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TACHYPHYLAXIS TO β-ADRENOCEPTOR AGONISTS IN HUMAN BRONCHIAL SMOOTH MUSCLE: STUDIES *IN VITRO*

C. DAVIS* & M.E. CONOLLY†

Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12

In studies on human isolated peripheral airway smooth muscle;

1 A concentration dependent β-adrenoceptor tachyphylaxis was observed to isoprenaline.

2 Cross desensitization to other β-adrenoceptor agonists was demonstrated.

3 The desensitization was reversible with time. Hydrocortisone appeared to accelerate the recovery from the desensitized state. Low concentration isoprenaline (10^{-9} mol l^{-1}) prevented recovery whereas cycloheximide 1.8×10^{-4} mol l^{-1} had no noticeable effect on recovery. Continued occupancy of the receptor appears to prevent recovery. The recovery from the desensitized state does not apparently require synthesis of new proteins.

4 Bronchial wall cyclic AMP response to isoprenaline was attenuated after isoprenaline induced desensitization whereas total phosphodiesterase activity of bronchial wall was not altered by desensitization. Thus by exclusion the adenylate cyclase receptor complex may be altered in human peripheral airway smooth muscle β-adrenoceptor tachyphylaxis.

Introduction

Considerable controversy exists over the occurrence and importance of β-adrenoceptor desensitization in asthmatic patients. A number of clinical reports have suggested that adrenergic bronchodilator aerosols may lead to a refractory state if they are used excessively (Keighley, 1966; Van Metre, 1969). Several clinical studies have provided data relevant to this problem, but the results have been conflicting. Thus, Choo-Kang, Simpson & Grant (1969) noted a diminution in response to inhaled salbutamol with repeated administration in asthmatic patients. Paterson, Courtnay Evans & Prime (1971) observed a marked rebound bronchoconstriction in 4 out of 15 asthmatics following a prolonged infusion of isoprenaline. Jenne, Strickland, Chick & Wall (1976) have observed a diminished β-adrenoceptor response in patients treated for long periods with terbutaline. In normal subjects, Holgate, Baldwin & Tattersfield (1977) demonstrated a waning bronchodilator response to inhalations of salbutamol during a 4 week period of exposure to that drug.

In contrast to these observations, Larsson, Svedmyr & Theringer (1974) demonstrated tachyphylaxis with respect to muscle tremor, but not to bronchodilator response in patients treated for 3 months with oral terbutaline 15 mg daily. Other authors, reporting similar long term studies in patients receiving adrenergic bronchodilators, have also concluded that no significant desensitization to their effects occurs (Formgren, 1976; Gibson, Tattersfield & Pride, 1972). The reasons for such discrepancy are not clear. It may be a reflection of dose of bronchodilator, individual susceptibility to desensitization or other factors such as dosage of concomitantly administered corticosteroids.

Experiments in animals or on animal isolated tissues under-taken to explore this phenomenon have also produced conflicting results, and there is considerable uncertainty about the interpretation of the results obtained (Conolly, Davies, Dollery & George, 1971; Benoy, El-Fellah, Schenider & Wace, 1976; Spilker & Tyll, 1976).

Thus there is a pressing need for studies in human isolated tissues to evaluate the extent of the occurrence of β-adrenoceptor desensitization, in order to evaluate its importance in bronchial asthma. This approach permits study under controlled conditions, free from artefacts such as concomitant administration of other drugs and devoid of problems caused by day to day variation in severity of underlying disease (asthma) (Tinkelman, Avner & Cooper, 1977), and avoids the difficulties of

Present addresses: *Department of Medicine, Hamilton General Hospital, Hamilton, Ontario, Canada.

†Division of Clinical Pharmacology School of Medicine, University of California, Los Angeles, California 90024, USA.

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interpretation which may arise when drugs exert indirect effects on the organ or tissue being studied—e.g., by centrally mediated reflex effects.

Methods

Lung tissue was obtained from patients undergoing surgery for bronchial carcinoma. These patients had no clinical or spirometric features of asthma. Macroscopically normal tissue was obtained from the surgical specimen, usually within 20 min of removal from the chest. It was then placed in ice cold Krebs-Henseleit solution which had previously been gassed with 95% O_2, 5% CO_2 and thereafter sealed.

Bronchi of 2–4 mm diameter were dissected free of parenchymal tissue and cut into spiral strips 2 mm wide and 2 to 3 cm long, by the method of Constantine (1965). It was found by experience that the muscle strips responded poorly on the day of resection, but performed well, if allowed to 'settle' overnight. Accordingly, each strip was left for 14 ± 2 h in Krebs-Henseleit solution at 4°C.

The strips were then hung in conventional 5 ml organ baths with a water jacket to maintain the temperature at 37 ± 0.1°C. The organ bath was filled with continuously gassed Krebs-Henseleit solution. The strips were hung under the presumed optimal passive tension of 200 mg (Stephens, Meyers & Cherniack 1968), using an isotonic optical density wedge transducer (Scientific Research Instruments Ltd, Model 7043). The output from these transducers was fed into the amplifiers of a Devices M4 recorder and the signals were recorded on a multichannel chart for subsequent analysis.

The tissues were allowed to equilibrate under these conditions for 60–90 min before any drugs were added. At the end of this time, the positions of the transducers were adjusted to restore the transducer arms to the horizontal position. The strips were then contracted with histamine, using a range of concentrations (10^{-9} to 10^{-4} mol l^{-1}) to produce a cumulative dose-response curve, and to establish for each tissue, the concentration (EC_{90}) of histamine required to produce 90% of the maximum attainable contraction. The 90% response was used as the contraction against which β-adrenoceptor agonists could be tested. In a separate study, it was shown that the contractile response to any given concentration of histamine was fully maintained for at least 45 min. Using the EC_{90} generated contraction, a cumulative dose-response relaxation curve for isoprenaline was then obtained. The next step in the procedure was the attempt to produce β-adrenoceptor desensitization. The tissues were once more washed and were then incubated with isoprenaline 10^{-6} or 4×10^{-4} mol l^{-1} for 1 h. Therafter, the tissues were washed and allowed to re-equilibrate for 1 h in drug-free Krebs-Henseleit solution. Following this, the original EC_{90} of histamine was added to the bath and a further isoprenaline dose-response curve was generated. At the end of this second dose-response curve, EDTA (4 mg/ml, final concentraion) was added to some of the baths to bring about full relaxation of the tissues in order to demonstrate that a comparable degree of contraction had been present at the start of both pre- and post-incubation dose response curves (Figure 1). Cumulative dose-response expressed as a percent of the maximal isoprenaline (10^{-5} mol l^{-1}) response curves for noradrenaline, adrenaline and salbutamol were also examined before and after incubation with isoprenaline. The response of each strip to these agents before and after isoprenaline incubation was expressed as a percent of the pre-isoprenaline incubation response to isoprenaline 10^{-5} mol l^{-1}. The pre- and post-incubation dose-response curves of strips from 15 lungs were compared, by t-test, and compared with the control response of strips from the same lung not incubated in isoprenaline with dose-response curves of control strips obtained from the same lung, but which had been incubated for the one hour period in Krebs-Henseleit solution without isoprenaline.

In addition, 3 of the 6 strips from each of 15 separate lungs were incubated overnight and for the duration of the above protocol, with hydrocortisone (1.5×10^{-4} mol l^{-1}).

In some of the experiments, the muscle strips were repeatedly washed after the post-incubation dose-response curve, and then left for a further hour to assess the degree of recovery of β-adrenoceptor responsiveness.

In a further series of experiments, the effect of a persistent low concentration of isoprenaline (10^{-9} mol l^{-1}) on restoration of responsiveness during the 'recovery' phase, was examined in muscle from 3 lungs.

In an attempt to define the mechanism of tachyphylaxis, we measured cyclic AMP generation in bronchial strips from 3 lungs, using a modified Gilman method, as previously described (Conolly & Greenacre, 1977). Bronchial strips were incubated in Krebs-Henseleit alone or with isoprenaline 4×10^{-4} mol l^{-1} for 1 h. The strips were then washed, repeatedly, in fresh gassed Krebs at 37°C. (It was found necessary to wash with 1 litre, using 15 cycles over 1 h to allow cyclic AMP levels in the isoprenaline treated strips to return to control levels seen prior to the isoprenaline dose response) and then incubated for 15 min with 10^{-8}, 10^{-6} and 10^{-4}M isoprenaline in the presence of 10^{-2} mol l^{-1} theophylline. The strips were boiled, homogenized and centrifuged. Aliquots of supernatant were taken for cyclic AMP assay.

To investigate the possibility that phosphodiesterase (PDE) activity might be increased

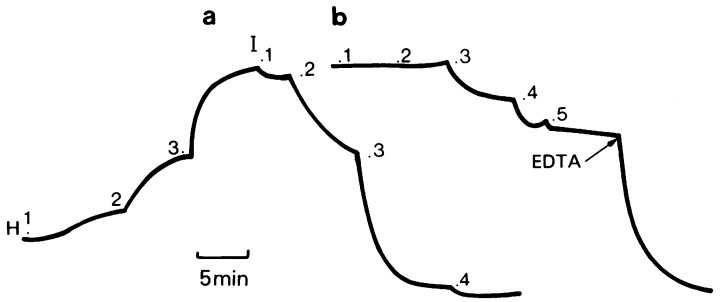

Figure 1 Isoprenaline dose-response curves a) pre- and b) post-4×10^{-4} mol l^{-1} isoprenaline incubation for 1 h. H histamine, I isoprenaline.

in the isoprenaline treated bronchi, muscle strips from 6 lungs were examined. PDE activity was measured by the technique of Thompson & Appleman (1971) as modified by Greenacre, Schofield & Conolly (1978). Bronchial muscle strips, again incubated either in Krebs-Henseleit solution alone, or with isoprenaline 4×10^{-4} mol l^{-1} for 1 h were homogenized and centrifuged. Aliquots of the supernatant were taken for PDE assay. Comparison of PDE activity between control and isoprenaline treated bronchi (expressed as nmol cyclic AMP hydrolyzed mg^{-1} protein $15\ min^{-1}$) was made after the data had been corrected for protein content, by the method of Lowry, Rosebrough, Farr & Randall (1951).

Statistical methods used were the pair and unpaired *t*-test where not specified.

Results

The group of bronchial strips showed a spectrum of susceptibility to desensitization induced by isoprenaline 10^{-6} mol l^{-1}. Between patients there was a wide and significant variation in the susceptibility of bronchi to desensitization ($P < 0.05$); but the variation between strips within the same lung was not significant ($P > 0.10$) on two way analysis of variance.

In contrast, following the 4×10^{-4} mol l^{-1} isoprenaline incubation, desensitization was uniformly present in all lungs studies. One hour after the 10^{-6} mol l^{-1} isoprenaline incubation the maximal isoprenaline response (mean value for all tissues studied) was 78.5% of the control response, whereas after 4×10^{-4} mol l^{-1} isoprenaline it was reduced to 22.1% (Table 1, Figure 1).

Whe the bronchial strips were relaxed with other β-adrenoceptor agonists (adrenaline, noradrenaline, salbutamol) 1 h after incubation with 4×10^{-4} mol l^{-1} isoprenaline marked attenuation of response was noted, indicating the development of cross tachyphylaxis to other β-adrenoceptor agonists (Table 2).

In those strips that were washed repeatedly and left for 2.5 h after the incubation, there was a partial recovery of responsiveness. The degree of recovery in those strips incubated with hydrocortisone was more significant ($P < 0.05$, $n = 6$) than those strips bathed in Krebs-Henseleit solution alone ($P > 0.1$, $n = 6$). The

Table 1 Desensitization of bronchial smooth muscle by incubation with isoprenaline 10^{-6} and 4×10^{-4} mol l^{-1} for 1 h. Values given are % of maximal relaxation (mean ± s.e. mean) induced by isoprenaline prior to desensitization ($n = 15$ lungs).

Isoprenaline concentration (mol l^{-1})	*Control tissues*		*Tissues incubated with isoprenaline* 10^{-6} *mol* l^{-1}		4×10^{-4} *mol* l^{-1}
10^{-9}	3.0 ± 1		1.1 ± 0.4		0
10^{-8}	22.0 ± 2.5	*	7.4 ± 2.0	*	2.5 ± 0.2
10^{-7}	62.0 ± 2.5	*	36.0 ± 8.0	*	4.1 ± 0.5
10^{-6}	85.0 ± 3.5	*	57.5 ± 9.5	*	9.1 ± 1.5
10^{-5}	86.0 ± 2.0	*	67.0 ± 9.0	*	18.5 ± 3.0

Mean ± s.e. ($n = 15$)
(*$P < 0.05$)

Table 2 Desensitization to other β-adrenoceptor agonists by 1 h incubation with isoprenaline 4×10^{-4} mol l^{-1}. Response (1 h after incubation) expressed as % of maximal relaxation (means ± s.e. mean) induced by isoprenaline 10^{-5} mol l^{-1} prior to desensitization.

Salbutamol concentration (mol l^{-1})	*Control response*	*Post-incubation response*	*Adrenaline concentration (mol l^{-1})*	*Control response*	*Post-incubation response*	*Noradrenaline concentration (mol l^{-1})*	*Control response*	*Post-incubation response*
10^{-7}	0	0	10^{-8}	1.6 ± 0.8	0	10^{-7}	0	0
10^{-6}	1.32 ± 0.7	0	10^{-7}	13.4 ± 4.1	0	10^{-6}	1.6 ± 0.6	0
10^{-5}	10.9 ± 2.1	0	10^{-6}	35.5 ± 8.5	0	10^{-5}	12.9 ± 1.8	0
10^{-4}	28.0 ± 3.4	4.8	10^{-5}	73.6 ± 16.5	3.3	10^{-4}	35.2 ± 9.5	0
10^{-3}	48.8 ± 4.0	6.0	10^{-4}	90.3 ± 8.0	3.3	10^{-3}	50.3 ± 10.5	8.5
	$n=5$	$n=2$		$n=5$	$n=2$		$n=5$	$n=2$

Table 3 Influence hydrocortisone (1.5×10^{-4} mol l^{-1}), cyclohexamide (1.8×10^{-4}mol l^{-1}) and isoprenaline (10^{-9} mol l^{-1}) on recovery of lung strips from isoprenaline (4×10^{-4} mol l^{-1}) induced desensitization. Responses are expressed as % (mean ± s.e. mean) of control response to each concentration of isoprenaline.

Isoprenaline concentration (mol l^{-1})	*Response at 60 min*		*Response at 150 min*			
	Krebs alone	*Krebs plus hydrocortisone*	*Krebs alone*	*Krebs plus hydrocortisone*	*Krebs plus cycloheximide*	*Isoprenaline (10^{-9} mol l^{-1})*
10^{-8}	6.5 ± 4.0	4.0 ± 2.0	31.5 ± 21.0 *	44.0 ± 15.0	5.0 ± 1	1.3 ± 1.3
10^{-7}	8.0 ± 5.0	9. ± 5.0	32.4 ± 18.0 *	42.0 ± 3.0	12.9 ± 1.0	3.4 ± 3.0
10^{-6}	12.0 ± 8.0	13.0 ± 6.0	52.0 ± 24.0 *	89.0 ± 20.0	58.0 ± 12.0	10.4 ± 4.0
10^{-5}	17.0 ± 7.0	26.0 ± 13.0	62.0 ± 24.0	96.0 ± 17.0	72.0 ± 10.0 *	13.3 ± 4.0
	$n=15$	$n=15$	$n=6$	$n=6$	$n=4$	$n=4$

*$P < 0.05$

strips incubated with hydrocortisone had regained 96.0% ± 17.0 of their original response, as opposed to 62.0% ± 28.0 in the untreated strips.

The persistent presence for 150 min of a low concentration of isoprenaline (10^{-9} mol l^{-1}) following the isoprenaline 4×10^{-4} mol l^{-1} incubation substantially retarded recovery of β-adrenoceptor responsiveness but the protein synthesis inhibitor cycloheximide (1.8×10^{-4} mol l^{-1}) did not retard recovery (Table 3).

Bronchial wall cyclic AMP response to isoprenaline (10^{-8} and 10^{-6} mol l^{-1}) was significantly blunted by the prior isoprenaline (4×10^{-4} mol l^{-1}) incubation ($P < 0.05$, $n = 4$), there being a roughly three-fold decrease in response (Table 4). Total bronchial wall cyclic AMP phosphodiesterase activity was not altered significantly (t–0.57, $\gamma = 5$, $P > 0.7$), (Table 5).

Discussion

In these experiments, we have demonstrated a concentration dependent desensitization by isoprenaline, of the β-adrenoceptor mechanism in human peripheral bronchial smooth muscle. A similar phenomenon has been observed in other species, notably the dog (Avner & Jenne, 1977) and guinea pig (Benoy *et al.*, 1976). In the rat, however, for technical reasons, such evidence has been difficult to obtain (Fleisch & Titus, 1972). It has also proved difficult to demonstrate desensitization convincingly *in vivo* in asthmatics treated with low doses of β-adrenergic receptor bronchodilator drugs. This may be for many reasons, including the doses used, the inherent variability of the disease and the insensitivity of many spirometric tests used to measure airway function. Using body plethysmography, Holgate *et al.* (1977) have shown a dose and time related decrease in bronchodilator responsiveness in normal subjects. Large doses of adrenergic drugs appear to impair bronchodilator function seriously in some subjects (Keighley, 1966; Van Metre, 1969; Paterson *et al.*, 1971).

Our data show (most markedly at the lower isoprenaline incubation concentration of 10^{-6} mol l^{-1}) that there is considerable variation in the interindividual susceptibility to desensitization. A clinical implication of this observation is that there might be subsets of the asthmatic population particularly liable to develop such resistance. The elimination of a portion of the asthmatic population by means of some constitutional abnormality has already been proposed as an explanation for the rise and fall of the United Kingdom asthma death rate in the 1960's (Inman, 1974).

The mechanism of this desensitization remains obscure. There appears to be a fairly readily reversible component since bronchial strips incubated with isoprenaline 4×10^{-4} mol l^{-1} had recovered over 60% of their original responsiveness within 3 h. Glucocorticoids appear to enhance this reversibility in that treatment with hydrocortisone was associated with a return to full responsiveness within 3 h.

Relaxation of smooth muscle is presumably a cyclic AMP dependent phenomenon, and we have demonstrated that the desensitization produced by isoprenaline incubation is associated with a reduced cyclic AMP response in the peripheral bronchial wall to isoprenaline, as observed in guinea pig, by El-Fellah, Marshall & Turnbull (1976). However, the reason for this diminution has not been elucidated. We have demonstrated that it cannot apparently be attributed to changes in phosphodiesterase activity. One cannot, however, exclude this possibility, since a local isoenzyme may be increased with no overall PDE activity change being demonstrable (Weiss & Hait, 1977). The most likely alternative mechanisms would therefore seem to be some derangement of the β-receptor-adenylate cyclase unit, or reduction of

Table 4 Bronchial wall isoprenaline cyclic AMP response after desensitization in three lungs (p mol mg^{-1} protein 15 min^{-1}).

Isoprenaline concentration (mol l^{-1})	*Control*	*Densensitized*	
10^{-8}	15.7	7.1	
	19.7	2.1	
	19	15	
Mean ± s.d.	18.13 ± 2.1	8.1 ± 6.5	$P < 0.05$
	71.0	228.3	
10^{-6}	72.0	2.4	
	74.3	28.7	
Mean ± s.d.	72 ± 1.8	19.8 ± 15	$P < 0.025$
10^{-4}	109.2	21.3	
	79	54.2	
Mean	94.1	37.8	

Table 5 Phosphodiesterase activity (expressed as a nmol cyclic AMP hydrolysed mg^{-1} protein 15 min^{-1}) in bronchial wall after 1 h incubation with Krebs alone or with isoprenaline (4×10^{-4} mol l^{-1}).

Experiment number	*Control tissue*	*Desensitized tissue (after 4×10^{-4} mol l^{-1}) isoprenaline*
1	9.5	17.7
2	13.3	27.2
3	49.3	48.4
4	12.1	6.1
5	1.8	2.5
6	11.9	6.9

availability of ATP by increased activity of ATP-ase. The latter mechanism has been demonstrated in leucocytes from asthmatic patients (Logsdon, Middleton & Coffey, 1972). More recently, studies with alprenolol of high specific activity, have provided evidence indicating reduced binding to β-adrenoceptors on the cell surface of several different tissues and in numerous species, after prolonged exposure to β-adrenoceptor agonists; recently a diminution of β-receptor sites on mononuclear cells from asthmatics has been reported (Kariman & Lefkowitz, 1977) and the suggestion has been made that this may be due to the formation of a stable receptor-agonist complex (Williams & Lefkowitz, 1977).

The ability of a persisting, but very low concentration of isoprenaline to prevent recovery so strikingly has previously been observed in fibroblasts (Morrison *et al.*, 1973) and has been presumed to indicate continuing inactivation or destruction of new receptor sites as they are formed to replace those lost during desensitization. If such an interpretation is correct, the inability of cycloheximide to delay recovery is rather surprising, the concentration used being adequate to reduce protein synthesis by about 90% (Browning, Brostrom & Groppi, 1976).

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