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INHIBITION OF EPINEPHRINE-INDUCED PLATELET AGGREGATION BY A DERIVATIVE OF WHEAT GERM AGGLUTININ

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SUMMARY

A nonagglutinating derivative of wheat germ agglutinin has been prepared and used as a probe to explore the initial events in platelet activation. The lectin derivative had no effect on platelet aggregation by adenosine diphosphate, collagen, ristocetin, wheat germ agglutinin or trypsin but aggregation induced by epinephrine or thrombin was inhibited. Unlike thrombin, the inhibition of aggregation by the derivative could not be overcome by increasing the concentration of epinephrine. The derivative did not affect the binding of [^{3}H]dihydroergocryptine to platelets. A 74,000 dalton protein isolated from platelet membranes by lectin affinity chromatography strongly inhibited platelet activation by thrombin but not by epinephrine. The receptors for thrombin and for epinephrine on platelets are different but they are closely linked.

Human platelets aggregate and undergo a secretion reaction when incubated with a number of agents including thrombin and catecholamines (1, 2). The initial step in platelet activation is believed to be binding of these ligands to specific receptors on the cell surface which leads to cell aggregation and secretion following a complicated series of events (3). While the broad characteristics of platelet aggregation are known, there is little information available on the molecular interactions involved. This paucity of information is primarily due to the rapidity of the interactions and our inability to distinguish between ligand-specific events from the general metabolic changes associated with cell activation. To explore these initial events, we have prepared a derivative of wheat germ agglutinin (WGA) which does not stimulate platelets but retains considerable cell binding capacity. This lectin derivative strongly inhibited platelet aggregation by thrombin while aggregation induced by a host of other agents was not affected (4). Further studies

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revealed that the derivative can block platelet aggregation by epinephrine giving rise to the possibility that there may be a single receptor for both epinephrine and thrombin on platelets. We present data which show that the platelet receptors for epinephrine and for thrombin are different but they may be closely coupled.

MATERIALS AND METHODS

Blood was collected from healthy volunteers in plastic syringes utilizing 0.1 volume of 3.8% sodium citrate as anticoagulant. The red cells were removed by low speed centrifugation and the platelets were isolated as described (5). Wheat germ agglutinin (WGA) was purchased from U.S. Biochemicals (Cleveland, Ohio). Data on its purity and interaction characteristics with platelets have been published (6-8). The WGA derivative was prepared by treating the lectin in 65% formic acid with excess CNBr for 20 hr (4). Epinephrine (1 mg/ml) in 0.15 M NaCl was purchased from Parke Davis (Detroit, Michigan).

Platelet aggregation was measured in a dual channel aggregometer (Payton, Buffalo, New York) (7). The experimental sample was analyzed in one channel while a control was run in the other. The amounts of stimulants shown are final concentrations. To measure secretion, platelets were loaded with [^{14}C]serotonin (59 mCi/mmol, Amersham, Arlington Heights, Illinois) and aggregation experiments were carried out as above. Five min after the addition of the stimulant, 0.1 ml of 10% formaldehyde was added to the platelet suspension. The samples were centrifuged and the radioactivity in an aliquot of the supernatant was measured and expressed as percent of appropriate controls (7).

Platelets suspended in phosphate-buffered saline (PBS) was first incubated for 10 min at room temperature with different amounts of the WGA derivative and then for another 15 min with a constant amount of [^{3}H]dihydroergocryptine (DHEC, 24 Ci/mmol, New England Nuclear, Boston, Massachusetts) in the dark. The platelet-bound DHEC was separated from free DHEC by filtration through 1.2 μ millipore filters under reduced pressure (9). The tubes and filters were then washed with 5 ml of buffer. The filters were dried and counted in a Packard liquid scintillation counter with an efficiency of 45%. Nonspecific binding was determined in the presence of 100 fold excess phentolamine (Ciba, Summit, N.J.) (10).

RESULTS AND DISCUSSION

Incubation of platelets with small amounts of epinephrine led to cell aggregation. The aggregation was characterized by a primary wave followed by a secondary wave after some delay. There was no detectable shape change prior to aggregation. The WGA derivative strongly inhibited platelet aggregation induced by epinephrine while aggregation by collagen, ADP, WGA, ristocetin and trypsin was not affected under the same conditions (Fig. 1) (4). Thus, the inhibition of epinephrine-induced aggregation was not due to a nonspecific perturbation of platelets by the WGA derivative. The inhibitory effect was

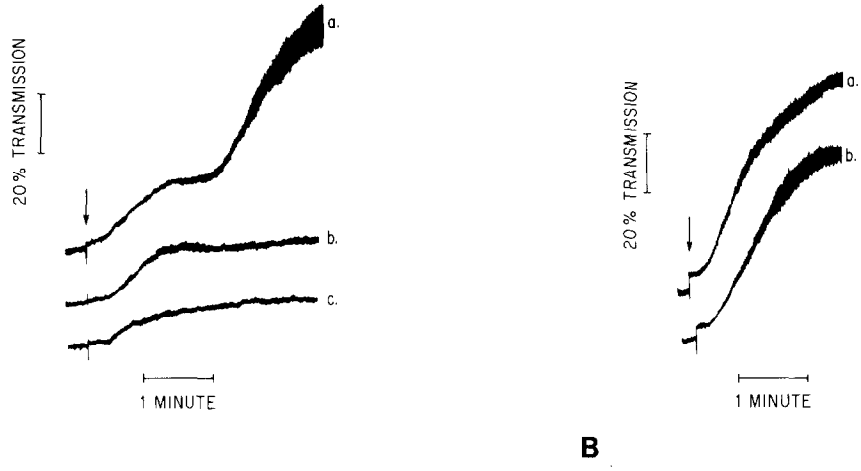

Fig. 1. (A) Inhibition of epinephrine-induced aggregation of human platelets in plasma (3 x 10^8/ml) by the derivative of wheat germ agglutinin. Pattern (a) shows the control while in patterns (b) and (c) platelets were pre-incubated with 100 µg/ml and 150 µg/ml of the lectin derivative respectively. Aggregation was initiated with 10 µg of epinephrine added at the arrow to a final volume of 0.5 ml of the platelet suspension. (B) Lack of effect of the lectin derivative on ADP-induced platelet aggregation. This experiment was done as in (A) by adding 1 x 10^{-5} M ADP at the arrow. Pattern (a) shows the buffer control while in pattern (b) the sample was preincubated with 100 µg/ml of the derivative.

more prominent on the secondary than on primary aggregation. Since secondary aggregation by epinephrine has been associated with materials secreted from platelets (11), we measured the release of serotonin from platelets. Under the conditions when the WGA derivative blocked aggregation, the release of serotonin from platelets was also inhibited. In Fig. 1, sample (a) showed 87% serotonin release while sample (b) or (c) had only 4% release. This inhibition of platelet aggregation or secretion by the WGA derivative could not be overcome by increasing the concentration of epinephrine.

One possibility is that the WGA derivative blocks platelet activation by competing with epinephrine for binding to its receptor on platelets. To explore this point, we measured the effect of different concentrations of the derivative on the binding of [^{3}H]DHEC to platelets. The WGA derivative did not affect the binding of DHEC to platelets suggesting that the inhibition of epinephrine-induced platelet aggregation by the derivative is a post-binding phenomenon (Fig. 2).

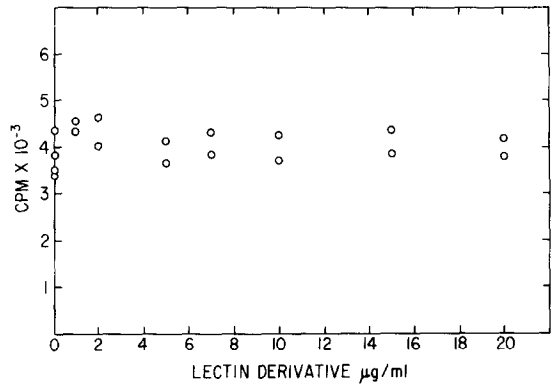

Fig. 2. Effect of increasing concentration of the derivative of wheat germ agglutinin on the binding of [^{3}H]dihydroergocryptine to platelets. Platelets in PBS were first incubated with different amounts of the lectin derivative for 10 min at room temperature and then a constant amount (1 nM) of DHEC was added. The amount of DHEC bound to platelets was determined after 15 min at room temperature by millipore filtration.

The above results indicate that the receptors for epinephrine and the WGA derivative are different which may be located on two different molecules or on two different sites of the same molecule. Affinity chromatography of solubilized platelet membranes utilizing the WGA derivative led to the isolation of a glycoprotein of apparent molecular weight of 74,000 (4). The effect of this isolated glycoprotein on platelet aggregation induced by several agents was tested. Similar to platelet aggregation caused by ADP, collagen or trypsin, the glycoprotein did not affect aggregation induced by epinephrine (Fig. 3). The glycoprotein blocked platelet aggregation only by thrombin under these conditions (4).

Catecholamine receptors on different cells have been classified as α-adrenergic, β-adrenergic or dopaminergic according to the potency of various catecholamines in elucidating a cellular response. There is some indirect evidence that aggregation of human platelets by catecholamines may be mediated through an α-adrenergic receptor although the identity and exact properties of the platelet receptor have remained unclear (11, 12, 13). We have recently prepared a nonagglutinating derivative of WGA and used it as a probe to explore the initial events in platelet activation (4). This derivative

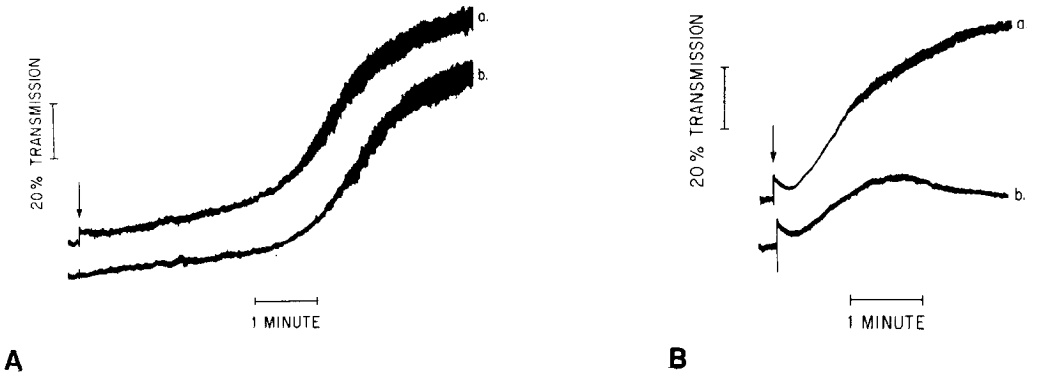

Fig. 3. (A) Lack of effect of the isolated receptor for the lectin derivative on epinephrine-induced aggregation of platelets in plasma (3 x 10^8/ml). Pattern (a) is the buffer control while in pattern (b) the ligand was first incubated for 10 min at room temperature with 4 μg/ml of the 74,000 dalton protein. Aggregation was initiated with 10 μg of epinephrine added at the arrow to a final volume of 0.5 ml. (B) Inhibition of thrombin-induced platelet aggregation by the 74,000 dalton protein of platelets. This experiment was done as in (A) but with 50 mU (1 nM) of thrombin which was added at the arrow. Pattern (a) is the buffer control while in (b) the thrombin sample was first incubated with 1.2 μg/ml of the protein for 10 min at room temperature before addition to the platelets.

blocked platelet stimulation by thrombin and by epinephrine while aggregation induced by a number of other agents was not affected (4). This study was undertaken to explore the hypothesis whether thrombin and epinephrine may act on platelets through a single receptor. The high degree of specificity observed in the inhibition reaction made it unlikely that the derivative exerts a nonspecific inhibitory effect only on epinephrine-platelet interaction without affecting any other agent except thrombin. The results of this study show that there are two important differences: (a) unlike thrombin, the inhibition of epinephrine-induced platelet aggregation by the derivative could not be reversed by increasing the epinephrine concentration and (b) the isolated glycoprotein blocked thrombin-induced platelet activation but had no effect on platelet aggregation by epinephrine even at a ten fold higher protein concentration. Thus, the lectin derivative inhibits platelet aggregation by epinephrine by interfering with the processing of the receptor which leads to signal generation. These observations suggest that the thrombin

receptor and epinephrine receptor on platelets are different but they may be closely linked.

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