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LACK OF CARDIAC OR BRONCHODILATOR TACHYPHYLAXIS TO ISOPRENALINE IN THE DOG

H. MINATOYA & B.A. SPILKER

Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144

1 Tachyphylaxis to heart rate and bronchodilator effects of (±)-isoprenaline was studied in anaesthetized open-chest dogs by 5 procedures.

2 Heart rate responses to a series of intravenous injections of isoprenaline were essentially unchanged before and after a 15 min infusion of isoprenaline (at 3 doses).

3 Heart rate and bronchodilator responses in the same animal to an intravenous injection of isoprenaline were not significantly different before and after a 30 min infusion of isoprenaline (at 2 doses).

4 Heart rate responses were relatively constant to an isoprenaline injection given every 30 min during a 4 h infusion of isoprenaline (at 3 doses), but the magnitude of the response was smallest for the largest infusion dose (highest background heart rate) and greatest for the smallest infusion dose (lowest background heart rate).

5 Heart rate and bronchodilator responses in the same animal to isoprenaline were relatively constant during a 5 h infusion of isoprenaline (at 2 doses).

6 Bronchodilator responses to intratracheally administered isoprenaline aerosol were essentially unchanged during a 4-5 h period using various doses and procedures.

7 Tachyphylaxis to the heart rate or bronchodilator effects of isoprenaline was not observed. The present data give no support to the hypothesis that tachyphylaxis to isoprenaline aerosols is an important mechanism in asthma mortality.

Introduction

Normal subjects have been reported to have a smaller heart rate increase to injected isoprenaline after receiving infusions of small amounts of isoprenaline (Conolly, Davies, Dollery & George, 1972). Kingsley, Littlejohns & Prichard (1972) also studied heart rate increases in normal subjects to isoprenaline and concluded that there was no tachyphylaxis in terms of the dose required to produce a given peak heart rate. Tachyphylaxis to heart rate effects of isoprenaline has also been reported in dogs (Conolly *et al.*, 1971) and cats (Atkinson & Rand, 1968).

Some asthmatic patients were reported to have no bronchodilator response to nebulized isoprenaline (Reisman, Friedman & Arbesman, 1968; Van Metre, 1969; Herxheimer, 1969, 1972a). It is not certain whether this lack of response was due to tachyphylaxis or to other causes (see Discussion). Tachyphylaxis to the bronchodilator effects of isoprenaline was observed in *in vivo* guinea pig experiments by Conolly *et al.* (1971) and Bouhuys, Douglas & Lewis (1972), but not by Pun, McCulloch & Rand (1971).

The purpose of the present investigation was to study the development of tachyphylaxis to intravenous isoprenaline by measuring both heart rate and bronchodilator effects in the same animal. Bronchodilator effects were also measured during repeated doses of an isoprenaline aerosol. A preliminary report of this work has been presented (Minatoya & Spilker, 1973).

Methods

A total of 81 mongrel dogs (9 to 13 kg) of either sex was anaesthetized with pentobarbitone Na (30 mg/kg, i.v.) followed 30 min later by morphine sulphate (3 mg/kg, i.m.). The open-chest dog was maintained by artificial respiration (14 to 22 strokes/min of 250 ml each). A specially designed non-rebreathing plastic valve was modified from one previously reported (Minatoya & Luduena, 1967) and was attached to a tracheal cannula so that expired air returned to the atmosphere. Previously conducted experiments using the non-rebreathing valve, demonstrated that open-chest dogs under artificial respiration gave

more consistent recordings of intratracheal pressure, bronchoconstriction and bronchodilation than did closed-chest dogs. CO_2 in expired air was continuously monitored in previous as well as present experiments by the Beckman model LB-1 CO_2 gas analyser and was in the range of 4.0 to 5.0%. A volume of 250 ml was used to respire dogs since this volume maintained blood pressure, heart rate and CO_2 in expired air at a nearly constant level throughout a 6 h experiment in many other dogs. A Statham P23B pressure transducer was attached to the side arm of the tracheal cannula and the intra-tracheal pressure recorded on a Grass Model 7 polygraph. Aerosols were delivered directly into the trachea during inspiration through an actuator attached to the cannula. Heart rate was monitored with a Sanborn model 300 portable ECG apparatus using lead II. Blood pressure was measured from the right femoral artery by a Statham P23A pressure transducer and recorded on the polygraph. The left femoral vein was cannulated for infusions and the right femoral vein cannulated for injections.

Bronchoconstriction was induced with histamine diphosphate. Doses ranged from 25 to 50 μg/kg i.v., but were kept constant during each experiment. The degree of bronchoconstriction was determined by measuring the area of the intratracheal pressure recordings above the baseline for a 5 min period after the histamine injection. Paper speed was 10 mm/min and a planimeter was used to measure this area. Bronchodilation was expressed as percentage inhibition of the two control histamine-induced bronchoconstrictions.

Drugs used were (±)-isoprenaline hydrochloride (Sterling-Winthrop) and histamine diphosphate (Schwarz-Mann). All doses are in terms of base. Drug solutions for injection were prepared daily in distilled water and kept on ice. Isoprenaline solutions for infusion were prepared in 0.9% w/v NaCl (saline) and infused at a rate of 0.1 ml/min using a Harvard model 600 constant infusion apparatus with a 10 ml syringe. Pressurized aerosol solutions of isoprenaline, delivering 63 or 250 μg per actuation, were used.

Analysis of variance determinations were performed on a PDP-15 computer (Digital Equipment).

Results

Heart rate responses to isoprenaline before and after a 15 min infusion of isoprenaline

The heart rate response to injections of different doses of isoprenaline was determined before and after its infusion for 15 minutes. Three injection doses of isoprenaline (0.0125, 0.05 and 0.2 μg/kg) were tested and three different infusions were given (0.007 μg kg^{-1} min^{-1}, $n = 5$; 0.021 μg kg^{-1} min^{-1}, $n = 6$; and 0.063 μg kg^{-1} min^{-1}, $n = 6$). Sufficient time was allowed between each of the three injections (10 to 15 min) for the heart rate to approximate control. After a 15 min infusion, the heart rate was allowed to return to within 10 beats of the control value just prior to infusion before the second dose-response was begun (usually 10 to 15 minutes). Each dose-response procedure took 30 to 40 min to perform. Heart rate increases to isoprenaline obtained before and after each isoprenaline infusion were essentially unchanged (Table 1).

Bronchodilation and heart rate responses to isoprenaline before and after a 30 min infusion of isoprenaline

Heart rate and bronchodilator responses to injections of isoprenaline (1 μg/kg, i.v.) obtained

Table 1 Heart rate responses to isoprenaline (i.v.) before and after a 15 min infusion of isoprenaline.

Infusion rate	*Pre-infusion increase in heart rate (beats/min)*			*Post-infusion increase in heart rate (beats/min)*		
(μg kg^{-1} min^{-1})	*0.0125 μg/kg*	*0.05 μg/kg*	*0.2 μg/kg*	*0.0125 μg/kg*	*0.05 μg/kg*	*0.2 μg/kg*
0.007	13 ± 2 (100)	42 ± 6 (104)	92 ± 17 (104)	16 ± 3 (113)	42 ± 9 (113)	90 ± 17 (111)
0.021	20 ± 3 (117)	48 ± 5 (121)	101 ± 10 (123)	24 ± 2 (128)	56 ± 6 (127)	94 ± 5 (129)
0.063	16 ± 3 (118)	59 ± 9 (112)	86 ± 13 (112)	17 ± 3 (128)	48 ± 10 (126)	77 ± 15 (125)

The numbers in parentheses are the heart rates just prior to the isoprenaline injection. Each value ± s.e.m. is derived from 5 or 6 experiments. The post-infusion increase in control heart rate was not significant when analysed by a paired *t* test.

in the same animal before and after isoprenaline infusions for 30 min were determined (Table 2). Two doses of isoprenaline were infused (0.007 μg kg^{-1} min^{-1}, $n = 6$; and 0.021 μg kg^{-1} min^{-1}, $n = 6$). Histamine was injected 45 s after the isoprenaline injection to allow time for the maximum increase in heart rate to be observed. Linear contrasts within a row by column analysis of variance demonstrated that there were no significant differences ($P > 0.05$) between the 10 and 30 min values for heart rate. Also, there were no significant differences between these values and the mean value of control heart rate ($P > 0.05$). Similarly, there were no significant differences in the degree of bronchodilation before and after the infusion of isoprenaline.

The control heart rates of dogs used in this experiment were higher than those used in experiments reported in Table 1. This may be due to the fact that the two experiments were conducted at different times of the year with dogs of different breeds.

Heart rate responses during a 4 h infusion of isoprenaline with a concomitant injection of isoprenaline every 30 minutes

Heart rate responses during a 4 h infusion of isoprenaline and to concomitant injections of isoprenaline (0.05 μg/kg i.v.) every 30 min during the infusion are shown in Figure 1. Three infusion doses were given ($n = 4$ per dose) and there were dose-related increases in heart rate which reached a plateau in approximately 15 minutes. The elevated heart rates remained relatively constant throughout the infusion period. Heart rate increases to single injections of isoprenaline given on top of the infusion also remained relatively constant and the amplitude was dependent upon the heart rate level (Figure 1). An infusion of 0.175 μg kg^{-1} min^{-1} caused the heart rate to plateau at approximately 220 beats/minute. At this heart rate, an increase of only 5 to 10 beats/min was obtained to isoprenaline injections, whereas an increase of 38 to 47 beats/min was obtained with the lowest infusion dose (0.007 μg kg^{-1} min^{-1}) where the heart rate was about 120 beats/minute.

Bronchodilation and heart rate responses during a 5 h infusion of isoprenaline

Heart rate and bronchodilator effects were studied concurrently in the same animal during a 5 h infusion. Responses to histamine were determined before, 5 min after and at hourly intervals during the infusion of isoprenaline. Heart rates were monitored 5 to 10 min prior to each injection of histamine. No decrease of the bronchodilator

Table 2 Heart rate and bronchodilator responses to isoprenaline (1 μg/kg, i.v.) before and after a 30 min infusion of isoprenaline.

	Heart Rate				*Bronchodilation*			
Infusion rate (μg kg^{-1} min^{-1})	*Pre-infusion* (beats/min)	*10 min. Post-infusion* (beats/min)	*30 min. Post-infusion* (beats/min)	*s.e.m.*	*Pre-infusion* (%)	*10 min. Post-infusion* (%)	*30 min. Post-infusion* (%)	*s.e.m.*
0.007	73 (154 ± 7)	68 (157 ± 7)	65 (160 ± 11)	±5.0	51	43	46	±2.5
0.021	88 (151 ± 13)	78 (155 ± 13)	76 (159 ± 10)	±4.3	53	44	40	±10.3

The numbers in parentheses are the heart rates ± s.e.m. ($n = 6$) just prior to the isoprenaline injection. The s.e.m. values for heart rate increases and % bronchodilation are based on pooled variance estimates from analysis of variance.

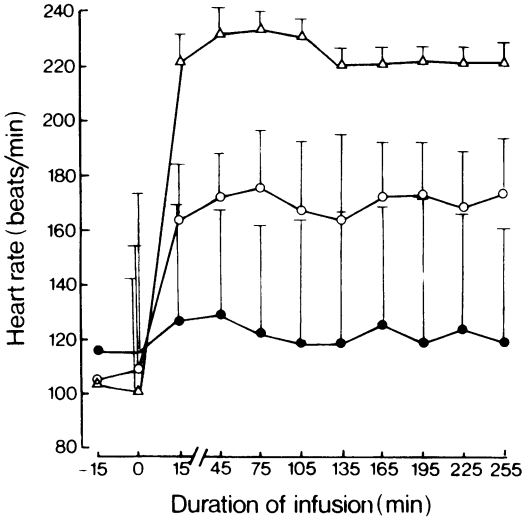

Figure 1 Heart rate responses to infusions of isoprenaline and to single injections of isoprenaline (0.05 μg/kg) every 30 min during the infusion. The effect of each injection of isoprenaline is shown as an upward line. (△) Isoprenaline (0.175 μg kg^{-1} min^{-1}, $n = 4$); (○) Isoprenaline 0.035 μg kg^{-1} min^{-1}, $n = 4$); (●) Isoprenaline (0.007 μg kg^{-1} min^{-1}, $n = 4$).

response to isoprenaline was observed (Figure 2). A consistent bronchodilation of about 75% was maintained for 5 h with the larger infusion dose (0.175 μg kg^{-1} min^{-1}, $n = 7$) and a bronchodilation of 15-30% with the smaller dose (0.007 μg kg^{-1} min^{-1}, $n = 4$). The elevated heart rate remained constant throughout the infusion period. Substituting physiological saline for histamine during the isoprenaline infusion did not alter the heart rate effect (Figure 2, $n = 4$).

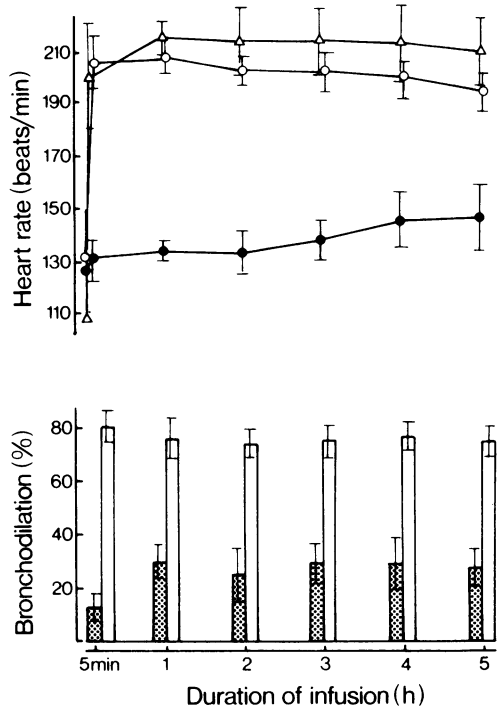

Figure 2 The upper trace shows heart rate increases ± s.e.m. to infusions of isoprenaline where injections of histamine or saline were made each hour. (△) Isoprenaline (0.175 μg kg^{-1} min^{-1}) with saline injections ($n = 4$); (○) Isoprenaline (0.175 μg kg^{-1} min^{-1}) with histamine injections ($n = 4$); (●) Isoprenaline (0.007 μg kg^{-1} min^{-1}) with histamine injections ($n = 4$). The lower trace shows bronchodilator effects (% antagonism of the histamine-induced bronchoconstriction) in the same animals. Open columns, isoprenaline (0.175 μg kg^{-1} min^{-1}); stippled columns, isoprenaline (0.007 μg kg^{-1} min^{-1}).

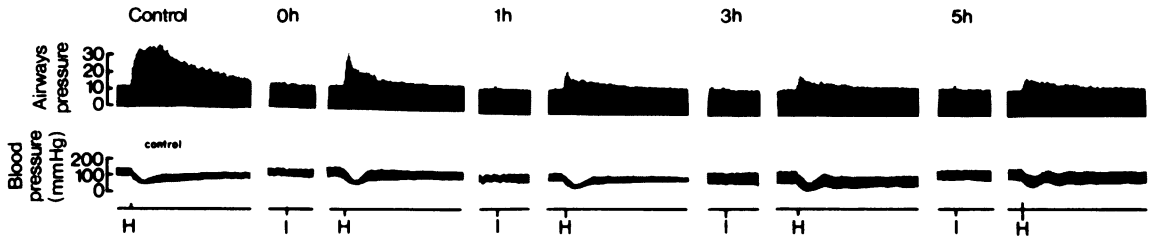

Figure 3 Bronchodilator effects of repeated intratracheal administrations of isoprenaline aerosol in an 11.2 kg female dog. A dose of 500 μg (2 actuations) was given each hour (I). Airways pressure is recorded as mm from polygraph trace. Histamine (45 μg/kg i.v.) was tested hourly 15 min after each aerosol medication (H). Respiration frequency was 15/minute.

Table 3 Bronchodilator effects of repeated intratracheal administrations of isoprenaline aerosol.

Isoprenaline Dose (μg)	Frequency (min)	Bronchodilation (%) at various times (min) after the first dose																
		2	15	45	62	75	80	105	122	135	165	180	182	195	225	242	255	280	302	315	320
250	Every 30		30	25		23		42		23	36			46	44						
500	Every 30		46	48		54		56		61	52			57	60		57				
1000	Every 30		47	51		53		52		59	58			48	54		62				
250	Every 60		12			29				28				28			26			61	
500	Every 60		58			69				52				68			58				
1000	Every 60		52			47				50				60			67			66	
2000	Every 60		72			74				71				72			71				
1000	Every 60	82			85				82				88			87			85		
2000	Every 60	97			95				95				88			90			89		
250	Every 20						30					32						33			32
63	Every 20						17					20						19		14	

Each value of bronchodilation represents percent inhibition of the histamine (i.v.) challenge.
Histamine injections were given at 2, 15, or 20 min after each medication.
The actuator delivered 250 μg/actuation except in the last experiment where it was 63 μg/actuation.
Each experiment was performed in 2-4 dogs (total = 25 dogs) and results were averaged.

Bronchodilation studies during repeated aerosol medications of isoprenaline

Figure 3 shows a polygraph tracing obtained in a dog (11.2 kg) receiving 500 μg of isoprenaline aerosol (2 actuations) each hour for 5 hours. The histamine challenge was given 15 min after each aerosol medication. At the initial measurement (zero hour) there was a 68% bronchodilation. Thereafter, consistent bronchodilations of about 75% were obtained.

Table 3 summarizes the aerosol results. Doses ranging from 63 to 2000 μg (1-8 actuations) were administered every 20 to 60 min for 4-5 hours. The intravenous histamine challenge was given 2, 15 or 20 min after each aerosol administration and the results were expressed as percentage inhibition (bronchodilation) of the control histamine response. No evidence of bronchodilator tachyphylaxis was observed ($n = 25$). There were no differences in the results irrespective of whether histamine was injected 2 or 15 min after each aerosol actuation(s) except that the degree of bronchodilation was greater when tested at 2 minutes.

Discussion

The present results showed no evidence of bronchodilator or heart rate tachyphylaxis to isoprenaline in healthy dogs using various experimental procedures. Conolly *et al.* (1971) reported that tachyphylaxis occurred to isoprenaline in dogs. This discrepancy could be due to differences in experimental conditions. For example, Conolly *et al.* (1971) used closed-chest dogs not under artificial respiration. The average dose of isoprenaline required to increase heart rate by 40 beats/min in control dogs was greater than 1.0 μg/kg in their experiments, but less than 0.05 μg/kg in the present experiments. Other reported values for this effect are about 0.1 μg/kg (Kofi Ekue, Shanks & Zaidi, 1971) and 0.7 μg/kg (Daly, Farmer & Levy, 1971). These doses are in terms of base.

There is considerable controversy concerning the cause of the increased death rate in asthmatic patients experienced in certain countries during the 1960's (Harris, 1971; Stolley, 1972; Silverglade, 1972; Herxheimer, 1972b; Gandevia, 1973; Spilker, 1973). Conolly *et al.* (1971) suggested that tachyphylaxis to the β-adrenoceptor effects of nebulized bronchodilators and a drug-induced cross tachyphylaxis to endogenous sympathetic stimulation could have led to deterioration of the asthmatic patient. One of us has previously questioned this hypothesis (Spilker, 1973).

Conolly *et al.* (1971) were unable to explain their finding that an infused dose of only 0.01 to 0.86 μg/kg isoprenaline caused a significant shift of the isoprenaline dose-response curve to the right in 9 of 11 dogs and caused a nearly 3-fold increase in the dose of isoprenaline required to increase the heart rate by 40 beats/minute. In humans, an infused dose of 0.055 to 0.45 μg/kg isoprenaline also caused a significant shift of the dose-response curve in 6 of 7 subjects and more than doubled the dose required to increase heart rate by 20 beats/minute. The magnitude of these effects was *not* related to the size of the infused dose. The infused dose of isoprenaline in our study varied from 0.105 to 52.50 μg/kg, but in no case was tachyphylaxis observed, either during the infusion or to a dose-response relationship for isoprenaline determined after the infusion.

Paterson, Conolly, Davies & Dollery (1968) reported that normal subjects experienced tachycardia to therapeutic doses of isoprenaline aerosol whereas asthmatic patients did not. However, it has often been shown that heart rate increases to isoprenaline aerosol are minimal or absent in asthmatics while bronchodilator responses are marked (Hambleton & Shinebourne, 1970; Streeton, 1970; Riding, Chatterjee, Bernstein & Dinda, 1971; Freedman & Hill, 1971). Further, Paterson *et al.* (1968) did not examine bronchodilator responses in their group of asthmatic patients. Thus, even if tachyphylaxis to heart rate effects of isoprenaline were valid, it could not be used to support the view that bronchodilator tachyphylaxis was also present and then to suggest that the observed rise in asthma deaths in England and Wales could be explained, as did Conolly *et al.* (1971), by tachyphylaxis to isoprenaline. Morton & Ostensoe (1963) and Pierson & Grieco (1969) reported that heart rate responses of normals and asthmatics to inhaled isoprenaline were equal. Cookson & Reed (1963) reported that there was no significant difference between heart rate responses of normals and asthmatics to three doses of infused (i.v.) isoprenaline. Kingsley *et al.* (1972) were unable to demonstrate tachyphylaxis to the acute heart rate effects of isoprenaline in normal human subjects.

Atkinson & Rand (1968) observed decreased heart rate effects to injected isoprenaline (as well as to other catecholamines) given during an isoprenaline infusion in cats (Figure 5 of their paper). The heart rate response to injected isoprenaline was also decreased when it was injected during orciprenaline infusion (Figure 6 of their paper). These reduced responses to isoprenaline injections would be expected on the basis of the increased background heart rates observed during the infusion and are not an example of tachyphylaxis. The reduced heart rate response to injected isoprenaline given while the heart rate was elevated is also seen in Figure 1 of this paper. A similar observation has been made in man (Kingsley *et al.*, 1972).

The possibility of isoprenaline-induced bronchodilator tachyphylaxis has been previously studied. Conolly *et al.* (1971) reported that guinea-pigs developed tachyphylaxis to isoprenaline after repeated injections over 5 hours. Tachyphylaxis was shown by the increased mortality of isoprenaline treated guinea-pigs to histamine challenge. This study was confirmed by Bouhuys *et al.* (1972). However, the increased mortality was statistically significant in only one of three groups of guinea-pigs treated with isoprenaline in both the study of Conolly *et al.* (1971) and that of Bouhuys *et al.* (1972). The degree of mortality after histamine may have been influenced by haemodynamic changes caused by the doses of isoprenaline used (4 μg/kg i.m.—every 20 min for 5 h in the study of Bouhuys *et al.* and every 20 to 45 min for 5 h in the study of Conolly *et al.*). Thus the relevance of these results to isoprenaline tachyphylaxis will be better understood when haemodynamic data are available. No evidence of tachyphylaxis to bronchodilator responses was observed upon repeated isoprenaline injections during a 2.5 h isoprenaline infusion in guinea-pigs (McCulloch, Pun & Rand, 1968: Pun *et al.*, 1971). Repeated applications of isoprenaline in concentrations greater than 5×10^{-5} M did *not* consistently produce tachyphylaxis in more than 50 tracheal preparations from both young and old rats (Fleisch & Titus, 1972). Sobol, Emirgil, Wadhwani & Goyal (1972) followed 32 patients with chronic obstructive pulmonary disease for 4 to 32 months (average 18 months) who used an isoprenaline nebulizer at least 5 times/day and found that bronchodilator effects did not diminish. Ayres (1973) studied 350 patients over a 4 year period and did not observe any evidence of tachyphylaxis to nebulized isoprenaline.

There are several reports of patients in status asthmaticus who do not respond favourably to sympathomimetic bronchodilators (Keighley, 1966; Reisman *et al.*, 1968; Van Metre, 1969). There are also some patients whose condition may deteriorate when such aerosols are used (Keighley, 1966). It is important to determine whether patients who do not respond to bronchodilator aerosols are tolerant to their effects because of over-use or for other reasons. Although this question cannot be definitively answered at present, there is some evidence that asthmatics are not tolerant solely because of aerosol over-use. An editorial in 'The British Medical Journal' (1972) points out that in cases of status asthmaticus

'Bronchodilator aerosols have ceased to be effective' and 'Sympathomimetic bronchodilators, by inhalation or by injection, should be avoided unless oxygen can be administered at the same time.' Possible reasons to explain the lack of effect obtained with bronchodilators in severe asthma include: impaired ventilation/perfusion ratios due to abnormal blood gas tensions which isoprenaline does not always correct (Palmer & Diament, 1967; Waddell, Emerson & Gunstone, 1967) and severe mucous plugs in the bronchi which are unaffected by isoprenaline (Harris, 1972). Severely asthmatic patients thus may reach a bronchodilator tolerant stage, independent of any prior use of aerosols.

Our conclusion, therefore, is that neither heart rate nor bronchodilator tachyphylaxis to isoprenaline has been unequivocally demonstrated in animals or man.

Note added in proof:
After this paper went to press, another paper appeared (McDevitt, Shanks & Swanton, *Br. J. Pharmac.* (1974), **50**, 335-344) in which no evidence of significant tachyphylaxis to isoprenaline in dogs was observed.

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References

ATKINSON, J.M. & RAND, M.J. (1968). Mutual suppression of cardiovascular effects of some β-adrenoceptor agonists in the cat. *J. Pharm. Pharmac.*, **20**, 916-922.

AYRES, S.M. (1973). The laboratory evaluation of the effects of isoproterenol in the asthmatic patient. *Ann. Allergy*, **31**, 3-8.

BOUHUYS, A., DOUGLAS, J.S. & LEWIS, A.J. (1972). Hypersensitivity to adrenoceptor agents in the guinea pig *in vitro* and *in vivo*. *Br. J. Pharmac.*, **46**, 520-521P.

BRITISH MEDICAL JOURNAL EDITORIAL (1972). Treatment of status asthmaticus. *Br. Med. J.*, **IV**, 563-564.

CONOLLY, M.E., DAVIES, D.S., DOLLERY, C.T. & GEORGE, C.F. (1971). Resistance to β-adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). *Br. J. Pharmac.*, **43**, 389-402.

COOKSON, D.U. & REED, C.E. (1963). A comparison of the effects of isoproterenol in the normal and asthmatic subject. *Am. Rev. Resp. Dis.*, **88**, 636-643.

DALY, M.J., FARMER, J.B. & LEVY, G.P. (1971). Comparison of the bronchodilator and cardiovascular actions of salbutamol, isoprenaline and orciprenaline in guinea pigs and dogs. *Br. J. Pharmac.*, **43**, 624-638.

FLEISCH, J.H. & TITUS, E. (1972). The prevention of isoproterenol desensitization and isoproterenol reversal. *J. Pharmacol. exp. Ther.*, **181**, 425-433.

FREEDMAN, B.J. & HILL, G.B. (1971). Comparative study of duration of action and cardiovascular effects of bronchodilator aerosols. *Thorax*, **26**, 46-50.

GANDEVIA, B. (1973). Pressurized sympathomimetic aerosols and their lack of relationship to asthma mortality in Australia. *Med. J. Aust.*, **1**, 273-277.

HAMBLETON, G. & SHINEBOURNE, E.A. (1970). Evaluation of the effects of isoprenaline and salbutamol aerosols on airway obstruction and pulse rates of children with asthma. *Arch. Dis. in Childhood*, **45**, 766-768.

HARRIS, M.C. (1971). Are bronchodilator aerosol inhalations responsible for an increase in asthma mortality? *Ann. Allergy*, **29**, 250-256.

HARRIS, M.C. (1972). Isoproterenol in chronic asthmatic patients who do not require hospital treatment. *Ann Allergy*, **30**, 315-320.

HERXHEIMER, H. (1969). Asthma mortality and pressurized aerosols. *Lancet*, **ii**, 642-643.

HERXHEIMER, H. (1972a). Asthma deaths. *Lancet*, **i**, 98.

HERXHEIMER, H. (1972b). Asthma deaths. *Br. Med. J.*, **II**, 795.

KEIGHLEY, J.F. (1966). Iatrogenic asthma associated with adrenergic aerosols. *Ann. Int. Med.*, **65**, 985-995.

KINGSLEY, P.J., LITTLEJOHNS, D.W. & PRICHARD, B.N.C. (1972). Isoprenaline-induced tachycardia in man. *Br. J. Pharmac.*, **46**, 539P-540P.

KOFI EKUE, J.M., SHANKS, R.B. & ZAIDI, S.A. (1971). Comparison of the effects of isoprenaline, orciprenaline, salbutamol and isoetharine on the cardiovascular system of anaesthetized dogs. *Br. J. Pharmac.*, **43**, 23-31.

McCULLOCH, M.W., PUN, L.-Q. & RAND, M.J. (1968). Deaths from Asthma. *Br. Med. J.*, **II**, 429.

MINATOYA, H. & LUDUENA, F.P. (1967). Effects of propranolol and guanethidine on the bronchodilation induced by sympathomimetic agents and by stimulation of the stellate ganglia in the anaesthetized dog. *Fed. Proc.*, **26**, 293.

MINATOYA, H. & SPILKER, B.A. (1973). Lack of cardiac or bronchiolar tolerance to isoproterenol in the anaesthetized dog. *The Pharmacologist*, **15**, 217.

MORTON, J.W. & OSTENSOE, L.G. (1963). A comparative study of aerosol, oral and intravenous administration of bronchodilators in asthma, with the use of isoproterenol (isuprel), Th 152, and aminophylline. *J. Allergy*, **34**, 16-25.

PALMER, K.N.V. & DIAMENT, M.L. (1967). Effect of aerosol isoprenaline on blood-gas tensions in severe bronchial asthma. *Lancet*, **ii**, 1232-1233.

PATERSON, J.W., CONOLLY, M.E., DAVIES, D.S. & DOLLERY, C.T. (1968). Isoprenaline resistance and the use of pressurized aerosols in asthma. *Lancet*, **ii**, 426-429.

PIERSON, R.N. Jr. & GRIECO, M.H. (1969). Isoproterenol aerosol in normal and asthmatic subjects. Time relationship of pulmonary and hemodynamic responses. *Am. Rev. Resp. Dis.*, **100**, 533-541.

PUN, L.-Q., McCULLOCH, M.W. & RAND, M.J. (1971). Bronchodilator effects of sympathomimetic amines given singly and in combination. *Eur. J. Pharmac.*, **14**, 140-149.

REISMAN, R.E., FRIEDMAN, I. & ARBESMAN, C.E. (1968). Severe status asthmaticus: prolonged treatment with assisted ventilation. *J. Allergy*, **41**, 37-48.

RIDING, W.D., CHATTERJEE, S.S., BERNSTEIN, A. & DINDA, P. (1971). A comparison of the cardiorespiratory effects of salbutamol and isoproterenol-phenylephrine aerosols in asthma. *Am. Rev. Resp. Dis.*, **104**, 688-693.

SILVERGLADE, A. (1972). Changing mortality from bronchial asthma. *Aerosol Age*, **17**, 24-27, 62.

SOBOL, B.J., EMIRGIL, C., WADHWANI, B. & GOYAL, P. (1972). Simple ventilatory tests for assessing response to bronchodilator in chronic obstructive pulmonary disease. *Anesth. Analg.*, **51**, 21-26.

SPILKER, B. (1973). Increase in asthma mortality. *Br. Med. J.*, **4**, 171-172.

STOLLEY, P.D. (1972). Asthma mortality. Why the United States was spared an epidemic of deaths due to asthma. *Am. Rev. Resp. Dis.*, **105**, 883-890.

STREETON, J.A. (1970). Salbutamol: a double-blind crossover trial. *Med. J. Aust.*, **2**, 1184-1187.

VAN METRE JR., T.E. (1969). Adverse effects of inhalation of excessive amounts of nebulized isoproterenol in status asthmaticus. *J. Allergy*, **43**, 101-113.

WADDELL, J.A., EMERSON, P.A. & GUNSTONE, R.F. (1967). Hypoxia in bronchial asthma. *Br. Med. J.*, **II**, 402-404.

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