Late phase of nitroglycerin-induced coronary vasodilatation blunted by inhibition of prostaglandin synthesis

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ABSTRACT In chloralose-anesthetized dogs with the left circumflex coronary artery perfused at constant flow, the effects of increasing doses of indomethacin or naproxen on the coronary and systemic hemodynamic responses to a 5 μg intracoronary injection of nitroglycerin (NTG) were evaluated. The integrated areas of NTG-induced coronary vasodilatation were reduced after administration of indomethacin or naproxen. The extent of this reduction was increased progressively by augmenting the dose of indomethacin and naproxen up to 1.5 and 7 mg/kg, respectively. We also assessed the extent of cyclooxygenase inhibition induced by indomethacin or naproxen through the radioimmunoassay of thromboxane B2, which reflects thrombin-induced activation of platelet thromboxane A2 production during whole blood clotting. The level of inhibition progressively increased and complete inhibition was attained with 1.5 mg/kg indomethacin and 7 mg/kg naproxen. Further increase in dosage failed to induce further reduction of integrated areas of coronary vasodilatation, and a correlation was found between the extent of the reduction of the integrated areas of coronary vasodilatation and the dose of indomethacin (r = .828, n = 35, p < .001) or naproxen (r = .729, n = 35, p < .001). Finally, the NTG-induced maximum fall in coronary perfusion pressure remained unmodified after inhibition of prostaglandin synthesis, but there was a faster return of the perfusion pressure to the basal value. Furthermore, additional experiments performed with constant intracoronary infusion of NTG (60 μg/min for 4 min) showed that when a steady-state condition was reached, the pretreatment with indomethacin or naproxen also reduced the first part of the integrated areas of coronary vasodilatation (from the beginning to the end of the NTG infusion) (indomethacin, 1868 ± 163 vs 2940 ± 251 mm Hg/sec, p < .001; naproxen, 1606 ± 132 vs 2787 ± 148 mm Hg/sec, p < .001) probably because of a slower decrease of the perfusion pressure (the slope of the regression line obtained plotting perfusion pressure vs time was 0.067 ± 0.005 before and 0.054 ± 0.006 after indomethacin, p < .05; 0.069 ± 0.002 before and 0.06 ± 0.003 after naproxen, p < .05). These data indicate a possible role of prostaglandins in NTG-induced coronary vasodilatation.


ALTHOUGH nitroglycerin (NTG) has been used clinically in the management of angina pectoris since 1855,1 its mechanisms of action remain incompletely understood. Morcillio et al.2 raised the hypothesis that myocardial E prostaglandins may participate in the mediation of the NTG-induced coronary vasodilatation. They demonstrated in dogs that intravenous infusion of NTG causes a decrease in coronary arterial resistances that is accompanied by an increase in concentrations of E series prostaglandins in the coronary sinus blood. Since indomethacin diminishes both these effects, these authors suggested that NTG acts in part through the prostaglandin system. In contrast, other authors3-5 have failed to demonstrate any relationship between NTG and the prostaglandin system. In particular, Forster,4,6 while able to show that NTG elicits an increase in coronary flow in isolated, perfused rat or guinea pig hearts, was unable to demonstrate an increase in PG12 in the coronary effluent. More recently, Levin et al.7 incubated cultured human endothelial cells with NTG at various concentrations and observed that NTG elicits dose-dependent increments in the synthesis of PG12 by the endothelial cells. Therefore they
suggested that NTG-induced vasodilatation might be, at least partially, caused by an altered PGI₂/thromboxane A₂ (TXA₂) ratio in favor of PGI₂ excess. Finally, Panzenbeck et al.⁸ have recently reported that pretreatment with indomethacin does not significantly modify the maximal increase in coronary arterial blood flow induced by intracoronary infusion of NTG.

This study was undertaken to further investigate the possibility that prostaglandins participate in the coronary hemodynamic response to NTG. We assessed in the dog the effects of different degrees of inhibition of prostaglandin synthesis on the response to intracoronary NTG administration to test the hypothesis that the prostaglandin system participates in the mediation of the vasodilatation induced by intracoronary infusion of NTG.

**Methods**

Mongrel dogs of both sexes weighing 10 to 20 kg were anesthetized with sodium thiopental (30 mg/kg iv) and α-chloralose (80 mg/kg iv). Throughout the experiment a stable level of anesthesia was maintained with supplemental doses of chloralose (10 mg/kg iv hourly). A cuffed endotracheal tube was inserted and the dogs were ventilated artificially with air supplemented with oxygen (2 to 3 liters/min) at a frequency of 13 cycles/min and a tidal volume determined by a nomogram based on body weight. Arterial PO₂, PCO₂, and pH were measured to maintain PO₂ at 30 to 40 mm Hg and arterial PCO₂ above 90 mm Hg in all experiments. When necessary, sodium bicarbonate (90 mM in 0.9% saline) was given intravenously in appropriate doses to maintain pH between 7.3 and 7.4. Muscle relaxation was induced by intravenous administration of succinylcholine (0.2 mg/kg) and was maintained by supplemental injection of 0.05 mg. Body temperature was maintained between 37° and 38° C by using a heated operating table.

**Preparation of the animal.** The chest was opened via a left thoracotomy. The pericardium was opened and its cut edges were sutured to the thoracotomy margin to create a cradle for the heart. The left atrial appendage was retracted and the circumflex coronary artery was exposed near its origin by blunt dissection. Care was taken to avoid damage to the neural supply to the vessel. After the administration of heparin (Liquemin, Roche) (500 U/kg iv), the left circumflex coronary artery was cannulated with a polyethylene catheter introduced through the right carotid artery and positioned under fluoroscopic guidance. The wall of the left circumflex coronary artery was ligated around the catheter positioned in the lumen of the vessel. The coronary artery was then perfused at constant flow with a Sigmaemotor T85 peristaltic pump with blood of the same animal obtained through a polyethylene catheter introduced through the external iliac artery in the abdominal aorta. Under such conditions, changes in vascular tone of the perfused district were reflected by proportional changes in perfusion pressure. Perfusion pressure was measured from a T connection on the outflow side of the pump. Blood flow rate was adjusted to give perfusion pressure close to systemic blood pressure. This perfusion pressure was maintained slightly higher than systemic blood pressure to compensate for the resistances of the tubing system, and flow rate was left unchanged throughout the experiment. In this way, the values of blood flow in the perfused district were comparable to those measured by means of an electromagnetic flowmeter (Micron Instruments) before implanting the perfusion system. After the administration of indomethacin or naproxen, coronary blood flow was not modified to counterbalance changes in baseline coronary resistance induced by the drugs. Arterial blood pressure was continuously monitored through a catheter placed in the thoracic aorta via the femoral artery. A Battaglia-Rongoni multichannel polygraph and Statham P23dB pressure transducers were used. The intra-arterial administration of drugs was made in the perfusion circuit beyond the pump and immediately before the perfused district. All drugs were injected into the coronary circulation in a volume of saline not exceeding 0.1 ml. A polyethylene catheter introduced through a femoral vein was positioned under fluoroscopic guidance in the right atrium for blood sampling.

At the end of the surgical procedure and before the beginning of the experiment, 20 min were allowed to elapse to reach a complete equilibration of the system.

**Analyses.** The extent of inhibition of prostaglandin synthesis induced by the different pharmacologic treatments was assessed through the radioimmunoassay of serum thromboxane B₂ (TXB₂), which reflects thrombin-induced activation of platelet TXA₂ production during whole blood clotting.⁹

Statistical analysis was performed by comparing the values of systemic and perfusion pressure recorded after drug injection with the basal value by analysis of variance and Duncan's test. Furthermore, to assess the effects of different pharmacologic treatments on the coronary response to intracoronary infusion of NTG, the integrated areas of coronary vasodilatation were measured by planimetry and the values before and after each pharmacologic treatment were compared. In the measurement of these areas, the end of the phenomenon was assumed to be the point at which the perfusion pressure returned within 10% of the basal value. The maximum fall in coronary perfusion pressure induced by infusion of NTG before and after each pharmacologic treatment was calculated and compared. Finally, we calculated the slope of the regression line obtained in each experiment by plotting the value of the perfusion pressure, recorded from the beginning to the end of the infusion of NTG, against time, and we used this parameter as an index of the rate of decrease of the perfusion pressure during NTG-induced coronary vasodilatation.

**Experimental protocol.** Preliminary experiments were performed by injecting different doses of NTG in the perfused coronary vessel to identify the dose able to induce a significant reduction in coronary perfusion pressure and small changes in systemic arterial pressure and heart rate. According to the results of these studies, we chose the dose of 5 μg for the subsequent experiments. Furthermore, since it has been shown¹⁰ ¹¹ that the coronary vasodilatation elicited by some naturally occurring substances such as norepinephrine, adenosine triphosphate, adenosine diphosphate, bradykinin, and angiotensin II is at least partially mediated through the biosynthesis and the release of prostaglandin-like substances, we chose to use the intracoronary injection of histamine (5 μg) to demonstrate that the vascular responsiveness to vasoactive substances was not nonspecifically reduced throughout the experiment by the pharmacologic treatment performed. However, since the involvement of the prostaglandin system in the mediation of the vascular effects of histamine cannot be ruled out a priori, a series of experiments was performed in which the hemodynamic response to intracoronary injection of histamine was assessed before and after the intravenous administration of different doses of indomethacin or naproxen. The experimental protocol and the doses of the two drugs tested in these experiments were the same as those used in the studies with NTG, which are specified below:

To investigate the relationship between NTG-induced coronary vasodilatation and the prostaglandin system, two series of
experiments were performed. In the first group (18 dogs) the effects on the hemodynamic responses elicited by the intracorony injection of NTG of seven different doses of indomethacin (0.1, 0.3, 0.5, 1.5, 3, 4, and 5 mg/kg body weight) administered intravenously were evaluated. In the second group of studies (18 dogs), naproxen was substituted for indomethacin as the agent inhibiting prostaglandin synthesis. With this drug seven doses were given intravenously (1, 3, 5, 7, 10, 12, and 15 mg/kg body weight). In both groups no more than two individual doses were given per dog. The dosage schedule for each dog was determined according to a computer-based randomization scheme, and they were not necessarily in a step-progressive fashion. The doses were cumulative, with the smaller dose administered before and the second dose obtained by adding the appropriate amount of drug to the first dose. Initially, in each dog a venous blood sample was withdrawn from the right atrium to assess the basal cyclooxygenase activity. Then, after a hemodynamic response to an intracoronary injection of histamine (5 \(\mu\)g) was recorded, the effects of intracoronary NTG (5 \(\mu\)g) were evaluated. Subsequently, indomethacin or naproxen was administered intravenously. Thirty minutes after the administration of the specific prostaglandin synthesis-inhibiting agent, a second blood sample was withdrawn for the assessment of the cyclooxygenase activity to calculate the percent inhibition of the basal cyclooxygenase activity induced. Then, after the vascular responsiveness had again been evaluated by histamine challenge, a second response to intracoronary NTG was elicited. The higher dose of indomethacin or naproxen was then administered and its effects on cyclooxygenase activity, vascular responsiveness, and NTG-induced hemodynamic response were assessed as indicated above.

Furthermore, two additional groups of experiments were performed in which a constant flow of NTG was infused in the coronary circulation. NTG was administered by a Harvard infusion pump at a rate of 60 \(\mu\)g/min (1 ml/min) over 4 min. This infusion period was chosen according to the results of preliminary experiments suggesting that this is the time required to reach a steady-state response to NTG infusion in our preparation. Furthermore, since this amount of NTG has been reported to induce relevant change in heart rate, which may influence the coronary vascular response, heart rate and the interval between atrial and ventricular pacing were maintained at constant. Atrioventricular pacing was performed as previously described.\(^1\)\(^2\) In the first group of experiments (n = 5), the infusion of NTG was performed under control conditions; then, after a hemodynamic response to histamine (5 \(\mu\)g ic) was recorded, indomethacin was administered at the dose of 1.5 mg/kg iv. This dose was found to induce a complete inhibition of cyclooxygenase activity (see Results). Thirty minutes after the administration of indomethacin, the vascular responsiveness was again evaluated by histamine challenge and a second response to NTG infusion was elicited. In the second group of experiments (n = 5), the same protocol was followed but indomethacin was replaced by naproxen as the agent inhibiting prostaglandin synthesis.

Finally, to exclude the possibility that time-dependent modification in the hemodynamic response to NTG might occur, five experiments were performed in which four responses were evoked at 30 min intervals without any pharmacologic treatment.

**NTG preparation.** A solution containing 5 mg of NTG and 45 mg of propylene glycol dissolved in 70% ethanol (volume 1 ml) was mixed in a glass vial with 9 ml of normal saline. The NTG concentration of this solution was 500 \(\mu\)g/ml.

**Results**

The intracoronary injection of NTG elicited consistent vasodilatation of the coronary bed (figure 1).

In the pilot experiments involving increasing doses of NTG, a significant correlation between the integrated areas of coronary vasodilatation and the doses of NTG was found (r = .802, n = 30, p < .001). In addition, it was found that 7 and 10 \(\mu\)g doses did not induce any further increment of the coronary response as compared with the 5 \(\mu\)g dose (table 1). Furthermore, the 5 \(\mu\)g dose did not elicit any significant change in systemic blood pressure and heart rate, whereas the doses of 7 and 10 \(\mu\)g induced a significant fall in systemic blood pressure (table 1). Therefore, the 5 \(\mu\)g dose was used in further experiments to minimize the consequences of changes in systemic hemodynamics on the coronary circulation.

Under control conditions the 5 \(\mu\)g dose of NTG, but not the same volume of saline or of the vehicle, induced a fall in coronary perfusion pressure of about 25% of the basal value. This fall was reached at about 10 sec after the intracoronary injection of the drug. Within 1 min the coronary perfusion pressure returned to values that were not statistically different from the baseline. Therefore we divided the pressure response curve to NTG into two phases: early phase (from the beginning until the nadir of the coronary vasodilatation) and the late phase (from the nadir of the fall in coronary perfusion pressure to the return to the base-
activity by 90%, and complete inhibition was reached with 1.5 mg/kg. Simultaneously, systemic and coronary perfusion pressure showed a progressive increase as compared with the value recorded in control conditions. In both cases, the increase was correlated with the percent of cyclooxygenase activity inhibition induced by the increasing doses of indomethacin administered (systemic blood pressure, r = .618, n = 35, p < .001; coronary perfusion pressure, r = .691, n = 35, p < .001). The increase in systemic blood pressure was accompanied by a presumed reflex reduction in control heart rate (data not shown). Furthermore, when the integrated areas of coronary vasodilatation were compared, a progressive decrease was detectable, which reached a significant level after the 1.5 mg/kg dose of indomethacin (figure 2). No further reduction in the integrated areas of coronary vasodilatation was detectable with the higher doses of indomethacin. When the reductions in the integrated areas of coronary vasodilatation induced by the different doses of indomethacin were plotted against the dose of drug administered, a significant correlation was detected (r = .828, n = 35, p < .001) (figure 3). The reduction in the integrated areas of NTG-induced coronary vasodilatation was not accompanied by a reduction in the maximum fall in perfusion pressure; for example, it was 33 ± 8 mm Hg before and 31 ± 8 mm Hg after the intravenous administration of 3 mg/kg indomethacin. On the other hand, after the complete inhibition of prostaglandin synthesis, the NTG-induced coronary vasodilatation was characterized by a faster return of the perfusion pressure to the basal value (figure 4). Furthermore, no

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Hemodynamic effects of increasing doses of NTG injected into the left circumflex coronary artery</strong></td>
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<tr>
<td>NTG (µg)</td>
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<td>2</td>
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<td>5</td>
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<td>7</td>
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<td>10</td>
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<tr>
<td>Each value represents the mean ± 1 SE of five observations.</td>
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<tr>
<td>The coronary response, expressed either as integrated areas of coronary vasodilatation or percent increase, and the systemic response, expressed as maximum fall in mean arterial pressure, were analyzed by the paired t test.</td>
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<td>*p &lt; .05; **p &lt; .01.</td>
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line). Furthermore, the lag between intracoronary administration of NTG and the first evidence of a systemic hemodynamic response ranged from 40 to 50 sec. After such an interval there was a slight and nonsignificant reduction in systemic blood pressure, which disappeared in about 5 min. Heart rate remained completely unmodified during the first 40 to 60 sec after NTG injection, then it showed a slight and nonsignificant increase (figure 1).

**Effects of indomethacin treatment.** Systemic cyclooxygenase activity was significantly reduced after administration of indomethacin. In particular, the percent of inhibition of the cyclooxygenase activity increased progressively with augmentation of the dose of indomethacin. The smallest dose inhibited cyclooxygenase activity by 90%, and complete inhibition was reached with 1.5 mg/kg. Simultaneously, systemic and coronary perfusion pressure showed a progressive increase as compared with the value recorded in control conditions. In both cases, the increase was correlated with the percent of cyclooxygenase activity inhibition induced by the increasing doses of indomethacin administered (systemic blood pressure, r = .618, n = 35, p < .001; coronary perfusion pressure, r = .691, n = 35, p < .001). The increase in systemic blood pressure was accompanied by a presumed reflex reduction in control heart rate (data not shown). Furthermore, when the integrated areas of coronary vasodilatation were compared, a progressive decrease was detectable, which reached a significant level after the 1.5 mg/kg dose of indomethacin (figure 2). No further reduction in the integrated areas of coronary vasodilatation was detectable with the higher doses of indomethacin. When the reductions in the integrated areas of coronary vasodilatation induced by the different doses of indomethacin were plotted against the dose of drug administered, a significant correlation was detected (r = .828, n = 35, p < .001) (figure 3). The reduction in the integrated areas of NTG-induced coronary vasodilatation was not accompanied by a reduction in the maximum fall in perfusion pressure; for example, it was 33 ± 8 mm Hg before and 31 ± 8 mm Hg after the intravenous administration of 3 mg/kg indomethacin. On the other hand, after the complete inhibition of prostaglandin synthesis, the NTG-induced coronary vasodilatation was characterized by a faster return of the perfusion pressure to the basal value (figure 4). Furthermore, no

**FIGURE 2.** Changes of the integrated areas of coronary vasodilatation after administration of increasing amounts of indomethacin and naproxen (n = 5). Each bar represents the mean ± 1 SE. Statistical analysis was performed by comparing the values obtained under control conditions and after each pharmacologic treatment. Statistical analysis and symbols as in figure 1.
significant change of the effect of NTG on systemic blood pressure and heart rate was caused by the inhibition of the prostaglandin synthesis (figure 4).

Infusion of NTG resulted in a progressive decrease in coronary perfusion pressure that reached its nadir after about 20 sec and then remained stable until 1 min after the end of the infusion (figure 5). Thereafter, coronary perfusion pressure increased and returned to baseline 4 min after the end of the NTG infusion (figure 5). Mean systemic blood pressure also showed a progressive decrease, which reached its nadir after about 4 min (−12 ± 2 mm Hg). Indomethacin administered intravenously at a dose of 1.5 mg/kg did not modify the maximum fall in coronary perfusion pressure (15 ± 1 mm Hg under control conditions vs 13 ± 1 mm Hg after indomethacin treatment; NS). However, the integrated areas of coronary vasodilatation were significantly reduced (control, 5578 ± 246 mm Hg/sec; after indomethacin, 3171 ± 370 mm Hg/sec; p < .001). This reduction was caused by a decrease in both phases of NTG-induced coronary vasodilatation. The integrated area of the early phase was 2940 ± 251 mm Hg/sec in control conditions and 1968 ± 163 after indomethacin (p < .001). Furthermore, the mean of the slopes of the regression lines obtained by plotting perfusion pressure vs time was significantly reduced after indomethacin (0.054 ± 0.006 vs 0.067 ± 0.005; p < .05). Finally, the late phase of the NTG-induced coronary vasodilatation was reduced from 2638 ± 247 to 1203 ± 209 mm Hg/sec (p < .001), since coronary perfusion pressure returned to values not different from the baseline after about 3 min.

**Effects of naproxen treatment.** The administration of different doses of naproxen inhibited cyclooxygenase activity to a different extent. There was a progressive increase in the percentage of inhibition of systemic cyclooxygenase activity (83% after 1 mg/kg, 96% after 3 mg/kg, 96% after 5 mg/kg), and complete inhibition was reached with the dose of 7 mg/kg. This drug, like indomethacin, elicited an increase in the basal value of systemic and perfusion pressure and a reduction in heart rate. The increases in both systemic and coronary perfusion pressure were correlated with the percentage of inhibition of systemic cyclooxygenase activity induced (systemic blood pressure, r = .430, n = 35, p < .01; coronary perfusion pressure, r = .651, n = 35, p < .001). In agreement with the results obtained with the administration of indomethacin, after naproxen there was a progressive reduction in the integrated areas of NTG-induced coronary vasodilatation with increasing doses up to 7 mg/kg (figure 2). A significant correlation was found when the decrease in the integrated areas of coronary vasodilatation observed were plotted against the dose of naproxen administered (r = .729, n = 35, p < .001) (figure 3). On the contrary, the maximum fall in perfusion pressure induced by NTG remained unmodified after administration of naproxen (38 ± 7 mm Hg before and 37 ± 7 mm Hg after 7 mg/kg), but the return of the perfusion pressure to the basal value was faster than that under control conditions (figure 4). The response of the systemic blood pressure and heart rate to NTG was not influenced by the pharmacologic treatment (figure 4). Finally, the changes in the coronary response to NTG induced by naproxen were similar to those observed after administration of indomethacin (figure 5). In particular, the maximum fall in coronary perfusion pressure was 15 ± 2 mm Hg under control conditions and 16 ± 1 mm Hg after naproxen (NS). The integrated areas of coronary vasodilatation were 5264 ± 218 mm Hg/sec under control conditions and 2984 ± 253 mm Hg/sec after naproxen (p < .001). As
in the case of indomethacin there was a decrease in the early phase of NTG-induced coronary vasodilatation (control, 2787 ± 148 mm Hg/sec; after naproxen, 1606 ± 132 mm Hg/sec; p < .001), in the mean of the slopes of the regression lines obtained by plotting the perfusion pressure vs time from the beginning to the end of NTG infusion (control, 0.069 ± 0.002; after naproxen, 0.06 ± 0.003; p < .05), and in the late phase (control, 2477 ± 217 mm Hg/sec; after naproxen, 1378 ± 256 mm Hg/sec; p < .001).

Specificity of the pharmacologic treatments. In no instance was the hemodynamic response induced by histamine modified by the various pharmacologic treatments (table 2). Furthermore, in the experiments in which four NTG-induced responses were evoked at 30 min intervals without any pharmacologic treatment, no statistically significant change in coronary vasodilatation occurred (first, 1940 ± 220; second, 1908 ± 270; third, 1972 ± 301; fourth 1928 ± 232 mm Hg/sec, NS).

### TABLE 2

<table>
<thead>
<tr>
<th>Indomethacin</th>
<th>Naproxen</th>
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<tr>
<td>Control</td>
<td>2750 ± 330</td>
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<tr>
<td>0.1 mg/kg</td>
<td>2460 ± 400</td>
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<tr>
<td>Control</td>
<td>2800 ± 300</td>
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<tr>
<td>0.3 mg/kg</td>
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<tr>
<td>Control</td>
<td>2660 ± 296</td>
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<td>0.5 mg/kg</td>
<td>2766 ± 400</td>
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<tr>
<td>Control</td>
<td>2716 ± 296</td>
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<tr>
<td>1.5 mg/kg</td>
<td>2277 ± 232</td>
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<tr>
<td>Control</td>
<td>2740 ± 290</td>
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<tr>
<td>3 mg/kg</td>
<td>2480 ± 110</td>
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<tr>
<td>Control</td>
<td>2511 ± 260</td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>2306 ± 190</td>
</tr>
<tr>
<td>Control</td>
<td>2566 ± 297</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>2055 ± 260</td>
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</table>

Each value represents the mean ± 1 SE (n = 5).
FIGURE 5. Effects of 4 min intracoronary infusion of NTG (60 μg/min) on coronary perfusion pressure (PP) and systemic blood pressure (BP) in control conditions (A and C) and after administration of indomethacin (1.5 mg/kg iv) (B) or naproxen (7 mg/kg iv) (D) (n = 5). Each point represents the mean ± 1 SE. Statistical analysis as in figure 1. *p < .05; # = p < .01.

Discussion

The observation by Ganz et al. and by other authors that NTG relieves myocardial ischemia primarily by reducing cardiac work as a result of peripheral vasodilatation demonstrates how much the changes in systemic hemodynamics may influence the effects of NTG on the coronary circulation. Therefore we looked for a dose of NTG that was large enough to markedly decrease the coronary perfusion pressure but too small to cause any significant change in systemic hemodynamics. The finding that the injection of 5 μg of NTG in the perfused coronary artery of the dog induces coronary vasodilatation that cannot be increased by doubling the dose is in keeping with the results of Feldman et al., who observed that in man the coronary vasodilatation elicited by the intracoronary injection of 5 μg of NTG was approximately two-thirds of the total dilatation obtainable. Two experimental approaches are available in evaluating the possible role of endogenous prostaglandins in a physiologic or pharmacologic phenomenon: (1) prostaglandin assay in blood, urine, or tissue specimens or (2) administration of drugs that inhibit prostaglandin synthesis. Measurements of prostaglandin concentrations may potentially provide a more complete elucidation of the role of these substances, since they indicate both the specific prostaglandins involved and the degree of the evoked change in their concentrations. However, the reproducibility and the sensitivity of prostaglandin assays are limited by several factors, including the method of assay itself, the handling and extraction of samples, and the study protocol with particular regard to fluid and solute intake. Furthermore, it remains uncertain whether an observed change in prostaglandin level represents a cause or merely a consequence of a given phenomenon.

Given the above limitations of prostaglandin assays, in this study we chose the use of prostaglandin synthesis inhibitors to test the hypothesis that prostaglandins play a role in the mediation of NTG-induced coronary vasodilatation. However, this method has its own pitfalls: the potential non-prostaglandin-mediated actions of these drugs and less-than-complete prostaglandin inhibition. To overcome the first obstacle, we used, according to the recommendations of Dunn and Hood, two different inhibitors of prostaglandin synthesis: indomethacin and naproxen. Since we were able to duplicate the results obtained with indomethacin by using naproxen, it seems reasonable to ascribe the change observed in our experiments with these drugs to concurrent changes in prostaglandin synthesis rather than to nonspecific pharmacologic properties of
these drugs independent of inhibition of prostaglandin synthesis.

With regard to the possibility of incomplete prostaglandin inhibition, the assay of TXB₂ served as a means of determining the extent of inhibition of platelet cyclooxygenase activity. We assumed that vascular cyclooxygenase was inhibited to the same extent, since a dissociation of platelet and vascular cyclooxygenase inhibition has never been reported for both indomethacin and naproxen.

The inhibition of prostaglandin synthesis induced a concomitant increase in coronary vascular resistances and in systemic blood pressure. Similar results after indomethacin were observed in other studies by dogs and humans, and these changes may represent the hemodynamic consequences of the suppression of basal levels of prostaglandins. However, the possibility that inhibition of prostaglandin synthesis may induce a nonspecific reduction in vascular responsiveness cannot be ruled out. Therefore we looked for a specific agent whose coronary hemodynamic effects were not reduced after inhibition of prostaglandin synthesis. Histamine fulfilled this requirement and was used for testing vascular responsiveness after the specific pharmacologic trials given in our experimental protocol. The lack of change in integrated areas of histamine-induced coronary vasodilatation after any of the pharmacologic challenges used in our protocol indirectly supports the hypothesis that prostaglandins specifically participate in the mediation of NTG-induced coronary vasodilatation. Moreover, the most convincing evidence supporting this hypothesis are the results of the dose-response curve obtained by plotting the reduction of the integrated areas of NTG-induced coronary vasodilatation vs the doses of indomethacin or naproxen. We found that the integrated areas of NTG-induced coronary vasodilatation were progressively reduced by increasing the amount of indomethacin or naproxen administered up to a dose of 1.5 or 7 mg/kg, respectively. These doses produced complete inhibition of cyclooxygenase activity. Further increases in the doses of both indomethacin and naproxen failed to further increase the reduction in the integrated areas of NTG-induced coronary vasodilatation. Finally, the results obtained with intracoronary infusion of NTG lend further support to the hypothesis that prostaglandins participate in the NTG-induced coronary vasodilatation. In fact, in agreement with the observation of Panzenbeck et al., they show that inhibition of prostaglandin synthesis does not modify the fall in coronary perfusion pressure during NTG infusion. However, when the integrated areas of coronary vasodilatation induced by NTG infusion before and after inhibition of prostaglandin synthesis were compared, a significant reduction was detected. This was caused by both a slower decrease of the coronary perfusion pressure in the early phase and a faster return of the coronary perfusion pressure to basal values during the late phase of NTG-induced coronary vasodilatation.

The basis for understanding these results suggesting that prostaglandins mediate, at least in part, the vascular effects of intracoronary NTG may be represented by the findings of Levin et al. These authors showed that intact monolayers of human endothelial cells in vitro release PGI₂ in response to clinically attainable concentration of NTG. Therefore, they speculated that vasodilating properties of NTG are, at least in part, indirect and are attributable to the induction by NTG of endothelial cell PGI₂ synthesis. This may induce coronary vasodilatation by altering the PGI₂/TXA₂ ratio in favor of PGI₂ excess. Our results fit well with this hypothesis and may represent the confirmation in a study in vivo that the increased release of PGI₂ induced by NTG administration plays a role in the hemodynamic response to intracoronary injection of NTG. However, the observation that the inhibition of prostaglandin synthesis reduces the coronary hemodynamic response to NTG does not necessarily imply that NTG stimulates the synthesis of prostaglandins from endothelial cells. Furthermore, it is also interesting that the inhibition of cyclooxygenase activity did not reduce the maximum fall in coronary perfusion pressure after NTG but induced a faster return of the perfusion pressure to the baseline as compared with control conditions. This finding seems to indicate that NTG has a direct and an indirect effect on the vascular wall. The direct effect is responsible for a marked initial decrease in coronary vascular resistance. Controversy remains concerning the mechanism underlying this effect, although evidence from studies in vitro suggesting that NTG induces an activation of guanylate cyclase, via a series of enzymatic reactions that are modulated by the availability of sulphhydril groups, have recently been confirmed in vivo. The indirect effect is mediated through the release of prostaglandins from the endothelial wall and accounts for the persistence of reduction in vascular resistance.

References


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