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# Comparative Effects of Nitroglycerin and Nitroprusside on Prostacyclin Generation in Adult Human Vessel Wall

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**The precise mechanism of vasodilatory actions of nitroso-compounds is not clear. It has been suggested that these drugs might modulate release of the vasodilator, prostacyclin, from cultured endothelial cells and bovine arteries or potentiate actions of prostacyclin. This study was designed to examine the effects of nitroglycerin and nitroprusside on prostacyclin release from adult human vasculature. Saphenous vein ring preparations were incubated with nitroglycerin or nitroprusside and arachidonic acid, the substrate for prostacyclin. Vascular rings incubated with nitroglycerin released significantly more prostacylin (measured as 6-keto-prostaglandin $F_{1\alpha}$, a stable hydrolysis product of prostacyclin by radioimmunoassay) compared with the control vascular rings ($p < 0.02$). This increase was observed at the therapeutic concentrations of nitroglycerin (5 to 10 ng/ml). However, incubation of saphenous vein rings with nitroprusside in concentrations as high as 1 $\mu$g/ml was not associated with any increase in prostacyclin release.**

**Prior incubation of vascular rings with the cyclooxygenase blocker, indomethacin, inhibited nitroglycerin-induced prostacyclin release. Incubation of vascular rings with the selective thromboxane $A_2$ blocker, OKY 1581, resulted in additional prostacyclin release with nitroglycerin treatment, presumably by inhibiting vessel wall-generated thromboxane $A_2$. Nitroprusside had no significant effect on prostacyclin release from indomethacin-treated or OKY 1581-treated vascular rings.**

**This study suggests significant stimulatory effects of nitroglycerin, but not of nitroprusside, on prostacyclin release from human saphenous vein. Nitroglycerin-induced prostacyclin release may be an important mechanism of its antiischemic actions in human subjects.**

Nitroglycerin and nitroprusside are potent vasodilators used in a variety of cardiovascular disorders. Nitroglycerin has preferential venodilatory actions, whereas nitroprusside has equipotent venodilatory and arteriodilatory actions. The precise mechanism by which these agents dilate human blood vessels is not known, although modulation of vessel wall cyclic nucleotides has been speculated to be a mechanism of their action (1–3).

During the last several years, an important role of the arachidonic acid metabolites, thromboxane $A_2$ and prostacyclin, in the pathogenesis of increased vascular resistance has been implicated (4–7). Thromboxane $A_2$, generated primarily by the platelets, is a potent vasoconstrictor and platelet proaggregant, whereas prostacyclin produced by the blood vessels is a potent vasodilator and platelet antiaggregant. An imbalance between these two major prostaglandins toward thromboxane $A_2$ or away from prostacyclin may be a cause of increased vascular resistance. Indeed, increased thromboxane $A_2$ synthesis and decreased prostacyclin generation have been identified in ischemic heart disease (6,8,9). Enhanced in vivo platelet activity has been observed in congestive heart failure (10) and hypertension (11,12). Nitroglycerin (13), but not nitroprusside (14), has been shown to stimulate prostacyclin synthesis by cultured umbilical vein endothelial cells.

Because vascular prostacyclin generation decreases with age (15,16), it is important to examine the effect of these agents on blood vessels obtained from adult patients who may receive these drugs. In previous studies, cultured cells after several passages were used, which may or may not indicate prostacyclin production by adult human vasculature in response to these agents. The present studies were performed to examine the production of prostacyclin by adult human vascular tissues in response to nitroglycerin and nitroprusside.

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## Methods

**Preparation of vascular tissues for prostacyclin generation.** The blood vessels examined were human saphenous veins that were obtained from six patients (aged 55 to 62 years) undergoing coronary bypass surgery immediately after anesthesia and leg dissection. Informed consent was obtained from all patients, none of whom had taken prostaglandin-active drugs during the previous 10 days. The tissues were collected in calcium- and magnesium-free physiologic buffer (described under reagents) at 4°C. All experiments were conducted within 3 hours of tissue collection.

*The technique for preparation of vascular tissue has been previously described (17,18).* In brief, the saphenous veins were gently dissected free from the surrounding tissue and cut into fine rings (0.5 to 1 mm thickness). Aliquots of vascular rings (wet weight 30 to 35 mg) were placed into small polypropylene vials containing 1 ml of calcium- and magnesium-free buffer. The rings were gently washed several times with buffer, and placed in 1 ml of Hanks buffered salt solution (HBSS, referred to as "buffer") containing calcium and magnesium for 15 minutes. The aliquots were then incubated with nitroglycerin or nitroprusside (5, 10, 100 and 1,000 ng/ml, final concentration) in buffer at 37°C for 15 minutes, followed by treatment with arachidonic acid (0.1 m*M*) for an additional 15 minutes.

*In each experiment, the following control incubations of vascular rings were performed:* buffer alone without nitroglycerin or nitroprusside, and buffer plus lactose or saline solution (vehicles for nitroglycerin or nitroprusside, respectively). In other experiments, vascular rings were first treated with the cyclo-oxygenase inhibitor, indomethacin, 0.1 m*M*, for 15 minutes before incubation with nitroglycerin or nitroprusside and then with arachidonic acid. Previous studies have indicated generation of thromboxane $A_2$ in addition to prostacyclin by human saphenous veins (17), and have shown that selective thromboxane $A_2$ inhibition may divert arachidonic acid metabolism to prostacyclin (19). Therefore, in some experiments, vascular rings were treated with a selective thromboxane $A_2$ inhibitor, OKY 1581 (20), 0.1 m*M*, for 15 minutes before incubation with nitroglycerin or nitroprusside plus arachidonic acid.

**6-keto-PGF$_{1\alpha}$ determination.** 6-keto-prostaglandin $F_{1\alpha}$, a stable hydrolysis product of prostacyclin, was measured by radioimmunoassay (17). Lyophilized standards, antibodies and $^3$H 6-keto-PGF$_{1\alpha}$ were obtained from New England Nuclear Corporation. The crossreactivity of 6-keto-PGF$_{1\alpha}$ antibody was 100% for 6-keto-PGF$_{1\alpha}$, 2.7% for prostaglandin $F_{2\alpha}$, less than 2% for prostaglandin $E_2$, and less than 0.1% for prostaglandin $A_2$ and thromboxane $B_2$ (21). All measurements in the supernates were made in duplicate and results expressed as pg/mg tissue wet weight. The duplicate 6-keto-PGF$_{1\alpha}$ values varied by 10%.

**Reagents.** Calcium- and magnesium-free buffer was prepared according to Jaffe et al. (22). Buffer consisted of 138 m*M* sodium chloride (NaCl), 4 m*M* potassium chloride (KCl), 0.5 m*M* sodium phosphate dibasic ($Na_2HPO_4$), 0.15 m*M* potassium phosphate monobasic ($KH_2PO_4$), 11.7 m*M* chloride dextrose and final pH 7.4. Calcium- and magnesium-containing buffer contained 1.3 m*M* calcium chloride dehydrase, ($CaCL_2 \cdot 2H_2O$), 5.4 m*M* KCl, 0.4 m*M* $KH_2PO_4$, 0.8 m*M* magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$), 136.9 m*M* NaCl, 4.2 m*M* sodium bicarbonate ($NaHCO_3$), 0.3 m*M* $Na_2HPO_4$, 5.6 m*M* dextrose adjusted to pH 7.4 with 1 N sodium hydroxide (NaOH). Indomethacin (Merck, Sharp, and Dohme) was dissolved in Tyrode solution (final pH 7.4) with gentle boiling. The selective thromboxane $A_2$ synthetase inhibitor, OKY 1581 (Ono Pharmaceutical Co., Ltd.) (20) was kept at −70°C until use and was dissolved in a normal saline solution just before use. Arachidonic acid (Sigma Chemical Co.) was kept at −70°C until use, dissolved in Tris buffer and kept under nitrogen before use (final pH 7.4). Nitroglycerin in lactose (Eli Lilly and Co.) was diluted with normal saline solution. Crystalline nitroprusside (Hoffmann La Roche) was dissolved in normal saline solution and was protected from light by covering the solution with aluminum foil.

**Statistical calculations.** All data were obtained from at least five experiments. All measurements were made in duplicate and averaged. The average values were used to calculate the mean ± standard error. The Student's *t* test (paired data) and multiple analysis of variance were used for statistical calculations. A probability (p) value less than 0.05 was considered significant.

## Results

**Effect of nitroglycerin on prostacyclin production.** After multiple washings and incubation of saphenous vein rings with buffer alone, the measurement of 6-keto-PGF$_{1\alpha}$ revealed concentrations in the supernates varying from 48 to 116 pg/mg (mean 72 ± 10; n = 6). Concentrations of 6-keto-PGF$_{1\alpha}$ in the supernates of different aliquots of vascular rings from the same saphenous vein varied by 9% (range 6 to 12).

Concentrations of 6-keto-PGF$_{1\alpha}$ in the supernates after incubation with buffer plus arachidonic acid varied from 108 to 612 pg/mg (mean 260 ± 45; $p < 0.01$ compared with buffer alone; n = 6). Treatment of vascular rings with lactose (vehicle for nitroglycerin) and then with arachidonic acid for 15 minutes did not significantly affect 6-keto-PGF$_{1\alpha}$ levels (mean 275 ± 51 pg/mg; n = 6). Vascular rings treated with nitroglycerin released significantly more ($p < 0.01$) 6-keto-PGF$_{1\alpha}$ on treatment with arachidonic acid than did those not treated with nitroglycerin (Fig. 1). An increase in 6-keto-PGF$_{1\alpha}$ was present in all supernates of vascular rings treated with nitroglycerin. Maximal increase was observed at 5 ng/ml in one experiment (mean increase 17%; n = 5), at 10 ng/ml in two experiments (mean increase 28%; n = 5), at 100 ng/ml in two experiments (mean increase 40%; n = 6) and at 1,000 ng/ml in one experiment

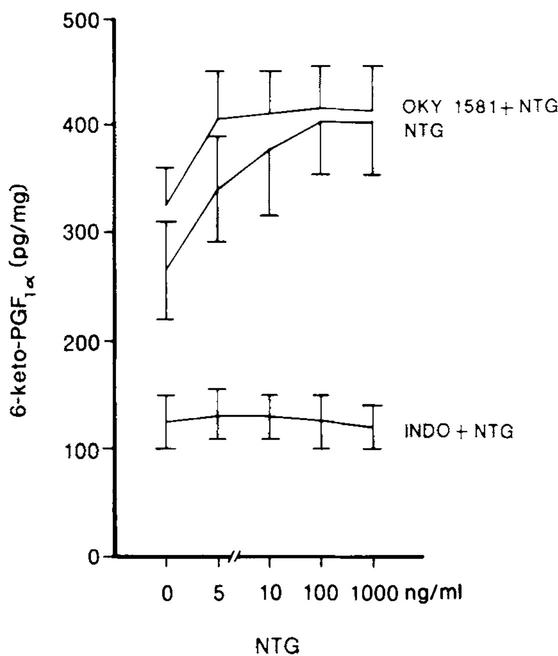
(mean increase 32%; n = 5). At 5 to 10 ng/ml concentrations of nitroglycerin, 6-keto-$PGF_{1\alpha}$ levels increased significantly ($p < 0.02$) compared with the control (buffer alone or buffer plus lactose). There were small additional increases when higher concentrations of nitroglycerin were used.

*In experiments in which vascular rings were first treated with indomethacin,* arachidonic acid-stimulated 6-keto-$PGF_{1\alpha}$ levels were significantly lower ($-54 \pm 7\%$; $p < 0.01$) than those from the vascular rings not treated with indomethacin (Table 1). Incubation with nitroglycerin and arachidonic acid of indomethacin-treated vascular rings failed to stimulate prostacyclin release. Incubation of OKY 1581-treated vascular rings with arachidonic acid resulted in 6-keto-$PGF_{1\alpha}$ concentrations in supernates significantly higher ($+26 \pm 5\%$; $p < 0.02$) than those from the rings not treated with OKY 1581. Nitroglycerin treatment resulted in significantly higher concentrations ($p < 0.05$) of 6-keto-$PGF_{1\alpha}$ in the supernates of vascular rings treated with OKY 1581 (Fig. 1). This was observed at 5 and 10 ng/ml concentrations of nitroglycerin (Table 1).

**Effect of nitroprusside on prostacyclin production.** Before incubation with arachidonic acid, supernates of vascular rings (not treated with nitroprusside) contained 48 to 194 pg/mg (mean 90 ± 16) of 6-keto-$PGF_{1\alpha}$ (n = 5). On incubation of vascular rings with arachidonic acid, the concentration of 6-keto-$PGF_{1\alpha}$ increased in each supernate compared with those incubated in buffer alone (range 150 to 366; mean 241 ± 36 pg/mg; $p < 0.01$). However, 6-keto-$PGF_{1\alpha}$ concentrations in the supernates of all vascular rings were similar, whether or not they had been treated with nitroprusside (Fig. 2).

*In experiments in which vascular rings were first treated with indomethacin,* arachidonic acid-stimulated 6-keto-$PGF_{1\alpha}$ concentrations were significantly lower ($-46 \pm 12\%$; $p < 0.02$) than those from the vascular rings not treated with indomethacin. Incubation with nitroprusside and arachidonic acid of indomethacin-treated vascular rings failed to stimulate prostacyclin release (Table 1). In contrast, vascular rings treated with OKY 1581 produced more prostacyclin ($+24 \pm 9\%$; $p < 0.05$) after treatment with arachidonic acid than did those not treated with OKY 1581. Nonetheless, nitroprusside treatment in any concentration failed to significantly affect 6-keto-$PGF_{1\alpha}$ concentrations.

**Figure 1.** Effect of nitroglycerin (NTG) on prostacyclin ($PGI_2$) release from the adult human saphenous vein rings. Nitroglycerin significantly increased ($p < 0.02$) 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) concentrations in the supernates at 5 to 10 ng/ml concentrations. Indomethacin (INDO) reduced, whereas OKY 1581 enhanced 6-keto-$PGF_{1\alpha}$ concentrations. Nitroglycerin had no effect on $PGI_2$ release from indomethacin-treated vascular rings, but enhanced $PGI_2$ release from OKY 1581-treated vascular rings. All aliquots of vascular rings were treated with arachidonic acid. Control values for nitroglycerin (0 ng/ml) refer to arachidonic acid in buffer plus lactose. The data are a summary of six separate experiments; values are expressed as mean ± standard error of the mean.




## Discussion

**Effect of nitroglycerin on prostacyclin release.** This study demonstrates that nitroglycerin stimulates prostacyclin release from human saphenous vein rings. This increase occurs in clinically achieved concentrations of 5 to 10 ng/ml (23). Stimulation of vascular prostacyclin release in response to nitroglycerin treatment is supported by the measurements of 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) in the vascular ring supernates. Pretreatment of the vascular rings with indomethacin inhibited prostacyclin release, whereas pretreatment with OKY 1581 stimulated prostacyclin in response to nitroglycerin. In contrast, nitroprusside had no significant effect on prostacyclin release from the saphenous vein rings. In addition, nitroprusside had no stimulatory effect on prostacyclin release even when vascular rings had been pretreated with OKY 1581.

It is evident that prostacyclin measured as 6-keto-$PGF_{1\alpha}$ was indeed released from the vascular rings. Indomethacin, by blocking cyclo-oxygenase enzyme, prevented the synthesis of prostacyclin. Nitroglycerin or nitroprusside treatment followed by incubation with arachidonic acid failed to stimulate prostacyclin (Table 1). Treatment with OKY 1581, a selective thromboxane $A_2$ synthetase inhibitor (20), resulted in an increase in prostacyclin release. It is known that human saphenous veins also synthesize thromboxane $A_2$ in addition to prostacyclin (17) and that selective thromboxane $A_2$ inhibition may divert cyclic endoperoxides toward prostacyclin (7,19). In this study, we did not measure thromboxane $B_2$ levels in the supernates, but the use of

**Table 1.** Effect of Prostaglandin Inhibitors On Prostacyclin ($PGI_2$) Release

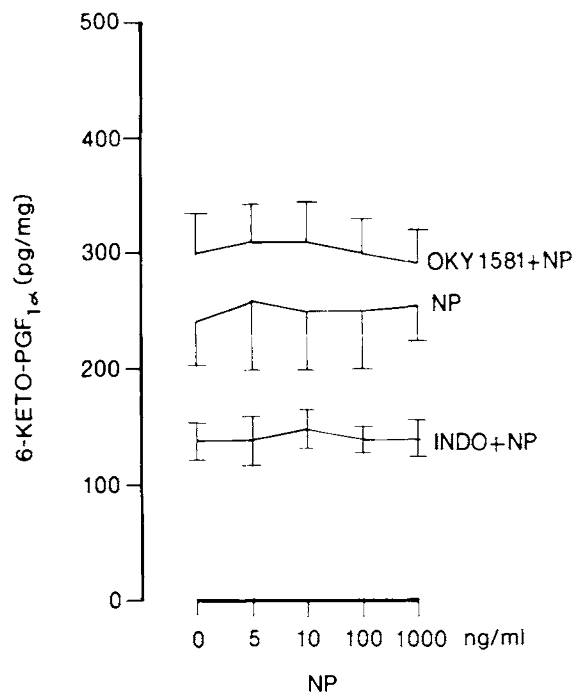
| | 6-keto-$PGF_{1\alpha}$(pg/mg) | |
|---|---|---|
| | NTG Experiments (n = 6) | NP Experiments (n = 5) |
| Vascular rings + buffer + AA | 260 ± 45 | 241 ± 36 |
| Vascular rings + NTG or NP + AA | 333 ± 50§ | 245 ± 45 |
| Vascular rings + indomethacin + AA | 120 ± 25‡ | 130 ± 29‡ |
| Vascular rings + indomethacin + NTG or NP + AA | 125 ± 30 | 139 ± 25 |
| Vascular rings + OKY 1581 + AA | 328 ± 49† | 300 ± 38† |
| Vascular rings + OKY 1581 + NTG or NP + AA | 410 ± 45* | 310 ± 40 |

Data refer to experiments with 10 ng/ml of nitroglycerin or nitroprusside.
* $p < 0.02$ compared with OKY 1581 + AA; † $p < 0.02$ compared with buffer + AA; ‡ $p < 0.01$ compared with buffer + AA; § $p < 0.01$ compared with buffer + AA.
AA = arachidonic acid; buffer = Hanks buffered salt solution; NP = nitroprusside, 10 ng/ml, NTG = nitroglycerin, 10 ng/ml.

OKY 1581 increased 6-keto-$PGF_{1\alpha}$ levels, probably by inhibiting thromboxane $A_2$ synthesis.

**Methodologic considerations.** As in several previous studies (17,18,24–26), we employed vascular rings that included all layers of the vessel wall, in contrast to cultured endothelial cells used by others (13,14). Some major methodologic differences of our experiments compared with those of others are noteworthy. First, prostacyclin measured in the vascular ring supernate by our method includes release from all layers of the vessel wall. Although the precise contribution of smooth muscle layers in the release of prostacyclin (27) cannot be ascertained, it is known that the endothelial cells generate much more prostacyclin than do the smooth muscle cells. Therefore, it can be assumed that the recovered prostacyclin was derived mostly from the endothelial cells. Second, the saphenous veins were obtained after anesthesia, which may influence the release of prostaglandins. Third, trauma to the blood vessels during preparation of vascular rings may also cause prostacyclin release (28). However, similarly prepared rings were used for studying the effects of nitroglycerin, nitroprusside and multiple control agents. Because prostacyclin release was observed only in nitroglycerin-treated rings, it is likely that nitroglycerin alone stimulated prostacyclin release.

**Figure 2.** Effect of nitroprusside (NP) on prostacyclin ($PGI_2$) release from the saphenous vein rings. Nitroprusside had no significant effect on 6-keto-$PGF_{1\alpha}$ concentrations in the supernates in concentrations up to 1,000 ng/ml. Indomethacin (INDO) reduced, whereas OKY 1581 enhanced prostacyclin release. Nitroprusside had no effect on indomethacin-treated or OKY 1581-treated vascular rings. All aliquots of vascular rings were treated with arachidonic acid. Control data for nitroprusside (0 ng/ml) refer to arachidonic acid in buffer plus normal saline solution. The data are a summary of five separate experiments; values are expressed as mean ± standard error of the mean.




Previous studies employing cultured umbilical vein cells (13) and bovine coronary arteries (29) have shown nitroglycerin-induced potentiation of prostacyclin release. We employed fresh human saphenous vein rings and studied them within 3 hours of collection to determine whether rings from the entire vessel would behave differently from isolated cultured endothelial cells after two or three serial passages. Comparison of our data with those of Levin et al. (13) shows that the magnitude of prostacyclin stimulation by human saphenous vein rings is the same as that by cultured endothelial cells at the same concentration of nitroglycerin. In studies by Schror et al. (29), maximal prostacyclin release from bovine coronary artery strips occurred at 0.3 to 3 ng/ml concentrations of nitroglycerin. Concentrations of 1 μg/ml or greater resulted in a decrease in prostacyclin release. Our data do not suggest any decrease in prostacyclin release with nitroglycerin up to 1 μg/ml, although maximal stimulation is observed at lower concentrations. These concentration-related differences in nitroglycerin-stimulated pros-

tacyclin release may relate either to differences in canine and human vessels or to the types of vessels used (that is, coronary artery versus saphenous vein). Nevertheless, data from all these studies indicate stimulation of prostacyclin release by nitroglycerin in therapeutic concentrations. In contrast, nitroprusside failed to stimulate prostacyclin release from human saphenous vein rings in any concentration. These data are in accord with a recent study (14) that failed to show significant effects of nitroprusside on prostacyclin release from cultured endothelial cells.

**Nitroglycerin versus nitroprusside.** In a previous study (30), nitroglycerin was shown to decrease coronary vascular resistance accompanied by release of prostaglandins of the E series in dogs. Both of these effects of nitroglycerin were blocked by indomethacin. Other investigators were not able to show any significant effects of nitroglycerin on prostacyclin release using pig aortic microsomes (31), or rat or guinea pig heart after high concentrations of nitroglycerin (32). A recent report (33), however, shows an increase in prostacyclin release in human coronary venous blood after the administration of sublingual nitroglycerin. The differences in these observations may relate to several factors: 1) very high concentrations of nitroglycerin may reduce prostacyclin release (29), 2) there may be specific species differences, and 3) endothelial cells are necessary for effects of nitroglycerin on prostacyclin release (13).

*Nitroprusside has been shown to inhibit platelet adhesion (34), platelet aggregation and thromboxane $A_2$ synthesis (10,14).* It has been suggested that nitroprusside may act by inhibiting platelet function. In studies in patients with congestive heart failure, nitroprusside reduced both ex vivo and in vivo platelet aggregation (10,35), but nitroglycerin had no significant effect on platelet aggregation in therapeutic concentrations (35). Although nitroglycerin inhibits platelet aggregation in supratherapeutic concentrations ($>$ 10 ng/ml) (36), human platelet thromboxane $A_2$ generation may be decreased by small concentrations of nitroglycerin (37). Levin et al. showed some potentiation of prostacyclin-induced platelet aggregation inhibition by nitroglycerin (13) and nitroprusside (14). Thus, it appears that nitroglycerin has important prostacyclin-stimulating effects but no significant effects on thromboxane $A_2$ in therapeutic concentrations, whereas nitroprusside lacks significant effects on prostacyclin release but has important effects on thromboxane $A_2$ generation.

**Clinical implications.** Nitroglycerin relieves myocardial ischemia by systemic venodilation (38), direct local coronary vasodilation (39,40) and redistribution of blood flow from nonischemic to ischemic areas (41,42). An increase in prostacyclin release by nitroglycerin in therapeutic concentration may be an important mechanism of its action in human beings. Although a potent vasodilator, nitroprusside does not appear to have significant effects on prostacyclin release from adult human saphenous vein rings. Prostacyclin has myocardial protective effects (43), probably related to its ability to alter cyclic nucleotide levels and calcium flux (44). The absence of significant beneficial effects of nitroprusside in myocardial ischemia or even a possible deterioration (45–47) may relate to its inability to potentiate prostacyclin release. However, reduction in platelet aggregation and thromboxane $A_2$ generation may be important mechanisms of action of nitroprusside in conditions of increased vascular resistance (10,35).

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