

EFFECT OF PHENTOLAMINE ON PLATELET AGGREGATION IN PATIENTS  
WITH PHEOCHROMOCYTOMA

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ABSTRACT

Since exogeneous catecholamines potentiate ADP-induced aggregation in vitro, and this effect is blocked directly by phentolamine, it was assumed that platelets from patients with pheochromocytoma would be unusually sensitive to ADP and that this sensitivity would be reduced in the presence of phentolamine. Findings in four patients with pheochromocytoma were compared to results in 20 normals. Phentolamine had no immediate effect in either group. Pheochromocytoma platelets became more responsive to ADP after standing and this increase in responsiveness was inhibited by phentolamine. These results: 1) suggest that catecholamine concentrations in the plasma of patients with pheochromocytoma are not high enough to potentiate ADP aggregation and 2) may be explained by assuming that pheochromocytoma platelets are saturated with catecholamines.

INTRODUCTION

Catecholamines induce (1) and potentiate (2) blood platelet aggregation when studied "in vitro". Whether this phenomenon occurs "in vivo" in humans is not known, although it has been suggested, particularly in discussions of factors precipitating acute myocardial infarction, and there is some indirect evidence to support this view (3). In considering strategies to get directly at this question, the study of patients with pheochromocytoma was selected as being a practical approach to obtain relevant information. Since catecholamines in nearly physiologic concentrations potentiate ADP-induced platelet aggregation, and this effect is prevented by the alpha-adrenergic blocking agent, phentolamine (2,4), it was hypothesized that platelets from patients with pheochromocytoma would be unusually sensitive to ADP and that this sensitivity could be unmasked by phentolamine. We report here the results of such studies in four patients with pheochromocytoma as contrasted to findings in a control group. Also reported are the results of preliminary studies which were required for the development of an appropriate experimental design.

### METHODS

#### Subjects

One patient with pheochromocytoma and twenty healthy adults provided blood for preliminary experiments and another twenty healthy adults were bled for the definitive experiment. Four patients with pheochromocytoma were the main subjects of investigation in the definitive experiment (Table 1). All subjects had taken no medication for at least seven days prior to venesection.

TABLE  
1

Clinical Data from Four Patients with Pheochromocytoma

| Patient | Age | Sex | Blood Pressure<br>mm Hg | Catecholamines<br>µg/24° Urine |                | Tumor Weight<br>(Grams) |
|---------|-----|-----|-------------------------|--------------------------------|----------------|-------------------------|
|         |     |     |                         | Epinephrine                    | Norepinephrine |                         |
| T.K.    | 19  | F   | 180/110                 | < 1.0                          | 838            | 49                      |
| R.T.    | 10  | M   | 190/150                 | 312                            | 1430           | 16                      |
| T.T.    | 9   | M   | 160/115                 | 163                            | 2266           | 12                      |
| R.A.    | 24  | M   | 155/100                 | 16                             | 757            | 25                      |

#### Platelet Aggregation

Studies were carried out on venous blood drawn into plastic or siliconized glass syringes containing 3.8% sodium citrate (9 parts blood to 1 part citrate). Tests were performed either at room temperature or at 37°C utilizing previously described turbidimetric techniques (5,6). Phentolamine (Regitine<sup>R</sup> CIBA) was diluted with normal saline solution to provide the concentrations indicated in the various experiments.

#### Preliminary Experiments

##### 1. Effect of phentolamine on platelet aggregation in one patient with pheochromocytoma.

Responses to  $1.0M \times 10^{-6}$  and  $5.0M \times 10^{-6}$  ADP were studied at room temperature. Aggregation was induced 1 minute after the addition of phentolamine  $10^{-4}M$  and  $10^{-6}M$  to the platelet rich plasma (PRP). Maximum aggregation in the presence of phentolamine was contrasted to that observed in the presence of an equal volume of saline. Phentolamine  $10^{-6}M$  had no effect but  $10^{-4}M$  produced a striking reduction of the maximum rise (MR) of the aggregation curve.

##### 2. Effect of phentolamine on platelet aggregation in normal subjects.

Aggregation with ADP  $10^{-6}M$  in the presence of saline or phentolamine was studied at room temperature. Response in the presence of phentolamine  $10^{-6}M$  was compared to saline controls. If no inhibition was apparent at  $10^{-6}M$  the procedure was repeated at  $10^{-5}M$ , and, if indicated, at  $10^{-4}M$ . It was found that phentolamine  $10^{-4}M$  consistently inhibited ADP aggregation, while lower concentrations did not.

##### 3. Phentolamine inhibition of adrenaline-potential of ADP aggregation: effect of prolonging PRP exposure to adrenaline.

Aliquots of PRP were incubated in the presence of  $1 \times 10^{-8}M$  adrenaline (Parke-Davis) for specified times at room temperature. Saline was then added, and aggregation was induced with  $10^{-6}M$  ADP 1 minute later. This procedure

was repeated on a duplicate sample of PRP, but phentolamine  $10^{-6}M$  was added in the place of saline prior to aggregation with ADP. Samples were incubated with adrenaline for 1, 3, 5, 15, and 35 minutes. Studies were performed at room temperature on PRP from three normal subjects. It was found that incubation with adrenaline for periods up to 35 minutes did not alter the potentiating effect of adrenaline on the ADP response or phentolamine's ability to block that potentiation (Fig. 1).

PHEOTOLAMINE INHIBITION OF ADRENALINE POTENTIATION  
OF ADP AGGREGATION  
(EFFECT OF DURATION OF ADRENALINE INCUBATION)

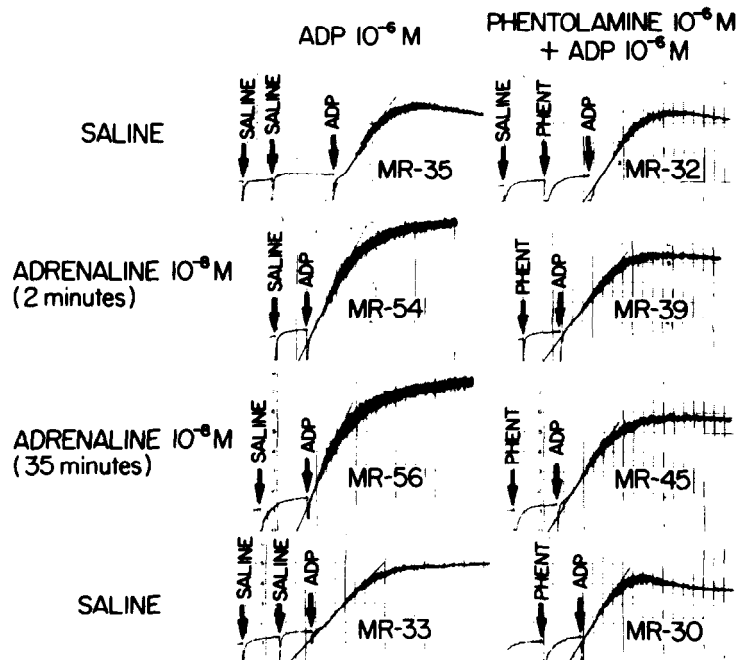


FIG. 1

ADP-induced aggregation in the presence of phentolamine after brief and prolonged exposure to a potentiating concentration of adrenaline. Studies were performed on aliquots from one plasma sample. MR = maximum rise.

## Experimental Design

### Aggregation at Room Temperature

Aggregation with ADP  $10^{-6}M$  in the presence of either saline or phentolamine  $10^{-6}M$  was compared. PRP was allowed to stand with saline for 1 minute before addition of ADP, and then another aliquot utilizing phentolamine in place of saline was studied. The same procedure was repeated with a pair of samples incubated at room temperature with saline and phentolamine respectively for a period of 30 minutes prior to aggregation.

### Aggregation at 37°C

Studies were performed in the same manner as those at room temperature, except that: 1) the incubation period was extended to 80 minutes, 2) the ADP concentration was individualized for each subject so that the minimal concentration of ADP needed to induce a second wave of aggregation was utilized (the "ADP endpoint" concentration).

## RESULTS

### Aggregation at Room Temperature

In control subjects aggregation was uninfluenced by phentolamine; even prolonged exposure to it (Table 2). In patients immediate aggregation was not significantly different from control subjects. However, the ADP response of pheochromocytoma platelets was more intense after 30 minutes of standing ( $MR = 68.3 \pm 14.2$  as compared to  $39.5 \pm 1.8$  without standing). This change on standing was not observed in control subjects and proved to be highly significant ( $p < 0.001$ ). Although aggregation was slightly less in patients after short exposure to phentolamine, this effect was not greater than that observed in control subjects under these conditions. However, aggregation of pheochromocytoma platelets was clearly inhibited by 30 minutes exposure to phentolamine.

### Aggregation at 37°C

#### "ADP End-point"

In control subjects end-point concentrations ranged from  $1.0M \times 10^{-6}$  to  $5.0M \times 10^{-6}$  with a mean of  $2.0M \times 10^{-6}$ . The mean value for patients with pheochromocytoma was also  $2.0M \times 10^{-6}$  with a range from 1.0 to  $4.0M \times 10^{-6}$ .

#### Effect of Phentolamine on ADP Aggregation

Immediate aggregation in saline was similar in both groups of subjects and there was no evidence of a direct inhibitory effect of phentolamine (Table 2). Responses in the groups were clearly different after prolonged standing in the presence of the alpha blocker. Aggregation after 80 minutes of phentolamine was inhibited in patients, but not among controls (Fig. 2). This phenomenon was not observed in one patient (R.A.) studied eight months after removal of his tumor (Fig. 2).

## DISCUSSION

Adrenaline (and nor-adrenaline) can influence human blood platelet aggregation in three ways when studied in vitro. In concentrations greater than  $10^{-6}M$  adrenaline causes biphasic aggregation (2,7) but in the  $10^{-7}M$  range a uniphasic response is typically all that is seen (2,8). At lower concentrations, usually not less than  $10^{-8}M$ , the presence of adrenaline is manifested by an increased sensitivity to aggregation induced by ADP (2).

Plasma concentrations of catecholamines in humans normally are in the

$10^{-9}$ M range (9). Higher concentrations are found in patients suffering from pheochromocytoma (10), but it is unlikely that these exceed the  $10^{-8}$ M range. We therefore designed our platelet aggregation studies in such a way as to uncover heightened sensitivity to ADP in patients with this disorder.

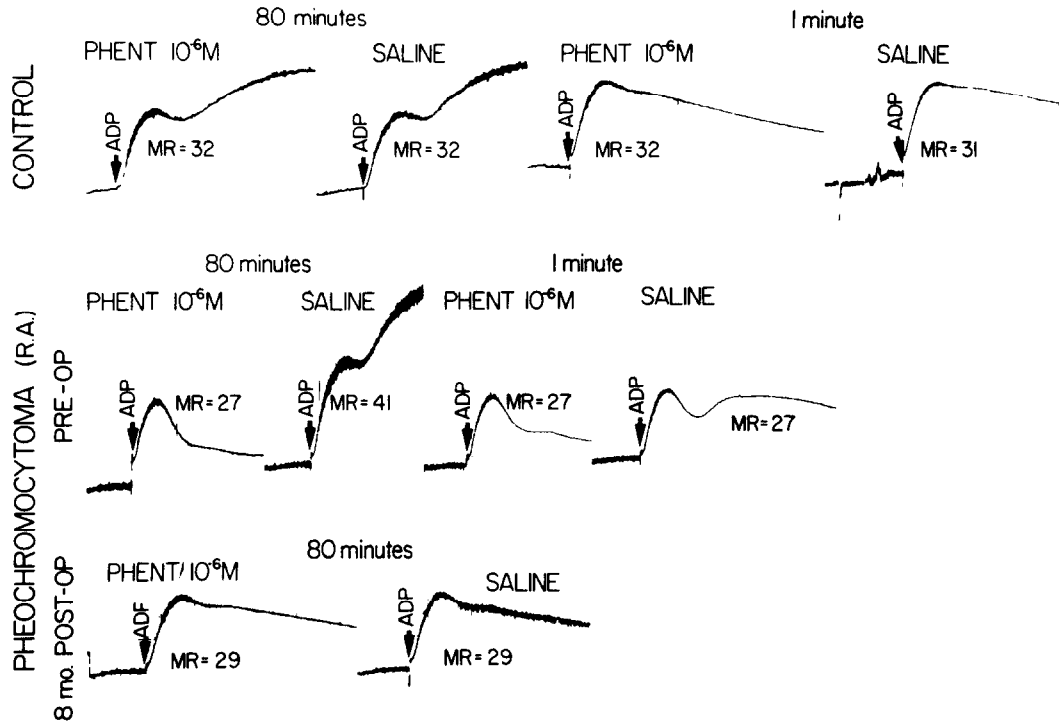


Fig. 2

ADP-induced aggregation in the presence of phentolamine in a normal subject and a patient with pheochromocytoma.

Preliminary investigation in one patient with pheochromocytoma revealed that  $10^{-6}$ M phentolamine did not have an immediate inhibitory effect, whereas  $10^{-4}$ M did. A subsequent experiment to find the optimal concentration of phentolamine to utilize in further studies of patients revealed that  $10^{-4}$ M was inhibitory to all platelets whereas  $10^{-6}$ M was not. A non-specific inhibitory effect of high concentrations of phentolamine has in fact been suggested by others (11,12). The concern that prolonged exposure of platelets to adrenaline might interfere with phentolamine's ability to block its potentiating effect on ADP aggregation led to a final preliminary experiment which demonstrated that this was not the case. Nevertheless, in view of the observation that  $10^{-6}$ M phentolamine had not had an immediate effect in our first patient, the definitive experiment was designed so as to study the effects of both immediate and prolonged exposure to phentolamine.

Analysis of the data collected in this study resulted in three major findings. First, pheochromocytoma platelets are no more aggregable in response to ADP than normal ones. This finding tends to deny the hypothesis that catecholamine-potentiated ADP aggregation occurs in patients with this clinical problem. However, in view of the considerable variability of ADP response, and the small number of patients included in this study, no firm conclusions can be drawn from this negative observation. Also, the only

TABLE

2

ADP-Induced Platelet Aggregation  
(maximum rise of 1st wave in O.D. units)

|          | Room Temperature<br>(ADP $10^{-6}$ M) |             |           |             | 37°C<br>(ADP $2M \times 10^{-6}$ ) |             |           |             |       |       |         |
|----------|---------------------------------------|-------------|-----------|-------------|------------------------------------|-------------|-----------|-------------|-------|-------|---------|
|          | Immediate                             |             | Incubated |             | Immediate                          |             | Incubated |             |       |       |         |
|          | Saline                                | Phent† Diff | Saline    | Phent† Diff | Saline                             | Phent† Diff | Saline    | Phent† Diff |       |       |         |
| Controls | 54.8                                  | -0.39       | 54.6      | 51.7        | 2.9                                | 44.2        | 43.5      | 0.71        | 43.8  | 43.4  | 0.41    |
| Mean     | (3.9)                                 | (4.1)       | (4.2)     | (4.0)       | (1.5)                              | (2.9)       | (2.7)     | (.85)       | (2.5) | (2.4) | (.05)   |
| <hr/>    |                                       |             |           |             |                                    |             |           |             |       |       |         |
| Patients |                                       |             |           |             |                                    |             |           |             |       |       |         |
| T.K.     | 39                                    | 38          | 106       | 64          |                                    | 30          | 30        |             | 42    | 23    |         |
| R.T.     | 35                                    | 32          | 50        | 33          |                                    | --          | --        |             | 51    | 38    |         |
| T.T.     | 40                                    | 35          | 43        | 19          |                                    | 57          | 46        |             | 48    | 30    |         |
| R.A.     | 44                                    | 38          | 74        | 60          |                                    | 30          | 30        |             | 41    | 35    |         |
| <hr/>    |                                       |             |           |             |                                    |             |           |             |       |       |         |
| Mean     | 39.5                                  | 35.8*       | 68.3      | 44.0        | 24.3***                            | 39.0        | 35.3      | 3.7         | 45.5  | 31.5* | 14.0*** |
| (SEM)    | (1.8)                                 | (1.4)       | (14.2)    | (10.8)      | (6.3)                              | (9.0)       | (5.3)     | (3.7)       | (2.4) | (3.3) | (3.0)   |

† Phent = Phentolamine  $10^{-6}$ M

\*\* = &lt;.01

\* = &lt;.05

\*\*\* = &lt;.001

report in the clinical literature which bears on the present investigation is in fact not in agreement with these results. Danta (13) has reported that platelet adhesiveness declines in patients with pheochromocytoma after tumor removal, suggesting the presence of heightened platelet responsiveness preoperatively.

The second noteworthy finding in this study is related to a pair of observations: the lack of inhibition of ADP aggregation of pheochromocytoma platelets by a 1 minute exposure to phentolamine  $10^{-6}M$  and the absence of a difference in ADP end-points between patients and controls. Both of these observations support the impression, already suggested, that there is not catecholamine potentiation of the ADP response in patients with pheochromocytoma.

The third interesting finding in the study is the demonstration that prolonged exposure to phentolamine is inhibitory to pheochromocytoma platelets. This might be explained by assuming that prolonged contact is needed for phentolamine to compete effectively with catecholamines that have had ample opportunity to saturate the alpha receptor sites. Another hypothesis is suggested, however, by the fact that ADP responses increase substantially after platelets from patients stand at room temperature for as little as 30 minutes. It is well known that the reactivity of normal platelets varies considerably during standing. Most observers have found a slight increase of either platelet adhesiveness or ADP response after 1 or 2 hours and then a gradual decline (14,15). Recently a time-dependent increase in responsiveness after incubation at  $37^{\circ}C$  to epinephrine and collagen also has been reported (16). The increment in ADP response has been attributed to alteration of platelet membrane permeability with associated leakage of ADP into the plasma (17).

Normal human platelets contain very small quantities of catecholamines, but there is indirect evidence to suggest that these may be of some importance in platelet aggregation (18). Also, there are data which indicate that there is a slow spontaneous release of catecholamines from platelets resuspended in artificial medium (19). It is conceivable that pheochromocytoma platelets contain more catecholamines than normal ones, since platelets have been found to actively absorb both adrenaline (12,19) and nor-adrenaline (20) in vitro. If it is assumed that pheochromocytoma platelets are rich in catecholamines and that these leak out in substantial quantity during standing, then it can be seen how a potentiated ADP response might result and that this would be inhibited by phentolamine. The difference in results of aggregometry performed at room temperature and  $37^{\circ}C$  may be explained by a combination of metabolic inactivation and reuptake of released epinephrine during incubation at  $37^{\circ}C$  prior to addition of ADP.

It appears then, that pheochromocytoma platelets behave differently than normal platelets, but not in the manner which one might have predicted. They do not behave as if they were surrounded by catecholamines in concentrations high enough to potentiate ADP aggregation, but they tend to become more reactive during standing. The results may be interpreted to suggest that these platelets contain large amounts of catecholamines. This hypothesis deserves direct testing, since, if correct, it could lead to the development of simplified blood tests for the detection of pheochromocytoma rather than present methods which require urine collection over a period of time.

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