Protein Kinase C-Dependent Desensitization of HDL₃-Activated Phospholipase C in Human Platelets

H. Nazih, F. Nazih-Sanderson, V. Magret, B. Caron, J. Goudemand, J.C. Fruchart, C. Delbart

Abstract
In isolated human platelets, exposure of subfraction 3 high-density lipoprotein (HDL₃) binding sites to high concentrations of HDL₃ (1 mg/mL) causes rapid desensitization of HDL₃ (50 μg/mL)-stimulated breakdown of phosphatidylcholine, as shown in approximately a 70% depression of the maximal 1,2-diacylglycerol release activity by phospholipase C. This desensitization is HDL₃ dose dependent (IC₅₀, 150±20 μg/mL, n=6) and time dependent (t₀₂₅, <30 seconds). It requires the binding of HDL₃, as pretreatment of HDL₃ by tetranitromethane does not cause the desensitization of HDL₃-induced phospholipase C activity. Permeabilization of human platelets with 10 μg/mL digitonin, used to permit access of charged inhibitors to the cytosol, does not interfere with the pattern of HDL₃ (1 mg/mL)-induced desensitization of HDL₃ (50 μg/mL)-stimulated phospholipase C. Inhibitors of protein kinase C [(100 μmol/L H-7 and 10 μmol/L staurosporine) markedly inhibit desensitization of HDL₃-induced phospholipase C activity, whereas cAMP-dependent protein kinase inhibitor (1 μmol/L), heparin (100 nmol/L), or concanavalin A (0.25 mg/mL) were ineffective. HDL₃-induced desensitization is accompanied at least by the phosphorylation of the 94- and 110-kD proteins. Inhibition of HDL₃-induced desensitization by 100 μmol/L H-7 or 10 μmol/L staurosporine is characterized by a marked reduction of the phosphorylation state of these proteins in permeabilized platelets. Whereas protein kinase C inhibitors fully inhibited the phosphorylation of the 94- and 110-kD proteins, inhibitors of protein kinase A were less effective. These data establish that phosphorylation by protein kinase C represent a step in the desensitization of HDL₃ binding sites in human platelets. (Arterioscler Thromb. 1994;14:1321-1326.)

Key Words • HDL₃ • desensitization • phospholipase C • platelets • protein kinase C

Previous studies have demonstrated that subfraction 3 high-density lipoprotein (HDL₃) binding sites are present on platelet membranes. Kolker et al. have reported that the glycoprotein IIb/IIIa complex, the inducible platelet fibrinogen receptor, is able to interact with lipoproteins. HDL₃ was found to bind to either the 95- to 110-kD glycoprotein IIa or to the 136- to 140-kD glycoprotein IIb. Glycoprotein IIb/IIIa acts until now as a platelet HDL₃ binding site. Furthermore, HDL₃ binding sites are coupled to phospholipase C (PLC) through pertussis toxin–sensitive GTP binding proteins. In human platelets, HDL₃ binding sites act via the PLC-mediated hydrolysis of phosphatidylinositol or phosphatidylcholine and the generation of 1,2-diacylglycerol, which activates the multifunctional enzyme protein kinase C (PKC). The HDL₃-induced signaling pathway is recorded only when platelets are stimulated by low concentrations of HDL₃ (50 μg/mL). Higher HDL₃ concentrations (1 mg/mL) are not able to trigger phosphatidylcholine hydrolysis, indicating a loss of responsiveness.

One major form of regulation of the activity of G protein–coupled receptors is desensitization, which is a general biological phenomenon whereby the response to a specific ligand wanes over time despite the continuous presence of the ligand. The impairment of receptor functions is linked to protein phosphorylation, which appears to be a key factor. Most often, homologous desensitization is accompanied by receptor phosphorylation, which triggers the process of functional uncoupling from G proteins. In addition, a rapid sequestration of the receptors away from the cell surface and a modulation of the expression of the receptor gene itself result in a net decrease in receptor number. Wherever desensitization of the adrenergic system is rather well understood, less is known about receptors coupled to phosphatidylinositol or phosphatidylcholine turnover. Moreover, until now little has been known about HDL₃ binding site regulation. Platelets therefore would seem to provide a good model for the study of putative HDL₃-induced desensitization. To investigate the regulation of HDL₃-induced signal transduction, we have studied the events surrounding the eventual protein phosphorylation induced by high concentrations of HDL₃. This report is the first demonstration of the possibility that in human platelets, a high concentration of HDL₃ (1 mg/mL) induces acute desensitization of the signaling pathway induced at a low concentration by a mechanism involving, at least in part, PKC.

Methods
L-α-Phosphatidylcholine, dipalmitoyl-[2-palmitoyl-9,10-³H]-adenosine 5'-(γ³P)triphosphate (³²P]ATP), and the ECL Western blotting detection system were from Amersham. Concanavalin A (ConA) was from IBF. cAMP-dependent protein kinase inhibitor (PKI), heparin, staurosporine, digitonin, phospholipase C, and concanavalin A markedly inhibit desensitization of HDL₃-induced phospholipase C activity, whereas cAMP-dependent protein kinase inhibitor (1 μmol/L), heparin (100 nmol/L), or concanavalin A (0.25 mg/mL) were ineffective. HDL₃-induced desensitization is accompanied at least by the phosphorylation of the 94- and 110-kD proteins. Inhibition of HDL₃-induced desensitization by 100 μmol/L H-7 or 10 μmol/L staurosporine is characterized by a marked reduction of the phosphorylation state of these proteins in permeabilized platelets. Whereas protein kinase C inhibitors fully inhibited the phosphorylation of the 94- and 110-kD proteins, inhibitors of protein kinase A were less effective. These data establish that phosphorylation by protein kinase C represent a step in the desensitization of HDL₃ binding sites in human platelets. (Arterioscler Thromb. 1994;14:1321-1326.)

Key Words • HDL₃ • desensitization • phospholipase C • platelets • protein kinase C

Received April 29, 1994; revision accepted May 19, 1994.
From Serlia, Institut Pasteur (H.N., F.N.-S., V.M., J.C.F., C.D.); and Service d'Hématologie, Hôpital C. Huriez (J.G.), Lille, France.
Correspondence to Dr C. Delbart, Serlia, U325 INSERM, Institut Pasteur, 1 rue du Pr Calmette, BP 245, 59019 Lille Cedex, France.
© 1994 American Heart Association, Inc.
nin, pararitrophenol phosphate, and phenylmethylsulfonyl fluoride were from Sigma Chemical Co and silica gel high-performance thin-layer chromatography (TLc) plates from Merck. H-7 was kindly provided by Dr Sergheraert. Other biochemical reagents were from Sigma.

**Lipoprotein Isolation**

HDL$_{3}$ was isolated from human serum by standard differential ultracentrifugal flotation[16] (HDL$_{3}$ d=1.125 to 1.210 g/mL). Its protein content was measured by the procedure of Peterson. The apolipoprotein E constituted less than 0.2% of the total HDL$_{3}$ protein. HDL$_{3}$ modified with tritiated methanol was prepared as described previously.[9] Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) performed on a 7% gel indicated that HDL$_{3}$ was pure from any plasmatic protein contaminants.

**Human Platelet Isolation**

Platelets were isolated from citrated blood drawn freshly from healthy donors. After centrifugation at 1500 rpm for 20 minutes, the platelet-rich plasma was centrifuged for 15 minutes at 900g, and the platelets were resuspended in 0.35% bovine serum albumin (wt/vol) buffer containing 137 mmol/L NaCl, 2.7 mmol/L KCl, 0.32 mmol/L Na$_2$HPO$_4$, 5.6 mmol/L glucose, 1.19 mmol/L NaHCO$_3$, 1.3 mmol/L HEPES, 100 μmol/L paranitrophenol phosphate, and 5 μmol/L phenylmethylsulfonyl fluoride, pH 7.4 (HEPES-BSA buffer) at a density of 2.5×10$^8$ cells per milliliter.

**[3H]Phosphatidylcholine-Prelabeled Platelets**

[3H]Phosphatidylcholine (1.25 μCi) was pipetted into vials and the solvent was evaporated to leave a thin film. Platelets (10$^9$) were then added and incubated for 60 minutes at room temperature. The specific activity of the preparation was 3500 cpm per 2.5×10$^9$ platelets unless otherwise specified. Less than 1/200 of the radioactivity was associated with free lipids and nearly all the radioactivity with the phosphatidylcholine fraction.[4,5]

**Permeabilization of Human Platelets**

Human platelets or [3H]phosphatidylcholine-prelabeled platelets were resuspended in HEPES-BSA buffer (2.5×10$^6$ cells per milliliter) were permeabilized with 10 μmol/L digitonin as described earlier[2] by stepwise addition until more than 95% of the platelets were trypan blue positive. Permeabilized cells retained their normal microscopic appearance.

**Desensitization of HDL$_{3}$ Receptors in Platelets**

Permeabilized platelets in HEPES-BSA buffer (or for controls, intact platelets) were incubated at a cell density of 2.5×10$^6$ cells per milliliter with or without 1 mg/mL HDL$_{3}$ for 5 minutes or the indicated time at 37°C.

Heparin (100 nmol/L), PKI (1 μmol/L), staurosporine (10 μmol/L), and H-7 (100 μmol/L) were added to permeabilized platelets 2 minutes before the HDL$_{3}$ stimulation. Pretreatment with ConA was realized before desensitization with 0.25 mg/mL ConA in HEPES-BSA buffer for 20 minutes at 37°C followed by two washes in HEPES-BSA buffer. Cells were then permeabilized and treated as described above. Desensitized platelets were collected by gentle centrifugation (15 minutes at 900g), the desensitization medium was discarded, and platelets were processed for 1,2-diacylglycerol release or protein phosphorylation assays.

**1,2-Diacylglycerol Release**

After permeabilization and desensitization, 2.5×10$^6$ [3H]phosphatidylcholine-prelabeled platelets suspended in 1 mL HEPES-BSA buffer were exposed to 50 μg/mL HDL$_{3}$ for 30 seconds. The incubations were terminated by immediate addition of 4 mL ice-cold trichloromethane/methanol (2:1 vol/vol). The lipids were extracted as described by Folch et al[22] and spotted on silica gel 60 thin-layer chromatography plates that were developed with petroleum ether/ethyl ether/acetic acid (90:10:5 vol/vol/vol) to separate 1,2-diacylglycerol. The radioactive spots corresponding to 1,2-diacylglycerol and phosphatidylcholine were cut and processed for radioactivity counting. A good correlation for 1,2-diacylglycerol release was found between the [3H]phosphatidylcholine method and the 1,2-diacylglycerol kinase assay.[23,24] 1,2-Diacylglycerol release recorded in unstimulated platelets after a 5-minute incubation at 37°C ranged around 100 cpm, for a specific activity of 3500 cpm per 2.5×10$^6$ cells.

**Protein Phosphorylation in Permeabilized Platelets**

Permeabilized platelets (2.5×10$^6$/mL) in HEPES-BSA buffer containing 6 μmol/L ATP, 3 Ci=110 GBq) of [γ-32P]ATP were incubated with or without 1 mg/mL HDL$_{3}$ (and staurosporine, H-7, heparin, PKI, or ConA when specified) for 5 minutes at 37°C. The reaction was terminated by being brought to 0°C and centrifuged at 900g for 15 minutes. After two washing steps in HEPES-BSA buffer, the pellet was resuspended in an identical volume of HEPES-BSA buffer. Proteins were precipitated by 5 vol cold acetone and incubation at −20°C for 10 minutes. Membrane proteins were pelleted by centrifugation (10 000g, 5 minutes). After two repeated precipitation and drying steps, the protein pellet was dissolved in 30 μL of Laemmli sample buffer[25] at 100°C for 3 to 5 minutes. SDS-PAGE was performed on 4% to 15% gradient gels using the discontinuous system of Laemmli.[26] Gels were stained with Coomassie brilliant blue, and radiolabeled bands were located by exposure with Kodak X-OMAT K film at −80°C.

As controls, permeabilized platelets were incubated only in the presence of the different kinase inhibitors without any desensitization or activation by HDL$_{3}$. No protein phosphorylation could be registered in such controls (data not shown).

**Results**

**HDL$_{3}$-Induced Desensitization**

To characterize the involvement of a desensitization phenomenon, we analyzed the effects of HDL$_{3}$ preincubation on HDL$_{3}$-induced intracellular signals in human platelets. Brief exposure (5 minutes) of [3H]phosphatidylcholine-prelabeled platelets to 1 mg/mL HDL$_{3}$ produced nearly a 70% decrease in the ability of subsequent applications of 50 μg/mL HDL$_{3}$ to induce hydrolysis of phosphatidylcholine into 1,2-diacylglycerol, relative to the control, represented by 50 μg/mL HDL$_{3}$-stimulated platelets. Fig 1 shows that desensitization was HDL$_{3}$-concentration dependent. This drop in 1,2-diacylglycerol production was not linked to [3H]phosphatidylcholine exchange between prelabeled platelets and high concentrations of HDL$_{3}$. The radioactivity of HDL$_{3}$ after a 5-minute incubation in the presence of prelabeled platelets represented less than 1% of the specific activity of the platelet preparation and was not significantly modified by increasing HDL$_{3}$ concentration. Desensitization of HDL$_{3}$-induced phosphatidylcholine breakdown was almost complete after a 5-minute exposure to 0.8 mg/mL HDL$_{3}$ (IC$_{50}$, 150±20 μg/mL, n=6). Fig 2 shows that the desensitization produced by HDL$_{3}$ was time dependent. With 1 mg/mL HDL$_{3}$, 1/10 for desensitization was 30 seconds, with maximal desensitization occurring at 5 minutes. To ascertain whether stimulation and desensitization of 1,2-diacylglycerol formation are mediated by the binding of HDL$_{3}$, [3H]phosphatidylcholine-prelabeled platelets were exposed to 1 mg/mL HDL$_{3}$ modified by...
tetranitromethane for 5 minutes before measurement of 50 μg/mL HDL₃-induced PLC activity (Fig 3). 1,2-Diacylglycerol release was similar to the 1,2-diacylglycerol control value of 50 μg/mL HDL₃-stimulated platelets. Tetranitromethane abolished HDL₃ binding to platelet membranes and the ability of 1 mg/mL HDL₃ to reduce PLC activity.

**Effects of Protein Kinase Modulators on HDL₃-Induced Desensitization**

To determine whether HDL₃-induced desensitization is associated with protein phosphorylation, we tested different inhibitors to analyze the contribution of different protein kinases to HDL₃-induced desensitization of PLC activity.

Permeabilization with digitonin (10 μg/mL) was performed to provide inhibitor access to the cytosol, while leaving HDL₃-induced desensitization unaltered. In digitonin-permeabilized platelets, the extent of maximal 1,2-diacylglycerol release over basal activity was reduced by HDL₃ (1 mg/mL) pretreatment to 50% of HDL₃ (50 μg/mL)-stimulated PLC activity. This result was similar to unpermeabilized control values, indicating that digitonin does not interfere with the pattern of HDL₃-induced desensitization (Fig 4).

To address the potential role of protein kinase-dependent phosphorylation of proteins in producing receptor desensitization, we tested the prevention of HDL₃ (1 mg/mL)-induced desensitization in the presence of 1 μmol/L PKI (protein kinase A [PKA] inhibitor), 100 μmol/L H-7 or 10 μmol/L staurosporine (PKC inhibitors), or 100 nmol/L heparin (low-density lipoprotein receptor kinase and β-adrenergic receptor kinase inhibitor). Results are summarized in Fig 5. PKI and heparin alone did not prevent desensitization or regulation of the HDL₃ binding site–coupled 1,2-diacylglycerol release. PKI even seemed to slightly potentiate desensitization; however, 1,2-diacylglycerol release under these conditions was not significantly different from 1,2-diacylglycerol content in unstimulated platelets. In sharp contrast, the HDL₃ (1 mg/mL)-induced desensitization of the HDL₃ binding site–coupled response was fully blocked by both H-7 and staurosporine. When desensitization was performed in the presence of H-7 or staurosporine, it did not modify the ability of subsequent applications of 50 μg/mL HDL₃ to induce hydrolysis of phosphatidylincholine into 1,2-diacylglycerol. Moreover, a slight increase in 1,2-diacylglycerol production was recorded. Incubation of platelets in the presence of the PKC inhibitors alone did not induce significant 1,2-diacylglycerol release (UC-ST or UC-H7).

---

**Fig 1.** Line graph shows dose-response relation for HDL₃-stimulated desensitization. [3H]Phosphatidylincholine-prelabeled platelets (2.5×10⁶ cells per milliliter) were incubated with various HDL₃ concentrations for 5 minutes at 37°C before measurement of 50 μg/mL HDL₃-induced 1,2-diacylglycerol (1,2-DAG) generation. Phospholipase C data are expressed as a percentage of maximal HDL₃ (50 μg/mL)-induced 1,2-DAG release in each experiment. Results are mean±SD of three separate determinations performed in duplicate.

**Fig 2.** Line graph shows time course for HDL₃-induced desensitization. [3H]Phosphatidylincholine-prelabeled platelets (2.5×10⁶ cells per milliliter) were incubated with 1 mg/mL HDL₃ for indicated times at 37°C. Subsequent to desensitization, samples were processed for measurement of 50 μg/mL HDL₃-induced 1,2-diacylglycerol generation. Data are expressed as a percentage of maximal desensitization in each experiment and are mean±SD of three separate determinations performed in duplicate.

**Fig 3.** Bar graph shows role of HDL₃ modified by tetranitromethane (TNM-D) in desensitization. [3H]Phosphatidylincholine-prelabeled platelets (2.5×10⁶ cells per milliliter) were desensitized with 0 mg/mL (control), 1 mg/mL HDL₃ (D), or 1 mg/mL TNM-D for 5 minutes at 37°C before measurement of 50 μg/mL HDL₃-induced 1,2-diacylglycerol (1,2-DAG) generation as described in Fig 1. Results are mean±SD of three separate determinations performed in duplicate.
Effects of ConA on HDL₃-Induced Desensitization

As a second step, we sought to determine whether HDL₃ binding sites are sequestered away from the cell surface into a membrane-associated compartment as for β-adrenergic receptors. HDL₃ (50 μg/mL)-induced 1,2-diacylglycerol production was measured in permeabilized platelets pretreated with 0.25 mg/mL ConA (known to block sequestration) before the desensitization step with HDL₃ (1 mg/mL). Fig 5 shows that in the context of the processes studied here, pretreatment by ConA does not modify or produce even a modest reduction in HDL₃ (50 μg/mL)-induced 1,2-diacylglycerol production compared with desensitized controls. This suggests that HDL₃ binding site sequestration is not the main event of HDL₃-induced desensitization.

Desensitization-Induced Protein Phosphorylation

Desensitization could be in general associated with either receptor, G protein, or effector phosphorylation. To show whether HDL₃-induced desensitization is linked to protein phosphorylation, digitonin-permeabilized platelets were incubated in the presence of 6 μmol/L [³²P]ATP (3 Ci=110 GBq) and 1 mg/mL HDL₃ for 5 minutes at 37°C. Membrane proteins were separated by SDS-PAGE and processed for autoradiography (Fig 6). Among the few protein bands (94, 110, 120, 140, 158 kD) that were detectable after autoradiography, two proteins (94 and 110 kD) were highly phosphorylated only in desensitized platelet extracts compared with platelets stimulated only by 50 μg/mL HDL₃.

The addition of different kinase inhibitors to the desensitization medium subsequently modified the phosphorylation state of the previously labeled proteins. When 1 μmol/L PKI was added, the apparent steady-state level of protein phosphorylation was slightly decreased. In contrast, the addition of both 100 μmol/L H-7 or 10 μmol/L staurosporine had a marked effect on the 94- and 110-kD protein phosphorylation. No phosphorylation of these proteins was found when desensitization was performed in the presence of PKC inhibitors.

These results again suggest that desensitization involves at least in part a PKC-dependent phosphorylation of both 94- and 110-kD proteins.

Discussion

In a previous article, we demonstrated that HDL₃ (50 μg/mL)-induced 1,2-diacylglycerol production in [³²P]-phosphatidylcholine-prelabeled platelets is not encoun-

[Graph showing effects of inhibitors on HDL₃-induced desensitization.]
tered with HDL₃ modified with tetranitromethane and is concentration dependent in the range of 0 to 0.7 mg/mL HDL₃, with half-maximal and maximal responses occurring at 0.15 and 0.60 mg/mL HDL₃, respectively. Stimulation with higher HDL₃ concentrations resulted in a gradual decline in 1,2-diacylglycerol production. Similar decreases in agonist-stimulated receptor activity have resulted in a loss of responsiveness of the system, also termed desensitization.

As HDL₃ promotes phosphatidylcholine hydrolysis, the accompanying release of 1,2-diacylglycerol may activate PKC and at the same time switch on a feedback loop that can uncouple ligand and binding sites from PLC activation. This idea is supported by the observations that (1) high concentrations of HDL₃ fail to activate PLC, (2) HDL₃ internalization within platelets is encountered for higher concentrations compared with other HDL₃-internalizing cells, and (3) platelet stimulation by phorbol esters can stimulate a delayed phosphorylation mechanism is operative.

HDL₃-induced desensitization in human platelets was assessed by quantifying the signal transduction efficacy of the system after exposure of HDL₃ binding sites to physiological agonist concentrations. A brief exposure of [³²P]ATP to platelets modified with HDL₃ (50 µg/mL HDL₃) induced phosphorylation of specific protein bands that activated PKC and at the same time switched on a feedback loop that can uncouple ligand and binding sites from PLC activation. This idea is supported by the observations that (1) high concentrations of HDL₃ fail to activate PLC, (2) HDL₃ internalization within platelets is encountered for higher concentrations compared with other HDL₃-internalizing cells, and (3) platelet stimulation by phorbol esters can stimulate a delayed phosphorylation mechanism is operative.

HDL₃-induced desensitization of PLC activity is mediated by the binding of HDL₃, as HDL₃ pretreatment with tetranitromethane abolishes both HDL₃ binding to the cell surface and desensitization of HDL₃-induced PLC activity. Indeed, a 5-minute preincubation of platelets in the presence of 1 mg/mL HDL₃ modified with tetranitromethane does not suppress the ability of 50 µg/mL HDL₃ to induce phosphatidylycholine breakdown.

Current studies suggest that protein phosphorylation by cellular kinases and receptor sequestration are important components of the early events of desensitization. In human platelets, recent investigations suggest that activation of PKC is able to desensitize the action of thrombin by applying different sequestration or protein kinase inhibitors to digitonin-permeabilized platelets, we investigated the contribution of these processes to HDL₃-induced desensitization. PKA-dependent phosphorylation could be selectively inhibited by PKI or sequestration by ConA. Despite the fact that H-7 and staurosporine lack specificity for PKC over other several kinases, they remain popular tools for examining the role of PKC in regulating cell functions. Heparin was purposely used as an inhibitor of β-adrenergic receptor or low-density lipoprotein receptor kinase and was not supposed to impair HDL₃-induced desensitization.

Impairment of HDL₃-induced desensitization by inhibitors was analyzed by 1,2-diacylglycerol assay to determine HDL₃-induced PLC activity and by the uptake of [³²P]ATP into platelets and incorporation into substrates of interest. To determine whether HDL₃-induced desensitization is associated with any change in protein phosphorylation, we incubated platelets during the desensitization step in the presence of [³²P]ATP. Autoradiography of SDS-PAGE-separated membrane proteins indicates that some protein phosphorylation is achieved only in desensitized platelets compared with control platelets stimulated only with 50 µg/mL HDL₃. The apparent molecular masses of the two major phosphorylated protein bands are 94 and 110 kD. Among the three candidate proteins (ie, HDL₃ binding sites, G proteins, and PLC) that could be phosphorylated during desensitization, G proteins and PLC do not fit with the molecular mass characteristics of the phosphorylated protein bands. Relevance of the 94- or 110-kD protein to HDL₃ binding sites is a conceivable hypothesis awaiting further confirmation.

Recently it has been reported that platelet activation induces tyrosine phosphorylation of three specific proteins with apparent molecular masses of 84, 95, and 97 kD, but it is likely that in our experiments protein phosphorylation was unrelated to inadvertent platelet activation during washing and centrifugation steps, as no protein labeling was recorded in controls. These findings clearly indicate that specific protein phosphorylation occurs during the desensitization process of HDL₃-induced 1,2-diacylglycerol generation.

Pretreatment with ConA, known to abolish sequestration, was unsuccessful in reducing HDL₃-induced desensitization of PLC activity, indicating that the contribution of sequestration to HDL₃ binding site regulation was not significant at 1 mg/mL HDL₃. Previously, Lohse et al demonstrated that sequestration of β-adrenergic receptors occurs at high concentrations of agonist and that pretreatment with ConA has no significant effect if the phosphorylation mechanism is operative.

Pretreatment of platelets with heparin does not impair desensitization of HDL₃ (50 µg/mL)-induced PLC activity, in any of its kinase inhibitor or other roles.
Among the all the kinase inhibitors, only PKC inhibitors are able to counteract HDL3-induced desensitization, which is fully blocked. After a 5-minute desensitization in the presence of H-7 or staurosporine, 50 \( \mu \)g/mL HDL3 is able to activate PLC activity and induce 1,2-diacylglycerol release from phosphatidylcholine. These findings suggest that the binding of HDL3 can produce 1,2-diacylglycerol in human platelets but that at a physiological concentration of the agonist, the signaling pathway may be desensitized acutely by a PKC-mediated feedback system. Moreover, H-7 and staurosporine fully block the phosphorylation of 94- and 110-kD proteins during desensitization of platelets to HDL3.

In sharp contrast, PKA inhibitors are not able to counteract HDL3-induced desensitization. Furthermore, a slight decrease in PLC activity was registered when desensitization was performed in the presence of PKI, suggesting that PKA could control HDL3-induced PLC activity. However, this speculation appears less likely, as the decrease in 1,2-diacylglycerol content is not significant when compared with the 1,2-diacylglycerol content of unstimulated platelets. Phosphorylation of 94- and 110-kD proteins is decreased when desensitization was performed in the presence of PKA inhibitors, whereas proteins of a higher molecular mass are slightly phosphorylated. Various forms of cross talk between kinases or phosphates and other signaling cascades have been observed in other situations, but it is far too speculative to ascertain the role of PKA, if any, in HDL3-induced desensitization of PLC activity in human platelets.

If the HDL3 binding proteins represent glycoprotein IIb/IIIa, one would anticipate a relation between HDL3 binding and glycoprotein IIb/IIIa complex activation. To ascertain whether HDL3 fulfills any role in controlling glycoprotein IIb/IIIa functions requires a better characterization of the HDL3 binding proteins that are phosphorylated in human platelets.

In summary, we have provided evidence that exposure of platelets to 1 mg/mL HDL3 impairs HDL3-induced PLC activity at least by a PKC-mediated feedback system and that the binding of high concentrations of HDL3 to its binding sites induces a mechanism that reduces platelet responsiveness to low-intensity stimulation.

Acknowledgments

This work was supported by grants from INSERM U 325. The authors are very grateful to Paul Kelly for his helpful editorial comments, J. Fremaux for his technical assistance, and S. Dyke for typing the manuscript.

References

Protein kinase C-dependent desensitization of HDL3-activated phospholipase C in human platelets.

H Nazih, F Nazih-Sanderson, V Magret, B Caron, J Goudemand, J C Fruchart and C Delbart

doi: 10.1161/01.ATV.14.8.1321

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/14/8/1321

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org/subscriptions/