Vasoactive Intestinal Peptide Receptors in Human Platelet Membrane

Characterization of Binding and Functional Activity

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Vasoactive intestinal peptide (VIP) mediated inhibition of platelet aggregation has been demonstrated in rabbit platelets.[1] While the mechanism by which VIP inhibits aggregation is thought to be via a specific receptor linked to adenylate cyclase, platelet receptors for VIP have not yet been demonstrated. The current studies were designed to test whether VIP regulates aggregation in human platelets and to determine whether specific, high-affinity VIP receptors are expressed on platelet membranes.

MATERIALS AND METHODS

Membrane Preparation

Platelet-rich plasma was isolated from peripheral blood of normal human subjects. Platelets were disrupted in lysis buffer (10 m*M* Tris, 5 m*M* EDTA, pH 7.4) with a polytron. Plasma membranes were isolated by differential centrifugation[2] and stored in Buffer A (20 m*M* Hepes, 150 m*M* NaCl, 2 m*M* $MgCl_2$, 5 m*M* EDTA, 1 m*M*-β-mercaptoethanol, 50 μg/ml phenylmethyl sulfonyl fluoride, pH 7.4).

[^{125}I]VIP Binding Studies

Membranes (200 μg protein) were incubated at 17°C with 100-150 p*M* [^{125}I]VIP (SA = 647 Ci/g), in the absence or presence of competing unlabeled peptide, in a final volume 0.5 ml. Membrane-bound [^{125}I]VIP was separated from free by filtration through Whatman GF/C filters presoaked in 0.3% polyethylenimine as described by O'Dorisio *et al.*[3] Time course and temperature studies demonstrated steady-state binding from 30 to 90 minutes at 17°C. The percentage of total counts bound to membrane protein ranged from 18% to 31% with 61% to 82% of bound ligand representing specific binding. Specific binding increased linearly with increasing concentration (50 to 500 μg) of membrane protein. Kinetic parameters were determined by a nonlinear computer-assisted fit of the competitive binding data.[4]

Platelet Aggregation Studies

Peripheral blood was obtained from healthy volunteers and slowly dispensed into 5-ml polystyrene tubes containing citrate (4.5 ml blood + 0.5 ml citrate). Platelet-rich plasma was prepared by centrifuging citrated blood at 25°C for six minutes at 400 × *g*. Platelet-poor plasma was obtained by recentrifuging the blood specimen 10

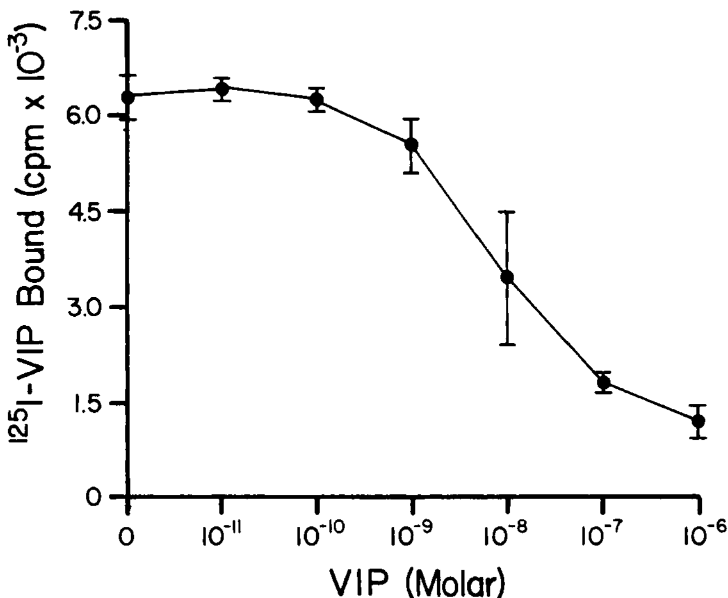

FIGURE 1. Competitive binding of [^{125}I]VIP to platelet membranes. 200 μg of membrane protein was incubated with 100 p*M* [^{125}I]VIP in the presence of indicated concentrations of unlabeled VIP for 45 minutes at 17°C. Computer-assisted fit of 12 competitive binding experiments was used to calculate affinity constants (K_D) and maximum number of binding sites (B_{max}).

TABLE 1. Effect of VIP on Platelet Aggregation[a]

Agent	Percent Transmission
ADP	80.5 ± 7
ADP + VIP	83.2 ± 5
PAF	75.8 ± 4.1
PAF + VIP	38.1 ± 2

[a] VIP-mediated inhibition of platelet aggregation using platelet-rich plasma from three healthy volunteers. Platelets were stirred in siliconized glass cuvettes for 60 seconds in the presence or absence of 1 μM VIP before addition of PAF or ADP. Values are mean ± SD for three experiments.

minutes at 1500 × *g*. Aliquots of platelet-rich plasma were stirred in siliconized glass cuvettes in a Bio/Data platelet aggregation profiler, Model PAP-3. PAF (1-alkyl-2-acetyl-glycero-3-phosphorylcholine) was purchased from Sigma and dissolved in modified Tyrode's solution[5] containing 5 mg/ml bovine serum albumin. VIP was dissolved in Tyrode's solution containing 0.5% BSA and 0.005% bacitracin. Platelet-rich plasma was incubated 60 seconds with or without VIP before addition of PAF or ADP.

RESULTS AND CONCLUSIONS

The specific binding (total bound [^{125}I]VIP minus label bound in the presence of 1 μM unlabeled VIP) increased linearly with membrane protein concentration. Computer analysis of the data from 12 separate experiments demonstrated a single class of high-affinity binding sites with a mean dissociation constant (K_D) of 23 ± 6.3 n*M* (FIG. 1). PHI, glucagon, and GHRF competed less effectively for [^{125}I]VIP binding sites, exhibiting K_Ds of 254 ± 51, 441 ± 21 and 135 ± 73 n*M*, respectively.

VIP effects on platelet aggregation were investigated as shown in TABLE 1. VIP inhibited PAF-induced platelet aggregation by 50%. PAF is a potent platelet aggregating agent.[6] This phospholipid has been shown to inhibit cyclic AMP accumulation in intact platelets and to inhibit adenylate cyclase activity in membranes of rabbit and human platelets.[7] VIP stimulates adenylate cyclase in intact platelets[1] and thus VIP-mediated inhibition of PAF-induced platelet aggregation may result from the opposing effects of VIP and PAF on adenylate cyclase.

In summary, platelet membranes have been shown to possess receptors for vasoactive intestinal polypeptide (VIP). Competitive binding experiments using 200 μg membrane protein demonstrated a K_D of 23 ± 6.3 n*M* for [^{125}I]VIP with a B_{max} of 3.9 ± 1.1 n*M*. The binding of [^{125}I]VIP to platelet membranes is highly specific; the homologous peptides, PHI, GHRF, and glucagon, have lower affinity than VIP. VIP antagonizes platelet aggregation induced by platelet-activating factor, but not ADP-induced aggregation. These results suggest that human platelets possess functional VIP receptors that modulate platelet aggregation.

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