

The effect of epinephrine on platelet aggregation in normal and atopic subjects

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The effect of epinephrine on platelet aggregation was determined in 25 patients with allergic rhinitis, 26 patients with asthma, and 23 normal subjects. Three doses of epinephrine (0.42, 0.83, 3.3 μ g) were evaluated in each preparation. Although 7 (28 per cent) of the patients with allergic rhinitis exhibited abnormalities in the pattern of platelet aggregation after addition of 0.83 μ g of epinephrine, the mean results in this patient group were not significant compared to results in 23 normal subjects. At a lower epinephrine concentration (0.42 μ g), 9 (36 per cent) of these patients showed abnormal responses that were significant. In asthmatic patients the mean aggregation-disaggregation curve differed significantly from the normal mean curve at all concentrations of epinephrine, and these abnormalities appeared 4 to 7 minutes after stimulation of platelets by epinephrine. It was demonstrated that humoral factors were involved in these reactions by incubating platelet-poor plasma (PPP) from 3 asthmatic patients with normal platelets prior to epinephrine stimulation. Normal platelet aggregation was subsequently impaired by this procedure. It was also shown that abnormal platelet aggregation in asthmatic patients could be partially reversed by prior incubation of their platelets with normal plasma. These results suggest that asthmatic patients have a humoral factor that specifically alters the receptor control mechanism of secondary platelet aggregation. However, since normal plasma partially restores a normal aggregation profile to asthmatic platelets, the platelet receptor in these patients is not permanently altered.

Although β -adrenergic receptor blockade mechanisms have been implicated in atopic sensitization, supporting data in single cell preparations are available only in the human leukocyte system.¹ In 1970 Fishel and Zwemer² reported that platelets of atopic patients exhibited unusual patterns of disaggregation after stimulation with adenosine diphosphate (ADP). These results resembled the effects of propranolol upon normal platelets, and it was proposed that β -adrenergic blockade was involved. Since ADP does not act directly on adenylyl cyclase or phosphodiesterase, this hypothesis must be tested further. However, some investigators have demonstrated that the aggregating effect of epinephrine³ does depend upon its ability to stimulate adenylyl cyclase and to increase cyclic adenosine monophosphate (AMP) levels.⁴ Blockade of adenylyl cyclase and the second messenger system has also been suggested as a possible

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mechanism in allergic diseases.⁵ Platelet response was therefore investigated as a model of β -adrenergic blockade in allergic subjects. In this study, it was shown that platelet responses to epinephrine were abnormal in a majority of asthmatic patients. Similar effects were observed in patients with allergic rhinitis, but to a lesser extent.

METHODS

Based on a modification of the technique of Bucher and Stucki,⁶ platelet aggregation was performed in an Aggregometer.*⁷ The degree of aggregation in experimental samples was determined by comparison with the subject's platelet-poor plasma (PPP), which served as the blank. Siliconized glassware, stirring bars, and plastic syringes were used throughout the procedure. Platelet-rich plasma (PRP) was prepared by centrifuging 9 ml. of whole blood in 1 ml. of 3.8 per cent trisodium citrate (pH 3.2) at 100 g for 15 minutes. Following an initial platelet count in a Coulter counter (Model B), the number of platelets was adjusted to 300,000 platelets per cubic millimeter by dilution with PPP obtained by spinning PRP at 1,000 g for 15 minutes. Aggregation was always performed within 30 minutes of collection. PRP, 0.5 ml., was placed in a cuvette, the stirring bar was added, and the platelet suspension was equilibrated at 37° C. until a stable baseline recording was observed at a wave length of 610 nm. Thereupon, dose response to epinephrine† was determined by adding varying amounts of the amine to each of 3 different equilibrated platelet suspensions, so that the final concentration in each cuvette (0.55 ml.) was 0.42 (2.3×10^{-6} M), 0.83 (4.5×10^{-6} M), 3.3 (1.8×10^{-5} M) μ g, respectively. The effect of prior addition of 0.33 μ g propranolol (7×10^{-6} M) was also monitored in each experiment.^{2, 6}

Two groups of allergic patients were included in this investigation. The first group consisted of 25 patients (16 males, 9 females, age range 23 to 52, mean 38.2) with seasonal rhinitis caused by ragweed, a diagnosis established by clinical history, positive skin tests, and quantitative nasal challenge by ragweed pollen.⁸ None of these patients had received specific immunotherapy. The second group was comprised of 26 patients (12 males, 14 females, age range 13 to 69, mean 38.1) with histories of perennial, episodic wheezing for at least one year, physical signs of wheezing, and objective evidence of reversible airways obstruction as recently as one month before testing. Normal volunteers included 23 (17 males, 6 females, age range 20 to 34, mean 24.0) medical students and laboratory personnel. Because platelet aggregation may be affected by many drugs (aspirin in particular), patients were instructed to avoid these drugs (Table I) for at least one week before performance of the test.

RESULTS

The pattern of typical epinephrine-induced aggregation of normal platelets is illustrated in Fig. 1. After an initial lag period, the slope of the aggregation curve appears. This is followed by a plateau phase and a second wave of platelet aggregation. This effect could be modified by prior addition of propranolol, which either abolishes or decreases the secondary aggregation phase.^{2, 6}

The effects of epinephrine upon platelet aggregation in the 2 patient groups and normal control subjects were analyzed separately for each epinephrine concentration. Abnormal responses were characterized either by a delay in the appearance time of the second wave of aggregation or its complete abolition 4 to 7 minutes after the addition of epinephrine. Fig. 2 summarizes results of platelet aggregation in all patients and normal subjects after the addition of 0.83 μ g epinephrine. The mean aggregation-disaggregation curve of patients with

*Chrono-Log Corp., 2883 West Chester Pike, Broomall, Pa.

†Parke, Davis & Co., Detroit, Mich.

TABLE I. Drugs affecting platelet aggregation

1. Aspirin	13. Reserpine
2. Indomethacin	14. Antimalarial compounds
3. Ethylenediaminetetraacetic acid	15. Antacids containing magnesium
4. Phenylbutazone	16. Beta blocking agents
5. Sulfinpyrazone	17. Colchicine
6. Persantin	18. Prostaglandin E ₁
7. Phenothiazines	19. Pyrimido-pyrimidine compounds
8. Tricyclic compounds	20. Heparin
9. Alpha adrenergic blockers	21. Mefenamic acid
10. Local anesthetics	22. Papaverine
11. Antihistamines	23. Glyceryl trinitrate
12. Azathioprine	

allergic rhinitis reveals alterations from the normal pattern 5 to 7 minutes after epinephrine was added to the platelet suspension. This variation was attributed to 7 (28 per cent) patients who exhibited definite abnormalities. When the mean results of patients with allergic rhinitis were compared to the mean results of normal subjects by Students' *t* test, these differences were not significant at any of the time intervals. At the highest concentration of epinephrine (3.3 μg), 5 or 20 per cent of patients demonstrated similar results. However, a decrease in epinephrine concentration to 0.42 μg appeared to increase the sensitivity of the procedure, since 9 or 36 per cent of the patients showed abnormal responses at this level. The latter results were significant ($p < 0.05$) 7 minutes after the addition of epinephrine.

In asthmatic patients (Fig. 2) it is apparent that the mean aggregation-disaggregation curve differs significantly from the normal mean curve, which begins to show aggregation approximately 2 minutes after the addition of epinephrine. Abnormalities in 15 or 58 per cent of the asthmatic patients were detected 4 to 7 minutes after platelet stimulation by 0.83 μg epinephrine. These results were significant at the fourth ($p < 0.02$), fifth ($p < 0.01$), sixth ($p < 0.005$), and seventh ($p < 0.005$) minute time intervals. Results of similar type were also observed in 16 (62 per cent) and 15 (56 per cent) of asthmatic patients at epinephrine concentrations of 0.41 μg and 3.3 μg , respectively. The lowest concentration of epinephrine (0.41 μg) also increased the sensitivity of response in asthmatic patients ($p < 0.001$ at the sixth and seventh minute time intervals). The abnormal pattern of platelet aggregation in a single asthmatic patient is shown in Fig. 3, *B* (left).

Asthmatic patients with normal platelet patterns could not be clearly differentiated from asthmatic patients with abnormal responses on the basis of clinical severity. The mean disability (school or work loss weeks) was 3.2 weeks in the nonresponder group and 3.8 weeks in the responder group. Mean duration of asthma in these groups was 10.2 and 12.4 years, respectively. Ten of 15 patients with abnormal platelet aggregation required steroids for control of symptoms as compared to 5 of 11 patients with normal platelet aggregation. In 6 patients who were classified as intrinsic, normal and abnormal platelet responses were evenly divided.

To determine if humoral factors were involved in these reactions, PPP from asthmatic patients was incubated with normal PRP in equal volumes for 30 min-

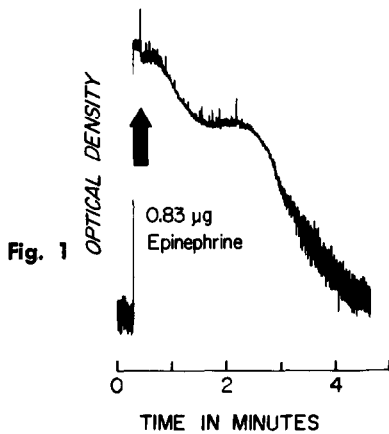


Fig. 1

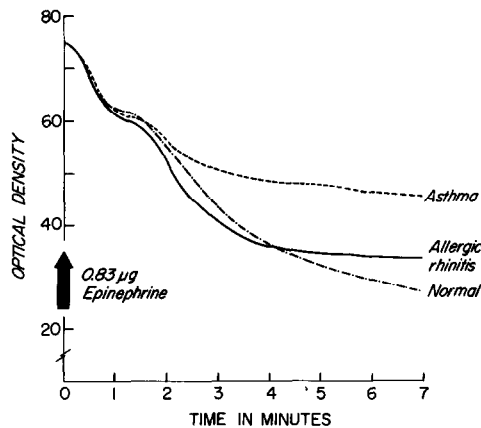


Fig. 2

FIG. 1. Pattern of platelet aggregation of normal platelets after addition of 0.83 μ g of epinephrine.

FIG. 2. Mean platelet aggregation responses to epinephrine (0.83 μ g) in 25 patients with seasonal allergic rhinitis and 26 asthmatic patients compared to 23 normal subjects.

utes at 37° C. prior to epinephrine stimulation. A typical result of one of these experiments is illustrated in Fig. 3, A. As expected, normal platelets aggregated normally either in their parent plasma or homologous normal PPP. When these same platelets were added to an asthmatic patient's plasma, aggregation was impaired. Similar observations were obtained when normal platelets were incubated with PPP from 2 other asthmatic patients. It was also demonstrated that abnormal platelet aggregation was partially reversible (Fig. 3, B). A typical abnormal aggregation pattern of PRP from an asthmatic patient after incubation with autologous PPP is represented in this figure. When this patient's platelets were resuspended in normal PPP, some improvement in aggregation was noted. This phenomenon was demonstrated in 3 different experiments. Finally, platelet aggregation remained impaired when PRP from an asthmatic patient was incubated with PPP from a different asthmatic patient whose intact platelet system was abnormal. Similar results were obtained in 3 separate experiments.

DISCUSSION

The mechanism of epinephrine-induced platelet aggregation is controversial. In a recent review Salzman⁹ stated that the phenomenon is an α -adrenergic effect that leads to a reduction in intracellular cyclic AMP. However, Haslam and Taylor⁴ described a stimulatory effect of epinephrine on intracellular platelet cyclic AMP. Although the α -adrenergic blocking agents, phentolamine and ergotamine, are capable of inhibiting the initial aggregation phase of epinephrine-induced platelet aggregation, they do not affect the second sequence of aggregation.¹⁰ Propranolol-treated platelets disaggregate faster, are more resistant to the release of intrinsic ADP, and aggregate poorly, if at all, after the initial

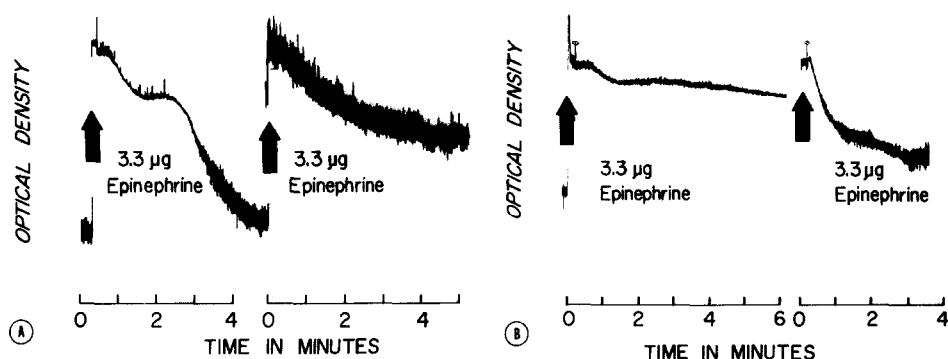


FIG. 3. **A**, The effect of platelet-poor "asthmatic plasma" on normal platelets. The platelet aggregation (left) was obtained from a normal subject. PPP from an asthmatic patient was added to normal platelets (right) before the addition of 3.3 μ g of epinephrine. Identical responses were also observed with smaller doses of epinephrine (0.42 and 0.83 μ g). **B**, Effect of normal PPP on the aggregation of platelets obtained from a patient with asthma. The platelet aggregation occurring after incubation of the patient's platelets with autologous plasma is shown on the left. The patient's platelets (right) are resuspended in normal PPP. Similar results were obtained with 0.42 and 0.83 μ g concentrations of epinephrine.

aggregation event.⁷ These results suggest that the two phases of platelet aggregation induced by epinephrine may be mediated by different receptors, α -adrenergic for initial aggregation and β -adrenergic for the second phase of disaggregation and subsequent aggregation. Results of this investigation support this idea inasmuch as abnormal platelet responses induced by epinephrine in patients with allergic rhinitis and asthma were confined to the phases of disaggregation and secondary aggregation.

Although the majority of patients with asthma exhibited platelet malfunction after stimulation with epinephrine, similar changes were observed in some patients with allergic rhinitis. Whether platelet abnormalities in allergic rhinitis might indicate a predisposition to asthma later in life is unknown, but sequential studies of this type are indicated. A retrospective analysis of several criteria of clinical severity in the asthmatic population did not suggest clear-cut distinguishing characteristics between patients with normal and abnormal aggregation patterns. It was evident that steroid therapy was required more often in patients with abnormal platelet reactions, but the significance of this observation must await extended studies in a larger sample of asthmatic patients.

The effect of PPP of asthmatic patients upon normal platelets suggests the presence of a humoral factor, which specifically alters the receptor control mechanism of secondary aggregation. However, since normal PPP partially restores a normal aggregation profile to platelets of asthmatic patients, the platelet receptor in these patients is not permanently altered. This could be considered analogous to the rise and ebb of β -adrenergic blockade effects observed in asthmatic patients after bouts of respiratory infection or other exacerbating stimuli. This technique offers a new approach to the study of the β -adrenergic mechanisms in patients with allergic disorders.

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