# Comparison of nitroprusside and nitroglycerin in inhibition of angiotensin II and other vasoconstrictor-mediated contraction in human coronary bypass conduits

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***Aims*** To compare the effect of nitroprusside (SNP) and nitroglycerin (NTG) on angiotensin II (ANGII), endothelin-1 (ET-1), and $\alpha_1$-adrenoceptor (phenylephrine, PE)-mediated contraction in internal mammary artery (IMA).

***Methods*** Human IMA segments ($n = 120$) taken from 37 patients were studied. Concentration-relaxation curves for SNP and NTG were established in IMA precontracted with these vasoconstrictors. Concentration-contraction curves were also constructed in IMA rings incubated with SNP and NTG (0.1 and 1 $\mu$M) for 10 min.

***Results*** Both SNP and NTG caused full relaxation with similar $EC_{50}$s except NTG was four-fold more potent than SNP in PE-induced contraction ($-7.92 \pm 0.06$ *vs* $-7.32 \pm 0.2$ log M, mean $\pm$ s.e. mean, $P < 0.01$; 95% confidence interval for the difference of the means: 0.19, 1.01 log M). Pretreatment with SNP (0.1 and 1 $\mu$M) significantly depressed the contraction by ANGII from $56.6 \pm 7.7\%$ (of 100 mM $K^+$-contraction) to $18.3 \pm 8.6\%$ and $3.9 \pm 2.1\%$ ($P = 0.0001$). In four rings treated with SNP, the contraction to ANGII was abolished whereas NTG did not depress ANGII-mediated contraction. Pretreatment with SNP (1 $\mu$M), but not NTG, significantly depressed the magnitude of the PE-induced contraction from $4.7 \pm 1.2$ to $1.7 \pm 0.4$ g ($P < 0.05$). Treatment with both SNP and NTG significantly increased the $EC_{50}$ ($-5.09 \pm 0.17$ log M, $P = 0.0007$ for SNP and $-5.40 \pm 0.06$ log M, $P = 0.02$ for NTG). Pretreatment with SNP did not significantly change either the magnitude or the $EC_{50}$ of the ET-1-induced contraction.

***Conclusions*** SNP may be advantageous compared with NTG in preventing coronary arterial graft contraction. However, once grafts have constricted to ANGII, $\alpha_1$-adrenoceptor agonists, and ET-1, NTG may be only marginally advantageous.

*Keywords:* nitroprusside, angiotensin II, nitroglycerin, endothelin 1, internal mammary artery

## Introduction

The peptide vasoconstrictors angiotensin II (ANGII) and endothelin-1 (ET-1) have been demonstrated to be produced not only from the plasma renin-angiotensin system and vascular endothelium but also produced independently from these sources [1, 2]. Local ANGII has been demonstrated to be important to conduit artery function in humans [3]. Both ANGII and ET-1 are potent vasoconstrictors. There is increasing evidence that these locally produced vasoconstrictor peptides may contribute to blood vessel homeostasis, as well as the development of vascular pathologic conditions [1].

In coronary artery bypass grafting (CABG) surgery, arterial grafts have been used with increased frequency because the long-term patency is expected to be superior to vein grafts. These include internal mammary artery (IMA), gastroepiploic artery, inferior epigastric artery, and radial artery. However, the use of arterial grafts has brought up the

question that hypoperfusion may occur due to the small diameter of the arterial grafts, the spastic characteristic, or both [4, 5]. In fact, recent studies have demonstrated that blood flow through arterial grafts may be inadequate for maximal exercise [6] and hence causes a hypoperfusion syndrome [7]. Severe hypoperfusion tends to occur early and may be worsened by high-dose-vasopressor therapy that could further reduce arterial graft flow [6, 7]. The cause for arterial graft vasoconstriction is still unknown. Many vasoconstrictor substances may be involved [4]. Of these, the peptide vasoconstrictors ANGII and ET-1 may be of importance. Plasma levels of ANGII and ET-1 are elevated after cardiopulmonary bypass [8–10]. Such an elevation of the plasma level may be one of the causes for coronary graft vasoconstriction immediately after CABG [11]. In addition, the $\alpha$-adrenoceptor has been demonstrated to be predominant in the human IMA [12] and circulating catecholamines mainly contract the IMA and other arterial grafts [4] so that the $\alpha_1$-adrenoceptor mechanism may be involved in the graft spasm.

However, the interaction between ANGII and vasodilator substances in the human coronary artery bypass conduits are

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not well understood. The use of vasodilators in treatment of cardiovascular diseases has recently increased. Many vasodilators such as calcium antagonists, ACE-inhibitors, long-lasting nitrates, and phosphodiesterase III inhibitors have been used in patients undergoing CABG [13–17]. Of these, two nitrovasodilators, sodium nitroprusside (SNP) and nitroglycerin (NTG), are commonly used during the postoperative period. The indications for the use of these two vasodilators are different. SNP is used immediately postoperatively for its antihypertensive effect as hypertension is frequently seen early after CABG. In contrast, NTG is indicated in ischaemic heart disease mainly because of its effects to dilate epicardial conductance arteries, to increase collateral blood flow to ischaemic myocardium, and to decrease left ventricular preload [13].

The present study was designed to investigate the interaction between nitrovasodilators and ANGII in arterial grafts with emphasis on the comparison between SNP and NTG. In addition, the effect of SNP on ET-1 and $\alpha_1$-adrenoceptor-mediated contraction was also studied with comparison to NTG. The most frequently used arterial graft, IMA, was investigated.

## Methods

One-hundred and twenty human IMA segments were collected from 37 patients undergoing IMA graft surgery. There were 30 males and 7 females. Approval to use discarded IMA tissue was given by the Hospital Ethics Committee. Any discarded distal IMA segments were collected and placed in a container with oxygenated, physiological solution (Krebs) maintained at $4^\circ$ C, and then transferred to the laboratory. The IMA was transferred into a glass dish and dissected out from its surrounding connective tissue. The vessels were cut into 3 mm long rings and suspended on wires in organ baths [14, 15]. The number of rings taken from each patient varied from 2–6. The Krebs' solution had the following composition (in mM): $Na^+$ 144, $K^+$ 5.9, $Ca^{2+}$ 2.5, $Mg^{2+}$ 1.2, $Cl^-$ 128.7, $HCO_3^-$ 25, $SO_4^{2-}$ 1.2, $H_2PO_4^-$ 1.2, and glucose 11. The solution was aerated with a gas mixture of 95% $O_2$–5% $CO_2$ at $37^\circ$ C.

### Organ-bath technique

A technique that allowed the vascular rings to normalize to a physiological pressure in the organ bath was used. This refers to set the vascular rings at a pressure comparable to that *in vivo*. The details of the technique have been published [14, 15]. Briefly, the rings were stretched-up in progressive steps to determine the length-tension curve for each ring. A computer iterative fitting program (VESTAND 2.1, Yang-Hui He, Princeton University, NJ) was used to determine the exponential line, pressure and the internal diameter. When the transmural pressure on the rings reached 100 mmHg, determined from their own length-tension curves, the stretch-up procedure was stopped and the rings were released to 90% of its internal circumference at 100 mmHg. This degree of passive tension was then maintained throughout the experiment.

The endothelium was intentionally preserved by cautiously dissecting and mounting the rings in our study since endothelium plays a modulatory role in the contractility of the human IMA. We previously found that this technique allowed the experiments to be carried out with an intact endothelium, as determined by the functional relaxation response to acetylcholine in the human isolated IMA rings ($63.0 \pm 10.0\%$ relaxation in endothelium-protected IMA and $6.8 \pm 3.0\%$ contraction in endothelium-denuded IMA, $P < 0.01$, [12]).

### Protocol

After the normalization procedure, the IMA rings were equilibrated at least for 45 min.

*Relaxation* SNP- or NTG-induced relaxation was studied in IMA rings contracted with ANGII (3 nM), ET-1 (10 nM), or PE (3 μM). The concentrations of these vasoconstrictor substances were submaximal as determined from the logistic-curve fitting equation [14]. These concentrations are equal to $EC_{50} - EC_{80}$ for the ANGII from the present study or ET-1 [16] and PE [17]-induced-contraction in the human IMA from previous studies. Cumulative concentration-relaxation curves to SNP or NTG were then established. Only one concentration-relaxation curve was obtained from each IMA ring. From 5–8 rings (taken from at least three patients), a mean concentration-relaxation curve was constructed.

### Depression of contraction by pretreatment with SNP or NTG

After equilibration, 100 mM $K^+$ was added into the organ bath and the contraction force was recorded. Rings were discarded if the contraction force to 100 mM $K^+$ was less than 1 g. The ring was frequently washed to restore the baseline. The contraction was expressed as percentage of the contraction force induced by 100 mM $K^+$, except when it was indicated as g.

*ANGII* Rings were allocated in three groups. Whenever possible, the rings taken from the same patient were allocated to three groups. One was used as a control and the other two were equilibrated for 10 min with one of the two concentrations (0.1 μM or 1 μM) of SNP or NTG. A cumulative concentration-contraction curve was then constructed for ANGII.

*PE* Three rings taken from the same patient were allocated in three groups. One of these rings was used as a control and the other two were equilibrated for 10 min with 1 μM SNP or NTG. A cumulative concentration-contraction curve was then constructed for PE.

*ET-1* Rings were allocated in three groups. Similar to ANGII experiments, whenever possible, the rings taken from the same patient were allocated to three groups. One of these rings was used as a control and the other two were equilibrated for 10 min with one of the two concentrations (0.1 μM or 1 μM) of SNP. A cumulative concentration-contraction curve was then constructed for ET-1. These results were compared with those of NTG published previously [16].

## Data analysis

The effective concentration of the constrictor (or dilator) agent that caused 50% of maximal contraction (or relaxation) was defined as $EC_{50}$. The $EC_{50}$ was determined from each concentration-contraction (or relaxation) curve by a logistic, curve-fitting equation: $E = MA^P/(A^P + K^P)$ where E is response, M is maximal contraction (or relaxation), A is concentration, K is $EC_{50}$ concentration, and p is the slope parameter [14]. A computerized program was used for the curve-fitting. From this fitted equation, the mean $EC_{50}$ value $\pm$ s.e. mean was calculated in each group. Data were expressed as mean $\pm$ s.e. mean with 95% confidence intervals where appropriate. Unpaired *t*-test or analysis of variance (ANOVA) were used to test statistical significance among different constrictors and dilators regarding the maximal response or $EC_{50}$. Scheffe's F test was used as a *post-hoc* test between groups. $P < 0.05$ was considered significant.

## Materials

Drugs used in this study and their sources were: angiotensin II (Sigma, St Louis, MO, USA); endothelin-1 (Peptides International, Louisville, Kentucky); nitroglycerin (SoloPak Laboratories, Franklin Park, IL); and sodium nitroprusside (F. Hoffmann-La Roche & Co. Ltd, Basle, Switzerland). Stock solution of endothelin-1 and angiotensin II was held frozen until required.

## Results

### Resting vessel parameters

The mean internal diameter of the 120 rings at an equivalent transmural pressure of 100 mmHg (D100) was $2.4 \pm 0.1$ mm as determined from the normalization procedure. When the IMA rings were set at a resting diameter of $0.9 \times D100$, the equivalent transmural pressure was $70.1 \pm 1.5$ mmHg, and the resting force was $3.7 \pm 0.3$ g.

### Relaxation by SNP or NTG in the IMA precontracted by ANGII, ET-1, or PE

Both SNP and NTG caused a full or nearly full relaxation in either ANGII (100%, respectively), ET-1 ($94.3 \pm 5.7\%$ *vs* $85.7 \pm 6.4\%$, $P = 0.2$, 95% confidence interval for the difference of the mean [95% CI]: 0.6, 20.7%), or PE (100% *vs* $99.0 \pm 1.0\%$, $P = 0.4$, 95% CI: $-1.6$, 3.7%)-precontracted IMA (Figure 1). There was no difference in the maximal relaxation between SNP and NTG.

In the ANGII-induced contraction the $EC_{50}$ was similar for SNP and NTG ($-7.69 \pm 0.18$ *vs* $-7.82 \pm 0.19$ log M, $P > 0.05$, 95% CI: $-0.44$, 0.71 log M). In ET-1-induced contraction, the $EC_{50}$ was $-6.93 \pm 0.12$ for SNP and $-6.98 \pm 0.22$ log M for NTG ($P = 0.8$, 95% CI: $-0.49$, 0.61 log M). However, in PE-induced contraction, the $EC_{50}$ for SNP was significantly higher than for NTG ($-7.32 \pm 0.2$ *vs* $-7.92 \pm 0.06$, $P < 0.01$, 95% CI: 0.19, 1.01 log M).
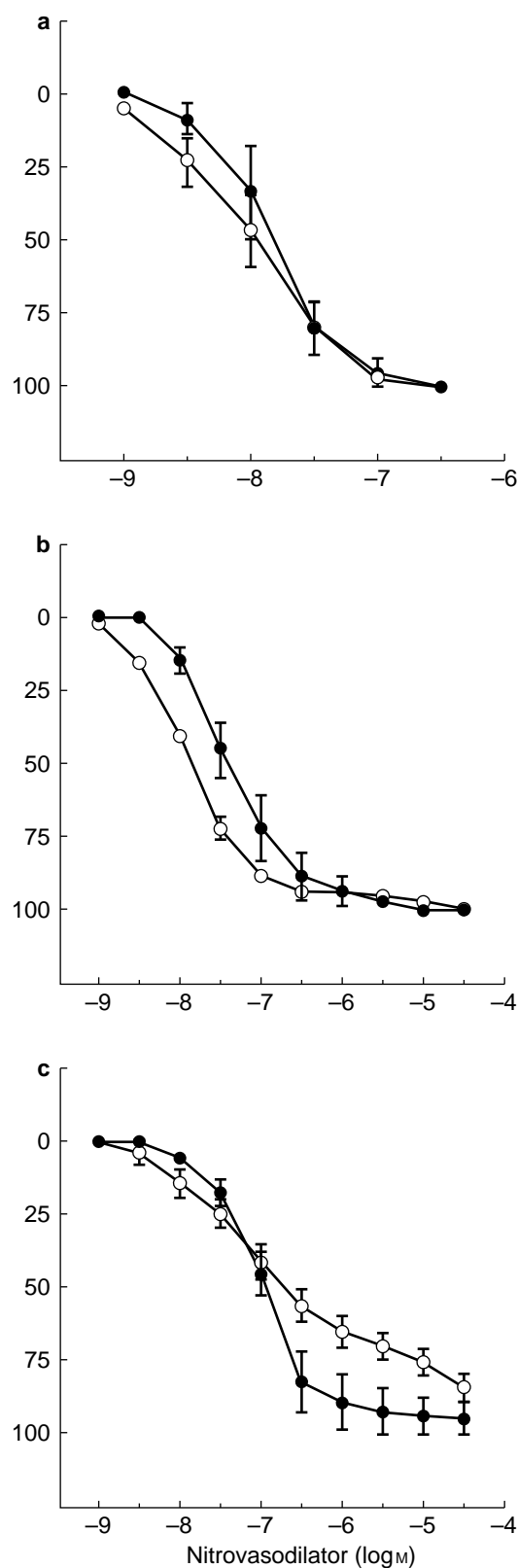


**Figure 1** Mean concentration (-log M)-response (% relaxation) curves for sodium nitroprusside (SNP, ●) and nitroglycerin (NTG, ○) in the human internal mammary artery precontracted by a) angiotensin II (ANGII, 3 nM, $n = 6$ in each group, b) phenylephrine (PE, 3 μM, $n = 6$ for SNP and $n = 8$ for NTG, and c) endothelin-1 (ET, 10 nM, $n = 6$ for SNP and $n = 5$ for NTG). Vertical error bars are 1 s.e. mean of mean values.

*Depression of contraction by pretreatment with SNP or NTG*

*ANGII* Pretreatment of IMA for 10 min with SNP significantly depressed the magnitude of the ANGII-induced contraction (Figure 2). In comparison with $56.6 \pm 7.7\%$ in the control, the maximal contraction was $18.3 \pm 8.6\%$ and $3.9 \pm 2.1\%$ ($P = 0.0001$) with the treatment of SNP at the concentration of 0.1 and 1 μM. The $EC_{50}$ was $-8.75 \pm 0.06$ log M in the control. In the one ring treated with 0.1 μM and three rings treated with 1 μM SNP, the $EC_{50}$ was not calculable because the contraction to ANGII was abolished by the treatment. In the rest of the rings, the $EC_{50}$ was significantly higher after treatment with SNP ($-8.32 \pm 0.08$



**a**

**b**
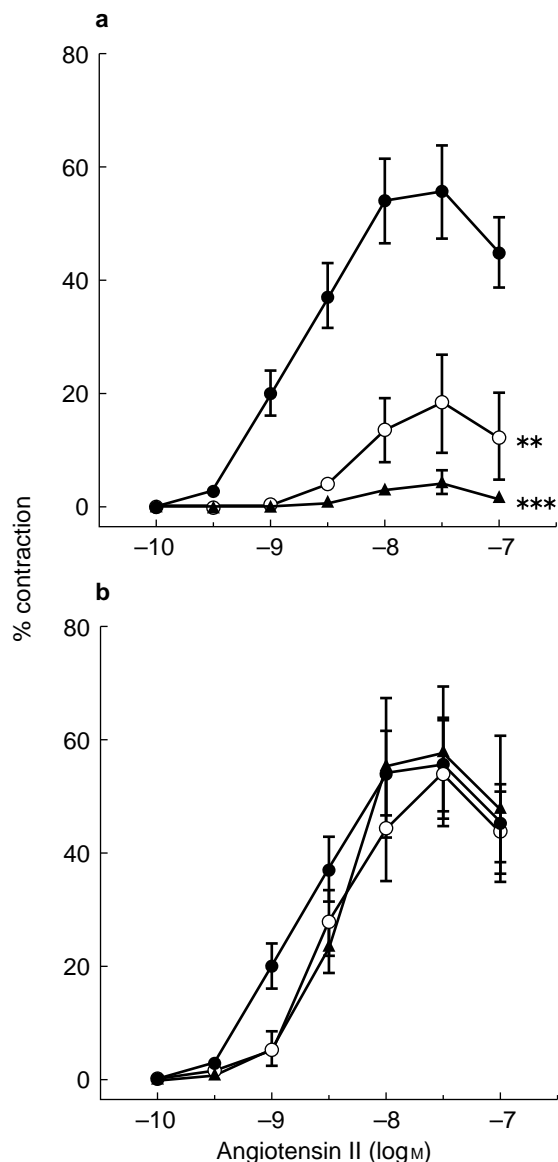
**Figure 2** Mean concentration (-log M)-contraction (percentage of 100 mM $K^+$-induced contraction) curves for ANGII. Rings were allocated to each treatment. One ring was a control (●, $n = 8$ for SNP and NTG) without pretreatment of nitrovasodilators. For the other two rings, SNP 0.1 (○, $n = 6$) or 1 (▲, $n = 6$) μM (a) or NTG 0.1 (○, $n = 7$) or 1 (▲, $n = 8$) μM (b) was added into the organ bath 10 min before the start of the ANGII curve. Vertical error bars are 1 s.e. mean of mean values. $P < 0.0001$, among the three groups. ★★$P < 0.01$ and ★★★$P < 0.001$, compared with the control at the maximal contraction.

for 0.1 μM SNP and $-8.15 \pm 0.07$ log M for 1 μM SNP, $P = 0.0002$).

In contrast, pretreatment with NTG did not depress the ANGII-mediated contraction. In the control rings, the maximal contraction was $56.6 \pm 7.7\%$, compared with $60.6 \pm 10.8\%$ and $60.8 \pm 13\%$ in the rings treated with 0.1 and 1 μM NTG, respectively. However, NTG treatment shifted $EC_{50}$ 2.6- and 2.2-fold higher ($P < 0.01$, Figure 2b). The $EC_{50}$ was $-8.75 \pm 0.06$ log M in the control. It increased to $-8.34 \pm 0.09$ and $-8.41 \pm 0.04$ log M after 0.1 and 1 μM NTG treatment, respectively ($P < 0.001$).

*PE* Pretreatment of IMA for 10 min with SNP (1 μM) significantly depressed the magnitude of the PE-induced contraction from $4.7 \pm 1.2$ to $1.7 \pm 0.4$ g ($P < 0.05$, Figure 3a) although the difference did not reach statistical significance if expressed as percentage of the $K^+$ (100 mM) (Figure 3b). In comparison, treatment with NTG (1 μM) only slightly depressed the PE-induced contraction ($4.7 \pm 1.2$ *vs* $2.1 \pm 0.4$ g, $P = 0.1$, Figure 4a). The $EC_{50}$ was $-6.0 \pm 0.15$ log M in the control. Treatment with both SNP and NTG significantly increased the $EC_{50}$ ($-5.09 \pm 0.17$, $P = 0.0007$ for SNP and $-5.40 \pm 0.06$, $P = 0.02$ for NTG).

*ET-1* Pretreatment of IMA for 10 min with SNP did not significantly depress the magnitude of the ET-1-induced contraction (Figure 4). In comparison with $210.7 \pm 5.3\%$ in the control, the maximal contraction was $232.4 \pm 28.4\%$ and $206.3 \pm 33.7\%$ ($P = 0.9$) in the treatment of SNP at the concentration of 0.1 and 1 μM. The $EC_{50}$ was $-8.32 \pm 0.10$ log M in the control, $-8.21 \pm 0.11$ log M in the rings treated with 0.1 μM and $-8.16 \pm 0.07$ log M in the rings treated with 1 μM SNP ($P = 0.45$).

## Discussion

In this study, we have found that in the human IMA, the major arterial graft for coronary artery bypass surgery, that 1) both SNP and NTG induced a maximal or near-maximal relaxation in either ANGII, ET-1, or PE-mediated contraction and 2) when applied prior to contraction, SNP showed inhibitory effect to ANGII superior to that of NTG.

These relaxation studies have demonstrated that both SNP and NTG are effective vasodilators in the human artery, tested in various vasoconstrictor-mediated contraction. This has important clinical implications with regard to the treatment of graft spasm after CABG. As aforementioned, both SNP and NTG are frequently used during the postoperative period. The present study demonstrates that these nitrovasodilators are potent in reversing graft vasospasm after CABG and there is no major difference between SNP and NTG although NTG is four-fold more potent than SNP in reversing PE-induced contraction. The action of these two vasodilators are through the production of nitric oxide (NO), which is identical to the endothelium-derived NO. NO stimulates soluble guanylyl cyclase resulting in an increase in the level of cyclic GMP in the vasculature.

The present study has demonstrated a major difference in the effect of inhibition of the ANGII-mediated contraction when applied prior to the contraction. Our study shows
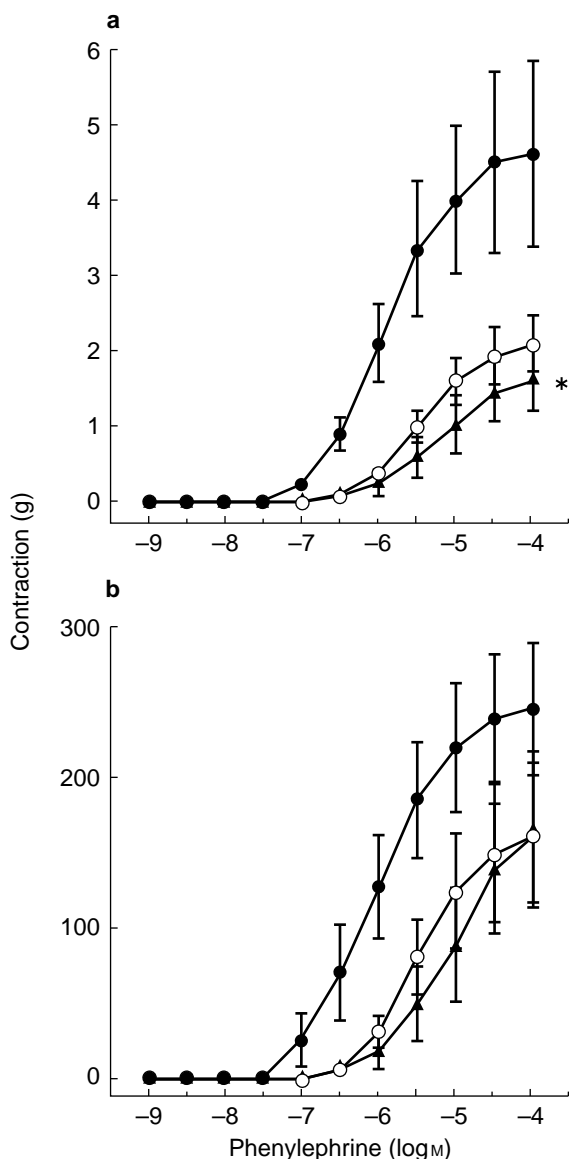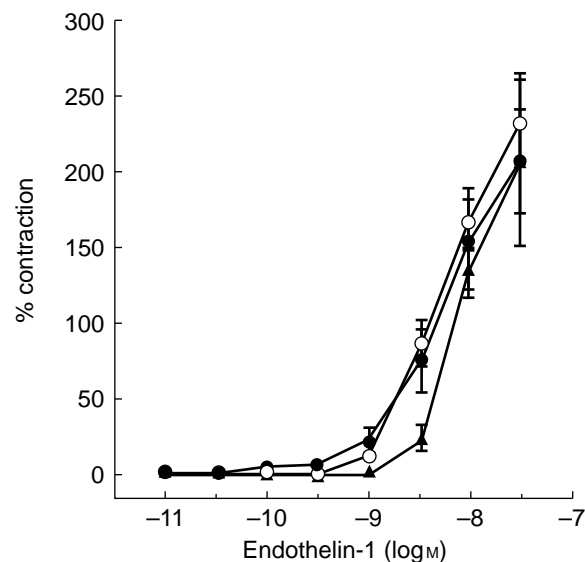
than for preventing contraction. However, the above explanation is obviously not the only reason because SNP, when applied prior to the ANGII-contraction, had a significant effect which was superior to NTG. Therefore, we speculate that there is a second reason accounting for the difference seen between NTG and SNP-rapid tolerance (tachyphylaxis) to NTG. In fact, it has been demonstrated that tolerance to NTG can be induced in an organ bath within 20 min in coronary arteries [26]. The results from the present study are in accordance with the previous findings that NTG, if applied prior to the vasoconstriction, has limited inhibitory effect [15–17]. Although SNP has been reported to show cross tolerance with NTG in a study [27], other studies have demonstrated that the inhibitory effect of SNP is only marginally suppressed in NTG-tolerant arteries [28]. Therefore, we hypothesize that the difference seen between SNP and NTG in inhibition of the ANGII-mediated contraction may be due to the difference related to the rapid tolerance between these two nitrovasodilators. The intrinsic cause for this, however, awaits further studies.

In the present study, the effect of SNP on the contraction mediated by ET-1 and the $\alpha_1$-adrenoceptor agonist PE was also investigated. There is a trend in inhibition of the PE-mediated contraction that SNP may be more effective than NTG. This is reflected by the results that SNP significantly suppressed the PE-induced contraction but the suppression by NTG was not significant, although when expressed as the percentage of $K^+$-induced contraction the difference could not be seen. Interestingly, SNP, at the concentrations tested in the present study, did not show any suppression on the ET-1-mediated relaxation, although higher concentrations may have such an effect. Compared with the effect of NTG on ET-1-mediated contraction previously published [16], the difference between SNP and NTG was not detectable.

In conclusion, the results of our study suggest that under certain circumstances, such as when ANGII is concerned in post CABG, SNP may be advantageous over NTG in preventing coronary arterial graft spasm or other conduit artery contraction. This may be also true in $\alpha_1$-adrenoceptor-mediated vasoconstriction in the human conduit arteries. In contrast, once grafts have constricted to ANGII, $\alpha_1$-adrenoceptor agonists, and ET-1, NTG may be marginally advantageous. This study, therefore, may provide useful information for the postoperative management in patients undergoing CABG.

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