose-related relaxation of the guinea-pig isolated trachea but did not significantly change responses to adrenaline, noradrenaline or salbutamol.

Isamoxole, at 1 μg/ml, inhibited SRS-A-induced contractions of human bronchial muscle strips by 60%, and at 10 μg/ml by 90%. Variable inhibitory effects (10 to 25%) were seen at 100 ng/ml.

On most preparations a dose-related relaxation was observed over the range 10 to 1000 ng/ml. This relaxation was occasionally preceded by a slight contraction. It was more noticeable on quiescent, rather than

spontaneously rhythmic tissue (Figure 4), and was maintained until the compound was removed. The relaxation occurred at concentrations that were considerably lower than those inducing inhibition of SRS-A-induced contractions.

Effect on prostaglandin synthetase

PGE_2, $PGF_{2\alpha}$ and PGD_2 were identified in the seminal vesicle enzyme experiments. At low substrate concentrations, Isamoxole in concentrations ranging from 0.1 to 10 μg/ml had no significant effect on prostaglandin formation (Table 9). At high substrate concentrations (2 μg/ml arachidonate) Isamoxole inhibited the synthesis of prostaglandins at concentrations of 1 and 10 μg/ml.

In contrast, at the same high substrate concentrations in the lung system, all concentrations of Isamoxole tested increased the total synthesis of prostaglandins (Table 10). No PGD_2-like material was produced by the lung synthetase.

Discussion

Isamoxole is one of the most potent members of a group of compounds which prevent the release of mediators associated with anaphylaxis. Recently, Oxatomide (Awouters, Niemegeers, Van den Berk, Van Neuten, Lenaerts, Borgers, Schellekens, Broechaert, De Cree & Janssen, 1977) FPL 52757 and 57787 (Augstein, Cairns, Hunter, Lee, Suschitsky,

Table 9 Effect of Isamoxole on prostaglandin synthetase prepared from ram seminal vesicles

Arachidonic acid (mg/ml)	*Isamoxole (mg/ml)*	*Total prostaglandins (ng)*	*% change*	P	*PGE_2 ct/min (ng)*	*$PGF_{2\alpha}$ ct/min (ng)*	*PGD_2 ct/min (ng)*
0.125	—	15.21	—	—	2085 (9.48)	291 (1.32)	971 (4.41)
0.125	0.1	15.35	2.4	NS	2079 (9.45)	302 (1.37)	995 (4.52)
0.125	1.0	13.21	11.9	NS	1790 (8.13)	257 (1.16)	859 (3.90)
0.125	10.0	11.38	24.0	NS	1516 (6.89)	221 (1.00)	768 (3.49)
0.5	—	68.94	—	—	2375 (43.2)	330 (6.0)	1086 (19.7)
0.5	0.1	69.24	0.4	NS	2315 (42.1)	330 (6.0)	1163 (21.1)
0.5	1.0	60.42	12.4	NS	1980 (36.0)	294 (5.3)	1049 (19.1)
0.5	10.0	55.23	19.9	<0.01	1879 (34.2)	279 (5.1)	878 (16.0)
2.0	—	230.3	—	—	1935 (141.2)	319 (23.3)	902 (65.8)
2.0	0.1	229.9	0.2	NS	1948 (142.2)	297 (21.7)	900 (65.7)
2.0	1.0	108.9	51.4	<0.001	826 (60.3)	139 (10.1)	527 (38.5)
2.0	10.0	78.6	65.9	<0.001	625 (45.6)	105 (7.7)	346 (25.3)

NS = not significant.

Table 10 Effect of Isamoxole on prostaglandin synthetase prepared from guinea-pig lung

Arachidonic acid (mg/ml)	*Isamoxole (mg/ml)*	*Total prostaglandins (ng)*	*% change*	P	*PGE-like ct/min (ng) R_F 0.45*	*PGF-like ct/min (ng) R_F 0.24*
0.125	—	4.18	—	—	849 (3.8)	70 (0.31)
0.125	0.1	4.51	8.0	NS	780 (3.54)	66 (0.30)
0.125	1.0	3.91	6.5	NS	787 (3.57)	63 (0.29)
0.125	10.0	3.41	18.3	NS	691 (3.14)	61 (0.28)
0.5	—	14.22	—	—	735 (13.36)	47 (0.85)
0.5	0.1	15.73	10.7	NS	808 (14.69)	53 (1.05)
0.5	1.0	13.55	4.7	NS	690 (12.54)	49 (0.89)
0.5	10.0	13.99	12.5	NS	818 (14.87)	62 (1.13)
2.0	—	17.44	—	—	171 (13.48)	67 (4.89)
2.0	0.1	28.63	64.2	<0.01	324 (23.6)	68 (4.96)
2.0	1.0	38.57	121.1	<0.001	491 (35.8)	38 (2.77)
2.0	10.0	35.28	102.2	<0.001	451 (32.9)	32 (2.34)

NS = not significant.

Altounyan, Jackson, Mann, Orr & Sheard, 1977) and Ketotifen (Martin & Römer, 1977) have been selected for clinical evaluation because of their ability to prevent the release of mediators. Isamoxole appears relatively selective in inhibiting the release of SRS-A and in addition has modest SRS-A antagonistic activity.

The importance of SRS-A in man was shown with the release of this substance from the lungs of asthmatic patients upon challenge with specific pollen *in vitro* (Brocklehurst, 1960). Human bronchial smooth muscle is contracted by SRS-A (Brocklehurst, 1956). Inhalation of an aerosol of crude SRS-A in a closed circuit spirometer reduced the vital capacity of asthmatic patients but not of control subjects (Herxheimer & Streseman, 1963).

Apart from its putative role as a bronchoconstrictor in asthma, SRS-A may potentiate the responses to other constrictors. It has long been recognized that in allergic disorders the bronchial tree is hypersensitive to histamine (Weiss, Robb & Blumgart, 1929) bradykinin (Simonsson, Anderson, Bergh, Skoogh & Svedmyr, 1970) 5-HT (Booij-Noord, Orie, Berg & De Vries, 1970) and particularly to $PGF_{2\alpha}$ (Hedqvist, Holmgren & Mathé, 1971). Whether this increase in sensitivity is due to SRS-A or some other agent or to a more profound change in the asthmatic lung, is not known.

SRS-A has been shown to be distinct from prostaglandins by Orange, Murphy, Karnovsky & Austen (1973) and this has been confirmed by our own work. Eicosatetraynoic acid (ETYA), a potent inhibitor of prostaglandin synthesis, does not modify the release of spasmogenic material on challenge (Dawson & Tomlinson, 1974), suggesting that, as prostaglandins are synthesized *de novo* within tissues, they are not released in sufficient quantity to contribute to the contractile response. However, Burka & Flower (1979) have used ETYA to modify SRS-A release. It is possible that some spasmogenic activity detected during bioassay could be due not to SRS-A but to rabbit aorta contracting substance releasing factor (RCS-RF) (Piper & Vane, 1969; Nijkamp, Flower, Moncada & Vane, 1976). Apart from SRS-A and RCS-RF, no other slow acting contractile materials have been shown to be released on immunological challenge from lung tissue. It may be assumed therefore, that the non-histamine contraction of the assay tissue is largely due to SRS-A itself.

In this study, Isamoxole inhibited the release of SRS-A in some animal models for several hours. It was active 3 h after intravenous or oral doses in rat peritoneal anaphylaxis, and at 4 h, allergic bronchospasm of the guinea-pig was reduced, particularly when the histamine response had been antagonized by mepyramine.

The anti-anaphylactic activity of Isamoxole was demonstrated in anaesthetized sensitized guinea-pigs challenged with antigen (Dixon & Brodie, 1903) and in *in vitro* lung systems. This activity did not appear to be due to phosphodiesterase inhibition or to the modification of prostaglandin biosynthesis.

The possibility that some of the apparent prevention of release of SRS-A was due to antagonism of SRS-A when bioassayed, was considered and accounted for a small proportion of the activity. Isamoxole is a moderately potent, selective pharmacological inhibitor of SRS-A activity on the guinea-pig ileum *in vitro* at concentrations that do not antagonize histamine, 5-HT, or bradykinin. It has slight antagonist properties against acetylcholine and PGE_2 and $PGF_{2\alpha}$ at high concentrations.

The prostaglandin synthetases in lung and seminal vesicles differed considerably in both the amount and types of prostaglandin formed. This may be related to availability of co-factor and may also reflect the high ability of the lung to metabolize the PGE_2 and $PGF_{2\alpha}$ rapidly to the 15-keto and dihydro 15-keto derivatives. Dawson, Lewis, McMahon & Sweatman (1974) showed that some of these metabolites act potently on bronchial muscle.

Isamoxole relaxed human bronchial muscle and guinea-pig isolated trachea and antagonized the bronchoconstriction induced by SRS-A in human tissue.

Both guinea-pig models involve heat-stable IgG antibody believed to be dissimilar to the heat-labile IgE reagin antibody present in human bronchial asthma. DSCG differs from Isamoxole in its failure to prevent the release of mediators in these models (Cox, 1967), although it is active in the guinea-pig if IgE-like antibodies are involved (Carney, 1976). In fact, DSCG can be shown to be active particularly when given intravenously, in most IgE mediated reactions whereas Isamoxole is active in all models described where SRS-A is released. In consequence, Isamoxole's inactivity in the PCA reaction suggests that the response is due not to SRS-A release but to histamine and 5-HT. The inference from this is that DSCG acts at a fairly early stage in the synthesis or release of mediators and Isamoxole at a later stage and mainly on SRS-A synthesis or release.

In conclusion, Isamoxole appears to have potential as an orally active anti-allergic compound with a novel mode of action. Its clinical efficacy as an orally active drug for the treatment of bronchial asthma is being studied.

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References

ALTOUNYAN, R.E.C. (1967). Inhibition of experimental asthma by a new compound, disodium cromoglycate, 'Intal'. *Acta. Allergol.* **22,** 487–488.

AUGSTEIN, J., CAIRNS, H., HUNTER, D., LEE, T.B., SUSCHITZKY, J., ALTOUNYAN, R.E.C., JACKSON, D.M., MANN, J., ORR, T.S.C. & SHEARD, P. (1977). New orally effective chromone derivatives for treatment of asthma *Agents & Actions*, **7,** 443–445.

AWOUTERS, F., NIEMEGEERS, C.J.E., BERK, J. VAN DEN, NUETEN, J.M., VAN, LENAERTS, F.M., BORGERS, M., SCHELLEKENS, K.H.L., BROECKAERT, A., CREE DE J. & JANSSEN, P.A.J. (1977). Oxatomide, a new orally active drug which inhibits both the release and the effects of allergic mediators. *Experientia*, **33,** 1657–1659.

BOOIJ-NOORD, H., ORIE, N.G.M., BERG, W. CHR. & DE VRIES, K. (1970). Results of provocation of human bronchial airways with allergic and non-allergic stimuli and of drug protection tests. In *Bronchitis III* ed. Orie, N.G.M. & Lende, R. van der. pp. 316–330. Assem., Royal Van Gorcum.

BROCKLEHURST, W.E. (1956). A slow reacting substance in anaphylaxis—"SRS-A". *Ciba Symposium on Histamine.* ed. Wolstenholme, G.E.W. & O'Conner, C.M. pp. 175–179. London: Churchill.

BROCKLEHURST, W.E. (1960). The release of histamine and formation of a slow-reacting substance (SRS-A) during anaphylactic shock. *J. Physiol.*, **151,** 416–435.

BROCKLEHURST, W.E. (1963). 'SRS-A' the slow reacting substance of anaphylaxis. *Biochem. Pharmac.*, **12,** 431–435.

BROOKER, G., THOMAS, JR. L.J. & APPLEMAN, M.M. (1968). The assay of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate in biological materials by enzyme radioisotopic displacement. *Biochem.*, **7,** 4177–4181.

BURKA, J.F. & FLOWER, R.J. (1979). Effects of modulators of arachidonic acid metabolism on the synthesis and release of slow-reacting substance of anaphylaxis. *Br. J. Pharmac.*, **65,** 35–41.

CARNEY, I.F. (1976). IgE-mediated anaphylactic bronchoconstriction in the guinea pig and the effect of disodium cromoglycate. *Int. Arch. Allergy, appl. Immun.*, **50,** 322–328.

COLLIER, H.O.J. & SWEATMAN, W.J.F. (1968). Antagonism by fenamates of prostaglandins $F_{2\alpha}$ and of slow reacting substance on human bronchial muscle. *Nature, Lond.*, **219,** 864–865.

CONSTANTINE, J.W. (1965). A spiral cut tracheal strip preparation. *J. Pharm. Pharmac.* **17,** 384–385.

COX, J.S.G. (1967). Disodium cromoglycate (FPL670) (Intal), a specific inhibitor of reaginic antibody-antigen mechanisms. *Nature, Lond.*, **216,** 1328–1329.

DAWSON, W., LEWIS, R.L., MCMAHON, R.E. & SWEATMAN, W.J.F. (1974). Potent bronchoconstrictor activity of 15-keto prostaglandin $F_{2\alpha}$. *Nature, Lond.*, **250,** 331–332.

DAWSON, W. & TOMLINSON, R. (1974). Effect of cromoglycate and eicosatetraynoic acid on the release of prostaglandins and SRS-A from immunologically challenged guinea pig lungs. *Br. J. Pharmac.*, **52,** 107–108P.

DIXON, W.E. & BRODIE, T.G. (1903). Contributions to the physiology of the lungs Part I: The bronchial muscles, their innervation and the action of drugs upon them. *J. Physiol.*, **29,** 97–173.

GREÉN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX T.L.C. of prostaglandins. *J. Lipid Res.*, **5,** 117–120.

HEDQVIST, P., HOLMGREN, A. & MATHÉ, A.A. (1971). Effect of prostaglandin $F_{2\alpha}$ on airway resistance in man. *Acta physiol. scand.* **82,** 29A.

HERXHEIMER, H. (1952). Repeatable 'microshocks' of constant strength in guinea pig anaphylaxis. *J. Physiol.*, **117,** 251–255.

HERXHEIMER, M. & STRESEMAN, E. (1963). The effect of slow reacting substance (SRS-A) in guinea pigs and in asthma patients. *J. Physiol.*, **165,** 78–79P.

MARTIN, U. & RÖMER, D. (1978). The pharmacological properties of a new, orally active antianaphylactic compound: ketotifen, a benzocycloheptathiophene. *Arzneim-Forsch.* **28,** 770–782.

MEACOCK, S.C.R. & WILLOUGHBY, D.A. (1965). Purification of serum proteins from lymph node cell extracts with permeability activity characteristic of the lymph node permeability factor. *Immunol.*, **15,** 101–113.

MONGAR, J.L. & SCHILD, H.O. (1956). Effect of antigen and organic bases on intracellular histamine in guinea pig lung. *J. Physiol.*, **131,** 207–219.

NIJKAMP, F.P., FLOWER, R.J., MONCADA, S. & VANE, J.R. (1976). Partial purification of rabbit aorta contracting substance-releasing factor and inhibition of its activity by anti-inflammatory steroids. *Nature, Lond.*, **263,** 479–482.

ORANGE, R.P., MURPHY, R.C., KARNOVSKY, M.L. & AUSTEN, K.F. (1973). The physiochemical characteristics and purification of slow reacting substance of anaphylaxis. *J. Immunol.* **110,** 760–770.

PIPER, P.J. & VANE, J.R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223,** 29–35.

ROSS, W.J., HARRISON, R.G., JOLLEY, M.R.J., NEVILLE, M.C., TODD, A., VERGE, J.P., DAWSON, W. & SWEATMAN, W.J.F. (1979). Antianaphylactic Agents. 1. 2-(Acylamino)oxazoles. *J. med. Chem.* **22,** 412–417.

SCHILD, H.O. (1947). pAx, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2,** 186–206.

SIMONSSON, B.G., ANDERSON, R., BERG, N.P., SKOOGH, B.E. & SVEDMYR, N. (1970). Results of provocation of human airways with allergic and non-allergic stimuli and drug protection tests. In *Bronchitis III* ed. Orie, N.G.M. & Lende, R. van der. pp. 334–342. Assem: Royal Van Gorcum.

WEISS, S., ROBB, G.P. & BLUMGART, H.C. (1929). The velocity of blood flow in health and disease as measured by the effect of histamine on the minute vessels. *Am. Heart J.* **4,** 664–691.

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