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FURTHER OBSERVATIONS ON THE CARDIOTOXICITY OF ISOPRENALINE DURING HYPOXIA

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1 In dogs respired with 10% oxygen : 90% nitrogen, only five out of 16 dogs survived repeated intravenous doses of isoprenaline (either 0.5 or 1.0 μg/kg) and only one out of six dogs survived repeated isoprenaline inhalations from a pressurized aerosol.

2 In dogs respired with 15% oxygen : 85% nitrogen, five out of six dogs survived repeated intravenous doses of isoprenaline (2.5 μg/kg).

3 The fatal response in these animals consisted of a fall in heart rate, arterial and pulse pressures. Sinus rhythm persisted even after the arterial pressure had fallen, though occasionally a slow A-V nodal rhythm or irregular ventricular ectopic beats occurred. Ventricular fibrillation did not occur.

4 Eight out of 10 dogs brought to the verge of a fatal response with 10% oxygen : 90% nitrogen and repeated doses of isoprenaline (2.5 μg/kg) were resuscitated by the administration of 100% oxygen and, when necessary, cardiac massage.

5 A group of five dogs survived the combined effects of repeated doses of isoprenaline (2.5 μg/kg) and respiration with 10% oxygen : 90% nitrogen when the time interval between doses was 11 min, instead of the usual 5 minutes.

6 Control of pH by infusion of sodium bicarbonate did not protect the dogs from the combined effects of hypoxia and repeated isoprenaline challenge.

7 After a 60 min period of continuous isoprenaline infusion in dogs breathing room air, only one of 10 dogs survived artificial respiration with 10% oxygen : 90% nitrogen and repeated challenge with intravenous isoprenaline (1.0 μg/kg) at 5 min intervals. At the higher infusion levels of isoprenaline (0.1 and 1.0 $\mu g\ kg^{-1}\ min^{-1}$), two dogs out of four died after the hypoxic mixture was started but before any isoprenaline challenge was given.

8 The possible relevance of these findings in dogs to the recently observed increase in mortality in young asthmatics is discussed.

Introduction

The observed increase in mortality from asthma between 1960 and 1966, particularly in the younger age groups, has been attributed to the excessive use of aerosols containing sympathomimetic amines, principally isoprenaline (Speizer, Doll, Heaf & Strang, 1968), and appears to have been geographically related to areas in which highly concentrated pressurized aerosols of isoprenaline were available (Stolley, 1972). Recognition of this possible relationship between isoprenaline inhalation and sudden death, and more disciplined use of this type of preparation has resulted in a reversal of the trend (Inman & Adelstein, 1969).

Various theories have been advanced to explain the possible mechanism of death, which is often sudden, in these patients. Initially it was suggested that it might result from sympathomimetic-induced cardiac arrhythmias, for example, ventricular tachycardia or fibrillation (Speizer *et al.*, 1968). Subsequently one of the metabolites of isoprenaline was found to have β-adrenoceptor blocking properties and it was thought that this might be harmful (Paterson, Conolly, Davies & Dollery, 1968) or that the heart might be sensitized to endogenous catecholamines by fluorinated hydrocarbons present in pressurized nebulizers (Taylor & Harris, 1970; Dollery, Draffan, Davies, Williams & Conolly, 1970). Lastly it has been postulated that patients may develop resistance to β-adrenoceptor stimulants by prolonged use of such agents thus depriving them of benefit from both endogenous and exogenous sympathetic stimulation (Conolly, Davies, Dollery & George,

1971). However, none of these ideas has received sustained or universal support.

In an earlier study, Collins, McDevitt, Shanks & Swanton (1969) described cardiotoxic effects from the administration of intravenous isoprenaline to hypoxic dogs, often resulting in their death. It was found that dogs breathing room air could withstand the administration of repeated small doses or single large doses of isoprenaline (up to 500 μg/kg) with no ill effects. However, dogs respired with 12% oxygen : 88% nitrogen, often died when given similar doses of isoprenaline. Death was from cardiac asystole, rather than from ventricular fibrillation and could be produced by giving four or five doses of isoprenaline (2.5 μg/kg) at 5 min intervals or by fewer doses of 25 μg/kg. These lethal effects of isoprenaline in hypoxaemia could be prevented by pretreatment with propranolol.

Since no other definitive pathogenesis has been established in sudden asthmatic deaths, we have extended our observations in an effort to elucidate the roles of isoprenaline and hypoxia in this situation and their possible relevance to sudden deaths in asthmatic patients.

Methods

Observations were made in greyhounds of either sex (19-35 kg) anaesthetized either by the subcutaneous injection of morphine sulphate (0.5 mg/kg) followed 1 h later by the intravenous injection of pentobarbitone (20 mg/kg) or by the intravenous injection of pentobarbitone (30 mg/kg). Anaesthesia was maintained by further doses of pentobarbitone as required. A cuffed endotracheal tube was inserted and the dogs were artificially respired with room air by means of a Starling Ideal pump adjusted to deliver a stroke volume of 13 ml/kg body weight at a rate of 18 strokes/minute. Some dogs were also respired with a mixture of 15% oxygen : 85% nitrogen and others with 10% oxygen : 90% nitrogen (British Oxygen Corporation). Drugs were administered through a catheter in a foreleg vein by injection or constant infusion. Inhalations were administered via a pressurized aerosol (Medihaler-Iso-Riker) which was incorporated into a special endotracheal tube. Arterial pressure was measured from the left carotid artery by means of a metal cannula leading to a pressure transducer (Consolidated Electrodynamics Type 4-327-L221), which was attached to a direct writing recorder (Model M4 or M8, Devices Ltd). The electrocardiogram was obtained from needle electrodes inserted under the skin; one lead (I, II or III) was recorded and another used to measure heart rate on a Nielsen instantaneous ratemeter (Devices Ltd), the output of which was recorded. Arterial pressure and the electrocardiogram were displayed on a four-channel oscilloscope (Airmec). Samples of arterial blood for gas analysis were obtained in heparinized syringes from the cannula in the left carotid artery. In most dogs a sample was taken during respiration with room air and thereafter at frequent intervals during the period of hypoxia. The Pa_{O_2} was measured with a Clark-type oxygen electrode used in conjunction with the Astrup apparatus. The Pa_{CO_2}, pH and base excess values were determined by the Astrup method (Siggaard Andersen, Engel, Jorgensen & Astrup, 1960).

The drug used was (±)-isoprenaline sulphate (Burroughs Wellcome and Co.). The doses are expressed in terms of the base. It was freshly prepared in a solution of 0.9% sodium chloride and acidified with 1 N HCl. A solution of sodium bicarbonate (1 M) was also infused in some experiments.

Results

I. The relationship between hypoxia and isoprenaline

Respiration with 10% oxygen : 90% nitrogen alone. Observations were made in 46 dogs which were respired with room air, after the induction of anaesthesia, until steady values for heart rate, arterial pressure and blood gases were obtained. Subsequently they were respired with 10% oxygen : 90% nitrogen for periods of at least 75 min unless death supervened. Forty-one dogs survived the total duration of hypoxia; the remaining five dogs died within 15 min of starting to breathe the gas mixture. Mean values of Pa_{O_2}, Pa_{CO_2}, pH and base excess before starting and after 40 min of hypoxia for the surviving dogs, or alternatively 1-5 min before death (in those dying), are shown in Table 1. The dogs which died appeared to have a greater degree of hypoxia and acidosis before death than those surviving had at 40 minutes. Hypoxia resulted in an increase in heart rate and both systolic and diastolic arterial pressure in the 41 dogs which survived.

Responses to repetition of the same dose of isoprenaline

Respiration with 15% oxygen : 85% nitrogen. In a previous study (Collins *et al.*, 1969), dogs failed to survive challenge with a series of doses of isoprenaline (2.5 μg/kg) given at 5 min intervals

Table 1 Average values (±s.e. mean) for PaO_2, $PaCO_2$, pH and base excess in 46 dogs respired with 10% oxygen : 90% nitrogen alone.

Group	T *(min)*	PaO_2 *(mmHg)*	$PaCO_2$ *(mmHg)*	*pH*	*Base excess*
41 dogs surviving hypoxia	0	92.0 ± 2.5	52.0 ± 1.2	7.31 ± 0.01	−1.50 ± 0.53
	40	34.0 ± 1.5*	39.0 ± 1.0*	7.36 ± 0.01*	−3.58 ± 0.47*
5 dogs which died	0	90.0 ± 8.3	54.0 ± 3.6	7.30 ± 0.03	−2.88 ± 1.64†
	last recorded value	27.0 ± 3.4	44.0 ± 4.0	7.23 ± 0.04	−10.22 ± 2.78†

T = time from commencing respiration with 10% oxygen : 90% nitrogen.
* = mean of 36 dogs.
† = mean of four dogs.

when artificially respired with 12% oxygen : 88% nitrogen.

Five minutes after starting artificial respiration with 15% oxygen : 85% nitrogen, five dogs were given a series of intravenous doses of isoprenaline (2.5 μg/kg) at 5 min intervals up to a maximum of 10 doses. Each isoprenaline challenge increased heart rate and decreased systolic and diastolic arterial pressure. There was a gradual rise in the resting heart rate and fall in the resting systolic and diastolic arterial pressure during the experiments and the responses after each isoprenaline dose diminished through the course of the experiment (Table 2). The dogs became moderately hypoxic (Table 3). Four of the five dogs survived the 10 doses of isoprenaline: the fifth died after the fifth dose with a rapid terminal fall in blood pressure, bradycardia and eventual cardiac asystole (Table 4).

Respiration with 10% oxygen : 90% nitrogen. Ten dogs were artificially respired with 10% oxygen : 90% nitrogen and, after breathing the hypoxic mixture for 5 min, they were challenged with a series of doses of isoprenaline (1.0 μg/kg) given at 5 min intervals. Six dogs, breathing the same hypoxic mixture, were given repeated doses of isoprenaline (0.5 μg/kg) at 5 min intervals. A maximum of 10 doses was given to any one dog. Both doses of isoprenaline increased heart rate and decreased systolic and diastolic arterial pressure in a manner similar to that shown in Table 2. In this experiment blood gases were monitored in only seven out of 10 dogs challenged with isoprenaline (1.0 μg/kg) and in only four out of six dogs given isoprenaline (0.5 μg/kg). After breathing the 10% oxygen mixture for 13 min, the mean PaO_2 had fallen to 35.3 and 21.7 mmHg (1 mmHg = 1.333 mbar) respectively in the two groups of dogs (Table 5) compared with 60.2 mmHg in the dogs respired with 15% oxygen (Table 3). Unfortunately, the two dogs that survived repeated isoprenaline challenge with 0.5 μg/kg were not having blood gases measured and the low PaO_2 in this group may be explained by the fact that it is the mean of three dogs, all of which died. Support for this idea is seen in the group given isoprenaline (1.0 μg/kg); at 13 min, the dogs which died had a mean PaO_2 of 28.7 mmHg in contrast to a mean of 44.0 mmHg in those which survived the period of hypoxia. The general impression gained was that dogs which died achieved low PaO_2 values more rapidly than those which survived.

Three out of 10 dogs survived repeated doses of isoprenaline (1.0 μg/kg) and two out of the six dogs survived the challenge with isoprenaline (0.5 μg/kg) (Table 4).

Response to repeated inhalation of an isoprenaline spray. Six dogs, artificially respired with 10% oxygen : 90% nitrogen, were given inhalations every 5 min down a specially adapted endotracheal tube. Each inhalation was two puffs of a Medihaler-Iso pressurized aerosol (Riker) and was equivalent to isoprenaline (200 μg per dose). Each inhalation of isoprenaline produced an increase in heart rate and a decrease in systolic and diastolic pressure but in contrast to the smallest intravenous dose of isoprenaline used, the increases and decreases were smaller and were achieved less rapidly. The PaO_2 values were similar to those described in the other studies. One dog survived the total period of hypoxia with a PaO_2 of 47 mmHg (Table 6). Only one dog survived three inhalations and it survived to the maximum of 10 doses (Table 4).

II. The effects on the fatal response of altering individual parameters

pH control. Isoprenaline administered to dogs breathing hypoxic gas mixture has been found consistently to produce a lowering of blood pH (e.g. see Tables 3 and 5).

Five dogs were respired with 10% oxygen : 90% nitrogen mixture and 5 min later an infusion of sodium bicarbonate, 1 M, was started at a rate of 0.1 ml kg^{-1} min^{-1} and was continued throughout the remainder of the experiment. The intravenous administration of a series of increasing doses of isoprenaline (0.1, 0.5, 2.5, 10.0, 50.0 and 100.0 μg/kg) was given at 6, 11, 16, 22, 30 and 41 min respectively after starting respiration with the hypoxic mixture. During the period of hypoxia and infusion of sodium bicarbonate, pH and base excess did not fall. All five dogs survived isoprenaline doses of 0.1, 0.5 and 2.5 μg/kg. One died after 10.0 μg/kg, two after 50.0 μg/kg and the remaining two after the 100 μg/kg dose.

Administration of oxygen and external cardiac compression. Ten dogs were respired with 10% oxygen : 90% nitrogen and repeated doses of isoprenaline (2.5 μg/kg) were administered intravenously at 6 min intervals. When a potentially fatal response had been produced, as judged by the development of sinus bradycardia, slow A-V nodal rhythm or irregular ventricular ectopic beats and continuously falling blood pressure (Collins *et al.*, 1969), pure oxygen was substituted for the 10%

Table 2 Average changes (±s.e. mean) in heart rate, systolic and diastolic arterial pressure produced by the intravenous injection of a series of doses of isoprenaline (2.5 μg/kg) given at 5 min intervals in dogs respired with 15% O_2 : 85% N_2.

Number of doses		1	3	5	7	9
Number of dogs		5	5	5	4	4
Heart rate (beats/min)	R	163.0 ± 6.2	175.0 ± 7.1	188.0 ± 3.7	183.8 ± 5.5	185.0 ± 5.4
	I	+93.0 ± 9.5	+67.0 ± 7.2	+55.0 ± 2.2	+56.3 ± 3.1	+52.5 ± 6.0
Systolic pressure (mmHg)	R	204.0 ± 21.6	202.0 ± 22.6	202.0 ± 29.8	198.0 ± 36.8	185.0 ± 34.3
	I	−63.0 ± 4.6	−31.0 ± 5.3	−31.0 ± 5.6	−31.3 ± 8.3	−30.0 ± 2.0
Diastolic pressure (mmHg)	R	168.0 ± 24.5	151.0 ± 17.1	144.0 ± 21.3	147.5 ± 26.3	137.5 ± 26.7
	I	−77.0 ± 7.0	−50.0 ± 9.4	−51.0 ± 4.9	−40.0 ± 6.5	−45.0 ± 5.4

Values for the 1st, 3rd, 5th, 7th and 9th doses are shown. R, Resting value; I, maximum change produced by isoprenaline.

Table 3 Average values (±s.e. mean) for PaO_2, $PaCO_2$ and pH in five dogs respired with 15% oxygen : 85% nitrogen and given a series of doses of isoprenaline (2.5 μg/kg) at 5 min intervals.

T *(min)*	*0*	*13*	*23*	*33*	*43*
PaO_2 (mmHg)	101.2 ± 3.9	60.2 ± 7.4	49.8 ± 8.1	44.0 ± 8.6*	52.3 ± 7.8*
$PaCO_2$ (mmHg)	45.2 ± 1.2	47.6 ± 3.2	51.4 ± 3.1	56.4 ± 3.5*	58.0 ± 4.1*
pH	7.34 ± 0.12	7.28 ± 0.09	7.22 ± 0.01	7.17 ± 0.09*	7.17 ± 0.10*

T = time in minutes from starting respiration with the mixture of 15% oxygen : 85% nitrogen.
* = mean of four dogs.

Table 4 Effects of repeated isoprenaline challenge at 5 min intervals on the survival of dogs respired with low oxygen mixtures.

Number of dogs	*Oxygen concentration (%)*	*Isoprenaline dose (μg/kg)*	*Number of doses of isoprenaline*									
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
5	15	2.5	5/5	5/5	5/5	5/5	5/5	4/5	4/5	4/5	4/5	4/5
10	10	1.0	10/10	9/10	7/10	7/10	5/10	3/10	3/10	3/10	3/10	3/10
6	10	0.5	6/6	5/6	3/6	2/6	2/6	2/6	2/6	2/6	2/6	2/6
6	10	200 μg (spray)	6/6	5/6	2/6	1/6	1/6	1/6	1/6	1/6	1/6	1/6

The isoprenaline doses are intravenous except for last column where they are by inhalation. For each dose the numerator is the number of animals surviving the isoprenaline challenge out of the number tested (denominator).

Table 5 Average values (±s.e. mean) for PaO_2, $PaCO_2$ and pH in (a) seven dogs respired with 10% oxygen : 90% nitrogen and challenged with isoprenaline (1.0 μg/kg) and (b) four dogs respired with 10% oxygen : 90% nitrogen and challenged with isoprenaline (0.5 μg/kg) at 5 min intervals.

(a) T *(min)*	*0*	*13*	*23*	*33*	*43*
PaO_2 (mmHg)	86.6 ± 4.1	35.3 ± 3.9	29.5 ± 3.4*	25.0 ± 4.7†	27.7 ± 3.9†
$PaCO_2$ (mmHg)	49.2 ± 1.7	43.8 ± 2.2	45.6 ± 5.0*	51.5 ± 6.5†	38.0 ± 5.0†
pH	7.35 ± 0.10	7.32 ± 0.10	7.24 ± 0.10*	7.21 ± 0.10†	7.15 ± 0.02†
(b) T *(min)*	*0*	*13*	*23*	*33*	*43*
PaO_2 (mmHg)	80.3 ± 2.1	21.7 ± 1.3†	+	+	+
$PaCO_2$ (mmHg)	50.0 ± 2.7	47.3 ± 2.4†	+	+	+
pH	7.32 ± 0.12	7.25 ± 0.15†	+	+	+

T = time in minutes from commencing respiration with 10% oxygen : 90% nitrogen.
* = mean of six.
† = mean of three.
+ = blood gases were not monitored on the two dogs which survived.

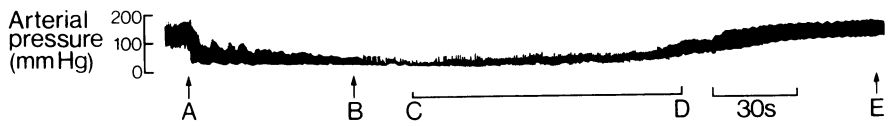

Fig. 1 Record of arterial pressure in a hypoxic dog showing a potentially fatal response produced by the third of a series of repeated doses of isoprenaline, 2.5 μg/kg, administered at point A. Oxygen, given at B, combined with external cardiac compression, C-D, resulted in resumption of cardiac activity. The dog was returned to respiration with room air at E.

oxygen : 90% nitrogen mixture and where cardiac standstill appeared imminent, external cardiac compression was performed. After being respired with oxygen for approximately 5 min, the animals were once again respired with room air.

Eight dogs out of 10 recovered from potentially fatal responses. The potentially fatal response occurred in four dogs after the third dose of isoprenaline (2.5 μg/kg), in three dogs after the fourth dose, in one dog after the sixth dose and in the remaining two dogs after the seventh dose. At the time of transfer from the hypoxic gas mixture to pure oxygen, the mean blood pressure was approximately 44/25 mmHg. Part of a typical tracing from one of these experiments is shown in Figure 1. After return to respiration with room air in the eight dogs which recovered, blood pressure and heart rate continued to rise, reaching pre-hypoxia control levels in from 7-30 minutes.

Extended time intervals between isoprenaline administration. Five dogs were artificially respired with 10% oxygen : 90% nitrogen. Five minutes later, doses of isoprenaline (2.5 μg/kg) were administered intravenously at 11 min intervals to a maximum of seven doses. The effects of the isoprenaline on heart rate and arterial pressure were similar to those described in previous experiments. The average values for blood gases and pH before and at intervals after commencing respiration with the hypoxic mixture are shown in Table 7. The PaO_2 fell markedly and the animals became hypoxic. All five dogs survived the administration of these seven doses of isoprenaline.

III. The effect of isoprenaline infusion on isoprenaline challenge

Observations were made in dogs in which isoprenaline was infused continuously for 60 min in doses ranging from 0.01 μg kg^{-1} min^{-1} to 1.0 μg kg^{-1} min^{-1}, whilst the animals were breathing room air. One dog was infused at 0.01 μg kg^{-1} min^{-1}, five dogs at 0.02 μg kg^{-1} min^{-1}, two dogs at 0.1 μg kg^{-1} min^{-1} and two more at 1.0 μg kg^{-1} $minute^{-1}$. In most instances, the infusion of isoprenaline caused an initial rise in heart rate and fall in arterial pressure but this had always returned to values close to the initial resting values before the period of hypoxia was begun. With the infusion continuing, each dog was then artificially respired with 10% oxygen : 90% nitrogen and after 5 min, regular doses of isoprenaline (1.0 μg/kg) were administered intravenously to a maximum of 10 doses. The isoprenaline challenge again caused increase in heart rate and decrease in systolic and diastolic arterial pressure but these changes were less than control responses carried out before the period of infusion and generally less than responses occurring in animals which were not infused continuously with isoprenaline. The response of PaO_2, $PaCO_2$ and pH was similar to

Table 6 Average values (±s.e. mean) for PaO_2, $PaCO_2$ and pH in six dogs respired with 10% oxygen : 90% nitrogen and challenged with isoprenaline inhalation, 200 μg every 5 minutes.

T (min)	0	13	23	33	43
PaO_2 (mmHg)	82.7 ± 3.9	26.8 ± 4.2	29.7 ± 9.2*	47.0†	47.0†
$PaCO_2$ (mmHg)	56.3 ± 4.9	51.5 ± 3.4	53.3 ± 7.4*	41.0†	39.0†
pH	7.29 ± 0.12	7.24 ± 0.12	7.21 ± 0.14*	7.30†	7.28†

T = time in minutes from starting respiration with the 10% oxygen : 90% nitrogen.
* = mean of three dogs.
† = one dog only.

Table 7 Average values (±s.e. mean) for PaO_2, $PaCO_2$ and pH in five dogs during a period of hypoxia when a series of repeated doses of isoprenaline, 2.5 μg/kg, was administered.

T (min)	0	15	26	37	48	59	70
PaO_2 (mmHg)	120 ± 4.7	56.0 ± 5.0	43.0 ± 5.3	38.0 ± 5.3	34.0 ± 4.7	34.0 ± 3.9	34.0 ± 8.0
$PaCO_2$ (mmHg)	48.0 ± 0.4	47.0 ± 4.7	49.0 ± 5.2	47.0 ± 2.7	42.0 ± 2.2	43.0 ± 1.5	46.0 ± 3.0
pH	7.31 ± 0.01	7.28 ± 0.02	7.25 ± 0.01	7.22 ± 0.01	7.21 ± 0.02	7.18 ± 0.02	7.17 ± 0.02

T = time in minutes from starting respiration with the mixture of 10% oxygen : 90% nitrogen. Doses of isoprenaline were given at 5, 16, 27, 38, 49, 60 and 71 minutes.

that obtained in other experiments with 10% oxygen : 90% nitrogen.

The survival of the dogs is shown in Table 8. Only one of the 10 dogs survived the period of isoprenaline infusion plus the repeated isoprenaline challenge. At the higher infusion levels of isoprenaline (0.1 and 1.0 μg kg^{-1} min^{-1}) two dogs out of four died after the 10% oxygen : 90% nitrogen mixture was started but before any isoprenaline challenge could be given: the other two died after the first 1.0 μg/kg dose. Two dogs out of five survived seven doses of isoprenaline (1.0 μg/kg) during hypoxia and infusion with 0.02 μg kg^{-1} $minute^{-1}$. There was no evidence that prolonged isoprenaline infusion at any dose level protected the animals from repeated isoprenaline challenge during hypoxia.

Discussion

It has previously been shown that dogs respired with 12% oxygen : 88% nitrogen respond to intravenous injections of isoprenaline differently from those breathing room air. In the former situation, the dogs die in cardiac asystole following a reduction in cardiac contractility (Collins *et al.*, 1969). Death was produced either by multiple doses of isoprenaline (2.5 μg/kg) or by larger and increasing doses; the lethal effects could be prevented by pre-treatment with propranolol.

The present experiments extend our knowledge of the inter-relationships of the factors involved. In the first place, the degree of hypoxaemia seems to be important. Thus, five out of six dogs breathing 15% oxygen : 85% nitrogen survived repeated challenges with a dose of isoprenaline which had previously been shown to be fatal in dogs respired with 12% oxygen : 88% nitrogen. Only five out of 16 dogs survived repeated doses of lower concentrations of isoprenaline (0.5 and 1.0 μg/kg) when the gas mixture respired was 10% oxygen : 90% nitrogen, and only one out of six dogs survived repeated isoprenaline inhalations when breathing this latter mixture. The PaO_2 was generally higher over the period of the experiments when the higher oxygen concentrations were used. Further support for the importance of hypoxaemia in this reaction is shown by the fact that eight out of 10 dogs brought to the verge of a fatal response with 10% oxygen : 90% nitrogen and repeated doses of isoprenaline (2.5 μg/kg) were resuscitated by the administration of 100% oxygen and, where thought necessary, external cardiac massage. However, it must be emphasized that hypoxaemia alone is not responsible in most instances as 41 out of 46 dogs survived a period of at least 75 min respiration with 10% oxygen : 90%

nitrogen. Similarly, isoprenaline in the absence of hypoxaemia is generally non-toxic in anaesthetized dogs and it would appear that the combination is necessary and that the degree of hypoxaemia induced is important. It is not clear whether this relates to the capacity of isoprenaline and other β-adrenoceptor stimulants themselves to worsen hypoxaemia (Field, 1967). It was a general impression that those animals which did worst were those which became severely hypoxaemic most rapidly.

The PaO_2 values obtained in these experiments were generally of the order of 20-40 mmHg when a fatal response was obtained. It could be argued that the PaO_2 levels obtained in these experiments are lower than those which occur in clinical practice. However, several investigators have reported PaO_2 levels below 40 mmHg in patients with status asthmaticus both in adults (Rees, Millar & Donald, 1968) and in children (Downes, Wood, Striker & Pittman, 1968). Rees *et al.* (1968) described five patients in whom the PaO_2 was 30, 39, 32, 31 and 35 mmHg respectively on admission to hospital and they also demonstrated that in hypoxaemic patients, the PaO_2 may fall even further when adrenaline or aminophylline was administered. If we deduce that the patients who die are those who are in severe status asthmaticus and who are endeavouring to use large amounts of β-adrenoceptor stimulants then the relationship of the experimental to the clinical situation may not be as distant as has been assumed.

The role of isoprenaline in this fatal response has been further elucidated. It has previously been shown that repeated doses of isoprenaline (2.5 μg/kg) given at 5 min intervals is likely to be fatal in the presence of hypoxaemia. It now appears that isoprenaline (1.0 μg/kg and even 0.5 μg/kg) will produce death in a majority of severely hypoxaemic dogs. This would be equivalent to 35-70 μg per dose for a 70 kg man. A pressurized aerosol inhaler such as the Riker 'Medihaler-Iso-Forte' delivers 500 μg isoprenaline per puff. Paterson *et al.* (1968) have shown that inhaled isoprenaline is between 40-400 times less effective than isoprenaline given intravenously in producing a tachycardia in normal and asthmatic subjects. Thus a single inhalation of such a spray might be equivalent to approximately 1-12 μg intravenous isoprenaline. Some hypoxic dogs died after a single dose of isoprenaline (Table 4) and in the worst possible human situation, this would compare to as little as three puffs of a high concentration isoprenaline inhaler in a 70 kg man (or less if he were lighter). In addition, it has been shown that repeated inhalations of isoprenaline (200 μg) produce a similar fatal response to that obtained by the intravenous route, though the response of heart rate and arterial pressure to each dose is, as might be expected, less rapid.

The combined effect of hypoxia and isoprenaline was to produce an acidosis, often severe; such a combination of acidosis and hypoxia has been shown to depress myocardial contractility (Downing, Talner & Gardner, 1966). In these experiments, the elimination of the acidosis by infusion of sodium bicarbonate did not prevent the development of the fatal response. There was additional evidence to suggest that systemic acidosis combined with hypoxia was not involved to any great extent in the production of fatal cardiac depression. Firstly, in experiments where repeated doses of isoprenaline were given, some animals showed fatal responses after the first few

Table 8 Effect of isoprenaline infusion on survival of dogs challenged by isoprenaline, 1.0 μg/kg, every 5 min whilst breathing 10% O_2 : 90% N_2 mixture.

Infusion rate ($\mu g\ kg^{-1}\ min^{-1}$)	*Number of dogs infused*	*Number of doses of isoprenaline (1.0 μg/kg)*									
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
0.01	1	0/1									
0.02	5	5/5	4/5	3/5	2/5	2/5	2/5	2/5	1/5	1/5	1/5
0.1	2	0/1*									
1.0	2	0/1*									
Total surviving	10	5	4	3	2	2	2	2	1	1	1

Repetitive doses of isoprenaline commenced after a 60-min period of isoprenaline infusion breathing room air. For each dose the numerator is the number of animals surviving the isoprenaline challenge out of the number tested (denominator).

* One dog died on the isoprenaline infusion alone when it started to inhale the hypoxic mixture, before any isoprenaline challenge.

doses when little change in systemic arterial pH had occurred. Secondly, when repeated 2.5 μg/kg doses were administered at 11 min intervals, a fatal effect was not produced, even though after seven doses acidosis was severe.

Another way in which the animals could be protected from the combined effects of repeated doses of isoprenaline (2.5 μg/kg) and hypoxia was by extending the time interval between doses from 5 to 11 minutes. This might be an indication that the toxic effect of isoprenaline was cumulative.

The nature of the fatal response appeared to be similar in most instances. Following the fatal dose, there was a steady fall in blood pressure, with a striking reduction in pulse pressure. After an initial increase, the heart rate declined to the resting level and then fell rapidly. The electrocardiogram showed that, in most cases, sinus rhythm persisted even after the arterial pressure had fallen, though occasionally a slow A-V nodal rhythm or irregular ventricular ectopic beats occurred. It has previously been shown that within seconds of the fatal dose, there was a sharp decrease in cardiac output and stroke volume, accompanied by increases in ventricular end-diastolic pressures, indicating a failure of myocardial contractility (Collins *et al.*, 1969; Swanton, 1972). The terminal response developed suddenly: cardiac output, stroke volume, and right and left ventricular responses to the penultimate dose have been shown to be relatively normal, though there was some reduction in heart rate and arterial pressure responses with repeated doses of isoprenaline.

The relevance of these observations in animals to the human situation is still not clear, although it now seems possible that the doses of isoprenaline used and the levels of hypoxia required to produce the fatal combination may not be dissimilar to those found in patients with very severe status asthmaticus who grossly abuse isoprenaline aerosols. The mechanism by which these patients die has seldom been observed. Recently, however, Dr I.W.B. Grant (personal communication) observed a patient in status asthmaticus who had expended at least half the contents of an isoprenaline and atropine spray in a period of 4 hours. A further 4 h later, after his admission to hospital, his condition had deteriorated so much that he was given 0.5% salbutamol aerosol in 40% oxygen by intermittent positive-pressure ventilation and almost immediately developed progressive bradycardia and cardiac asystole. No information was available on his Pa_{O_2} before salbutamol was given. Cardiac action returned following external cardiac massage and ventilation with a high concentration of oxygen. Apart from the use of salbutamol, which has previously been shown to produce a fatal response in hypoxic dogs identical to that observed with isoprenaline (Shanks & Swanton, 1971), the similarity between this episode and that described in the present experiments with hypoxic dogs can be seen, even to recovery with oxygen and external cardiac massage.

Recently, it has been suggested that prolonged exposure to β-adrenoceptor stimulants in bronchodilator aerosols may lead to the development of resistance to such agents in asthmatic patients and that such resistance will interfere with endogenous sympathetic stimulation (Conolly *et al.*, 1971). Atkinson & Rand (1968) had previously found that, during infusions of sympathomimetic amines, cats showed decreased sensitivity to cardiac and depressor effects of single injections of adrenaline, isoprenaline and orciprenaline. Conolly *et al.* (1971), confirmed these findings in dogs but also claimed that similar resistance could be demonstrated in man with isoprenaline, terbutaline and isoetharine. However, further studies in man have not shown any evidence of resistance to isoprenaline in terms of the dose required to produce a given peak heart rate, but only that the dose needed to produce a given increment in heart rate may be raised if the pre-isoprenaline heart rate is increased (Kingsley, Littlejohns & Prichard, 1972). In the present study, isoprenaline infusions did not appear to protect hypoxic dogs from the cardiotoxicity of isoprenaline challenge. Indeed, some of the animals succumbed to the effect of the infusion itself, before any further isoprenaline challenge was given, once they became hypoxic. No evidence of significant resistance was seen, measured in terms of protection from mortality.

Conolly *et al.* (1971) reported that pretreatment of guinea-pigs with sympathomimetic agents increased mortality provoked by histamine and it seemed to be the development of resistance by respiratory β_2-adrenoceptors in humans about which the authors were principally concerned. However, Pun, McCulloch & Rand (1971), also using histamine-induced bronchospasm in guinea-pigs, could find no evidence of the development of tachyphylaxis with prolonged infusions of sympathomimetic amines, including isoprenaline. Although the investigation of tolerance to the respiratory effects of these drugs in man is still in progress, Parker, Choo-Kang, Cooper, Cameron & Grant (1971) have shown that oral salbutamol maintains its effectiveness, measured by increase in peak expiratory flow rate, for at least four weeks in patients with chronic asthma.

In addition, on theoretical grounds, it might be expected that the development of resistance to β-adrenoceptor stimulants by prolonged exposure to bronchodilator aerosols would produce gradual deterioration of respiratory function rather than sudden death.

The relationship between the rise in death rate from asthma and the use of bronchodilator pressurized aerosols of β-adrenoceptor agonists now seems to be established (British Medical Journal, 1972). However, it would appear that the mechanism by which these drugs caused death still lacks definitive explanation and that serious doubt must now be cast on the relevance of resistance to β-adrenoceptor stimulants. The present study would suggest that the cardiotoxic effects of isoprenaline in hypoxic situations is worth further consideration.

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