

Effect of beta adrenergic stimulation and blockade on immediate hypersensitivity skin test reactions

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The effects of isoproterenol (a β -adrenergic stimulating agent) and propranolol (a β -adrenergic blocking agent) were studied on immediate hypersensitivity skin test reactions in 15 atopic subjects. Forearm skin of these subjects was pretreated with these agents by local iontophoretic application and compared to areas locally pretreated with saline or diphenhydramine hydrochloride. Following pretreatment of skin, scratch or intradermal tests were performed with the most reactive antigen for each patient. A significant increase of skin reactivity occurred in areas of skin pretreated with propranolol. A significant decrease in reactivity followed pretreatment with isoproterenol. Thus the iontophoresis of β -adrenergic agents can alter the immediate hypersensitivity skin test reactions of atopic patients. This alteration is consistent with a modification of the local synthesis of cyclic adenosine monophosphate (CAMP) induced by these pharmacologic agents. It is not clear whether this presumed alteration in CAMP is exerting its effect on mediator release from dermal mast cells or directly on the dermal blood vessels or both.

Catecholamines, presumably by their effects on the adenyl cyclase CAMP system, appear to be important mediators in influencing in vitro mast cell histamine release in allergic reactions. It has been demonstrated in vitro that β -adrenergic agents prevent this release, whereas β -adrenergic blockade enhances histamine release.^{1, 2} This work is of clinical significance since there is evidence that alterations in the β -adrenergic system may play a role in the pathogenesis of atopic diseases.³⁻⁹ The present study was undertaken to determine if the in vitro effects of β -adrenergic manipulation could be demonstrated in vivo. To accomplish this, iontophoresis was employed to administer adrenergic agents locally into the skin in order to assess their effect on immediate hypersensitivity skin tests in atopic individuals. To our knowledge, this is the first application of iontophoresis, the introduction of charged molecules into dermal tissues by electromotive force, specifically for this purpose.

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TABLE I

Drug	Propranolol hydrochloride	Isoproterenol hydrochloride	Diphenhydramine hydrochloride	Physiologic saline
Concentration	1 mg./ml.	1:200 w/v aqueous solution	10 mg./ml.	0.9% solution
pH	Adjusted to 4.0 with citric acid	2.5	4.5	7.0
Net ionic charge in solution	Positive	Positive	Positive	
Polarity of "applicator" electrode	Positive	Positive	Positive	Applied to all negative electrodes for electrical conductivity and to one positive electrode as control for experimental drugs
Rationale for inclusion in study	β -adrenergic blocking agent	β -adrenergic stimulating agent	Iontophoresis and skin test technique control (decreases white dermographism when iontophoresed into atopic skin ¹²)	
Amount applied to each electrode	0.75 ml.	0.75 ml.	0.75 ml.	0.75 ml.
Suppliers	Ayerst Labs., 685 Third Ave., New York, N. Y. (Inderal)	City of Memphis Hospital, 860 Madison Ave., Memphis, Tenn. (supplied for inhalation therapy)	Parke, Davis & Co., Detroit, Mich. (Benadryl)	

MATERIALS AND METHODS

Patient selection

Fifteen subjects were chosen from volunteers who gave a history of perennial allergic rhinitis or asthma or both, and who had at least three positive immediate hypersensitivity skin tests (scratch or intradermal) to a battery of standard antigens. These antigens included tree, grass, and ragweed pollens and animal dander (Greer Laboratories, Lenoir, North Carolina). No patient participated in the study during the same week that initial skin tests were performed. Patients were not allowed any medication for 48 hours prior to skin testing.

Iontophoresis (apparatus and technique)

The apparatus used was a custom-designed unit isolated from wall current. It was fused on both primary and secondary sides of the circuit, included current-regulating and current-controlling circuits, and was housed in a grounded chassis and case (Fig. 1). The output of this unit was 0 to 26 ma. at 170 v DC. Electrodes were silver-plated 55 × 31 mm. electrocardiogram electrodes fitted with underlying (55 × 31 mm.) filter paper rectangles (fashioned from Whatman No. 1, 12.5 cm. circles, W. R. Balston, Ltd., England) saturated with amounts of the experimental drugs, as seen in Table I. The electrodes were strapped in place over the saturated filter paper on the subject's forearm. Each experimental trial included 4 electrodes on the volar surface of the forearm for simultaneous iontophoresis of the 4 experimental drugs. Two negatively charged electrodes were placed at either extreme of the dorsal aspect of the forearm to complete the electrical circuit. In this fashion 4 drugs could be studied simultaneously. The current employed was the lowest milliamperage producing a subjective "tingling" sensation in each subject. This threshold current level varied from 0.75

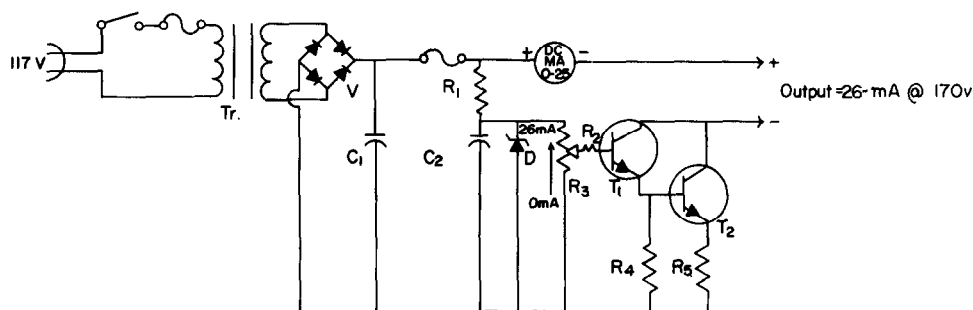


FIG. 1. Schematic diagram of the iontophoresis apparatus (Model UT-1) designed and used in this study. *Tr.*, Isolation transformer, 10 w or larger; *V*, Varo bridge; *C*₁, 200 mf, 150 v; *C*₂, 300 mf, 35 v; *R*₁, 15 K Ω , 2 w; *R*₂, 10 K Ω , 2 w; *R*₃, 0-10 K Ω ; *R*₄, 10 K Ω , 1/2 w; *R*₅, 1 K Ω , 1 w; *D*, 1N 4750; *T*₁, 2N 3440; *T*₂, 2N 3738.

ma. to 1.5 ma. Current in excess of 2 ma. caused sufficient local erythema to interfere with evaluation of scratch test results. The threshold current was maintained for 45 minutes, which was sufficient time for iontophoresis of the appropriate drugs used to penetrate the forearm skin of each subject.

Drugs

The drugs used were applied to the appropriate electrodes according to the net electric charge of the agent in solution. Positively charged molecules, for example, were applied to positively charged electrodes. The concentration, pH, and net charge of the drugs used, as well as the rationale for their use, are seen in Table I.

Skin testing

The initial and experimental allergy skin tests were performed using standard techniques.¹⁰ The scratch tests were done using a 2 pronged smallpox scarifying needle. The skin was punctured by the "prick" technique. Intradermal tests were performed using a 27 gauge tuberculin syringe. Care was taken to inject 0.03 ml. of each antigen with each test. The concentration of each antigen used was kept constant. A 1:20 (w/v) concentration was used for the prick tests and a 1:1,000 (w/v) concentration was used for the intradermal tests. The antigen chosen for testing was the one that induced the largest whealing response in the initial skin tests for each subject.

Immediately following iontophoresis, each of the 4 areas of skin simultaneously pretreated was tested with an experimental scratch or intradermal skin test using the predetermined antigen. A saline sham test was also applied to the same area under investigation. After a 15 minute period, skin tests on each of the 4 areas were simultaneously read. The results were recorded as the greatest diameter of the wheal. Each skin test was evaluated by 2 independent observers without prior knowledge of the drugs used to pretreat the skin.

Analysis of data

The differences among the drug-induced effects on skin reactivity were analyzed by a one-way analysis of variance (Table II). Using Duncan's Multiple Range Test¹¹ (Table III), further analysis was made to determine if the mean values for each drug differed significantly from each other.

RESULTS

The study revealed differences ($p < 0.01$) in skin test reactivity to antigenic stimuli in skin pretreated with propranolol, isoproterenol, diphenhydramine,

TABLE II. Immediate hypersensitivity skin test reaction

Skin test	Allergic history	Propranolol (mm.)	Saline (mm.)	Diphenhydramine (mm.)	Isoproterenol (mm.)
ID	AR	12*	8*	5	3
S	AR	10	7	6	3
ID	AR	10	8	7	4
S	AR	8*	8	5	4
S	AR	10*	9*	7	5
S	A + AR	5	3	2	0
S	A + AR	3	2	0	0
S	AR	11*	5	2	3
S	A + AR	5	2	1	1
ID	AR	5	2	1	1
S	AR	12*	5*	3	4
S	A + AR	5*	2	2	0
S	AR	12*	8*	5	4
S	AR	8*	5	3	3
S	AR	7	6	4	3
Mean		8.2†	5.3†	3.3†	2.5†

AR: allergic rhinitis.

S: scratch test.

A: asthma.

*With pseudopod.

ID: intradermal skin test.

†Diameter of wheal in mm.

TABLE III. Analysis of data by Duncan's Multiple Range Test

Drugs compared	Difference	SSR*	Confidence level
Propranolol-isoproterenol	5.534	2.5	$p < 0.01$
Propranolol-diphenhydramine	4.734	2.43	$p < 0.01$
Propranolol-saline	2.934	2.33	$p < 0.01$
Saline-isoproterenol	2.6	2.43	$p < 0.01$
Saline-diphenhydramine	1.8	1.75 (0.05)	$p < 0.05$
Diphenhydramine-isoproterenol	0.8	1.75 (0.05)	Not significant

*Significant studentized range for 0.01 level except where p value is indicated in parentheses.

and saline (Table II). Propranolol-pretreated skin responded to antigenic challenge with a wheal-and-flare reaction significantly larger ($p < 0.01$) than that occurring in the area pretreated with saline, diphenhydramine, or isoproterenol. Conversely, skin pretreated with diphenhydramine or isoproterenol exhibited a significantly smaller wheal ($p < 0.01$) than that pretreated with saline or propranolol. In the present evaluation there was no statistical difference in the efficacy of diphenhydramine and isoproterenol regarding their effect on wheal suppression at the 15 minute post-skin test reading. However, the effect of isoproterenol appeared to be more sustained. Re-evaluation at 30 minutes after skin testing revealed progressive diminution of the wheal in isoproterenol-pretreated skin, whereas the diphenhydramine-pretreated wheals had increased in size. This increase approached control levels.

DISCUSSION

This investigation was intended to assess the effect of β -adrenergic manipulation on the immediate hypersensitivity skin test. To accomplish this, the technique of iontophoresis was employed. This method was utilized because relatively high concentrations of pharmacologically active agents could be delivered to

discrete, localized areas of the skin. Because of this feature of iontophoresis, it was hypothesized that several drugs could be delivered simultaneously in pharmacologically active local concentrations without significant elevation of systemic levels. This appeared to be the case since none of the patients studied exhibited any clinical evidence of systemic effects from the agents used. Iontophoresis, therefore, appeared to be a safe and effective technique to permeate human skin with β -adrenergic and other pharmacologic agents. After iontophoresis of a β -adrenergic blocking agent (propranolol), atopic individuals had increased wheal formation upon antigenic challenge. Conversely, in the same subjects, the iontophoresis of a β -adrenergic agonist (isoproterenol) decreased antigen-induced wheal formation. The iontophoresis of a competitive inhibitor of histamine (diphenhydramine) also produced a suppression of wheal formation equal to that of isoproterenol. However, the suppression induced by diphenhydramine was of shorter duration than that following the iontophoresis of isoproterenol.

The current hypothesis of adrenergic involvement in the immediate hypersensitivity reaction involves the modulation of CAMP synthesis by β -adrenergic agents. CAMP has been demonstrated to have a profound effect on the release of mediators of the immediate hypersensitivity reaction in many experimental systems.^{1, 2} Ishizaka and associates³ have demonstrated in sensitized human leukocytes in vitro that either CAMP or β -adrenergic agents can block histamine release upon antigenic challenge. In this in vitro human leukocyte system, the addition of β -adrenergic blocking drugs prevents this effect. Lichtenstein and De Bernardo,¹ using an in vitro sensitized monkey lung system, noted similar results not only for the release of histamine but also for slow-reacting substance of anaphylaxis (SRS-A).

Decreased mediator release due to stimulation of CAMP synthesis and availability is the most plausible explanation for the decreased wheal formation after antigenic challenge in the β -adrenergic stimulated skin of our atopic patients. Increased wheal formation in immediate hypersensitivity skin tests of skin locally pretreated with a β -adrenergic blocking agent may similarly be explained by inhibition of CAMP synthesis. However, recent evidence suggests that CAMP has direct action on the vascular system itself.¹⁴⁻¹⁷ Therefore, these drugs may be acting wholly, or in part, at the level of the target organ, the small blood vessels.

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