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Resistance to β-adrenoceptor stimulants (a possible explanation for the rise in asthma deaths)

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Summary

1. Resistance to isoprenaline has been produced in man and dog by prolonged exposure to the same, or the pharmacologically similar compounds, terbutaline and isoetharine.

2. In guinea-pigs, pretreatment with these agents increases asthma mortality provoked by histamine which suggests that this resistance may reduce the effectiveness of endogenous sympathetic drive to the bronchi.

3. Possible mechanisms by which the asthma death rate could be increased are discussed.

4. It is suggested that a drug-induced cross resistance to endogenous sympathetic stimulation could have led to a deterioration of the asthmatic state in patients using the pressurized aerosols of sympathomimetic bronchodilators and that this might account for the rise in asthma death rate.

Introduction

Pressurized aerosols of β-adrenoceptor agonists were introduced into the treatment of bronchial asthma about 10 years ago. They were effective in bringing considerable symptomatic relief to many asthmatic patients, and their use increased rapidly. However, in 1968 Speizer and his colleagues drew attention to a 3-fold rise in the death rate from asthma, particularly among younger patients. Asthma became the fourth commonest cause of death in the age range 5–34 years (Speizer, Doll & Heaf, 1968a). Many of these deaths occurred suddenly at home. Autopsy showed the classical changes of asthma in 91% of a series of 113 postmortems.

Circumstantial evidence pointed strongly to an association between excessive use of pressurized aerosols of sympathomimetic drugs and the rise in mortality (Speizer, Doll, Heaf & Strang, 1968b). Since this trend was recognized the use of these preparations has been reduced, and the death rate has fallen sharply (Inman & Adelstein, 1969). Nevertheless these agents continue to be used, and newer, relatively selective β-adrenoceptor agonists are being introduced. It is therefore important to establish the mechanism behind the rise in death rate.

In an earlier study (Paterson, Conolly, Davies & Dollery, 1968) we found that patients inhaling large doses of isoprenaline were relatively resistant to the cardiac

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effects of intravenously administered isoprenaline. We have now extended these observations in man and animals, and put forward a possible explanation for the rise in death rate in asthmatic patients.

Methods

All observations were made in man or intact animals. The drugs used were isoprenaline sulphate, terbutaline sulphate, salbutamol sulphate, isoetharine hydrochloride and histamine acid phosphate. All doses are expressed in terms of the free base.

Man

The heart rate response to rapid intravenous injections of isoprenaline was examined in eight subjects before and after intravenous infusions of isoprenaline in doses ranging from 0·002 to 0·01 (μg/kg)/min over periods ranging from 15 to 45 minutes. In three of these subjects the effects of infusing even smaller doses over a comparable period of time were also measured.

The subjects were studied in the supine position and all drugs were administered via a scalp-vein needle inserted into a forearm vein under local anaesthetic. Rapid injections of isoprenaline were given in a constant volume (2 ml) via the side arm of a three-way tap; immediately after each injection the tubing was flushed through with saline.

Heart rate was measured on a Nielson-type instantaneous rate meter, the signal being displayed on a Devices M2 or M4 recorder, or else a specially designed digital rate meter (Emons & Conolly, 1971) was used, the output being displayed on a Servoscribe recorder. In each case the rate meter was triggered by the R wave of the electrocardiogram obtained from the Devices recorder, using chest electrodes.

Decrease in responsiveness to isoprenaline (isoprenaline resistance) was recognized by a rightward shift of the second log dose-response curve. In every case the pairs of dose-response curves were analysed by computer, according to the method set out in appendix A to determine whether or not a statistically significant displacement had occurred. In addition, the dose required to increase the heart rate by twenty beats per minute was derived from the regression lines calculated by the method of least squares, and the logarithmic values of the differences between the control and postinfusion doses were used to perform a paired t test, and the ratio of the two values were used as an index of isoprenaline resistance.

Dogs

Mongrel dogs, weighing 16–25 kg were used. They were anaesthetized with thiopentone and pentobarbitone. The responsiveness of heart rate and diastolic pressure to isoprenaline was measured before and after:

(a) rapid intravenous injections of 10–20 μg isoprenaline;

(b) intravenous infusions of isoprenaline in doses ranging from 0·0007 to 0·38 (μg/kg)/min over periods ranging from 15 to 210 minutes;

(c) the following dosage of isotopically labelled long acting β-adrenoceptor stimulant drugs: (1) intraduodenal terbutaline, 2 mg/kg; (2) a 25 min infusion of terbuta-

line (0·004 μg/kg)/min ; (3) a rapid intravenous injection of terbutaline, 4·0 μg/kg ; (4) a rapid intravenous injection of isoetharine, 8·0 μg/kg.

Heart rate was measured as in the human studies. Arterial blood pressure was measured using a Consolidated Electrodynamics transducer (type 4-3276 L221) connected to a PE160 polythene cannula 20 cm long, introduced into the femoral artery. The signal from the transducer was displayed on a Devices M2 or M4 recorder.

The data were analysed in the same way as in the human studies, except that comparisons were made of the dose required to increase heart rate by forty beats/min, or to lower diastolic pressure by 40 mmHg (1 mmHg≡1·333 mbar).

Guinea-pigs

The mortality from histamine-induced bronchospasm was measured in matched groups of guinea-pigs after intramuscular injections of saline (six groups), isoprenaline, 4 μg/kg (three groups), terbutaline, 15 or 20 μg/kg (one group at each dose), or salbutamol, 4 μg/kg (one group). The injections were given at 25–40 min intervals over about 5 hours. The dose of isoprenaline was chosen as approximating to the dose an asthmatic patient might use in an attack. Equivalent bronchodilator doses of the other agents were used in the other studies. The dose of histamine used in the challenge (0·6–1·1 mg/kg) was determined by treating comparable animals from the same population with a range of histamine doses to determine which dose gave a mortality of 10–40% in the control state. Two hours were allowed to elapse between the end of the treatment with the β-adrenoceptor stimulants and the histamine challenge, since preliminary experiments had indicated that the mortality was highest at this time. The difference between the death rates in the control groups and those treated with β-adrenoceptor agonists were analysed by means of the test using Yates' modification for non-continuously distributed data, and at the end of the experiment, the data from all six studies were combined and examined to determine whether treatment with β-adrenoceptor stimulants had a significant influence on mortality, the test used being set out in appendix B.

Results

Man

In four subjects the isoprenaline infusion increased the heart rate by five–seventeen beats/minute. In the other four, lower doses were used, and these had no chronotropic effect. After the infusions, there was a statistically significant parallel rightward displacement of the isoprenaline dose-response curves in seven out of eight cases (Table 1). In two of the three subjects who were infused with lower doses on another occasion a rightward shift was still observed, though it failed to achieve statistical significance (Table 2). In the eight original studies the ratio of the doses of isoprenaline required to increase the heart rate by twenty beats/min before and after the infusion was increased on average 3·93 times. The mean increase in dose required to achieve an increase of twenty beats/min (excluding subject 8, in whom the increase of 6·8 μg was abnormally large). This increase was highly significant ($P<0·001$). The duration of this resistance was studied in only one subject, in whom it persisted unchanged for a further 65 min after the second dose-response curve. Aminophylline (3·5 mg/kg) injected intravenously after

resistance had been produced, caused no consistent alteration in isoprenaline responsiveness in the three subjects to whom it was given.

Dogs

Isoprenaline studies

Heart rate data. As can be seen from Table 3, there was a parallel rightward displacement in all thirteen experiments, which achieved statistical significance in eleven animals. The dose of isoprenaline required to increase the heart rate by 40 beats/min after the infusion was increased on average by 2·80 times. The mean

TABLE 1. *Human pulse rate data*

Subject	Infused dose (μg/kg)	Duration of infusion	Infusion rate ((μg/kg)/min)	Shift in dose-response curve	Dose to increase heart rate by 20 beats/min		Dose ratio
					Control	After	
1	0·45	45	0·010	Sig	0·28	0·88	3·14
2	0·45	45	0·010	Sig	0·75	1·72	2·29
3	0·45	45	0·010	N. Sig	1·60	1·74	1·09
4	0·24	15	0·016	Sig	1·13	2·25	1·99
5	0·055	30	0·002	Sig	0·59	1·06	1·80
6	0·072	30	0·002	Sig	1·21	2·00	1·65
7	0·067	30	0·002	Sig	0·56	1·83	3·27

Sig=Significant ($P \leqslant 0{\cdot}05$). N. Sig=Non-significant. Increase in dose required to increase heart rate twenty beats/min=0·77 μg. $t=7{\cdot}4700$; $P<0{\cdot}001$.

TABLE 2. *Human pulse rate data*

Subject	Infused dose (μg/kg)	Duration of infusion	Infusion rate ((μg/kg)/min)	Shift in dose-response curve	Dose to increase heart rate by 20 beats/min		Dose ratio
					Control	After	
1	0·45	45	0·010	Sig	0·28	0·88	3·14
	0·35	46	0·008	N. Sig	1·32	1·48	1·12
4	0·24	15	0·016	Sig	1·13	2·25	1·99
	0·056	20	0·003	N. Sig	3·08	2·00	0·65
6	0·072	30	0·002	Sig	1·21	2·00	1·65
	0·032	30	0·001	N. Sig	0·61	0·70	1·15

Sig=Significant ($P \leqslant 0{\cdot}05$). N. Sig=Non-significant. Three subjects studied under varying conditions.

TABLE 3. *Dog pulse rate data*

Dog no.	Infused dose (μg/kg)	Duration of infusion	Infusion rate ((μg/kg)/min)	Shift in dose-response curve	Dose to increase heart rate by 40 beats/min		Dose ratio
					Control	After	
49	5·76	15	0·38	Sig	0·19	0·38	2·00
50	0·76	32	0·024	Sig	0·50	3·19	6·38
56	0·86	60	0·014	N. Sig	0·84	5·47	6·51
60	11·17	210	0·053	Sig	2·24	4·00	1·79
62	0·11	35	0·003	Sig	0·25	0·82	3·28
63	0·011	15	0·0007	Sig	1·73	6·29	3·64
64	0·10	15	0·007	Sig	0·53	0·96	1·81
65	0·11	15	0·007	Sig	0·34	0·61	1·79
67	0·38	15	0·025	Sig	0·67	0·90	1·34
68	0·10	15	0·007	Sig	1·01	1·53	1·51
72	0·10	15	0·007	Sig	2·16	7·03	3·25
74	0·10	15	0·009	Sig	2·29	3·93	1·72
75	0·17	15	0·011	N. Sig	0·86	1·22	1·42

Sig=Significant ($P \leqslant 0{\cdot}05$). N. Sig=Non-significant. Mean increase in dose required to produce 40 beats/min increase=1·75 μg. $t=6{\cdot}2119$; $P<0{\cdot}001$.

increase in dose required was 1·53 μg. This increase was highly significant ($P < 0.001$). In three experiments resistance persisted for periods of 50–120 min without diminution. As with the human studies intravenous administration of aminophylline in doses of 5–10·1 mg/kg (average 8·5 mg/kg) produced no consistent modification of the isoprenaline resistance. The effect of increasing the duration of the infusion 2–3 fold was examined in six animals. The results (Table 4) are shown in Fig. 1. In four out of six animals there was a further increase in resistance, while in two a slight decrease was seen.

Diastolic pressure data. The changes produced in diastolic pressure responses were less consistent than the changes in heart rate. As shown in Table 5 in five experiments a significant decrease in response to isoprenaline was shown by a rightward displacement of the dose-response curve. In five others the change was in the same direction, but failed to achieve statistical significance. For the group as a whole the dose required to lower the diastolic pressure by 40 mmHg was increased 2·36 times. This represents a mean increase in dose of 0·35 μg which is a significant change ($0.05 > P > 0.025$). No significant correlation between duration of isoprenaline infusion and increase in isoprenaline resistance could be demonstrated in relation to diastolic response.

Rapid injections of isoprenaline 2–34·8 times the size of the subsequently infused dose produced no alteration in heart rate and blood pressure responsiveness. Similarly, infusions of normal saline were without effect on these variables.

Studies with long acting β-adrenoceptor stimulants

The number of animals used in these studies is too small to allow for statistical analysis. Each study is therefore reported individually.

(1) Intraduodenal terbutaline, 2 μg/kg (one dog, Fig. 2). The peak increase in heart rate of twenty-one beats/min occurred 7 min after dosing, whereas the peak plasma concentration of terbutaline (652·6 ng/ml) occurred at 46 min and declined with a half-life of 175 minutes. The heart rate initially fell in parallel with the decline in plasma concentration of terbutaline, but after 125 min fell away more rapidly to baseline. Isoprenaline resistance was measured after this point, at 230, 300 and 475 minutes. Over this period of time the resistance declined, roughly in parallel with the decline in plasma terbutaline, falling from 28·5 times to 10 times the control value.

(2) Two dogs were given a 20 min infusion of terbutaline at 0·004 (μg/kg)/minute. Although this caused no increase in heart rate, isoprenaline resistance was produced. There was no obvious relationship between the plasma concentration of terbutaline

TABLE 4. *Effect of prolonging isoprenaline infusions*

Dog	Increase in duration of infusion	Increase in dose ratio
65	×3	×3·28
67	×1·3	×1·49
68	×2	×0·89
72	×2	×1·45
	×3	×1·80
74	×2	×0·72
75	×2	×2·27

and isoprenaline resistance, peak resistance being observed at 57 min in one experiment and at 200 min in the other.

(3) After rapid injections of terbutaline (4·0 μg/kg) in two dogs, there was an increase in heart rate of thirty-five and forty-one beats/minute. Plasma concentrations of terbutaline declined exponentially and isoprenaline resistance declined in parallel with them.

(4) After rapid injection of isoetharine (8·2 μg/kg) in one dog, the plasma concentration of the unchanged drug declined in a biphasic manner, the first component having a half life of 1 min, the second being too long to estimate from the graph (Fig. 3). The heart rate response declined with a half life of 10 minutes. The isoprenaline resistance declined from 5·7 at 80 min to 4·3 times at 150 min, roughly

TABLE 5. *Dog diastolic pressure data*

Dog no.	Infused dose (μg/kg)	Duration of infusion	Infusion rate ((μg/kg)/min)	Shift in dose-response curve	Dose to lower diastolic pressure by 40 mmHg		Dose ratio
					Control	After	
49	5·76	15	0·38	N. Sig	0·2	0·25	1·25
50	0·76	32	0·024	N. Sig	0·41	0·51	1·24
56	0·86	60	0·014	Sig	0·53	2·84	5·36
60	11·17	210	0·053	N. Sig	0·37	1·85	5·00
62	0·11	35	0·003	Sig (-ve)	0·28	0·09	0·32
63	0·011	15	0·0007	Sig	1·19	4·47	3·75
64	0·10	15	0·007	N. Sig	1·35	0·87	0·64
65	0·11	15	0·007	N. Sig	0·31	0·28	0·91
67	0·38	15	0·025	Sig (-ve)	0·40	0·17	0·43
68	0·10	15	0·007	N. Sig	0·48	0·44	0·92
72	0·10	15	0·007	Sig	0·50	3·00	6·00
74	0·13	15	0·009	Sig	1·10	2·79	2·53
75	0·17	15	0·011	Sig	0·47	1·08	2·30

Sig=Significant ($P \leqslant 0{\cdot}05$). N. Sig=Non-significant. Mean increase in dose required to lower diastolic BP 40 mmHg=0·35 μg. $t=2{\cdot}5493$; $0{\cdot}05>P>0{\cdot}025$.

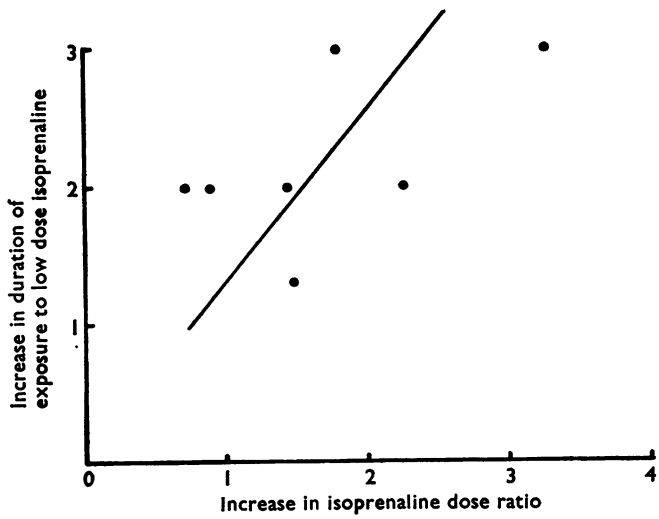

FIG. 1. Relationship between isoprenaline resistance and duration of exposure to isoprenaline.

in parallel to the very slow decline in the plasma concentration of isoetharine in the late phase of the experiment. In the second dog, the isoprenaline resistance was 1·6 at 66 min, rising to 2·1 at 140 min, finally falling to 0·7 by 190 minutes.

Guinea-pigs

In preliminary experiments, the mortality from histamine-induced bronchospasm was increased maximally 2 h after the end of the period of prolonged β-adrenoceptor stimulation. For this reason, and also because of the late appearance of maximum resistance in the dog experiments described above, in subsequent experiments guinea-pigs were challenged with histamine at 2 h after the various β-adrenoceptor stimulant regimes described above. An increase in mortality was found in all six groups, regardless of whether the agent used was isoprenaline, terbutaline or salbutamol (Fig. 4). The increase in mortality achieved statistical significance in the group treated with salbutamol and in one isoprenaline and one terbutaline treated group (Table 6). Failure to achieve statistical significance in the other terbutaline group and one of the isoprenaline groups in which the magnitude of the increase in mortality was of the same order is probably due to the relatively small numbers of animals used in each experiment. The test of significance of the trend observed (an increase in mortality in six out of six groups treated with β-adrenoceptor stimulants) yields a highly significant result ($P<0{\cdot}001$).

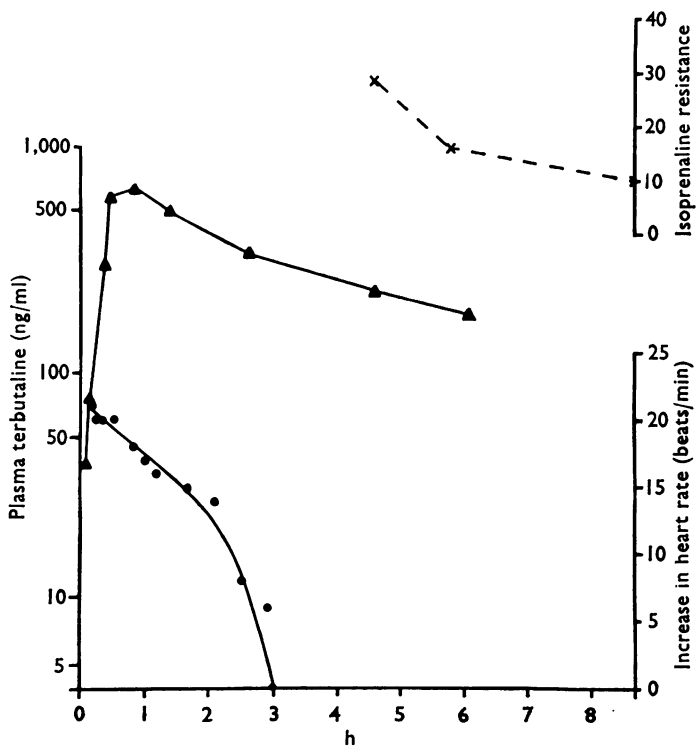

FIG. 2. Plasma radioactivity, pharmacological response and isoprenaline resistance after intraduodenal administration of terbutaline. (▲——▲), Plasma concentration; (●——●), heart rate.

Discussion

The increase in unexpected deaths from acute asthma, particularly in young patients, poses a serious problem. Several potentially reversible factors other than bronchial muscle activity may contribute to a worsening of the asthmatic state. Among these, mucosal oedema and mucus plugging are of great importance.

TABLE 6. *Mortality from histamine-induced bronchospasm in guinea-pigs after prolonged treatment with β-adrenoceptor stimulant drugs*

Drug	Dose given in each repeated injection	No. animals	Challenge dose of histamine (base)	Increase in % mortality	χ	P
Isoprenaline	4·0 μg/kg	10	0·8 mg/kg	35	2·018	0·2 $>P>$0·1
	4·0 μg/kg	15	1·1 mg/kg	5·5	0·250	—
	4·0 μg/kg	28	0·85 mg/kg	39·5	95·48	$P<$0·0005
Terbutaline	20·0 μg/kg	10	0·8 mg/kg	45	3·75	0·1 $>P>$0·05
	15·0 μg/kg	15	0·7 mg/kg	40	5·1675	0·025$>P>$0·01
Salbutamol	4·0 μg/kg	12	0·8 mg/kg	57·6	6·930	0·01 $>P>$0·005

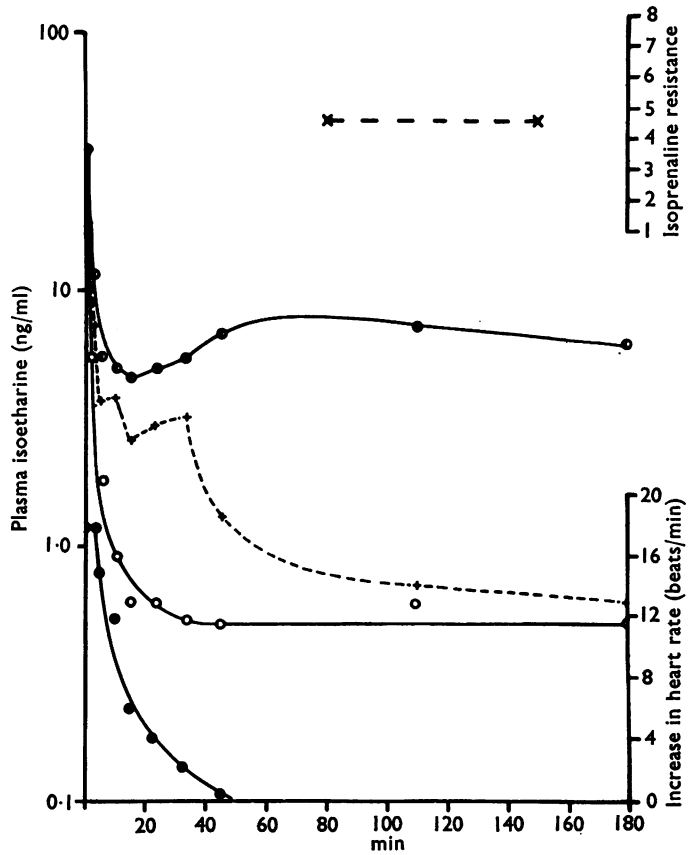

FIG. 3. Pharmacological activity of a single rapid injection of isoetharine and modification of isoprenaline responsiveness. (◐——◐), Total plasma ^{14}C; (+---+), 3-*O*-methylisoetharine; (○——○), isoetharine; (●——●), heart rate.

Bronchial muscle tone is also a major factor in asthma and it is important to look for mechanisms which may compromise bronchial muscle responsiveness in this condition.

Several mechanisms whereby β-adrenoceptor stimulants administered from pressurized aerosols might be responsible for the increase in the asthma death rate have been suggested.

(I.) One of the earliest explanations advanced was that excessive use of β-adrenoceptor stimulant drugs led to the production of a fatal arrhythmia (Greenberg & Pines, 1967). As none of these patients were monitored at the time of death, it is impossible to disprove this theory. However, in most patients reported by Speizer *et al.* (1968a and b) in whom autopsies were performed, the appearances were those of an asthmatic death with overdistension of the lungs and mucus plugging.

Although this does not disprove the arrhythmia theory, and while in elderly subjects, or those in whom arrhythmias were already present, β-adrenoceptor stimulation may worsen the abnormal rhythm, in younger subjects with normal hearts, ectopic rhythms caused by isoprenaline are very rare in our experience.

More recently, Bass (1970) has pointed out that some of the fluorocarbons used as propellent agents in the aerosols are chemically closely related to the short chain halogenated hydrocarbon anaesthetics such as chloroform and halothane, whose

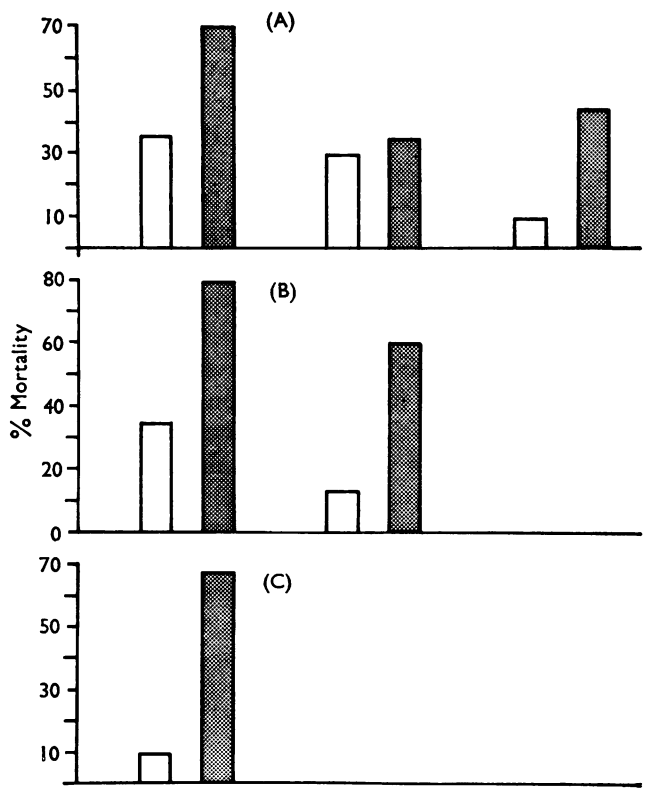

FIG. 4. Percentage mortality from histamine-induced bronchospasm in six mixed groups of guinea-pigs treated with saline (open columns) and β-adrenoceptor stimulants (shaded columns). (A) Isoprenaline (10×4 μg/kg); (B) terbutaline (10×20 and 10×15 μg/kg); (C) salbutamol (10×4 μg/ml). Each pair of histograms refers to the results of a single experiment.

ability to sensitize the myocardium to the arrhythmia-generating properties of catecholamines is well known. However, we have observed normal subjects taking doses of up to eight inhalations, repeated every 30 min, from a Medihaler Iso Forte, and under these conditions, no arrhythmias were encountered. In the course of investigations on subjects taking even larger doses from a placebo inhaler, which contained propellents but no isoprenaline, plasma concentrations of the alkyl fluorocarbons were measurable (Dollery, Draffan, Davies, Williams & Conolly, 1970) but these were only about one-fifth to one-tenth of those required in dogs to sensitize to arrhythmias caused by adrenaline (Clark, personal communication). On balance this observation, coupled with the fact that so little of the inhaled isoprenaline enters the circulation and exerts an effect on the heart (Blackwell *et al.*, 1970) makes this interaction seem unlikely to be of importance in asthma.

(II) It has been suggested that the reduced arterial oxygen saturation which is found in some severely asthmatic patients may be responsible for an altered response to isoprenaline. It has been shown (Collins, McDevitt, Shanks & Swanton, 1969) that dogs are able to tolerate repeated injections of up to 100 μg/kg isoprenaline when fully saturated with oxygen but will die in ventricular asystole if given repeated doses of 2·5 μg/kg of isoprenaline while breathing a 12% oxygen/88% nitrogen mixture which lowers the arterial oxygen tension to 40–45 mmHg.

It is difficult to be sure of the relevance of these observations to the clinical situation, since, in order to produce this effect large doses of isoprenaline (total dose 10–50 μg/kg) were used and the arterial oxygen tension was reduced more than is commonly seen even in severe asthmatics. While isoprenaline often lowers arterial oxygen tension significantly (Field, 1967) the fall is usually small, and tends to occur in the less hypoxic patients (Tai & Read, 1967) and does not reach levels generally regarded as dangerous from the point of view of reduction of arterial oxygen saturation (Campbell, 1967). Although the total doses are comparable with those which might be taken by an asthmatic patient, less than 5% of the inhaled dose exerts a pharmacological effect on the heart (Paterson *et al.*, 1968) and is absorbed over 10–15 minutes.

(III) An alternative explanation advanced in this paper is as follows.

Man and experimental animals can be made resistant to β-adrenoceptor stimulant drugs by prolonged exposure to such agents even in very low doses, and this resistance may last several hours. This phenomenon has been shown to apply to β_1-adrenoceptors (heart rate in man and dog), vascular β_2-adrenoceptors (change in diastolic pressure in the dog) and bronchial β_2-adrenoceptors (increased histamine sensitivity in the guinea-pig). The mechanism for the resistance described here and also that reported elsewhere is unknown. In an earlier study we hypothesized that the formation of a metabolite, 3 *O*-methyl isoprenaline, which has weak β-adrenoceptor blocking properties, might be responsible. However, its blocking properties are very weak, and the amounts in which it is formed are small (Morgan *et al.*, 1969). Further, it leaves unexplained the resistance to other β-adrenoceptor stimulants from which no blocking metabolite is formed. On the other hand the inconsistent relationship between the degree of isoprenaline resistance and the prevailing plasma concentrations of the various long acting β-adrenoceptor stimulants tested makes it very improbable that they produce resistance by competitive antagonism to isoprenaline at the level of the β-receptor. However, whatever mechanism is responsible the implications set out below remain the same.

Our results and some of the reports cited above show that cross resistance to various exogenous β-adrenoceptor stimulants occurs in man and animals and they also seem to indicate that prolongation of the duration of exposure to β-adrenoceptor stimulants is the most important factor in the production of resistance.

The change in responsiveness seen in the human and dog experiments is 2·5 to 3·9 times control values, and is comparable with that reported previously in subjects taking large doses of isoprenaline from pressurized aerosols (Paterson *et al.*, 1968).

If resistance to isoprenaline or related substances is accompanied by cross resistance to endogenous stimulation of the β-adrenoceptors, and the guinea-pig experiments suggest that this is so, then an explanation for the rise in asthma deaths is available since asthmatic patients rely heavily on their adrenergic drive to maintain bronchodilation. If the response of bronchial β-receptors is inhibited with β-adrenoceptor blocking drugs given by inhalation a much sharper increase in airway resistance occurs in asthmatics than in normal subjects (McNeil & Ingram, 1966).

We argue that prolonged β-adrenoceptor stimulation may cause such a degree of resistance both to exogenous sympathomimetic agents and to the natural transmitter released by the adrenergic nerves. If this occurs, then asthmatic patients could well deteriorate after prolonged use of a drug whose initial administration had caused relief of symptoms.

It is of interest to note that there have been reports of patients whose asthma was severe and failed to respond to steroids and other measures until adrenergic bronchodilator therapy was withdrawn (Keighley, 1966; Van Metre, 1967).

The data presented above suggest that the selective long acting agents, such as terbutaline, salbutamol or isoetharine, may be more potent in producing this resistance than isoprenaline. Thus, if excessive doses of these drugs (which can also be slowly absorbed from the gut in an active form) were used, these compounds might be as dangerous or conceivably more so to the asthmatic patient than isoprenaline.

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Appendix A

Statistical analysis of pairs of regression lines

A computer programme was written to examine each pair of regression lines firstly for parallelism and secondly for coincidence.

In performing these calculations, the following quantities were calculated.

For individual regression lines:

$$SXX=\Sigma(x_i-\bar{x})^2 \equiv \Sigma x_i^2-\frac{(\Sigma x_i)^2}{n}$$

$$SYY=\Sigma(y_i-\bar{y})^2 \equiv \Sigma y_i^2-\frac{(\Sigma y_i)^2}{n}$$

$$SXY=\Sigma(x_i-\bar{x})\,(y_i-\bar{y}) \equiv \Sigma x_i y_i-\frac{(\Sigma x)\,(\Sigma y)}{n}$$

$$\text{Slope, } b=\frac{SXY}{SXX}$$

For two lines a common slope is calculated as:

$$b\,(p)=\frac{SXY\,(1)+SXY\,(2)}{SXX\,(1)+SXX\,(2)}$$

For all data from both lines pooled to make one common regression line:

$$SXX(CO)=({}_1\Sigma x_i^2+{}_2\Sigma x_i^2)-\frac{({}_1\Sigma x_i+{}_2\Sigma x_i)^2}{n_1+n_2}$$

$$SYY(CO)=({}_1\Sigma y_i^2+{}_2\Sigma y_i^2)-\frac{({}_1\Sigma y_i+{}_2\Sigma y_i)^2}{n_1+n_2}$$

$$SXY(CO)=({}_1\Sigma x_i y_i+{}_2\Sigma x_i y_i)-\frac{({}_1\Sigma x_i+{}_2\Sigma x_i) * ({}_1\Sigma y_i+{}_2\Sigma y_i)}{n_1+n_2}$$

$$\text{Slope, } b_{(CO)}=\frac{SXY(CO)}{SXX(CO)}$$

Residual sums of squares were calculated as follows:—

1. For two separate lines,

Pooled RSS=SYY(1)+SYY(2)−b (1) * SXY(1)−b (2) * SXY(2)

Degrees of freedom=n (1)+n (2)−4

$$\text{Mean square, } S^2=\frac{\text{Pooled RSS}}{n\,(1)+n\,(2)-4}$$

2. For two parallel lines,

$$\text{Total RSS}=SYY(1)+SYY(2)-b_{(p)}(SXY(1)+SXY(2))$$

Degrees of freedom=n (1)+n (2)−3

3. For one common line,

$$RSS=SYY(CO)-b_{(CO)} * SXY(CO)$$

Degrees of freedom=n (1)+n (2)−2

In the test for parallelism the residual sums of squares about the two lines is compared with the residual sums of squares about two lines fitted to a common slope (b_p), and the ratio,

$$\frac{\text{Increase in RSS/Increase in degrees of freedom}}{S^2}$$

is compared with values from tables of the upper significance limits of the F distribution for P=0·05, where:—

v_1=increase in degrees of freedom=1

v_2=the degrees of freedom for the two lines using a common slope=n (1)+n (2)−3

In the test for coincidence the residual sums of squares for the one common line is compared with that for the data fitted round two lines with a common slope, $b_{(p)}$

The ratio:

$$\frac{\text{Increase in RSS/Increase in degrees of freedom}}{S^2}$$

is compared with values from the tables of the upper significance limits of the F distribution for P=0·05, where:—

v_1=increase in degrees of freedom=1

v_2=degrees of freedom for the data grouped about one common line=n (1)+n (2)−2

Appendix B

When all the experiments had been completed, the six sets of data were examined collectively to test for any consistent trend in mortality in relation to exposure to β-adrenoceptor stimulant drugs. The test employed utilizes a logistic transformation of the death rate data from each 2 × 2 contingency table. This method is appropriate provided there is homogeneity among the groups with respect to the percent mortality on a logit scale. Initially therefore it is necessary to examine by means of a χ squared test the null hypothesis that the logistic differences in each group (Δ_j) do not differ greatly from that of the whole body of data ($\hat{\Delta}$) (Cox 1970).

For each of k experiments the observations are as follows

Outcome	Group receiving β-adrenoceptor stimulant	Group receiving saline injections
Deaths	R_{1j}	R_{2j}
Survivors	$n_{1j}-R_{1j}$	$n_{2j}-R_{2j}$
Totals	n_{1j}	n_{2j}

From the k sets of data the following are calculated.

$$\Sigma\, \omega j(\mu j-\Delta)^2=X^2$$

Where: $j=1$ to k

$i=1$ or 2

$$Wj=\frac{1}{V_{1j}+V_{2j}}$$

$$V_{ij}=\frac{n_{ij}}{R_{ij}(n_{ij}-R_{ij})}$$

$$uj=Z_{2j}-Z_{1j}$$

$$Z_{ij}=\text{logit } R_{ij}$$

$$\hat{\Delta}=\frac{\Sigma\, W_{juj}}{\Sigma\, wj}$$

If X^2 is less than χ^2 on $(k-1)$ degrees of freedom the null hypothesis is not rejected, and it is permissible to test the significance of the increase in mortality for the whole body of data by examining the statistic

$$\frac{\hat{\Delta}}{\text{variance } \hat{\Delta}} \qquad \text{where variance } \hat{\Delta} = \frac{1}{\Sigma\omega j}$$

This statistic is a normal deviate and significance at the 5% level is indicated by a value exceeding 1·96.

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