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Loss of high-affinity prostacyclin receptors in platelets and the lack of prostaglandin-induced inhibition of platelet-stimulated thrombin generation in subjects with spinal cord injury

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ABSTRACT **Coronary artery disease is a leading cause of death in individuals with chronic spinal cord injury (SCI). However, platelets of those with SCI (n = 30) showed neither increased aggregation nor resistance to the antiaggregatory effects of prostacyclin when compared with normal controls (n = 30). Prostanoid-induced cAMP synthesis was similar in both groups. In contrast, prostacyclin, which completely inhibited the platelet-stimulated thrombin generation in normal controls, failed to do so in those with SCI. Scatchard analysis of the binding of [^{3}H]prostaglandin E_1, used as a prostacyclin receptor probe, showed the presence of one high-affinity (K_{d_1} = 8.11 ± 2.80 nM; n_1 = 172 ± 32 sites per cell) and one low-affinity (K_{d_2} = 1.01 ± 0.3 μM; n_2 = 1772 ± 226 sites per cell) prostacyclin receptor in normal platelets. In contrast, the same analysis in subjects with SCI showed significant loss ($P < 0.001$) of high-affinity receptor sites (K_{d_1} = 6.34 ± 1.91 nM; n_1 = 43 ± 10 sites per cell) with no significant change in the low affinity-receptors (K_{d_2} = 1.22 ± 0.23; n_2 = 1820 ± 421). Treatment of these platelets with insulin, which has been demonstrated to restore both of the high- and low-affinity prostaglandin receptor numbers to within normal ranges in coronary artery disease, increased high-affinity receptor numbers and restored the prostacyclin effect on thrombin generation. These results demonstrate that the loss of the inhibitory effect of prostacyclin on the stimulation of thrombin generation was due to the loss of platelet high-affinity prostanoid receptors, which may contribute to atherogenesis in individuals with chronic SCI.**

Premature coronary artery disease (CAD) is one of the major causes of death in individuals with chronic spinal cord injury (SCI) (1). Although there is a clustering of risk factors known to be associated with atherosclerosis in those with chronic SCI, the mechanism of the increased incidence of CAD in these subjects is poorly understood (2).

Aggregation of platelets is critically important in the events of normal blood coagulation as well as those of thrombosis and atherosclerosis (see refs. 3 and 4 for review). The aggregation of platelets is induced by several agonists, such as ADP, L-epinephrine, collagen, or thrombin, and the process is believed to be mediated, at least in part, through intracellular formation of prostaglandin G_2 and thromboxane A_2 (5). Homeostasis is achieved by the countervailing effects of several humoral factors, most notably by prostacyclin (prostaglandin I_2; PGI_2) through the inhibition of platelet aggregation (6). The inhibition of platelet aggregation by PGI_2 is achieved by increasing the intracellular level of cAMP, an effect of the prostanoid binding to specific receptors on the cell surface, which results in the activation of adenylate cyclase (7–10). It has been shown that the platelet surface contains one high-affinity, low-capacity receptor population and one low-affinity, high-capacity receptor population for PGI_2 (8–10). The binding of agonist to the low-affinity receptors, it is generally believed, leads to the increase of cAMP level (8–10). The physiological role of high-affinity binding sites remains uncertain.

Hyperactive platelets are known to play an important role in the pathophysiology of CAD (11–13). The synthesis of prostaglandin-induced synthesis of cAMP in CAD is decreased due to the impairment of prostaglandin E_1 (PGE_1)/PGI_2 receptor activity in platelets (14–16). Since the prevalence of vascular disease is increased in those with SCI, we investigated the role of PGI_2 in the inhibition of platelet aggregation in relation to the prostanoid receptor function and platelet-stimulated thrombin generation.

MATERIALS AND METHODS

Patient Selection. Male patients with SCI (n = 30) (18, paraplegia; 12, quadriplegia), with durations of injury >5 yr, between the ages of 35 and 60 yr, were age-matched with 30 able-bodied, male controls, ranging in age from 28 to 58 yr. None of the volunteers had taken any medication, including aspirin, 2 weeks prior to the study. All volunteers were human immunodeficiency virus negative and had no known history of CAD or diabetes mellitus. The protocol was approved by the Institutional Review Board for Clinical Research, Veterans Affairs Medical Center, Bronx, NY, prior to recruitment of subjects.

Collection of Blood and Platelet Aggregation. Blood samples (40–50 ml) were collected in plastic tubes containing sodium citrate (0.013 M final concentration) by using 19-gauge siliconized needles. Platelet-rich plasma (PRP) was prepared by centrifuging the blood samples at 200 × g for 15 min at 23°C. Platelet-poor plasma (PPP) was prepared by centrifuging PRP at 10,000 × g for 15 min at 23°C.

Aggregation of platelets was studied by placing 0.5 ml of PRP in a silicon-coated cylindrical cuvette (8 mm in diameter) containing a Teflon-coated stirring bar. Aggregation was studied by adding different aggregating agents in an aggregometer (Chrono-Log, Broomall, PA) and stirring the PRP at a rate of 1200 rpm at 37°C, as described (16). The aggregometer was calibrated so that the difference in light transmission between PRP and PPP was defined as 100% (16).

Inhibition of platelet aggregation by PGI_2 was determined by incubating PRP with different amounts of the prostanoid for 1 min at 37°C before the aggregation of platelets was initiated by adding ADP. Minimal inhibitory concentration

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Abbreviations: SCI, spinal cord injury; CAD, coronary artery disease; PRP, platelet-rich plasma; PFP, platelet-free plasma; PPP, platelet-poor plasma; PGI_2, prostacyclin (prostaglandin I_2); PGE_1, prostaglandin E_1; MIC, minimal inhibitory concentration.

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(MIC) of PGI_2 is defined as the minimal amount of the prostaglandin that completely inhibited the maximal aggregation induced by the minimal amount of ADP.

Preparation of Washed Platelets. Platelets from PRP were washed twice with 2 volumes of Tyrode's buffer (without Ca^{2+}, pH 7.5) containing 1.0 mM EDTA as described (8). Since the presence of Mg^{2+} is essential for the binding of prostaglandin to the receptors (8–10), platelets were washed with the chelating agent to release any bound prostanoid from the receptor sites that might have been occupied by the agonist *in vivo*. The platelets were then washed with 2 volumes of the same buffer (without EDTA) containing 5 mM $MgCl_2$. The platelet number was adjusted to 6.6×10^8 cells per ml by using a Coulter Counter. Gel-filtered platelets were prepared as described (17).

Platelet Prostacyclin (PGI_2) Receptor Assay. Since PGI_2 and PGE_1 bind to the same receptor on the platelet surface (8–10), and radiolabeled PGI_2, as free-acid form, is not commercially available, [^{3}H]PGE_1 {[5,6-^{3}H(N)]PGE_1; specific activity, 55 Ci/mmol (1 Ci = 37 GBq); New England Nuclear} was used as a stable probe to determine the PGI_2 receptor binding in platelets.

Unless otherwise stated, $\approx 10^8$ platelets washed in Tyrode's buffer (pH 7.5) containing 5 mM $MgCl_2$ were incubated with 3 nM [^{3}H]PGE_1 (60,000 dpm) in a total volume of 200 μl at 23°C for 15 min to attain equilibrium. After incubation, the reaction mixture was diluted to 1.2 ml with the buffer and filtered on glass microfiber filters (GF/C, Whatman), presoaked with the same buffer, under mild vacuum. The filters were then washed with 5 ml of Tyrode's buffer for a second time. Under these conditions, the platelets remained adsorbed to the membrane filter and the free ligand (that did not bind) was removed by washing. The filters were subsequently dried, and the radioactivity was determined by suspending them in ACSII solvent (Amersham) in a liquid scintillation counter (Isocarp/300, Searle) with 60% efficiency for ^{3}H. The nonspecific binding was determined by adding excess (15 μM) unlabeled prostaglandin to the assay mixture. The specific binding was calculated by subtracting the nonspecific binding from the total binding.

For the studies in which an insulin effect on platelets was investigated, PRP was incubated with 200 microunits of insulin per ml (Humulin R; Eli Lilly) for 2.5 hr at 23°C as described (17). After incubation, platelets were washed, as described above, and used for PGI_2 receptor assay.

Scatchard Analysis of [^{3}H]PGE_1 Binding to Platelets. The dissociation constants (K_d values) and the capacities (n, receptors) of PGE_1/PGI_2 receptors were determined by Scatchard analysis (18). Platelets were incubated with 3 nM [^{3}H]PGE_1 plus 0–3 μM unlabeled PGE_1 for 15 min at 23°C. The binding of [^{3}H]PGE_1 to platelets was determined for each concentration of the prostaglandin by calculating the specific activity of the ligand obtained by diluting [^{3}H]PGE_1 with a known concentration of the unlabeled prostanoid. The dissociation constants and the capacities were obtained from a nonlinear regression analysis of equilibrium binding by a nonweighted iterative, least-squares algorithmic analysis using a microcomputer (Elsevier, BIOSOFT, Cambridge, U.K.).

Determination of the Rate of Thrombin Generation. The rates of thrombin generation in PRP and PPP from SCI and normal volunteers were measured by determination of the recalcification time as described by Hougie (19). Typically, 0.2 ml of serially diluted plasma or PRP with 0.85% NaCl was incubated at 37°C for 1 min. After incubation, 0.1 ml of 0.025 M $CaCl_2$ was added to the mixture at 37°C and the recalcification time was determined (19). The rate of thrombin generation was calculated from the first-order reaction kinetics of clot formation and verified by adding various amounts of pure thrombin to the above mixture without added $CaCl_2$ under identical conditions. The rate was expressed in percent increase or decrease over that of the plasma alone, which was arbitrarily defined as 100% for each blood sample.

The effect of PGI_2 on thrombin generation was determined by adding different quantities of prostaglandin to PRP to plasma and incubating for 2 min at 37°C before the recalcification time was determined as described above. Appropriate control experiments were carried out with vehicle only.

cAMP Assay. The basal cAMP level and the increase of intracellular cAMP content by PGI_2 in platelets was determined by the protein kinase binding method (20) as described (21).

Statistical Analysis. Results are shown as mean ± SD or mean ± SE. The data were analyzed by paired or unpaired Student's t test, applied appropriately. Probability values of $P < 0.01$ were considered significant.

RESULTS

Aggregation of Platelets from Subjects with Chronic SCI by Various Aggregating Agents. Aggregation profiles of platelets from subjects with SCI by various aggregating agents, such as ADP, L-epinephrine, collagen, or thrombin, were similar to the aggregation profiles of platelets from age- and gender-matched non-SCI controls. The minimum amounts of aggregating agents needed for maximal aggregation of platelets from subjects with SCI were 3.5 ± 1.1 μM for ADP, 2.2 ± 1.5 μM for L-epinephrine, 2.0 ± 0.1 μg/ml for collagen, and 0.2 ± 0.05 unit/ml for thrombin and were not significantly different compared with platelets from non-SCI controls (ADP, 4.1 ± 1.6 μM; L-epinephrine, 2.5 ± 1.0 μM; collagen, 2.0 ± 0.2 μg/ml; thrombin, 0.20 ± 0.04 unit/ml).

Inhibition of Platelet Aggregation and the Synthesis of cAMP by PGI_2 in Platelets from Subjects with SCI and Controls. Inhibition of platelet aggregation by PGI_2 of volunteers with SCI was similar to that of platelets of non-SCI controls (Table 1). The minimum inhibitory concentrations of PGI_2 were almost identical in both groups. Furthermore, the stimulation by equimolar quantities of PGI_2 of cAMP synthesis in platelets of subjects with SCI and non-SCI controls was essentially similar (Table 1). The basal cAMP level was also the same in the two groups.

Effect of PGI_2 on the Inhibition of Platelet-Stimulated Thrombin Generation in Subjects with SCI. The presence of platelets in recalcified plasma is known to stimulate the rate of thrombin generation through the intrinsic pathway (19). Inhibition of platelet aggregation has been demonstrated to inhibit the stimulatory role of platelets (19).

Addition of PGI_2 to recalcified normal PRP effectively prevented the platelet-stimulated rate of thrombin generation when compared with the control rate (Fig. 1*A*). However, similar treatment with the prostanoid of PRP from subjects with SCI failed to show any inhibitory effect of the agent on platelet-stimulated thrombin generation time (Fig. 1*B*). Although the rate of thrombin generation in plasma from control and SCI subjects demonstrated individual variation, these values were not significantly different when the two groups were compared (163 ± 21 and 171 ± 30 sec, respectively). In

Table 1. Effect of prostacyclin on the aggregation of platelets and synthesis of cAMP in platelets from subjects with SCI and controls

PRP	MIC of PGI_2,* nM	cAMP, pmol per 10^8 cells
Control	No addition	1.91 ± 0.51
Control	6.1 ± 0.9	26.89 ± 8.21
SCI	No addition	2.22 ± 0.34
SCI	5.6 ± 0.3	20.25 ± 4.10

Values are expressed as mean ± SD. PRP from normal (n = 10) and SCI subjects (n = 10) was studied.

*Synthesis of cAMP by the prostanoid (100 nM) was determined by the protein kinase method (20, 21).

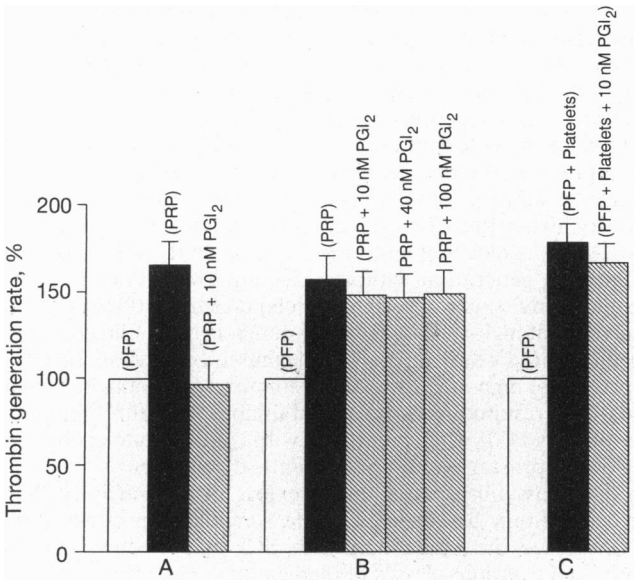

FIG. 1. Effect of PGI_2 on platelet-stimulated thrombin generation in PRP from subjects with SCI and controls. Open bars, platelet-free plasma (PFP); black bars, PRP in the absence of PGI_2; hatched bars, PRP in the presence of PGI_2. (*A*) Normal PRP. (*B*) PRP from subjects with SCI (n = 10). (*C*) Gel-filtered platelets from subjects with SCI suspended in plasma from control (non-SCI) volunteers (n = 10).

both groups, the addition of PGI_2 to PFP had no effect on the recalcification time.

To determine if the failure of PGI_2 to inhibit platelet-stimulated thrombin generation in the subjects with SCI was related to the platelets themselves, gel-filtered platelets prepared from PRP of volunteers with SCI were suspended in control plasma and then treated with PGI_2. No inhibition of platelet-stimulated thrombin generation time by PGI_2 could be demonstrated (Fig. 1*C*).

Prostacyclin Receptors in Platelets from Subjects with SCI and Controls. Since the prostanoids have been shown to mediate their effects through the interaction of the agonists to cell surface receptors (8–10), the binding of PGI_2 to platelet receptors was studied by using $[^3H]PGE_1$ as a stable probe (8–10). Equilibrium binding of the tritiated probe to the platelets from those with SCI and controls showed that the binding of $[^3H]PGE_1$ to the PGI_2 receptors on platelet surface from SCI subjects was persistently <50% when compared with controls (Fig. 2).

The Scatchard plot (18) of $[^3H]PGE_1$ binding in the platelets from controls was typically curvilinear, indicating heterogeneity of the receptor molecules. However, whether the curvilinearity of the Scatchard plot was due to negative cooperativity within the same molecules is not known. In contrast, the same plot of $[^3H]PGE_1$ binding to SCI platelets showed almost a linear curve with marked loss of high-affinity binding (Fig. 3). Computer analysis of the binding characteristics showed the presence of one high-affinity, low-capacity PGI_2 receptor population and one low-affinity, high-capacity receptor population in platelets from both groups. However, the number of high-affinity binding sites of the receptors was significantly decreased ($P < 0.001$) without any significant change in the affinity of the binding sites in the platelets from subjects with SCI when compared with the controls (Table 2).

Increase of High-Affinity PGI_2 Receptor Numbers in Platelets from Subjects with SCI by Insulin and Restoration of the Inhibitory Effect of the Prostanoid on Thrombin Generation Rate. The above results indicated that the failure of PGI_2 to inhibit platelet-stimulated thrombin generation was not due to the decreased cAMP synthesis (Table 1) but may have been related to the impairment of the binding of the prostanoid to

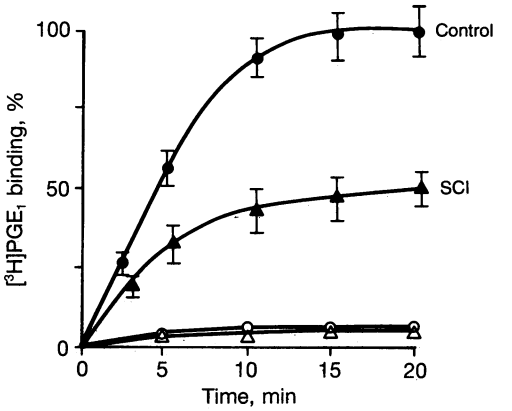

FIG. 2. Time course of binding of $[^3H]PGE_1$ to platelets from subjects with SCI and controls. Washed platelets were incubated with $[^3H]PGE_1$ (3 nM) at 23°C and, at different times, the binding of the radioligand to the platelets from control (●) and subjects with SCI (▲) was determined. ○ and △, nonspecific binding to control and SCI platelets, respectively. Results are expressed as mean ± SE of three experiments, each in triplicate.

the high-affinity receptor sites on the platelet surface. If this were the case, then restoration on the high-affinity PGI_2 receptor numbers in platelets from those with SCI should restore the inhibitory effect of the autacoid on thrombin generation time. Since treatment of platelets with physiologic amounts of insulin has been shown to increase the high- and low-affinity PGI_2 receptor numbers without affecting their affinities (17), platelets from those with SCI were treated with insulin as described (21). After incubation, the binding of $[^3H]PGE_1$ to the platelets was analyzed by Scatchard plot. In parallel experiments, the effect of PGI_2 on the inhibition of thrombin generation was determined. The incubation of platelets from SCI subjects with insulin not only increased the high-affinity PGI_2 receptor numbers to "normal ranges" but also resulted in the prostanoid-induced inhibition of thrombin generation (Table 3). In the control experiments, when non-SCI platelets were incubated with the hormone, the high-affinity PGI_2 receptor numbers were increased, along with an increase of the low-affinity receptor numbers. The increase of high-affinity receptor numbers on platelets from those with SCI resulted in increased inhibition of thrombin generation by

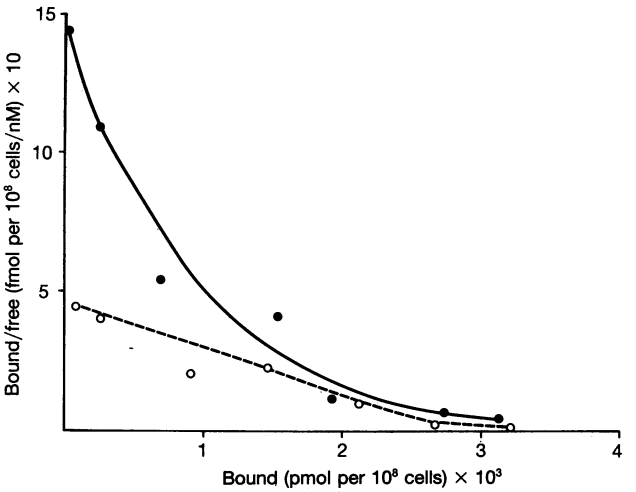

FIG. 3. Scatchard plot of the binding of $[^3H]PGE_1$ to platelets from subjects with SCI and controls. Shown is a representative Scatchard plot of the nine other experiments performed in each of the two groups. Each point in the plot is the average of three experiments. ○, Volunteers with SCI; ●, control subjects.

Table 2. PGE_1/PGI_2 receptor numbers and affinities of the platelets from subjects with SCI and controls

	PGE_1/PGI_2 receptors			
	High-affinity		Low-affinity	
Platelets	K_{d_1}, nM	n_1, sites per cell	K_{d_2}, μM	n_2, sites per cell
Control (non-SCI)	8.11 ± 2.80	172 ± 23	1.01 ± 0.30	1772 ± 226
SCI	6.34 ± 1.91	43 ± 10	1.22 ± 0.23	1820 ± 421

Results are expressed as mean ± SD of six experiments, each performed in triplicate.

PGI_2 when compared with platelets from those with SCI that were not insulin-treated (Table 3); these insulin-treated platelets of SCI subjects resulted in an inhibition similar to that of untreated platelets of controls.

DISCUSSION

Our results demonstrate that platelets from subjects with chronic SCI developed resistance to the inhibitory effect of PGI_2 in the platelet-induced stimulation of thrombin generation. Interestingly, however, this failure of the prostanoid in the control of thrombin generation was not related either to the inhibition of platelet aggregation or to the synthesis of cAMP (Table 1). Indeed, platelets from those with SCI did not show any hyperactivity that could be related to the increased resistance of these platelets to the antiaggregatory effects of PGI_2, leading to the impairment of PGI_2 effect on the platelet-induced increase of thrombin generation. The inhibition of platelet aggregation, it has been generally believed, results in the inhibition of platelet-induced stimulation of thrombin generation (19). Our results are contrary to this hypothesis and indicate that inhibition of platelet aggregation does not necessarily lead to the inhibition of platelet-stimulated thrombin generation in plasma. Thus, these two processes are under separate control mechanisms. Furthermore, these results also demonstrate that not all effects of PGI_2 are mediated through cAMP in platelets.

The existence of high- and low-affinity PGI_2/PGE_1 receptors on the platelet surface is well described. It is generally accepted that the interaction of PGI_2 or PGE_1 (both of which bind to the same receptor) with the low-affinity receptor results in the activation of adenylate cyclase, which consequently increases the intracellular level of cAMP in platelets (8–10). However, to our knowledge, the role of high-affinity receptors of PGI_2 or PGE_1, in relation to the effects of the prostanoid, has not been described previously. The binding of the prostaglandin to the high-affinity receptors on platelets has been reported to be controlled by cAMP (22). Since there is no method currently available to specifically block only one group of PGI_2 receptor population, it has not been possible until now to differentiate between the activities of high- or low-affinity PGI_2/PGE_1 receptors. The specific decrease of high-affinity receptors in platelets from those with SCI presented a unique opportunity to investigate the role of high-affinity PGI_2 receptors in platelet function. Three lines of evidence indicate that the binding of PGI_2 to its high-affinity receptors on the platelet surface resulted in the inhibition of platelet-induced stimulation of thrombin generation. (*i*) The loss of high-affinity PGI_2 receptors in platelets from those with SCI was associated with the lack of PGI_2 effect on the thrombin generation without affecting low-affinity PGI_2 receptor activity with regard to platelet cAMP synthesis. (*ii*) The increase of high-affinity receptor numbers by insulin corrected the impaired effect of PGI_2 on thrombin generation. (*iii*) The increase of high-affinity receptor numbers by insulin in normal platelets resulted in an increased inhibition of thrombin generation by PGI_2 when compared with control platelets treated with the prostaglandin under identical conditions.

The possibility exists that the loss of high-affinity PGI_2 receptors may be consequence to a medication effect, especially since the individuals with SCI in our study may be prescribed various classes of medications. The effects of some of these compounds on the PGE_1/PGI_2 receptors in platelets from normal controls have been determined (16). Incubation of control platelets with various medications does not interfere significantly with the binding of $[^3H]PGE_1$ (16).

The mechanism of PGI_2, through its binding to high-affinity receptors, in the inhibition of thrombin generation is not known. However, our preliminary data (unpublished) have shown that incubation of platelets from subjects with SCI with a calcium channel blocker (10 μM verapamil) inhibited platelet-induced stimulation of thrombin generation in the absence of intracellular increase of cAMP. These results might indicate that the interaction of PGI_2 with high-affinity platelet receptors could result in the inhibition of Ca^{2+} influx. Although it has been reported that cAMP itself stimulates Ca^{2+} extrusion from platelets (23), the PGI_2-induced increase of cAMP in platelets from those with SCI, however, did not result in the inhibition of thrombin generation. It remains to be ascertained whether the lack of cAMP effect was due to a "compartmentalized" increase of the nucleotide level.

Although the mechanism for the specific loss of high-affinity PGI_2 receptors in platelets from subjects with SCI is not known, the lack of inhibitory effect of the prostanoid on the regulation of platelet-induced thrombin generation in the system may explain, at least in part, the increased occurrence of CAD and other vascular disease in individuals with SCI. In addition to the function of thrombin in converting fibrinogen to fibrin and as a powerful platelet-aggregating agent, it is a potent mitogenic agent for human arterial smooth muscle cells. Thrombin has been shown to induce mRNA synthesis encoding platelet-derived growth factor, a well-known protein that has significant influence in the development of atherogenesis

Table 3. Insulin-induced increase of high-affinity PGE_1/PGI_2 receptor numbers in platelets from subjects with SCI and the effect of PGI_2 on platelet-stimulated thrombin generation

	PGE_1/PGI_2 receptors				
	High-affinity		Low-affinity		
Platelets	K_{d_1}, nM	n_1, sites per cell	K_{d_2}, μM	n_2, sites per cell	Rate of thrombin generation,* % increase over plasma alone
Control					
No PGI_2	7.25 ± 0.12	48 ± 11	1.91 ± 0.29	1739 ± 323	67 ± 5.0
PGI_2	—	—	—	—	68 ± 4.0
Insulin					
No PGI_2	5.67 ± 0.13	142 ± 36	2.11 ± 0.51	3214 ± 451	72 ± 3.0
PGI_2	—	—	—	—	2 ± 1.0

Values are expressed as mean ± SD of six experiments, each performed in triplicate.
*Rate of thrombin generation in PFP is arbitrarily defined as 100%.

(24–26). The enzyme, by activating endothelial cells, also stimulates the attachment of monocytes to the vessel wall, and it has also been implicated in atherosclerotic plaque formation (27). Furthermore, thrombin-generated fibrin degradation products have been shown to enhance atherosclerotic plaque growth through focal smooth cell proliferation (28).

1. Borges, P. M. & Hacker, R. H. (1982) *J. Urol.* **127,** 710–721.
2. Samsa, G. P., Patrick, C. H. & Feussner, J. R. (1993) *Arch. Neurol.* **50,** 909–914.
3. Lasslo, A. (1984) *Blood Platelet Function and Medicinal Chemistry* (Elsevier Science, New York).
4. Fuster, V., Steele, P. H. & Cheesbro, J. H. (1985) *J. Am. Coll. Cardiol.* **5,** 175B–184B.
5. Hamberg, M., Svenson, J. & Samuelsson, B. (1975) *Proc. Natl. Acad. Sci. USA* **74,** 2994–2998.
6. Moncada, S., Gryglewski, R. J. & Bunting, S., Vane, J. R. (1976) *Nature (London)* **263,** 663–665.
7. Haslam, R. J. (1975) *Ciba Found. Symp.* **35,** 121–151.
8. Dutta-Roy, A. K. & Sinha, A. K. (1987) *J. Biol. Chem.* **262,** 12685–12691.
9. Schafer, A. I., Cooper, B., O'Hara, D. & Handin, R. I. (1979) *J. Biol. Chem.* **254,** 2914–2917.
10. Siege, A. M., Smith, J. B., Silver, M. J., Nicolua, K. C. & Ahern, D. (1979) *J. Clin. Invest.* **63,** 215–220.
11. Smitherman, T. C., Halam, A., Woo, J., Willerson, J. T. & Frenkel, E. P. (1981) *Am. J. Cardiol.* **48,** 395–402.
12. DeBoer, A. C., Turpie, A. G. C., Butt, R. W., Johnston, R. V. & Genton, E. (1982) *Circulation* **66,** 327–333.
13. Dewood, M. A., Spores, J., Notsker, P., Burroughs, R., Golden, M. S. & Lang, H. T. (1980) *N. Engl. J. Med.* **303,** 897–902.
14. Neri Serneri, G. G., Fortini, A., Lombardi, A., Modesti, P. A., Abbate, R. & Gemseni, G. F. (1984) *Lancet* **ii,** 838–841.
15. Jachonek, K., Karch, K. R., Weisenberger, H., Tidow, S. & Faul, C. (1986) *J. Am. Coll. Cardiol.* **8,** 259–266.
16. Kahn, N. N., Mueller, H. S. & Sinha, A. K. (1990) *Circ. Res.* **66,** 932–940.
17. Kahn, N. N. & Sinha, A. K. (1990) *J. Biol. Chem.* **265,** 4976–4981.
18. Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51,** 660–670.
19. Hougie, C. (1977) in *Hematology*, eds. William, W. J., Butler, E. B., Erstlev, A. J. & Rundles, R. W. (McGraw–Hill, New York), pp. 1642–1645.
20. Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 305–312.
21. Kahn, N. N., Muellar, H. S. & Sinha, A. K. (1991) *Circ. Res.* **68,** 245–254.
22. Kahn, N. N. & Sinha, A. K. (1988) *Biochim. Biophys. Acta* **972,** 45–53.
23. Johnsson, J. S., Neid, L. E. & Hynes, D. H. (1992) *Biochim. Biophys. Acta* **1105,** 19–28.
24. Hedin, U., Frebelius, S., Sanchez, J., Dryjski, M. & Swedenborg, J. (1994) *Arterioscler. Thromb.* **14,** 254–260.
25. Shultz, P. J., Knauss, T. C., Mene, P. & Abboud, H. E. (1989) *Am. J. Physiol.* **257,** F366–F374.
26. Ross, R. (1986) *N. Engl. J. Med.* **314,** 488–500.
27. DiCorleto, P. E. (1993) *Am. J. Hypertens.* **6,** 314S–318S.
28. Thomson, W. D., Smith, E. B., Strik, C. M. & Kochhar, A. (1993) *Blood Coagulation Fibrinolysis* **1,** 489–493.