Int. J. Immunopharmac., Vol. 7, No. 6, pp. 833–838, 1985.
Printed in Great Britain.

0192–0561/85 $3.00+ .00

INHIBITION OF PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) BY AZELASTINE: DISSOCIATION OF ITS ANTIALLERGIC ACTIVITIES FROM ANTIHISTAMINIC AND ANTISEROTONIN PROPERTIES

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(*Received* 21 *September* 1984 *and in final form* 12 *March* 1985)

Abstract — Azelastine and methysergide injected i.v. 5 min prior to antigen challenge and disodium cromoglycate (DSCG) injected i.v. immediately before antigen challenge produced dose-dependent inhibition of IgE-mediated 72 h passive cutaneous anaphylaxis (PCA) responses with ID_{50}s of 0.3, 0.2 and 1.0 mg/kg, respectively. Thus, azelastine is about three times as effective as DSCG (a mast cell stabilizing agent) and somewhat less active than methysergide (a specific serotonin "D" receptor antagonist). Oral administration of azelastine and other drugs 2 h prior to antigen challenge produced strong inhibitory effects on PCA. The ID_{50}s (mg/kg) were as follows: azelastine = 1.4; astemizole = 1.6; ketotifen = 2.0; aminophylline = 4.6; and diphenhydramine = 10.9. After 4 h of oral administration, azelastine and other drugs inhibited PCA responses with the following ID_{50}s (mg/kg): azelastine = 1.8; astemizole = 2.3; ketotifen = 2.3; and aminophylline = 12.5. Azelastine administered orally 24 h before antigen challenge was still capable of exerting significant anti-PCA activity with an ID_{50} of 2.6 mg/kg, whereas none of the other drugs tested produced any significant inhibitory effects on PCA.

In subsequent experiments, it was established that the antiallergic and antihistaminic activities of azelastine are inseparable 2 h after oral administration (ID_{50} of azelastine mg/kg, p.o., 2 h: PCA = 2.6 and histamine = 3.1). However, the persistence of the oral antiallergic (anti-PCA) effects of azelastine for 24 h (ID_{50} = 3.7 mg/kg) does not seem to be associated with its antihistaminic or antiserotonin activities. The persistence of antiallergic (anti-PCA) effects of azelastine may be associated with its ability to inhibit the synthesis and/or release of chemical mediators and to block receptor sites for the released mediators in the rat skin.

Azelastine [4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepine-4-yl)-1-(2H)-phthalazinone hydrochloride] is a long-acting antiallergic agent with histamine H_1-receptor blocking properties (Diamantis, Harrison, Melton, Perhach & Sofia, 1981; Katayama, Akimoto, Shionoya, Morimoto & Katoh, 1981; Mandi, Galgoczy, Galambos & Aurich, 1981; Zechel, Brock, Lenke & Achterrath-Tuckermann, 1981; Chand, Nolan, Diamantis, Perhach & Sofia, 1983; Perhach, Connell, Hamilton, Diamond, Weiler & Melvin, 1984). Azelastine has been shown to inhibit allergic bronchospasms in guinea pigs (Zechel *et al.*, 1981; Chand *et al.*, 1983) and passive cutaneous anaphylaxis (PCA) in rats and guinea pigs (Katayama *et al.*, 1981). Azelastine exerts strong inhibitory effects on the release of chemical mediators, e.g. leukotrienes (Diamantis, Chand, Harrison, Pillar, Perhach & Sofia, 1982) and histamine (Katayama *et al.*, 1981; Fischer & Schmutzler, 1981; Chand, Pillar, Diamantis, Perhach & Sofia, 1983a; Chand, Pillar, Natarajan, Diamantis & Sofia, 1983b; Fields, Pillar, Diamantis, Perhach, Sofia & Chand, 1984). In addition to strong antihistamine and antiallergic properties, azelastine also possesses leukotriene (SRS-A) and serotonin (5-HT) receptor blocking activities (Katayama *et al.*, 1981; Diamantis *et al.*, 1982; Chand, Diamantis & Sofia, 1984).

In this investigation the antiallergic activity of azelastine in IgE-mediated 72 h PCA in rats was studied and compared to selected drugs. Furthermore, additional experiments were performed to dissociate the anti-PCA (antiallergic) activity of azelastine from its antihistaminic and antiserotonin properties, i.e. the ability of azelastine to influence weal and flare responses to antigen (PCA), histamine and 5-HT in rat skin was studied and compared with diphenhydramine (a selective H_1-histamine receptor antagonist) and methysergide (a highly specific D-serotonin receptor antagonist).

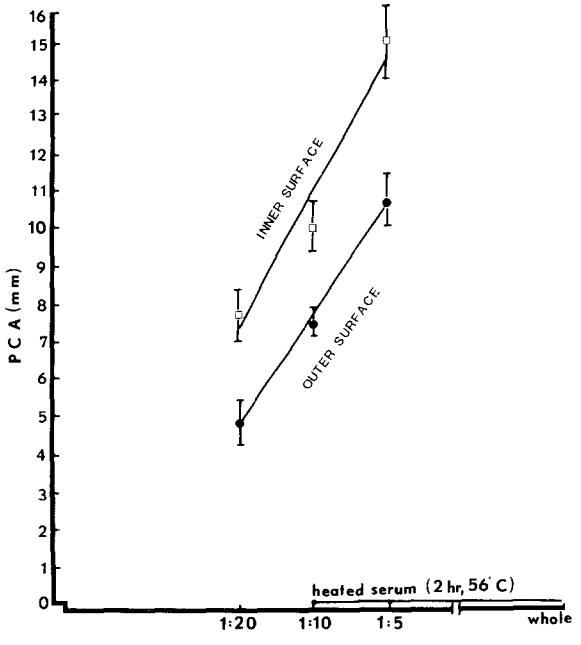

Fig. 1. Homologous IgE-mediated 72 h passive cutaneous anaphylaxis (PCA) in rats. The PCA activity of reaginic sera (antiovalbumin serum and its dilutions) was lost by heating at 56 °C for 2 h. Each point represents the mean ± S.E.M. of four to seven sites from different animals.

EXPERIMENTAL PROCEDURES

Drugs

Aminophylline, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulfate (Sigma), azelastine (Wallace Laboratories), astemizole (Janssen Pharmaceutica, Belgium), diphenhydramine HCl (Parke, Davis & Co.), cyproheptadine (Merck, Sharp & Dohme), disodium cromoglycate (Fisons), methysergide and ketotifen (Sandoz) were used in this study.

Methods

Preparation of reaginic serum. Adult, male Sprague-Dawley rats (Charles River Breeding Laboratories, North Wilmington, MA) were sensitized by i.p. injection of 1 ml suspension containing 1 mg of ovalbumin (OA) and 10 mg of alum (aluminum potassium sulfate) and intramuscular injection of killed *Bordetella pertussis* vaccine (1.1×10^{10} organisms/0.5 ml) (Becker & Austen, 1966). On day 18 of sensitization, rats were lightly anesthetized with carbon dioxide and bled by cardiac puncture. Blood samples were stored at room temperature for 30 min and then centrifuged at 3000 rotations/min for 15 min. Serum was separated, pooled and 1 ml aliquots stored in glass vials at −70 °C until used.

Passive cutaneous anaphylaxis (PCA). A modification of the PCA technique reported by Mota (1964) and Watanabe & Ovary (1977) was used. The dorsal area of Sprague-Dawley rats weighing 170−345 g was shaved using an Oster-Electric hair clipper.

Diluted antiovalbumin serum was injected intradermally in a volume of 0.1 ml at three sites on each side of the back of the rats. After 72 h of sensitization period, the intradermal injections of 0.05 ml of saline alone or containing histamine (20 μg base) and 5-HT (0.2 μg base) were made at three different sites on both sides of the back immediately prior to intravenous injection of 5 mg OA together with Evans blue dye (2.5 mg) in 1 ml saline into the dorsal penile vein. Thirty min later the rats were euthanized by exposure to carbon dioxide. The dorsal skin was cut and reflected, and the diameter of the blue weals (weal and flare response) was measured in mm.

Experiments in which the responses to histamine or 5-HT (minus the saline response) and PCA responses in control animals were less than 5 mm diameters were not included in this study. The intradermal injections of histamine and 5-HT were only included in experiments where dissociation of antiallergic activity of azelastine from its antihistaminic and antiserotonin properties was performed. In initial experiments it was demonstrated that the PCA activity of the anti-OA serum and its dilutions were lost by heating at 56 °C for 2 h (Fig. 1). This procedure established the involvement of heat-sensitive reaginic (IgE-containing) serum in the mediation of 72 h PCA. A serum dilution of 1:20, producing a weal diameter of at least 5 mm (averaging 7 ± 2 mm over the course of this study), was selected and injected at two sites 72 h prior to antigen challenge for the evaluation of the anti-PCA activities of the drugs.

Disodium cromoglycate (DSCG) was injected i.v. immediately before antigen challenge. Azelastine, diphenhydramine, methysergide and cyproheptadine were administered i.v. 5 min before OA injections. Azelastine, ketotifen, diphenhydramine, cyproheptadine, astemizole and aminophylline were also administered orally 2, 4 and 24 h before OA challenges.

Drugs for oral administration were dissolved or suspended in distilled water. Astemizole was

suspended in 1% acacia solution or Tris-Gel buffer. For intravenous injections, drugs were dissolved in physiological salt solution (0.9% NaCl).

The inhibition of PCA, histamine and 5-HT-induced weal and flare responses by each dose of a drug in rats was determined by the following formula:

$$\% \text{ inhibition} = \frac{C - T}{C} \times 100$$

C = mean of PCA or histamine or 5-HT-induced weal and flare responses (mm) in control (vehicle-treated) rats each day; T = PCA or histamine or 5-HT-induced weal and flare responses (mm) in individual drug-treated rats.

Statistical analysis

The significance of the observations was determined by comparing responses (mm weal diameter) in control (vehicle-treated) and drug-treated animals by Student's t-test. The ID_{50}, i.e., the dose of each drug producing 50% inhibition of weal and flare responses, and 95% confidence limits were also calculated.

RESULTS

Effects of azelastine and selected drugs on PCA in rats

The ID_{50}s of azelastine and other drugs against 72 h PCA in rats have been summarized in Table 1.

DSCG injected i.v. immediately before antigen challenge produced dose-dependent inhibition of PCA in rats with an ID_{50} of 1 mg/kg. Only the highest dose of DSCG (10 mg/kg) produced a significant ($P < 0.05$) reduction in PCA responses as compared to control.

The drugs injected i.v. 5 min prior to antigen challenge inhibited PCA responses. The ID_{50}s (mg/kg) were as follows: methysergide = 0.2; azelastine = 0.3; diphenhydramine = >1. The effects of diphenhydramine and methysergide on PCA were not dose related.

All the drugs administered orally 2 h before antigen challenge inhibited PCA responses in rats with the following ID_{50}s (mg/kg): azelastine = 1.4; astemizole = 1.6; ketotifen = 2.0; aminophylline = 4.6; and diphenhydramine = 10.9 mg/kg. Azelastine (1 to 10 mg/kg), astemizole (1 and 5 mg/kg), aminophylline (50 mg/kg), ketotifen (0.2 and 5 mg/kg) and diphenhydramine (10 mg/kg) produced significant ($P < 0.05$) reduction in PCA responses.

Drugs administered orally 4 h before antigen challenge also produced dose-dependent inhibition of PCA responses. The ID_{50}s (mg/kg) were as follows: azelastine = 1.8; ketotifen = 2.3; astemizole = 2.3; and aminophylline = 12.5. Azelastine (3, 5, 10 mg/kg), ketotifens (3 and 10 mg/kg) and astemizole (10 and 30 mg/kg) exerted significant ($P < 0.05$) inhibitory effects on PCA. Diphenhydramine (1 – 10 mg/kg, p.o., 4 h) also exerted 25.8 – 43.5% ($P > 0.05$) inhibition of PCA responses. These effects were neither dose related nor statistically significant. Interestingly, diphenhydramine at higher doses (50 mg/kg) was found to enhance ($P < 0.05$) PCA responses.

Azelastine (0.3 – 30 mg/kg) administered orally 24 h before antigen challenge produced inhibition of PCA with an ID_{50} of 2.6 mg/kg. The inhibitory effects of azelastine as well as all the other drugs tested were not dose related. With the exception of azelastine (1 – 30 mg/kg), none of the other drugs examined (diphenhydramine, ketotifen, aminophylline and astemizole) produced a significant inhibition of PCA responses in rats. Diphenhydramine (10 – 50 mg/kg) produced 26 – 51% ($P > 0.05$) inhibition of PCA.

Dissociation of anti-PCA activity of azelastine from its antihistaminic and antiserotonin activities

The oral administration of azelastine 2 h prior to intravenous challenge with antigen and intradermal injections of histamine and 5-HT produced significant inhibition of PCA and histamine responses with ID_{50}s of 2.6 and 3.1 mg/kg, respectively (Table 2). It exerted little or no antiserotonin activity (ID_{50} = >10 mg/kg). Diphenhydramine (1 – 10 mg/kg, p.o., 2 h) exerted no significant inhibition of PCA, histamine and 5-HT (ID_{50}s = >10 mg/kg). Methysergide (0.1 – 10 mg/kg, p.o., 2 h) produced significant inhibition of cutaneous responses to 5-HT only (ID_{50} = 0.8 mg/kg). Methysergide failed to exert any antihistaminic and anti-PCA activities (ID_{50} = >10 mg/kg) (Table 2).

The oral administration of azelastine 24 h prior to intradermal injections of histamine and 5-HT and intravenous antigen challenges produced marked anti-PCA activity with an ID_{50} of 3.7 mg/kg but failed to exert any antihistaminic and antiserotonin activities (ID_{50}s = >10 mg/kg) (Table 2).

Diphenhydramine (p.o., 24 h) failed to exert any significant inhibition of PCA, histamine and 5-HT

Table 1. Summary of the inhibitory activities (ID_{50}) of azelastine and selected drugs in the IgE-mediated 72 h passive cutaneous anaphylaxis (PCA) in rats

Drug	ID_{50} (mg/kg) (95% confidence limits) Time of drug administration prior to antigen challenge				
	i.v., (min)		Oral (h)		
	0	5	2	4	24
Azelastine	—	0.3 (0.19 – 0.60)	1.4 (0.5 – 4.2)	1.8 (1.0 – 3.3)	2.6 (1.3 – 5.4)
Diphenhydramine	—	>1.0	10.9 (0.6 – 208.4)	>50	– 50
Ketotifen	—	—	2.0 (0.06 – 58.9)	2.3 (1.0 – 5.6)	>10
Aminophylline	—	—	4.6 (1.1 – 19.0)	12.5 (0.6 – 274.0)	>100
Astemizole	—	—	1.6 (0.7 – 3.5)	2.3 (0.7 – 7.9)	>30
DSCG	1.0 (0.4 – 2.9)	—	—	—	—
Methysergide	—	0.2 (0.02 – 2.0)	—	—	—

(ID_{50}s = >10 mg/kg). Methysergide (p.o., 24 h) had no significant effect on PCA or histamine-induced responses (Table 2) but produced significant inhibition of 5-HT-induced cutaneous responses at 10 mg/kg, p.o.

DISCUSSION

The data obtained in this study demonstrated that 2 h after oral administration azelastine exerted approximately equal antiallergic (anti-PCA) and antihistaminic activities in rat skin but lacked any significant antiserotonin activity. In other words, 2 h after oral treatment in rats the antiallergic and antihistaminic activities of azelastine were not dissociable. Twenty-four h after oral administration the anti-PCA activity of azelastine still persisted whereas antihistaminic and antiserotonin activities were not detectable. In contrast, several other drugs, e.g., ketotifen, diphenhydramine, cyproheptadine, aminophylline and astemizole, administered orally 24 h prior to antigen challenge failed to exert any anti-PCA activity in the rats. Therefore, based on these findings it is concluded that the long-lasting antiallergic effects of azelastine are not associated with its antihistaminic and antiserotonin activities.

Table 2. Dissociation of antiallergic (anti-PCA) activities of azelastine from its antihistaminic and antiserotonin activities in rat skin

Drug	ID_{50} (mg/kg) p.o. (confidence limits)					
	2 h			24 h		
	PCA*	Histamine	Serotonin (5-HT)	PCA*	Histamine	Serotonin (5-HT)
Azelastine	2.6 (0.4 – 16.4)	3.1 (1.4 – 7.1)	>10	3.7 (1.1 – 12.1)	>10	>10
Diphenhydramine	>10	>10	>10	>10	>10	>10
Methysergide	>10	>10	0.8 (0.2 – 3.3)	>10	>10	>10

Drugs were administered orally 2 or 24 h immediately before intradermal injection of histamine and 5-HT or intravenous injection of antigen.

* IgE-mediated 72 h-PCA.

It has been suggested that azelastine interferes with 5-lipoxygenase pathway of arachidonic acid metabolism (Diamantis *et al.*, 1982; Chand *et al.*, 1983) and also antagonizes receptor sites for SRS-A (leukotrienes) (Diamantis *et al.*, 1982; Chand *et al.*, 1984). Therefore, the persistence of antiallergic (anti-PCA) effects of azelastine for at least 24 h may be attributed to the blockade of synthesis and/or release of chemical mediators, e.g., the product of 5-lipoxygenase pathway of arachidonic acid, i.e., 5-HPETE, 5-HETE, SRS-A (leukotrienes) and/or long-lasting blockade of leukotriene receptors in the rat skin.

At an ID_{50} level azelastine is about three and seven times as effective as aminophylline at 2 and 4 h post-treatment time intervals, respectively. DSCG, a mast cell stabilizing agent, exerts inhibitory effects on PCA responses in rats with an ID_{50} of 1.0 mg/kg (i.v., 0 min). DSCG is inactive orally. Azelastine (i.v., 5 min) is about three times as effective as DSCG (i.v., 0 min) against PCA responses in rats. The ID_{50} of methysergide (a selective "D" serotonin receptor antagonist) against PCA in rats is 0.2 mg/kg (i.v., 5 min). The blockade of PCA by methysergide suggests that 5-HT is the major mediator of allergic cutaneous responses in rats.

Azelastine (5 – 25 mg/kg, p.o., 1 h) inhibits serotonin-induced cutaneous responses by 40 – 60% in rat skin (Katayama *et al.*, 1981). Therefore, the early anti-PCA effects of azelastine may in part be mediated by the antagonism of 5-HT in rat skin and/or by the inhibition of the allergic release of biogenic amines (5-HT and histamine) from the mast cells (Chand *et al.*, 1983a, b; Fields *et al.*, 1984; Chand *et al.*, 1984).

Ketotifen and astemizole, both orally effective antihistamine-antiallergic drugs, were also found to inhibit cutaneous anaphylactic responses. After 2 – 4 h of post-treatment time intervals, the oral anti-PCA activity of astemizole (a newer antihistaminic drug) was found to be somewhat less than that of azelastine. In contrast to azelastine, the antiallergic effects of ketotifen and astemizole declined with the prolongation of post-treatment time intervals.

In conclusion, azelastine exerts long-lasting, antiallergic effects against IgE-mediated PCA responses in rats. The anti-PCA effects of azelastine may be mediated by the inhibition of the synthesis and/or release of chemical mediators and by the blockade of the receptor sites for leukotrienes (SRS-A).

Acknowledgments — We thank the following companies for the generous supply of drugs: Janssen Pharmaceutica for astemizole; Parke, Davis & Co. for diphenhydramine; Fisons Corp. for disodium cromoglycate; and Sandoz Pharmaceuticals for ketotifen and methysergide maleate. The authors wish to thank Mrs. Carolyn Denham and Mrs. Yvette McKnight for excellent secretarial assistance and Mr. Leo R. Maestripieri for editorial assistance.

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