Importance of β_2 -adrenoceptor stimulation in the suppression of intradermal antigen challenge by adrenaline

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1 Seven atopic subjects received two injections of antigen and one of saline intradermally in the back on each of 4 separate days. They were pretreated with four different drug combinations: (a) adrenaline 0.3 mg subcutaneously over the deltoid muscle (b) subcutaneous adrenaline preceded by 5 mg of the specific β_2 -adrenoceptor antagonist ICI 118,551 orally (c) 8 mg of salbutamol orally (d) placebo. Tablets were given 2 h before and subcutaneous injections 15 min before the intradermal injections of saline and antigen.

2 The median flare response to intradermal low dose antigen and high dose antigen after pretreatment with adrenaline was 4% and 49% of the response seen following pretreatment with placebo ($P < 0.001$). When adrenaline was preceded by ICI-118,551, the corresponding median flare responses were 2% and 44% ($P < 0.001$) of the placebo response. The flare response after pretreatment with salbutamol was not significantly different from placebo.

3 Adrenaline suppressed the median weal response to the higher dose of antigen to 52% of the response after pretreatment with placebo ($P < 0.05$). This suppression by adrenaline was blocked by pretreatment with ICI 118,551. The median weal response after the highest dose of antigen was suppressed by salbutamol to 66% of the response seen after placebo, although this was not significant even when a further three subjects were studied with either salbutamol or placebo.

4 These results confirm that a small dose of systemic adrenaline attenuates the weal and flare response to intradermal antigen. This suppression of the weal response is blocked by a β_2 -adrenoceptor antagonist although it is only partially mimicked by high dose oral salbutamol. The suppression of the flare response may involve α -adrenoceptor stimulation.

Keywords adrenaline β_2 -adrenoceptor antagonists antigen salbutamol

Introduction

It has previously been shown that the skin response to antigen may be suppressed if 0.3 mg of adrenaline is injected systemically before the challenge (Warren *et al.*, 1988). This is an important observation because the ability to suppress both the weal and flare of the immediate hypersensitivity reaction to antigen,

with even a relatively small dose of adrenaline, underlies the rationale for using this drug in acute anaphylaxis.

The major clinical indication for β_2 -adrenoceptor agonists is for the treatment of asthma where they are thought to bronchodilate by relaxing airway smooth muscle. However, it

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has been suggested that much of the airway narrowing of asthma is attributable to inflammatory oedema (Hogg *et al.*, 1987). The attenuation by adrenaline of both oedema formation and the associated flare of injected antigen is of additional theoretical benefit in the treatment of asthma as well as to the known β_2 -receptor mediated relaxation of smooth muscle.

The importance of β_2 -adrenoceptor stimulation in the suppression of intradermal antigen response has been investigated in the present study by comparing the efficacy of systemic adrenaline to oral salbutamol and by giving a specific β_2 -adrenoceptor antagonist, ICI 118,551 before the administration of adrenaline.

Methods

Seven atopic healthy men, aged 21–30 years, who gave informed consent were studied. The protocol was approved by the hospital Ethics Committee. For the pre-study assessment, increasing doses of house dust mite or grass pollen antigen (Pharmacia, Sweden) were given as 50 μ l intradermal injections with a 27 gauge needle into the skin of the back. The antigen solutions were diluted with 0.9% saline to give concentrations of 0.5, 1, 5, 10, 50 and 100 units ml^{-1} . Increasing doses of antigen were given until a flare reaction of at least a 2 cm diameter was obtained. This dose of antigen, together with a dose one tenth the concentration, and a saline control, were used to produce a dose response. Any subject with a history of asthma was excluded.

All subjects were pretreated with a tablet by mouth followed by a subcutaneous injection at 1 h 45 min and intradermal injections of antigen at 2 h. The four treatment days were:-

Oral	Injection
Placebo	Placebo
ICI 118,551 25 mg	Adrenaline 0.3 mg
Placebo	Adrenaline 0.3 mg
Salbutamol 8 mg	Placebo

and these were given in randomized order on four separate occasions 1 week apart. The flare was measured 15 min after the intradermal injections by tracing the area onto a perspex sheet. At 20 min after the intradermal injections, the weal was traced with ink and this was removed by adhesive tape. The areas of weal and flare were later determined by planimetry. The traces were drawn by an independent observer who was blind to the protocol. It was originally intended to study 10 subjects but ICI-118,551 was

withdrawn from clinical trials by the manufacturer after only seven subjects had completed the protocol. Therefore, a further three atopic healthy men were recruited (aged 28–33 years) and they were studied according to the above protocol except that they only completed the placebo and salbutamol day.

Significance was tested by 2-tailed Wilcoxon matched pairs signed rank sum test.

Results

The subjects experienced no side effect from the study apart from an increase in physiological tremor induced by adrenaline and salbutamol and some itch over the skin test site. Table 1 shows the dose and type of antigen used. Table 2 and Figure 1 show the effect of pretreatment with placebo, systemic adrenaline, systemic adrenaline with ICI-118,551 or salbutamol on the weal and flare response. The low dose of antigen had little effect on weal size but caused a 34 fold increase in flare response compared with the flare after intradermal saline. The flare response was significantly suppressed by adrenaline in the presence or absence of ICI-118,551 ($P < 0.001$ in each case). Of the weal responses, only the higher dose of antigen was suppressed by adrenaline ($P < 0.05$). Table 3 shows the data for the ten subjects who took salbutamol or placebo and includes data on three individuals who are not included in Table 2.

Discussion

The present study confirms our previous finding that a small dose of adrenaline given systemically is capable of protecting against both the weal and flare caused by intradermal antigen (Warren *et al.*, 1988). Adrenaline caused a marked suppression of the flare response and this was unaffected by pretreatment by the specific β_2 -adrenoceptor antagonist, ICI-118,551.

Table 1 Type and dose of antigen used in the 10 subjects.

Subject	Type and dose of antigen (units ml^{-1})	
1–4	GP	1,10
5	GP	10,100
6–9	HD	10,100
10	HD	0.5,5

HD = house dust mite; GP = grass pollen

Table 2 Effect of pretreatment with placebo, adrenaline 0.3 mg systemically, adrenaline 0.3 mg systemically with 5 mg ICI-118,551 orally or salbutamol 8 mg orally on the weal and flare response of the seven subjects. Data are median (95% confidence intervals)

		Placebo	Adrenaline	Adrenaline + ICI-118,551	Salbutamol
Weal	Saline	0.83 (0.47-0.95)	0.99 (0.76-1.10)	0.81 (0.73-0.89)	0.86 (0.71-1.01)
	Ag	1.02 (0.68-1.55)	1.02 (0.75-1.10)	0.98 (0.82-1.24)	1.02 (0.89-1.12)
	10x Ag	2.28 (1.20-3.26)	1.19* (0.98-1.92)	1.67 (1.24-2.72)	1.51 (0.99-2.33)
Flare	Saline	0.35 (0-1.71)	0* (0-0.17)	0* (0-0.09)	0.61 (0.03-1.51)
	Ag	11.92 (4.79-14.97)	0.49* (0-2.09)	0.29* (0-3.45)	5.35 (0.53-11.62)
	10x Ag	19.98 (11.77-31.35)	9.77* (4.57-20.27)	8.84* (3.20-22.25)	17.72 (7.15-30.49)

* = $P < 0.05$ compared with placebo. **Figure 1** Median areas of weal and flare for the seven subjects in response to intradermal saline, antigen and tenfold dose of antigen. (□) placebo, (●) adrenaline 0.3 mg systemically, (■) adrenaline 0.3 mg systemically with 5 mg ICI-118,551 orally, (◆) salbutamol 8 mg orally.

The flare response to intradermal antigen is thought to be mediated by an axon reflex but the exact mechanism is unknown (Chapman, 1977; Barnes *et al.*, 1986). Vasodilation in the skin is inhibited by noradrenaline released locally by sympathetic nerves and therefore the injected

Table 3 Effect of pretreatment with placebo or salbutamol 8 mg orally on the weal and flare response of the ten subjects. Data are median (95% confidence intervals)

		Placebo	Salbutamol
Weal	Saline	0.79 (0.60-0.82)	0.85 (0.77-0.93)
	Ag	0.92 (0.72-1.22)	0.99 (0.87-1.03)
	10x Ag	1.33 (1.19-2.43)	1.28 (1.07-1.83)
Flare	Saline	0.25 (1.12-1.22)	0.80 (0.43-1.69)
	Ag	6.67 (4.15-10.69)	4.05 (2.11-7.97)
	10x Ag	13.81 (12.50-23.88)	15.28 (11.09-22.97)

Differences between the groups were not significant.

adrenaline could mimic this action by stimulation of vasoconstrictor α_1 -adrenoceptors. However, intravenous phenylephrine in a dose which has significant cardiovascular effects, does not suppress the flare response (Kram *et al.*, 1975) and this does not fit the hypothesis that adrenaline inhibits flare by α -adrenoceptor stimulation. In contrast, clonidine, an α_2 -adrenoceptor agonist that decreases central sympathetic outflow, does inhibit both the weal and flare response to antigen in patients with asthma (Lindgren *et al.*, 1987). It was argued that α_2 -adrenoceptor stimulation of sensory C fibres in the skin explained this observation (Lindgren *et al.*, 1987) and therefore adrenaline may inhibit flare by α_2 -adrenoceptor rather than α_1 -adrenoceptor stimulation.

The inhibition of weal formation by adrenaline with the higher dose of antigen was blocked by pretreatment with ICI-118,551 suggesting that this is mediated via β_2 -adrenoceptors. Antigen induced weal formation in the skin is a consequence of mediator release from mast cells activated by IgE. Salbutamol is a potent inhibitor (some 10^2 – 10^3 more potent than cromoglycate) of IgE mediated mediator release from the lung *in vivo* (Howarth *et al.*, 1985) and *in vitro* (Church & Young, 1983; Butchers *et al.*, 1979). In the present study salbutamol showed a similar trend to adrenaline in reducing the weal response, although this did not reach significance.

It may be that a higher dose of salbutamol is needed since previous studies with oral β -adrenoceptor agonists have shown no suppression of skin test responses (Galant *et al.*, 1973; Spector, 1978; Abramowitz *et al.*, 1980), although the topical application or local injection of these drugs attenuates the response to intradermal antigen presumably by higher local concentrations (Mosko & Marshall, 1954; Ting *et al.*, 1983; Gronneberg *et al.*, 1980). However, the dose of salbutamol used in the present study caused greater systemic side effects than adrenaline and the difference in efficacy between adrenaline and salbutamol in suppressing weal remains unexplained.

The systemic absorption of this dose of adrenaline has been previously studied and the dose used causes a peak venous plasma concentration 15 min after injection of approximately 2 nmol l^{-1} , which remains raised for at least 40 min (Warren *et al.*, 1986). The only previous work to

study the effect of systemic adrenaline on the response to intradermal antigen used much higher doses of intravenous adrenaline, but found a similar suppressive effect (Kram *et al.*, 1975).

A 0.3 mg dose of adrenaline was chosen because this is still widely used in North America for the treatment of acute asthma and we had previously determined its pharmacokinetics (Warren *et al.*, 1986). A dose of salbutamol of 8 mg gives some normal subjects an uncomfortable tremor, but we were reluctant to use a smaller dose because 8 mg of the slow release preparation of salbutamol by mouth does not influence the skin weal and flare response to antigen (P. W. Ind, personal communication). ICI 118,551 has been shown to antagonize the β_2 effects of infused isoprenaline but not the β_1 effects in an optimum dose range of 25–50 mg (J. D. Harry, data on file, ICI Pharmaceuticals Division, Macclesfield).

It was our original intention to study 10 subjects but because ICI-118,551 has been withdrawn from clinical trials it was necessary to curtail the investigation after only seven volunteers had completed the protocol.

In summary, the small dose of adrenaline used in the present study significantly inhibited the flare response to intradermal antigen in man and this may be by α_2 -adrenoceptor stimulation. The weal response was also suppressed, and this was antagonized by a specific β_2 -adrenoceptor antagonist, although not mimicked by salbutamol.

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