Role of PGI$_2$ in the formation and maintenance of hyperdynamic circulatory state of portal hypertensive rats

Zhi-Yong Wu, Xue-Song Chen, Jiang-Feng Qiu, Hui Cao

Zhi-Yong Wu, Xue-Song Chen, Jiang-Feng Qiu, Hui Cao, Department of General Surgery, Renji Hospital, Shanghai Second Medical University, Shanghai 200127, China. Correspondence to: Dr. Zhi-Yong Wu, Department of General Surgery, Renji Hospital, 1630 Dongfang Road, Shanghai 200127, China. zhengwk@online.sh.cn Telephone: +86-21-50905336 Received: 2004-04-24 Accepted: 2004-05-09

Abstract

AIM: To investigate the role of prostacyclin (PGI$_2$) and nitric oxide (NO) in the development and maintenance of hyperdynamic circulatory state of chronic portal hypertensive rats.

METHODS: Ninety male Sprague-Dawley rats were divided into three groups: intrahepatic portal hypertension (IHPH) group by injection of CCl$_4$, prehepatic portal hypertension (PHPH) group by partial stenosis of the portal vein and sham-operation control (SO) group. One week after the models were made, animals in each group were subdivided into 4 groups: saline controlled group ($n=23$), NO-nitro-L-arginine (L-NNA)group ($n=21$) group, indomethacin (INDO) group ($n=22$) and high-dose heparin group ($n=24$). The rats were administrated 1mL of saline, L-NNA (3.3 mg/kg.d) and INDO (5 mg/kg.d) respectively through gastric tubes for one week, then heparin (200 IU/Kg/min) was given to rats by intravenous injection for an hour. Splanchnic and systemic hemodynamics were measured using radioactive microsphere techniques. The serum nitrate/nitrite(NO$_2$/NO$_3$) levels as a marker of production of NO were assessed by a colorimetric method, and concentration of 6-keto-PGF$_1$$\alpha$ was determined by radioimmunoassay.

RESULTS: The concentrations of plasma 6-keto-PGF$_1$$\alpha$ (pg/mL) and serum NO$_2$/NO$_3$ (µmol/L) in IHPH rats (1123.85±153.64, 73.34±4.31) and PHPH rats (891.88±83.11, 75.21±6.89) were significantly higher than those in SO rats (725.53±105.54, 58.79±8.47) ($P<0.05$). Compared with SO rats, total peripheral vascular resistance (TPR) and splanchnic vascular resistance (SVR) decreased but cardiac index (CI) and portal venous inflow (PVI) increased obviously in IHPH and PHPH rats ($P<0.05$). L-NNA and indomethacin could decrease the concentrations of plasma 6-keto-PGF$_1$$\alpha$ and serum NO$_2$/NO$_3$ in IHPH and PHPH rats ($P<0.05$). Meanwhile, CI, FPP and PVI lowered but MAP, TPR and SVR increased ($P<0.05$). After deduction of the action of NO, there was no significant correlation between plasma PGI$_2$ level and hemodynamic parameters such as CI, TPR, PVI and SVR. However, after deduction of the action of PGI$_2$, NO still correlated highly with the hemodynamic parameters, indicating that there was a close correlation between NO and the hemodynamic parameters. After administration of high-dose heparin, plasma 6-keto-PGF$_1$$\alpha$ concentrations in IHPH, PHPH and SO rats were significantly higher than those in rats administrated vehicle ($P<0.05$). On the contrary, levels of serum NO$_2$/NO$_3$ in IHPH, PHPH and SO rats were significantly lower than those in rats administrated Vehicle ($P<0.05$). Compared with those rats administrated vehicle, the hemodynamic parameters of portal hypertensive rats, such as CI and PVI, declined significantly after administration of high-dose heparin ($P<0.05$), while TPR and SVR increased significantly ($P<0.05$).

CONCLUSION: It is NO rather than PGI$_2$ that is a mediator in the formation and maintenance of hyperdynamic circulatory state of chronic portal hypertensive rats.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: Portal hypertension; Prostacyclin; Nitric oxide; Hyperdynamic circulatory

Wu ZY, Chen XS, Qiu JF, Cao H. Role of PGI$_2$ in the formation and maintenance of hyperdynamic circulatory state of portal hypertensive rats. World J Gastroenterol 2005;11(5):752-755
http://www.wjgnet.com/1007-9327/11/752.asp

INTRODUCTION

It is well established that systemic and splanchnic hyperdynamic circulatory state plays an important role in maintaining and aggravating the high portal venous pressure. However, the underlying mechanisms have not been completely understood. Recent studies suggest that vasodilators such as nitric oxide (NO) and prostacyclin (PGI$_2$) contribute much to hyperdynamic circulation$^{[1,2]}$.

NO may be a mediator in the pathogenesis of hyperdynamic circulatory state, but whether PGI$_2$ plays the same role is still controversial$^{[3,4]}$. In order to elucidate the relative contribution of PGI$_2$, NO and the possible interaction between these two vasodilators in the development of hyperdynamic circulatory state of chronic portal hypertensive rats, we designed the experiment to detect the plasma PGI$_2$, NO level and hemodynamic effects of NO inhibitor (L-NNA), COX$_2$ inhibitor (indomethacin) and high-dose heparin on IHPH, PHPH and SO rats.

MATERIALS AND METHODS

Experimental model

Ninety adult male Sprague-Dawley rats (weighing 300±50 g) were used in all experiments. Animals were housed in an environmentally controlled vivarium with light control (12 h light-dark cycle) and allowed free access to standard pellet diet and water. Survival surgery and hemodynamic studies were performed in strict sterile conditions under ketamine hydrochloride anesthesia (100 mg/kg, im). The temperature of rats was maintained at 37±0.5 °C by a heating lamp and
monitored by a rectal probe. Experimental animals were randomly divided into three groups: IHPH, PHPH and SO.

IHPH was induced in 22 rats by injection of CCl₄ according to a previously reported method[5]. Briefly, the rats were injected 15 times intramuscularly with (0.3 mL/100 g, first time injection of 0.5 mL/100 g) 60% CCl₄ in mineral oil, once every four days, and were given 10% alcohol instead of water. PHPH was induced in 20 rats by partial portal vein ligation according to a previously reported method[6]. In brief, the portal vein was isolated and a calibrated stenosis was performed with a single 3-0 silk ligature around a 20-gauge blunt-tipped needle. The needle was then removed, and the portal vein was allowed to reexpand. The viscera were placed back into the abdomen, and the incision was closed in two layers with suture. Antibiotic ointment was applied to the surgical wound. SO was matted in 24 rats.

**Experimental scheme**

One week after the models were made, animals in each group were subdivided into 4 groups: saline controlled group (n = 23), NO-nitro-L-arginine (L-NNA) group (n = 21), indomethacin (INDO) group (n = 22) and high-dose heparin group (n = 24). Rats were administrated 1 mL of saline, L-NNA (dissolved in 1 mL saline at the dose of 3.3 mg/kg·d) and INDO (dissolved in 1 mL saline at the dose of 5 mg/kg·d) respectively through gastric tubes for one week, then 1 mL heparin solution at the dose of 200 IU/Kg/min and 1 mL saline were given to high-dose heparin group rats and saline controlled rats respectively by intravenous injection for an hour.

**Hemodynamic study**

Splanchnic and systemic hemodynamic parameters including mean arterial blood pressure (MAP), free portal pressure (FPP), cardiac index (CI), portal venous inflow (PVI), total peripheral vascular resistance (TPR) and splanchnic vascular resistance (SVR) were measured using radioactive microsphere techniques[7].

**Assay of 6-keto-PGF₁α and NO2/NO3**

The concentration of 6-keto-PGF₁α in the plasma, a stable metabolite of PGI₂, was measured by radioimmunoassay as described previously, and NO₂(NO₃) were determined in serum by a colorimetric method according to a previous method[8].

**Statistical analysis**

All data were expressed as mean±SD. Statistical analysis was performed using SPSS10.0 package of statistical programs. Comparisons of means in three models and subgroups were performed by one-way ANOVA. Pearson’s correlation analysis was performed for selected variables. P<0.05 was taken as statistically significant.

**RESULTS**

**Effects of L-NNA and INDO on plasma PGI₂ and NO concentrations**

The concentrations of plasma 6-keto-PGF₁α (pg/mL) and NO₂(NO₃) in both IHPH and PHPH rats were significantly higher than those in SO rats (P<0.05). Compared with controlled group, both L-NNA and indomethacin reduced the plasma 6-keto-PGF₁α (pg/mL) and NO₂(NO₃) concentrations (Table 1) (P<0.05).

**Effects of L-NNA and INDO on systemic and splechnic hemodynamics**

In basal state, MAP and TPR were significantly decreased but CI was increased in IHPH and PHPH rats when compared with SO rats (P<0.05). Compared with controlled group, the CI decreased while the MAP and TPR in IHPH and PHPH rats elevated after L-NNA or INDO was given (Table 2) (P<0.05).

---

### Table 1 Effects of L-NNA and INDO on plasma PGI₂ and NO concentrations (mean±SD)

| Group   | Vehicle (n = 23) | L-NNA (n = 21) | Indo (n = 22) | Heparin (n = 24) |
|---------|-----------------|----------------|---------------|-----------------|
| 6-keto-PGF₁α (pg/mL) |                   |                |               |                 |
| SO      | 725.53±105.54   | 748.48±67.68   | 336.91±37.05  | 3 965.96±976.82 |
| IHPH    | 1 123.85±153.64 | 494.74±145.98  | 342.86±104.79 | 2 930.61±400.38 |
| PHPH    | 891.88±83.11    | 386.54±98.44   | 266.94±57.63  | 2 766.47±506.95 |
| NO₂/NO₃ (μmol/L) |                   |                |               |                 |
| SO      | 58.79±8.47      | 21.31±1.76     | 55.72±5.33    | 45.28±4.398     |
| IHPH    | 73.34±4.31      | 40.17±10.32    | 46.42±7.43    | 54.02±11.89     |
| PHPH    | 75.21±6.89      | 30.45±3.28     | 54.74±4.39    | 62.06±3.56      |

*P<0.05 vs SO; *P<0.05 vs control.

### Table 2 Effects of L-NNA and INDO on systemic hemodynamics (mean±SD)

| Group   | Vehicle (n = 23) | L-NNA (n = 21) | Indo (n = 22) | Heparin (n = 24) |
|---------|-----------------|----------------|---------------|-----------------|
| MAP (mmHg) |                   |                |               |                 |
| SO      | 141.86±3.02     | 159.29±1.98    | 145.86±3.72   | 157.33±3.77     |
| IHPH    | 134.00±1.83     | 161.50±6.14    | 161.14±4.45   | 137.88±2.17     |
| PHPH    | 130.29±1.89     | 142.50±4.37    | 147.71±2.75   | 138.29±4.39     |
| CI (mL/min) |                  |                |               |                 |
| SO      | 28.46±0.58      | 25.34±2.22     | 27.20±0.61    | 23.52±0.72     |
| IHPH    | 32.03±1.34      | 25.03±1.76     | 28.66±1.63    | 23.89±2.25     |
| PHPH    | 33.19±0.66      | 28.13±0.51     | 29.62±1.16    | 26.67±0.66     |
| TPR (mmHg/mL·min) |             |                |               |                 |
| SO      | 4.99±0.13       | 6.33±0.60      | 5.36±0.13     | 6.70±0.33      |
| IHPH    | 4.19±0.18       | 6.48±0.53      | 5.64±0.42     | 4.88±0.64      |
| PHPH    | 3.93±0.06       | 5.07±0.19      | 5.00±0.33     | 5.18±0.21      |

*P<0.05 vs SO; *P<0.05 vs control.
INDO had no impacts on the CI and TPR in SO rats. In basal state, FPP and PVI were significantly increased but SVR was lowered in IHPH and PHPH rats when compared with those in SO rats. Both L-NNA and INDO reduced the PVI but enhanced the SVR in IHPH, PHPH and SO rats (Table 3) ($P<0.05$).

**Effects of high-dose heparin on plasma PGI$_2$ and NO concentrations and systemic and splanchnic hemodynamics**

After administration of high-dose heparin, plasma 6-keto-PGF$_{1\alpha}$ concentrations (pg/mL) in IHPH, PHPH and SO rats were significantly higher than those in rats administrated vehicle. On the contrary, serum NO$_2$/NO$_3$ (μmol/L) concentrations in IHPH, PHPH and SO rats were significantly lower than those in rats administrated vehicle (Table 1) ($P<0.05$). Compared with the rats administrated vehicle, the hemodynamic parameters of portal hypertensive rats such as CI and PVI were declined significantly after the administration of high-dose heparin, while TPR and SVR were increased significantly (Tables 2, 3) ($P<0.05$).

**Correlation analysis**

There was a significant positive correlation between plasma 6-keto-PGF$_{1\alpha}$ and NO$_2$/NO$_3$ ($r=0.3939$, $P=0.01$). There were no significant correlations between plasma PGI$_2$ level and hemodynamics parameters such as CI, TPR, PVI and SVR after deduction of the action of NO, but after deduction of the action of PGI$_2$, NO still correlated highly with those hemodynamic parameters (Table 4).

**DISCUSSION**

Whether PGI$_2$ plays a role in formation and development of hyperdynamic circulatory state in portal hypertensive rats has not been specifically verified. Hamilton et al.$^{[8]}$ found that the plasma concentration of 6-keto-PGF$_{1\alpha}$, a stable hydrolytic product of PGI$_2$, was markedly elevated in PHPH rats by partial stenosis of the portal vein and was positively correlated to portal venous pressure (PVP). Sitzmann et al.$^{[10]}$ found that the concentration of PGI$_2$ in systemic artery circulation had a close relationship to the enhanced PVP, the increased mesenteric artery flow (MAF) and the decreased resistance of mesenteric artery in portal hypertensive rats. The hyperdynamic circulatory state in portal hypertensive rats was significantly alleviated after administration of COX inhibitor (indomethacin), which could be reversed after infusion of extrinsic PGI$_2$, thus postulating that PGI$_2$ contributes to the formation of hyperdynamics as a systemic mediator via escaping the hepatic hydrolysis through portal systemic shunt. The following findings that PGI$_2$ is positively related to PVP in portal hypertension and Budd-chiari syndrome patients and COX-1 mRNA transcription is elevated in superior mesenteric artery and thoracic aortic artery in PHPH rats also support the above hypothesis.$^{[11]}$

However, Blanchart et al.$^{[4]}$ did not find the above-mentioned effects of INDO on hyperdynamics of portal hypertensive rats, considering that the formation of collateral circulation in PHPH rats was a result of vascular dilation adapted to the high PVP. Our previous studies have shown that the magnitude of systemic and splanchnic hyperdynamics as well as portal systemic shunt was in the order of PCS>PHPH>IHPH, whereas the concentration of 6-keto-PGF$_{1\alpha}$ was in the order of PHPH>IHPH>PCS. Moreover, the concentration of 6-keto-PGF$_{1\alpha}$ was higher in PCS rats than in SO rats, but was lower than in PHPH and IHPH rats, namely the dynamics in PCS rats increased most but PGI$_2$ elevated least. After administration of NOS inhibitors, hyperdynamic state in PCS, PHPH and IHPH rats was reversed to the basic state of SO rats while PGI$_2$ level was ascended, especially in PCS and SO rats (there was no statistic difference when compared with PHPH and IHPH rats).

### Table 3 Effects of L-NNA and INDO on systemic and splanchnic hemodynamics (mean±SD)

| Group       | Vehicle (n = 23) | L-NNA (n = 21) | Indol (n = 22) | Heparin (n = 24) |
|-------------|-----------------|----------------|---------------|-----------------|
| FPP (mmHg)  |                 |                |               |                 |
| SO          | 6.93±0.35       | 7.29±0.39      | 7.07±0.35     | 7.06±0.39       |
| IHPH        | 10.29±0.39*     | 8.75±0.46*     | 9.28±0.57*    | 9.25±0.65*      |
| PHPH        | 13.71±0.49*     | 10.05±0.45*    | 11.57±0.53*   | 11.07±0.79*     |
| PVI (mL/min)|                 |                |               |                 |
| SO          | 2.35±0.27       | 1.06±0.20      | 1.63±0.17     | 1.57±0.31       |
| IHPH        | 3.83±0.64*      | 2.34±0.50*     | 2.16±0.59*    | 2.05±0.62*      |
| PHPH        | 7.37±1.56*      | 3.71±0.53*     | 3.77±0.81*    | 2.35±0.25*      |
| SVR (mmHg/mL-min) |           |                |               |                 |
| SO          | 58.06±6.95      | 96.18±13.71c   | 85.56±8.13c   | 99.36±20.26c    |
| IHPH        | 32.96±5.09*     | 67.83±14.33ac  | 74.75±19.34c  | 68.51±22.26w    |
| PHPH        | 16.39±3.23*     | 36.21±5.21ac   | 37.52±8.10ac  | 54.85±6.71w     |

*P<0.05 vs SO; †P<0.05 vs control.

### Table 4 Pearson partial correlations between PGI$_2$, NO levels and hemodynamic parameters

| Hemodynamic parameters | PGI$_2$ (deduction of the action of NO) | NO (deduction of the action of PGI$_2$) |
|------------------------|---------------------------------------|---------------------------------------|
| CI                     | $-0.0259$                             | $0.5520$                              |
| MAP                    | $-0.1335$                             | $-0.4572$                             |
| TPR                    | $0.0122$                              | $-0.6053$                             |
| FPP                    | $0.2598$                              | $0.4659$                              |
| PVI                    | $0.1536$                              | $0.3579$                              |
| PVR                    | $-0.0523$                             | $-0.2280$                             |
| SVR                    | $-0.1216$                             | $-0.4325$                             |

However, Blanchart et al.$^{[4]}$ did not find the above-mentioned effects of INDO on hyperdynamics of portal hypertensive rats, considering that the formation of collateral circulation in PHPH rats was a result of vascular dilation adapted to the high PVP. Our previous studies have shown that the magnitude of systemic and splanchnic hyperdynamics as well as portal systemic shunt was in the order of PCS>PHPH>IHPH, whereas the concentration of 6-keto-PGF$_{1\alpha}$ was in the order of PHPH-IHPH-PCS. Moreover, the concentration of 6-keto-PGF$_{1\alpha}$ was higher in PCS rats than in SO rats, but was lower than in PHPH and IHPH rats, namely the dynamics in PCS rats increased most but PGI$_2$ elevated least. After administration of NOS inhibitors, hyperdynamic state in PCS, PHPH and IHPH rats was reversed to the basic state of SO rats while PGI$_2$ level was ascended, especially in PCS and SO rats (there was no statistic difference when compared with PHPH and IHPH rats).
In our previous experiments, we also found that 3 and 7 d after orthotopic liver transplantation in IHPH rats, plasma PG\textsubscript{1}\textalpha concentration was obviously lower, but was still higher than in normal controlled rats 3 d after operation and so did the PVP. Seven days after operation, there was no difference in PG\textsubscript{1}\textalpha concentration between PPHPH and normal controlled rats. Nevertheless, 3 and 7 d after orthotopic liver transplantation in IHPH rats, hyperdynamics still existed, verifying that it is the enhanced PVP that causes the increase of PG\textsubscript{1}\textalpha, and PG\textsubscript{1}\textalpha does not play a role in hyperdynamic circulatory state. Recent data also show that PG\textsubscript{1}\textalpha could not modulate the vascular tension to the normal level in eNOS deleted mice\cite{1}

The findings of this study demonstrate that there are some hyperdynamic characteristics in IHPH and PPHPH rats such as the increase of cardiac output, PVI, and the decrease of SVR and MAP. L-NNA and INDO could lower the CI and PVI, but elevate the MAP and TPR in both IHPH and PPHPH rats, thereby improving the hyperdynamic circulatory state, which seems that both NO and PG\textsubscript{1}\textalpha are mediators in the pathogenesis of hyperdynamics. However, Hardy \textit{et al}\cite{2} found that while INDO inhibited the synthesis of PG\textsubscript{1}\textalpha, it could simultaneously inhibit the release of NO, which is consistent with our results in this study (Table 1). In another word, when INDO reduces the plasma PG\textsubscript{1}\textalpha level in IHPH and PPHPH rats, it decreases the serum NO level as well. Pearson partial correlation analysis between PG\textsubscript{1}\textalpha, NO levels and hemodynamic parameters manifests that after deduction of the action of NO, there is no significant correlation between plasma PG\textsubscript{1}\textalpha level and hemodynamic parameters such as CI, TPR, PVI and SVR. However, after deduction of the action of PG\textsubscript{1}\textalpha, NO still correlates highly with those hemodynamic parameters. Therefore, it is NO rather than PG\textsubscript{1}\textalpha that is a mediator in the formation and development of hyperdynamic circulatory state in chronic portal hypertensive rats.

It was reported that high-dose heparin could make physical changes of endothelial cell membranes to enhance intracellular PLA\textsubscript{2} activity, thus increasing the production of PG\textsubscript{1}\textalpha\cite{3,4}. Meanwhile, high-dose heparin could decrease the production of NO in endothelial cells by decreasing expression of eNOS or concentration between PHPH and normal controlled rats. Nevertheless, 3 and 7 d after orthotopic liver transplantation in IHPH rats, hyperdynamics still existed, verifying that it is the enhanced PVP that causes the increase of PG\textsubscript{1}\textalpha, and PG\textsubscript{1}\textalpha does not play a role in hyperdynamic circulatory state. Recent data also show that PG\textsubscript{1}\textalpha could not modulate the vascular tension to the normal level in eNOS deleted mice\cite{5}.

Increased endothelial nitric oxide synthase activity in the hyperdynamic vessels of portal hypertensive rats. \textit{J Hepatol} 1996; 25: 370-378

1. Casadevall M, Panes J, Pique JM, Marroni N, Bosch J, Whittle BJ. Involvement of nitric oxide and prostaglandins in gastric mucosal hyperemia of portal-hypertensive anesthetized rats. \textit{Hepatology} 1993; 18: 628-634
2. Oberti F, Sogni P, Caillaim S, Moreau R, Pippy B, Lebrec D. Role of prostacyclin in hemodynamic alterations in conscious rats with extrahepatic or intrahepatic portal hypertension. \textit{Hepatology} 1993; 18: 621-627
3. Blanchart A, Hernando N, Fernandez-Munoz D, Hernandez L, Lopez-Novoa JM. Lack of effect of indomethacin on systemic and splanchnic haemodynamics in portal hypertensive rats. \textit{Clin Sci (Lond)} 1985; 66: 605-607
4. Chojkier M, Groszmann RJ. Measurement of portal-systemic shunting in the rat by using gamma-labeled microspheres. \textit{Am J Physiol} 1981; 240: G351-G355
5. Wu Y, Burns RC, Sitzmann JV. Effects of nitric oxide and cyclooxygenase inhibition on splanchnic hemodynamics in portal hypertension. \textit{Hepatology} 1993; 18: 1416-1421
6. Pizcueta MP, Pique JM, Bosch J, Whittle BJ, Moncada S. Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension. \textit{Br J Pharmacol} 1992; 105: 184-190
7. Guerner C, Soriano G, Such J, Teixido M, Ramis I, Bulbena O, Rosello J, Guerner F, Gelpi E, Balanzo J. Systemic prostacyclin in cirrhotic patients. Relationship with portal hypertension and changes after intestinal decontamination. \textit{Gastroenterology} 1992; 102: 303-309
8. Hamilton G, Phing RC, Hutton RA, Dandona P, Hobbs KE. The relationship between prostacyclin activity and pressure in the portal vein. \textit{Hepatology} 1992; 2: 236-242
9. Sitzmann JV, Campbell K, Wu Y, St Clair C. Prostacyclin production in acute, chronic, and long-term experimental portal hypertensin. \textit{Surgery} 1994; 115: 290-294
10. Hou MC, Cahill PA, Zhang S, Wang YN, Hendrickson RJ, Redmond EM, Sitzmann JV. Enhanced cyclooxygenase-1 expression within the superior mesenteric artery of portal hypertensive rats: role in the hyperdynamic circulation. \textit{Hepatology} 1998; 27: 20-27
11. Brandes RP, Schmitz-Winnenthal FH, Feletou M, Godecke A, Huang PL, Vanhouette PM, Fleming I, Busse R. An endothelium-derived hyperpolarizing factor distinct from NO and prostacyclin is a major endothelium-dependent vasodilator in resistance vessels of wild-type and endothelial NO synthase knockout mice. \textit{Proc Natl Acad Sci USA} 2000; 97: 9747-9752
12. Hardy P, Abran D, Hou X, Lahaie I, Peri KG, Asselin P, Varma DR, Chemtob S. A major role for prostacyclin in nitric oxide-induced ocular vasorelaxation in the piglet. \textit{Circ Res} 1998; 83: 721-729
13. Nakamura H, Kim DK, Philbin DM, Peterson MB, Debros F, Koski G, Bonventre JV. Heparin-enhanced plasma phospholipase A2 activity and prostacyclin synthesis in patients undergoing cardiac surgery. \textit{J Clin Invest} 1995; 95: 1062-1070
14. Itoh F, Kaji T, Hayakawa Y, Oguma Y, Sakuragawa N. Heparin enhances thrombin-stimulated prostaglandin I2 production by cultured endothelial cells. \textit{Thromb Res} 1990; 57: 481-488
15. Upchurch GR, Welch GN, Freedman JE, Fabian AJ, Pigazzi A, Scribner AM, Alpert CS, Keaney JF, Loscalzo J. High-dose heparin decreases nitric oxide production by cultured bovine endothelial cells. \textit{Circulation} 1997; 95: 2115-2121
16. Boitano S, Dirksen ER, Sanderson MJ. Intercellular propagation of calcium waves mediated by inositol trisphosphate. \textit{Science} 1992; 258: 292-295

\textbf{REFERENCES}
1. Cahill PA, Redmond EM, Hodges R, Zhang S, Sitzmann JV.