Role of Phosphoinositides in the Regulation of Endothelial Prostacyclin Production

Haraldur Halldórsson, Matthias Kjeld, and Gudmundur Thorgeirsson

To elucidate the role of the phosphoinositide signal transduction system in endothelial prostacyclin production, endothelial cells from human umbilical veins previously labelled with 1-14C inositol were incubated with thrombin or histamine. Water-soluble inositol phosphates were separated on anion exchange columns. Both agonists evoked transient bursts of inositol phosphate production with inositol trisphosphate peaking at 15 seconds in histamine-stimulated cells and at 60 seconds in thrombin-stimulated cells. The inositol phosphate production was closely linked to prostacyclin production. After stimulation, there was concurrent densensitization to prostacyclin production and formation of inositol phosphates. Arachidonic acid and the Ca²⁺ ionophore A23187 did not affect inositol phosphate production in concentrations sufficient to increase prostacyclin production 20-fold, and they did not affect desensitization to a subsequent thrombin stimulation. The phorbol ester 12-0-tetradecanoyl phorbol 13-acetate, a stimulator of protein kinase C, inhibited thrombin-induced generation of inositol phosphates; enhanced A23187-mediated prostacyclin production, and had complex effects on thrombin-mediated prostacyclin production, but had no effect on its production from extrinsic arachidonic acid. The current data suggest that production of inositol phosphates is a link in receptor stimulation of endothelial cells to produce prostacyclin and that associated activation of protein kinase C affects both the generation of second messengers and the release of arachidonic acid. (Arteriosclerosis 8:147–154, March/April 1988)

Prostacyclin (PGI₂) is a potent vasodilator and inhibitor of platelet aggregation and is produced by endothelial cells in response to a variety of receptor-mediated stimuli. It is synthesized from the universal precursor of prostaglandins, arachidonic acid, that is stored in cell membrane phospholipids and released by activation of phospholipase A₂ or by the sequential action of phospholipase C and diacylglycerol lipase or by both. While several substances are known to stimulate endothelial cells to produce prostacyclin, much less is known about the actual mechanism of regulation. There are several potential points at which regulation may be affected, including the receptor, the amount of releasable arachidonic acid in cell membrane, the cyclooxygenase, and the prostacyclin synthetase. However, the most important controlling point and the step affected by stimulants is believed to be the release of arachidonic acid by the calcium-dependent enzymes phospholipase A₂ or diacylglycerol lipase, or both. In recent years, Ca²⁺ has been shown to be affected in a number of cell types by a complex signal transduction system which generates intracellular messengers from inositol phospholipids. The binding of an agonist to a receptor on the cell surface activates phospholipase C, which then splits phosphatidyl inositol bisphosphate (PIP₂) into inositol trisphosphate (IP₃) and diacylglycerol (DG). In many cell types, IP₃ induces Ca²⁺ release into the cytoplasm and DG activates protein kinase C that phosphorylates several cell proteins with potentially varied and cell type specific results.

Recently Derian and Moskowitz have shown that bradykinin stimulates the formation of inositol phosphates in bovine aortic and cerebral microvascular endothelial cells and segments of canine carotid artery. In the bovine aortic endothelial cells, synthesis of 6-keto-prostaglandin F₁α, the stable metabolite of PGI₂, closely paralleled the formation of inositol phosphates.

In the present article, we report on the effects of thrombin, histamine, arachidonic acid, the Ca²⁺ ionophore A23187, and the phorbol ester 12-0-tetradecanoyl phorbol 13-acetate (TPA) on PGI₂ production and formation of inositol phosphates in cultured human umbilical vein endothelial (HUVE) cells. We demonstrate concurrent desensitization of both responses after thrombin or histamine stimulation and examine the possible role of protein kinase C.

Methods

Materials

Myo-(2-³H)-inositol (specific activity 15.6 Ci/mmol), ³H-inositol phosphate mixture (1.0 Ci/mmol), and tritiated 6-keto-prostaglandin F₁α (6-keto PGF₁α) were obtained from Amersham International; Morgan's medium 199, Earle's balanced salt solution, and fetal calf serum (FCS)
were obtained from Gibco; collagenase, 12-O-tetradecanoylphorbol 13-acetate (TPA), thrombin, histamine, arachidonic acid, acetylsalicylic acid (ASA), and adenosine were from Sigma; clemastine was from Sandoz; cimetidine was from Delta, Iceland; Ionophore A23187 was from Calbiochem; 6-keto-PGF₁α was from Upjohn; anion exchange resin was from Bio-Rad; and tissue culture plates were from Flow Laboratories. Anti-6-keto-PGF₁α antiserum was a generous gift from Michael Dunn, Department of Medicine, Case Western Reserve University, Cleveland, Ohio.

**Endothelial Cell Culture**

As previously described, endothelial cells were harvested from human umbilical veins by collagenase digestion (0.125% collagenase, Clostridium histolyticum type 1A) and seeded on multiwell tissue culture plates (area per well 2.0 cm²) in 0.5 to 1.0 ml Morgan's medium 199 with 20% FCS and antibiotics. Meticulous care was taken in obtaining similar seeding density in each well (approximately 100 cells per mm²). The culture plates were incubated at 37°C in humidified air with 5% CO₂. Cultures were fed every other day with a complete change of medium and were used for experiments 2 to 4 days after reaching confluence.

**Prostacyclin Production**

Before use, the culture medium was removed and the cell monolayer was washed twice with 1 ml of Earle's solution. The cells were then incubated for 5 minutes at 37°C with Earle's solution and stimulants as indicated in the Results section. In some experiments, the cells were washed twice with Earle's solution after the first treatment and were then restimulated. To study the effects of TPA on PGI₂ production, the cells were preincubated at 37°C with TPA and then incubated for 5 minutes at 37°C with TPA and other stimulants as indicated.

After each incubation, the medium was removed and its PGI₂ content was measured by a radioimmunoassay for 6-keto-PGF₁α, a stable catabolic product of PGI₂.

**Radioimmunoassay for 6-keto-PGF₁α**

Anti-6-keto-PGF₁α antiserum, tritiated 6-keto-PGF₁α, and standard solutions of 6-keto-PGF₁α or the test samples (all in a final dilution in 0.1 M phosphate buffer, pH 7.4, containing 0.2% bovine serum albumin) were incubated together overnight at 4°C. Antibody-bound and free prostaglandins were separated by the addition of dextran-coated charcoal suspension. After centrifugation at 3000 g for 10 minutes, the radioactivity in the supernatant (bound fraction) was determined by scintillation counting. All samples were assayed in duplicate. Intra-assay imprecision was 8.5% and detection limits, 5 pg. The antiserum is highly specific, showing 0.24% cross-reactivity with 6-keto-PGF₁α at 50% B/BO.

**Formation of Inositol Phosphates**

Confluent cells were washed twice with inositol-free medium and were then incubated in 0.4 ml inositol-free medium with 20% dialyzed FCS and 3 μCi/ml ³H-inositol for 48 hours. After washing, the cells were exposed to stimulants in 0.5 ml of Earle's balanced salt solution for 0 to 300 seconds. The solution was then removed, 1 ml of ice-cold 10% trichloroacetic acid was added, and the cells were scraped off the culture surface with a rubber policeman. In some experiments, the production of inositol phosphates was examined in cells that had been pretreated with various agents as indicated in the Results section. After centrifugation, trichloroacetic acid was removed from the supernatant by extracting it four times with 1.5 ml of diethyl ether. After adjusting the pH to 7 by addition of Tris buffer, the supernatants were applied to columns (2 cm × 0.6 cm) of anion exchange resin (AG1-×8, 200 to 400 mesh, formate form) and inositol-containing compounds were separated by the method of Bone et al. Six ml of each solution were passed through the columns and the eluates were collected directly into scintillation vials in 1 ml fractions, were mixed with 3 ml of scintillation fluid, and counted. The separation of inositol mono- (IP), bis- (IP₂), and trisphosphates (IP₃) was verified using tritiated standards of inositol phosphates.

**Results**

**Production of Prostacyclin and Inositol Phosphates**

The endothelial PGI₂ production in response to treatment with various agonists is summarized in Table 1 and is expressed as the concentration of the stable metabolite 6-keto-PGF₁α in each culture well. Exposure to histamine, thrombin, or arachidonic acid led to a burst of PGI₂ production. When the cells were washed and restimulated by either thrombin or histamine, they did not respond by another such burst. However, arachidonate elicited a partial response when used as a second stimulant after previous stimulation by either histamine or arachidonate (or thrombin; data not shown). Adenosine did not stimulate endothelial prostacyclin production and did not desensitize the cells to subsequent stimulation by histamine. Figure 1 demonstrates the response of cells to variable concentrations of histamine and repeat stimulation with thrombin. There is a dose-dependent relationship between histo-

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>First stimulation</th>
<th>Second stimulation</th>
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<tbody>
<tr>
<td>Buffer-histamine</td>
<td>2.4</td>
<td>34.6</td>
</tr>
<tr>
<td>Histamine-histamine</td>
<td>39.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Thrombin-thrombin</td>
<td>40.3</td>
<td>3.1</td>
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<tr>
<td>Arachidonate- arachidonate</td>
<td>40.0</td>
<td>17.7</td>
</tr>
<tr>
<td>Histamine- arachidonate</td>
<td>32.5</td>
<td>16.3</td>
</tr>
<tr>
<td>Adenosine-histamine</td>
<td>1.8</td>
<td>31.0</td>
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The results are expressed as the mean concentrations of the stable metabolite 6-keto-PGF₁α in the medium of two cultures. Each culture was stimulated twice with a 5-minute interim period. The following concentrations of stimulants were used: histamine 5.5 μM, thrombin 0.5 U/ml, arachidonate 20 μM, and adenosine 1 mM.
Figure 1. Histamine-induced synthesis of 6-keto-prostaglandin F1α, the effects of repeated stimulation by thrombin. Endothelial cells were incubated with variable concentrations of histamine for 5 minutes. 6-keto-prostaglandin F1α was measured in the medium by radiolmmunoassay (○). The cells were then washed twice with buffer and stimulated with thrombin (1 U/ml) and 6-keto-PGF1α was again determined in the medium (×). Data are expressed as ng/ml. Each point is the mean from duplicate cultures.

mine and the PGI2 response. When the same cultures were restimulated with thrombin, there was a reciprocal relationship between the first and the second responses. The cells exposed to the lowest concentration of histamine gave the highest response to thrombin, while the cells that had responded maximally to histamine were refractory to the second stimulation. The total PGI2 in response to two sequential stimuli was thus fairly constant.

To elucidate the role of phosphoinositides in endothelial PGI2 production, the water-soluble inositol phosphates were monitored after stimulation by histamine or thrombin. A representative elution profile is shown in Figure 2 for the separation of inositol-containing compounds by anion exchange chromatography in unstimulated cells, in cells stimulated for 15 seconds with histamine, and in cells stimulated for 15 seconds with histamine after previous stimulation for 5 minutes with the same agent. Histamine stimulation caused a marked rise in IP2 and IP3 as well as some rise in IP. By contrast, a second stimulation produced only a small rise in IP2 and IP3. Pretreatment of cells for 5 minutes with the histamine H1 receptor antagonist clemastine (1 μM) abolished the burst of inositol phosphate production in response to histamine incubation, while the histamine H2 receptor antagonist cimetidine (1 μM) had no effect (data not shown). The relative levels of the inositol phosphates varied with the length of incubation with histamine (Figure 3). There was rapid rise of all the fractions, with IP3 peaking at 20 seconds, falling down to one-third its peak level at 60 seconds and down to control value at 2 minutes. IP2 rose more slowly but also peaked before one
minute although the exact timing of the peak may not have been caught in this experiment. The decay, however, was clearly slower than for IP₃ and the level was still relatively high at 2 minutes. IP had the slowest and most variable response, although in an experiment with 10 mM of L-PLC in the medium, it continued to rise for 5 minutes (data not shown). In Figure 4, analogous time curves for inositol phosphates after incubation with thrombin are depicted. While again there was rapid and transient rise of both IP₂ and IP₃, the time course differed in that IP₃ reached peak level at 60 seconds and not at 15 seconds as after stimulation with histamine. The effects of both histamine and thrombin were concentration-dependent (Figure 5), and the dose-response curves for the inositol phosphates were similar to those for PGI₂ production.

As after histamine stimulation (see Figure 2), endothelial cells previously stimulated by thrombin did not respond to a repeat stimulation with another burst of inositol phosphosphate production (Figure 6). To further evaluate the role of phosphoinositides in the regulation of PGI₂ production, the effects of extrinsic arachidonic acid, the Ca²⁺ ionophore A23187, and the cyclooxygenase inhibitor ASA on inositol phosphate production and subsequent response to thrombin stimulation were evaluated. Arachidonic acid, itself a precursor of PGI₂, did not stimulate the production of inositol phosphates and only minimally affected a subsequent response to thrombin stimulation (Figure 6). Similarly, A23187, which stimulates phospholipase A₂-mediated arachidonate release by increasing the concentration of cytoplasmic Ca²⁺, did not trigger the phosphoinositol signal and left the response to a subsequent thrombin stimulation unaffected (Figure 6). The blocking of cyclooxygenase by ASA (Figure 7) did not prevent (or enhance) the inositol phosphate production initiated by thrombin and did not prevent the cells from becoming desensitized to a repeat stimulation by thrombin. Finally, in this series of ex-
periments, the relative levels of inositol phosphates at various times after histamine stimulation were compared in cells stimulated for the first time to cells stimulated for the second time (Figure 8). Simultaneous assessment of PGI₂ accumulation was carried out. While histamine triggered PGI₂ accumulation and a transient peak of inositol phosphates at 15 seconds when applied for the first time, no inositol phosphate peak was detected at either 15 seconds or 5 minutes after the second stimulation, and no PGI₂ accumulation occurred.

Effects of TPA on the Production of Prostacyclin and Inositol Phosphates

To investigate the functional relationship between the two arms of the phosphoinositide signal transduction sys-

tem, the Ca²⁺ ionophore A23187 and the phorbol ester TPA were used to mimic the roles of IP₃ and DG, respectively. Figure 9 shows the levels of 6-keto-PGF₁α produced in response to various concentrations of A23187 with or without TPA. The ionophore caused a dose-dependent response. TPA alone did not stimulate the cells to produce PGI₂ but enhanced the PGI₂ production in response to all

![Figure 6. Comparison of the effects of thrombin, arachidonic acid, and the Ca²⁺ ionophore A23187 on the generation of inositol phosphates in HUVE cells. Prelabelled cells were incubated with either thrombin (1 U/ml), arachidonic acid (20 μM), or A23187 (0.4 μM) for the indicated times. Other cultures were pretreated for 5 minutes with one of these agonists and after washing were incubated with thrombin for 1 minute. Methods are described in the legend to Figure 3. The data are expressed as cpm in IP₂ plus IP₃ relative to control. Each bar represents the mean of duplicate cultures.](http://atvb.ahajournals.org/)

![Figure 7. The effects of ASA on thrombin-induced inositol phosphate accumulation in HUVE cells. Prelabelled cells were incubated with or without ASA (100 μM) for 10 minutes before exposure to thrombin (1 U/ml) for 1 minute or thrombin for 5 minutes followed by repeat exposure to thrombin for 1 minute. The methods are as described in the legend to Figure 3. The data are expressed as cpm in IP₂ plus IP₃ relative to control, and each bar represents the mean of duplicate cultures.](http://atvb.ahajournals.org/)

![Figure 8. The time course of histamine-induced accumulation of inositol phosphates after first and second stimulations of HUVE cells. Prelabelled cells were incubated with or without histamine (5.5 μM) for 5 minutes and then again with histamine for 15 seconds or 5 minutes. The methods are as described in the legend to Figure 3. The data are expressed as cpm in IP₂ plus IP₃ relative to control, and each column represents the mean of duplicate cultures. At 5 minutes after the second treatment, 6-keto-PGF₁α was measured in the medium and expressed as ng/ml.](http://atvb.ahajournals.org/)

![Figure 9. The synergistic effects of A23187 and TPA on PGI₂ production. HUVE cells were incubated with or without TPA (0.1 ng/ml) for 10 minutes. A23187 was then added at the concentrations indicated. After 5 minutes the medium was removed and its 6-keto-PGF₁α concentration was determined by radioimmunoassay. Data are expressed as ng/ml, and each point represents the mean of duplicate cultures. • = with TPA; O = without TPA.](http://atvb.ahajournals.org/)
Figure 10. The effects of TPA on thrombin-induced prostaglandin (PG) production. HUVE cells were incubated with TPA (0.1 ng/ml) for variable times as indicated. Thrombin was then added at either 0.1 U/ml (A) or 1.0 U/ml (B). After 5 minutes the medium was removed and its 6-keto-PGF₁α concentration was determined by radioimmunoassay. Data are expressed as PGF₁α in TPA-treated cultures relative to control (thrombin only).

Figure 11. The effects of TPA on thrombin-induced production of inositol phosphates. Prelabelled cells were incubated with TPA (0.1 ng/ml) for variable times as indicated. Thrombin was then added for 1 minute. Methods are as described in the legend to Figure 3. Data are expressed as percent inhibition of thrombin-induced generation of IP₃ or IP₂ in TPA-treated cultures. Each point is the mean ± SEM of four experiments, each done in duplicate.

Discussion

Increased turnover of inositol lipids after activation of plasma membrane receptors is associated with various cell functions in numerous tissues. Although much remains unknown concerning this association, it is now generally accepted that the initial response is the hydrolysis of PIP₂ by phospholipase C producing IP₃ [most likely the isomer Ins (1,4,5)P₃] and DG. These compounds are short-lived and operate as intracellular messengers; IP₃ releases Ca²⁺ from the endoplasmic reticulum and DG activates protein kinase C. The messengers interact in a number of ways and both have effects on phospholipid metabolism which have proven hard to separate from the initial response triggering their production. This complex signal transduction system has been reviewed extensively.

In many cell types, receptor-mediated inositol lipid turnover has been associated with prostaglandin production. In stimulated platelets, arachidonic acid is released either from the DG produced by the stimulus or from phospholipids due to activation of phospholipase A₂, which, in turn, is caused by IP₃-mediated rise in Ca²⁺. The thromboxane thus produced takes part in amplifying the primary stimulus by causing further breakdown of PIP₂. PG₂, the major prostaglandin produced by endothelial cells, has the opposite effect in platelets. It prevents hydrolysis of PIP₂, apparently by elevating cAMP levels.

The purpose of the present work is to provide insights into the role of this signal transduction system in endothelial PG₂ production. Hong and Deykin have demonstrated that endothelial PG₂ production involves formation of DG. Since the PG₂ production also is Ca²⁺-dependent, involvement of the phosphoinositide system was strongly suggested. Recently Derian and Moskowitz found that bradykinin stimulates the formation of inositol phosphates in bovine aortic and cerebral microvascular endothelial cells and segments of canine carotid artery. The results of our study show that in HUVE cells exposed to thrombin or...
histamine, there is a short-lived burst of inositol phosphate production closely linked to PG\(_I_2\) production. The effects of both agonists were concentration-dependent with respect to both the production of inositol phosphates and PG\(_I_2\), and the dose-response curves for inositol phosphates were similar to those for PG\(_I_2\). Histamine \(H_2\) receptor antagonist inhibited both PG\(_I_2\) production and the formation of inositol phosphates, while a histamine \(H_2\) receptor antagonist affected neither. Although ASA inhibited PG\(_I_2\) production, it did not affect the burst of phosphoinositides following agonist stimulation, excluding the possibility that cyclooxygenase products formed in the endothelial cells exposed to agonists may stimulate the phosphoinositide turnover. The rapid rise and transient presence of IP\(_3\) are consistent with its proposed role as a second messenger and suggest it mainly consists of the isomer Ins(1,4,5)P\(_3\) rather than Ins(1,3,4)P\(_2\). In other cell types, the latter has been found to be less transient and to outlast the Ca\(^{2+}\) signal by several minutes. High-performance liquid chromatography analysis of the IP\(_3\) isomers in agonist-stimulated endothelial cells, as well as search for such polyphosphoinositides as IP\(_6\), IP\(_5\), and IP\(_4\), await further work.

Following stimulation by thrombin or histamine, HUVE cells became concurrently desensitized to a repeat stimulation with respect to both PG\(_I_2\) production and inositol phosphate production (Figures 2, 6, and 8). In contrast, arachidonic acid and the Ca\(^{2+}\) ionophore A23187, which are both activators of PG\(_I_2\) production, do not activate the phosphoinositide turnover and do not abolish a subsequent rise of inositol phosphates in response to stimulation by thrombin or histamine (Figure 6).

The mechanism of desensitization of endothelial cells that have been stimulated to produce PG\(_I_2\) is unknown and clearly is a complex phenomenon involving cyclooxygenase and interaction of external factors with receptors. Our findings that the messenger system of phosphoinositides is also involved adds to the complexity. The findings that ASA (Figure 7) and prior stimulation of PG\(_I_2\) production by extrinsic arachidonic acid or A23187 (Figure 6) do not affect the desensitization of the phosphoinositide response exclude the involvement of a cyclooxygenase product. In contrast, the inhibition of inositol phosphate production by pretreatment of the cells with TPA (Figure 11) suggests that protein kinase C is involved.

Since protein kinase C represents one of the two arms of the inositol messenger system and the two arms interact in many ways, its activation provides for complex effects on PG\(_I_2\) production. The most commonly described interaction has been synergism with respect to the eventual cell response, but the activation of protein kinase C by DG also seems to have a role in terminating the response in several systems. In still other systems that show a biphasic response, i.e., an initial and a sustained response, the former can be mimicked by Ca\(^{2+}\) ionophore whereas DG causes the latter.

The mechanism of these interactions is still unclear and may involve both direct effects of protein kinase C on Ca\(^{2+}\) transport and feedback effects on further messenger generation by receptor inactivation, modulation of coupling proteins, and activation of inositol kinases.

The TPA inhibition of agonist-induced generation of inositol phosphates that we have shown in HUVE cells (Figure 11) is similar to TPA effects in many other systems and may mimic a negative feedback signal induced by protein kinase C activation by DG. Conversely, TPA applied for 10 minutes amplified the effects of A23187 on PG\(_I_2\) synthesis (Figure 9) but had no effect on PG\(_I_2\) production from extrinsic arachidonic acid. This suggests that the stimulatory effects of short-term TPA treatment are on arachidonic acid release (as has been found in MDCK cells\(^{37}\)) rather than on the cyclooxygenase. Our finding that the effects of TPA on thrombin-induced PG\(_I_2\) synthesis is dependent on both concentration of the agonist and on duration of pretreatment with TPA (Figure 10) has to be viewed with these conflicting effects of TPA at different steps in PG\(_I_2\) production in mind. At low concentrations of thrombin, the inhibitory effect on the phosphoinositide signal seems to be dominating and results in a reduced PG\(_I_2\) production. At a higher concentration, this same effect dominates after a brief (3-minute) TPA exposure, but after a 25-minute exposure, the stimulating effect dominates the outcome, possibly due to enhanced release of arachidonic acid.

The responses of endothelial cells previously treated with variable concentrations of one agonist to a repeat stimulation with another agonist were also examined. As indicated in Figure 2, the quantity of PG\(_I_2\) produced in response to the second stimulation with thrombin is inversely proportional to the quantity produced by the first stimulation with histamine. Similar results were obtained when cells previously treated with variable concentrations of A23187 or TPA and A23187 were incubated with histamine (data not shown). The total amount of PG\(_I_2\) that HUVE cells could produce in these two bursts seemed to be fixed. This control might be operating at the level of the cyclooxygenase, since after the cells have produced a certain quantity of PG\(_I_2\) they will not even respond to arachidonic acid. Still, it is clear that PG\(_I_2\) production is not only controlled by inactivation of cyclooxygenase but also by other factors involved in the signal transduction process.

The responses of endothelial cells previously treated with a combination of TPA and an agonist show that the effects of TPA on PG\(_I_2\) production are potentiated by substances that are included in our list of cell treatment agents. Since protein kinase C represents one of the two arms of the phosphoinositide cycle this suggests that the effects of TPA on PG\(_I_2\) production are potentiated by substances that are included in our list of cell treatment agents. Since protein kinase C represents one of the two arms of the phosphoinositide cycle this suggests that the effects of TPA on PG\(_I_2\) production are potentiated by substances that are included in our list of cell treatment agents.

Acknowledgments

We thank Amdis Theodore and Asún Kristmundsdóttir for skilled technical assistance and Elisabet Snorradóttir for manuscript preparation. Special thanks go to Valgardur Egilsson for valuable support.

References

3. Hong SL, Deykin D. Activation of phospholipases A2 and C.

4. Irvine RF. How is the level of free arachidonic acid controlled in mammalian cells? Biochem J 1982;204:3–16


28. Limes CJ. Phosphorylation of cardiac sarcolemmal reticulum by a calcium-activated phospholipid-dependent protein kinase. Biochem Biophys Res Commun 1986;137–1383


Index Terms: phosphoinositides • vascular endothelium • prostacyclin production
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Arterioscler Thromb Vasc Biol. 1988;8:147-154
doi: 10.1161/01.ATV.8.2.147

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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