B. Drug Effects on HFC without antigen

Exp. No		Drug M	Drug (No Antigen)	Controls (No Antigen)
I	Propanolol	10^{-5}	132±63	392±23
II		10^{-6}	617±33	481±20
III		10^{-6}	194±10	125±4
IV		10^{-6}	116±9	55·7±6
		10^{-7}	80·4±5	55·7±6
V	Phenylephrine	10^{-5}	181±14	125±4
VI		10^{-5}	147±16	91·1±13
		10^{-6}	120±4	91·1±13
VII	Phentolamine	10^{-5}	247±21	392±23

whether this effect on HFC by propranolol (and by other drugs acting on α- and β-adrenoceptors) could be obtained in the absence of antigen as well.

It was found (Table 1B) that propranolol greatly stimulated HFC in human leucocytes even in the absence of antigen, and in low concentrations (i.e. 10^{-7}M). Drugs acting on α-adrenoceptors appeared to produce effects opposite to those of drugs acting on β-adrenoceptors, phenylephrine stimulating HFC, and phentolamine inhibiting it. The possible significance of these results will be discussed.

Supported by The Wellcome Trust and the Asthma Research Council.

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Hypersensitivity to adrenoceptor agents in the guinea-pig *in vitro* and *in vivo*

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Increased mortality in patients using bronchodilator sympathomimetic aerosols over extended periods (Speizer, Doll, Heaf & Strang, 1968) may be the result of hyposensitization to both endogenous and exogenous sympathetic stimulation as a consequence of excess usage of these sprays (Conolly, Davies, Dollery & George, 1971). Conolly *et al.* (1971) have described cardiovascular resistance to β-adrenoceptor stimulant drugs after prolonged exposure to these agents in man and dog, and increased histamine susceptibility (expressed as increased mortality after histamine administration) in the guinea-pig after similar treatment. We reproduced an analogous situation *in vitro* and repeated the *in vivo* mortality experiments in guinea-pigs.

Female guinea-pigs (Hartley strain ; 200–250 g) were used. Spirally cut tracheal strips (Constantine, 1965) were suspended in 10 ml organ baths containing Tyrode solution (containing 17 μg ascorbic acid/ml) at 38° C, aerated with 95% O_2 and 5% CO_2. Contractions were recorded isometrically on a pen recorder. In addition, several preparations were examined isotonically. Immediately after incubation with (−)-isoprenaline hydrochloride (50 ng/ml for 20 min) the contractile

response to histamine was abolished (time 0 min). This response to histamine returned to normal within 30 to 60 min after the incubation, while the ability of isoprenaline to inhibit histamine contractions remained impaired at 60–90 min. The inhibition by isoprenaline returns to its pre-incubation level at 120 to 180 min. During the phase of inhibition of isoprenaline relaxation, pre-incubation isoprenaline relaxations were produced if concentrations were increased 10 to 15-fold. Isometrically recorded relaxations to isoprenaline were independent of contraction height when contractions were produced on the linear portion of the histamine dose-response curve. However, the corresponding relaxations measured under isotonic conditions were dependent on histamine contraction heights. Theophylline (100 μg/ml), dibutyryl cyclic 3′,5′-AMP (dbcAMP; 250 μg/ml) and (−)-noradrenaline bitartrate (2 μg/ml) incubations for 20 min produced similar hyposensitization to isoprenaline. Isoprenaline incubation (50 ng/ml) also produced hyposensitization to (−)-adrenaline bitartrate and noradrenaline mediated relaxations superimposed on histamine contractions.

For *in vivo* experiments, guinea-pigs were randomly allocated to six groups (13/group); three of these were given 0·9% saline I.M.; the other groups were treated with isoprenaline (4 μg base/kg) every 20 min for 5 h. The six groups of animals were challenged I.P. with histamine 2 h after the last injection (Table 1).

TABLE 1. *Effect of prolonged administration of isoprenaline on mortality after histamine challenge*

Group	Treatment (every 20 min for 5 h)	Histamine Challenge (base I.P.)	Mortality
1	0·1 ml saline	1·5 mg/kg	0/13
2	4 μg/kg isoprenaline	1·5 mg/kg	4/13
3	0·1 ml saline	3·0 mg/kg	1/13
4	4 μg/kg isoprenaline	3·0 mg/kg	10/13
5	0·1 ml saline	5·0 mg/kg	8/13
6	4 μg/kg isoprenaline	5·0 mg/kg	8/13

Mortality was significantly higher in isoprenaline-treated than in saline-treated animals ($\chi^2=12{\cdot}76$, $P>0{\cdot}0005$ for 3 mg/kg group). All animals appeared to die from respiratory obstruction. In the 5 mg/kg group, saline- and isoprenaline-treated animals had equal mortalities but the isoprenaline animals died later (approx. 15 min) than the saline animals (<5 min). The reason for this finding is not clear at present but it may be related to increased endogenous catecholamine release produced by the highest dose of histamine administered. The *in vivo* results reinforce the findings of Conolly *et al.* (1971) and further emphasizes the problems of chronic administration of bronchodilators.

The actions of β-adrenoceptor agonists are believed to be mediated via increased synthesis of cyclic 3′,5′-adenosine monophosphate. These agents and dbcAMP, the synthetic analogue, and theophylline, a phosphodiesterase inhibitor, produced hyposensitization. Our data suggest that hyposensitization to isoprenaline may be mediated via changes in cyclic nucleotide content. We conclude that airway smooth muscle of guinea-pigs can be partially desensitized against the relaxant effect of isoprenaline *in vivo* as well as *in vitro*.

This work was supported in part by US PHS Grants HE 14534 and HE 14179.

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Antagonism of some spasmogens of the rat seminal vesicle

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The rat seminal vesicle was studied to see if it could be used to differentiate mechanisms of action of smooth muscle relaxation and to determine the type of adrenoceptors present. Concentration response curves were obtained with three agonists, noradrenaline bitartrate (NA), acetylcholine bromide (ACh) and potassium chloride (KCl). ACh and KCl spasm was not attributable to α-adrenoceptor activation as shown by the effects of phentolamine (20–200 nM) and cocaine (0·1–1,000 μM). Using phentolamine (20–200 nM), propranolol (0·1 mM) and practolol (0·01 mM) evidence was obtained that the adrenoceptors appeared to be entirely α. Since the rat seminal vesicle responds by contraction to these three agonists this property was used to compare the differential potency of four smooth muscle relaxants (see Table 1). These were papaverine (PAP), procaine hydrochloride (PR), theophylline (TH—as aminophylline) and isoprenaline sulphate (ISO). The failure of propranolol and practolol to inhibit the relaxant action of ISO (against KCl) strongly suggests that this effect was not due to activation of β-adrenoceptors.

TABLE 1. *Differential potency of smooth muscle relaxants*

	NA	ACh	KCl	IC_{50} mM (KCl)
Pap	0·5	1·0	1·0	0·03
Pr	2·4	25·8	1·0	1·7
Th	7·4	27·7	1·0	25·2
Iso	23·9	0·7	1·0	20·3
Na_2SO_4	0·3	1·2	1·0	34·3
EC_{50}	100 μM	100 μM	150 mM	

IC_{50}=Concentration producing 50% of maximum inhibition.
EC_{50}=Concentration producing 50% of maximum effect.
Each value is the mean of six experiments.

The mechanism of ISO relaxation was investigated by comparing its action with sodium sulphate (Na_2SO_4) to test the hypothesis that ISO produced relaxation because of its sulphate anion. The differential potency of Na_2SO_4 did not support this hypothesis for inhibition of NA. By comparing the inhibition of KCl (150 mM) and potassium sulphate (K_2SO_4) (150 mM) spasm by Na_2SO_4 it was obvious that Na_2SO_4 relaxation was independent of sulphate concentration over a five-fold range. IC_{50} for Na_2SO_4 against KCl was 34 mM and for K_2SO_4 was 40 mM. The inhibitory action of ISO cannot be explained by the sulphate anion. Changes in pH and osmolarity comparable to those produced by ISO did not inhibit seminal vesicle spasm.

It is concluded that the rat seminal vesicle contains adrenoceptors of the α type alone and the inhibitory action of isoprenaline remains unexplained.

This work was supported by a grant from the M.R.C.