NT-702, a Selective Phosphodiesterase 3 Inhibitor, Dilates Rabbit Spinal Arterioles via Endothelium-Dependent and Endothelium-Independent Mechanisms

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Abstract: We investigated the effects of NT-702, a selective phosphodiesterase (PDE) 3 inhibitor, on arterioles isolated from rabbit lumbar spinal cords. NT-702 caused a dose-dependent dilation of the isolated spinal arterioles. The disruption of endothelium produced a significant reduction of higher concentrations (10^{-7} and 10^{-6} M), but not lower concentrations (less than 10^{-8} M), of NT-702–induced vasodilation. The NT-702–induced vasodilation of the arterioles with endothelium was not affected by pretreatment with an inhibitor of nitric oxide, cyclooxygenase, or cytochrome P-450 monooxygenase. In contrast, catalase reduced significantly the higher concentrations of NT-702–induced vasodilation only. Tetraethylammonium (TEA) completely reduced the lower concentrations of NT-702–induced vasodilation, but decreased only partially the higher concentrations of NT-702–induced vasodilation of the arterioles with endothelium. Hydrogen peroxide dilated significantly the isolated arterioles with endothelium, the response of which was reduced significantly by TEA. KT5720 (a selective protein kinase inhibitor) significantly decreased both the lower and higher concentrations of NT-702–induced vasodilation of the arterioles with endothelium. The findings suggest that NT-702 dose-dependently dilated the isolated spinal arterioles of rabbits via endothelium-dependent and endothelium-independent mechanisms. Protein kinase A (PKA)- and TEA-sensitive K^+ channels may be involved in the NT-702–induced vasodilation. Moreover, hydrogen peroxide may contribute in part to the endothelium-dependent higher concentrations of NT-702–induced vasodilation.

Key words: NT-702, spinal cord, arteriole, K^+ channels, hydrogen peroxide.

It is widely accepted that resistance vessels such as small arteries and arterioles play significant roles in the regulation of local blood flow in the central nervous system [1]. However, there have been relatively few studies regarding the mechanical properties of resistance vessels in the spinal cord circulation. Our previous studies demonstrated that vasoactive physiological substances significantly contribute to the regulation of mechanical activity in resistance vessels isolated from spinal cords of dogs and rabbits via endothelium-dependent and endothelium-independent mechanisms [2–7]. It has also been found that spinal cord blood flow is controlled by the autonomic nervous system; α-adrenergic stimulation increases the blood flow in the rat spinal cord [8]. Thus humoral and neural factors are considered to play significant roles in the regulation of spinal cord circulation in the physiological conditions.

Numerous hydrophilic physiological substances bind to specific receptors located on the plasma membrane and subsequently increase or decrease the intracellular concentration of second messengers such as cAMP and cGMP. Changes in the concentration of cyclic nucleotides activate/inactivate the downstream protein kinases that directly influence cellular functions. The cyclic nucleotides are degraded by 3',5'-cyclic nucleotide phosphodiesterases (PDEs), whose activity is therefore important for the modulation of the cellular functions. PDE inhibitors have been clinically used for antithrombosis and vasodilation treatment in cardiovascular disease [9–11]. NT-702 (paragrelil hydrochloride, NM-702, 4-bromo-6-[3-(4-chlorophenyl)propoxy]-5-[pyridin-3-ylmethyl] amino)pyridazin-3(2H)-one hydrochloride) is a selective inhibitor of PDE 3 [12, 13]. NT-702 also inhibits platelet thromboxane A_2 synthesis and aggregation at concentrations that inhibit rabbit phosphodiesterase [12]. Recently, it has been reported that NT-702 experimentally and clinically improves intermittent claudication in model animals [13] and humans [14], suggesting that it could be useful for the treatment of peripheral arterial disease. However, there has been no report of the effects of NT-702 on the mechanical activity of resistance vessels in the central nervous system.

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The purpose of the present study was to investigate the effects of NT-702 on the mechanical activity of isolated rabbit spinal arterioles [5–7]. We further examined the involvement of endothelium-derived substances, including nitric oxide (NO), prostaglandins (PGs), and cytochrome P-450 monooxygenase–dependent molecules in the NT-702–mediated responses of the isolated spinal arterioles.

MATERIALS AND METHODS

Animals. Japanese white rabbits (male, 1.5–2.6 kg, n = 43, Kitayama Rabes, Japan) were used for the present study. The rabbits were housed in an environmentally controlled vivarium and fed a standard pellet diet and water ad libitum. The Animal Ethics Committee at Shinshu University School of Medicine approved all experimental protocols in accordance with the principles and guidelines of the Japanese Physiological Society.

Preparation of isolated spinal arterioles. The rabbits were anesthetized with pentobarbital sodium (40 mg/kg, i.v.) and killed by bleeding. The spinal cord (lumbar portion) was rapidly removed and placed in a cooled (4°C) dissection chamber filled with 3-(N-morpholino)propanesulfonic acid (MOPS)–buffered solution (145 mM NaCl, 4.70 mM KCl, 2.0 mM CaCl₂, 1.17 mM MgSO₄, 1.2 mM NaH₂PO₄, 5.0 mM glucose, 2.0 mM MOPS) containing bovine serum albumin (BSA, 1%). The pH of the MOPS solution with or without BSA was adjusted to 7.40 ± 0.02 by using a pH meter (F21 Horiba, Japan). The spinal arterioles (~90 μm in passive diameter and ~2 mm long) were carefully dissected and transferred to a vessel chamber with two glass micropipettes containing the MOPS/BSA solution. After each arteriole was mounted on a proximal pipette and secured with sutures, the perfusion pressure was raised to 20 cmH₂O to clear the intraluminal space of the spinal arteriole. Then the distal micropipette was connected via Tygon tubing; the outflow tubing was closed with the stopcock throughout the experiments. The perfusion pressure was kept at a 10 ml syringe and a stopcock, respectively. The intraluminal space in the spinal arterioles was kept at 80 cmH₂O by elevating the 10 ml syringe connected to the inflow tubing; the outflow tubing was closed with the stopcock throughout the experiments. The pressure was optimal to produce intrinsic myogenic activity of the isolated rabbit spinal arterioles [5–7]. The spinal arterioles were superfused with MOPS solution at a constant flow rate of 9 ml/min throughout the experiment. The spinal arterioles were then warmed slowly to 37°C and allowed to equilibrate for ~60 min.

Measuring the diameter of spinal arterioles. Images of the spinal arterioles were obtained by using an objective lens (10×), a photo-eyepiece lens (3.3×), and a monochrome charge-coupled device camera (KCB-270A KOKOM, Korea). Images were displayed on a monochrome TV monitor (C1846-03 Hamamatsu Photonic, Japan). Changes in the diameter of the spinal arterioles were manually measured with a diameter-detection device [15]. The images were also recorded on a video cassette recorder (VRS-800 Victor, Japan) for an off-line analysis.

Experimental protocols. To evaluate functional viability of the endothelial cells of the spinal arterioles, acetylcholine (ACh, 10⁻⁶ M) was first perfused extraluminally over all of the spinal arterioles before the experiments were started [5–7].

In the first protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁵ M), beraprost sodium (BPS, an analogue of PGI₂, 10⁻¹⁰ to 10⁻⁴ M), limaprost (an analogue of PGE₁, 10⁻¹⁰ to 10⁻⁵ M), and sodium nitroprusside (SNP, 10⁻⁶ to 10⁻⁴ M) on spinal arterioles with endothelium were examined. Our preliminary experiments indicated that the NT-702–mediated responses of the spinal arterioles with endothelium at concentrations ranging from 10⁻¹⁰ to 10⁻⁶ M was reproducible, whereas NT-702–mediated responses at 10⁻⁵ M prevented a return to control diameter even after rinsing the arterioles for 120 min. Therefore we subsequently used NT-702 concentrations ranging from 10⁻¹⁰ to 10⁻⁴ M.

In the second protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁶ M) on the spinal arterioles with or without endothelium were examined. To disrupt the endothelium in the spinal arterioles, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, 0.6%, 500–1,000 µl) was infused into the intraluminal space of the spinal arterioles for 120 s, and the lumen was then rinsed with MOPS solution for 10 min. CHAPS is a detergent that disrupts or eliminates the endothelial functions without changing the responsiveness of the smooth muscle cells of arterioles and microlymphatics [16–19]. We could not succeed in studying the NT-702–mediated responses between before and after the treatment with CHAPS because of a methodological limitation. Thus we compared the NT-702–mediated responses between the spinal arterioles with and without endothelium in this protocol. The endothelial viability was confirmed by the endothelium-dependent ACh-induced dilation of the spinal arterioles [7].

In the third protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁶ M) on the spinal arterioles with endothelium were examined in the absence or presence of Nω-nitro-L-arginine methyl ester, (an inhibitor of NO synthase, L-NAME, 3 × 10⁻⁵ M), indomethacin (an inhibitor of cyclooxygenase, 10⁻⁵ M), or sulfinaphazol (an inhibitor of cytochrome P-450 monooxygenase, 10⁻⁵ M). The concentration of L-NAME, indomethacin, or sulfinaphazol is sufficient to selectively inhibit the enzyme activity [5–7, 20].

In the fourth protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁶ M) on the spinal arterioles with endothelium were ex-
amined in the absence or presence of catalase (1,250 U/ml) [21].

In the fifth protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁶ M) on the spinal arterioles with endothelium were examined in the absence or presence of KT5720, (a selective PKA inhibitor, 3 × 10⁻⁷ M) [22].

In the final protocol, the effects of NT-702 (10⁻¹⁰ to 10⁻⁶ M) on the spinal arterioles with endothelium were examined in the absence or presence of tetraethylammonium, (TEA, a nonselective blocker of K⁺ channels, 3 × 10⁻³ M), iberiotoxin (IBTx, a selective blocker of large conductance of Ca²⁺-activated K⁺ channels, 10⁻⁷ M), or glibenclamide (a selective blocker of ATP-sensitive K⁺ channels, 10⁻⁵ M). Moreover, the effects of hydrogen peroxide (10⁻⁸ to 10⁻⁴ M) on the spinal arterioles with endothelium were examined in the absence or presence of TEA (3 × 10⁻³ M). At the end of each experiment, all arterioles were dilated completely with EGTA (5 × 10⁻³ M)-Ca²⁺-free MOPS solution containing nifedipine (10⁻⁵ M) to obtain the maximum diameter [5–7].

Drugs. NT-702 was donated by Taisho Pharmaceutical Co. Ltd. (Japan). ACh (Daiichi Sankyo Co. Ltd., Japan); BPS (Chinoin Co., Hungary); limaprost, KT5720 (Biomol International Inc., USA); SNP (Merck kGaA, Germany); MOPS, CHAPS, nifedipine, sulfaphenazol, indomethacin, L-NAME, catalase (Sigma, USA); EGTA (Dojindo Laboratories, Japan); and all salts and hydrogen peroxide (Wako Pure Chemical Industries Ltd., Japan) were used in the present study. The indomethacin and sulfaphenazol were dissolved in ethanol, and NT-702 and glibenclamide were dissolved in DMSO as stock solutions. These stock solutions were further diluted with MOPS solution. Each solvent at the final concentration was confirmed to produce no significant effect on the diameter of the spinal arterioles. The other drugs were directly dissolved in the MOPS solution. Drugs used in the present study, except for CHAPS, were perfused extraluminally over the spinal arterioles with and without endothelium.

Data analyses. One or two arterioles were used for each rabbit, and n indicates the numbers of the arterioles. Experimental data in the text, figures, and table are expressed as mean ± standard error of mean. Changes in the diameter of the spinal arterioles before and after the suction of drugs were normalized against the maximum diameter [5–7]. The E_max denotes the drug-induced maximum response. The pD₂ value represents the negative logarithm of ED₅₀, the concentration of drugs causing 50% of the maximum response [19]. The negative logarithm of ED₅₀ (–ED₅₀) was also calculated as the concentration of drugs causing 10% of the maximum response. Significant differences (p < 0.05) were determined by one-way ANOVA, followed by a Student-Newman-Keuls post hoc test or paired or unpaired Student’s t-test, as appropriate.
caused significant and dose-dependent vasodilation of the spinal arterioles with endothelium. Table 1 shows summarized data for the PD_2 value, –ED_{10}, and E_{max} values during drug-induced dilation of the spinal arterioles with endothelium. We defined the nearly maximum as the 10^{-5} M NT-702–induced vasodilation because more than 10^{-5} M NT-702 produced an irreversible vasodilation of the arterioles. The PD_2 value for BPS was significantly or slightly less than that obtained with NT-702, limaprost, or SNP. The –ED_{10} of NT-702 was significantly or slightly larger than that obtained with BPS, limaprost, or SNP. There were no significant differences in E_{max} between NT-702, BPS, limaprost, and SNP.

**Effects of the endothelium on NT-702–induced dilation**

ACh (10^{-6} M) significantly dilated the spinal arterioles with endothelium (67 ± 8%, n = 4), whereas the spinal arterioles without endothelium showed no significant dilation in response to 10^{-6} M ACh (1 ± 2%, n = 4, p < 0.05 v.s. with endothelium).

Figure 3a shows the summarized data for the effects of NT-702 on the spinal arterioles with (open circles, n = 4) or without (closed circles, n = 4) endothelium. The higher concentrations (10^{-7} and 10^{-6} M) of NT-702–induced dilation of the spinal arterioles without endothelium was significantly less than those obtained with the endothelium; at 10^{-7} M NT-702, the spinal arterioles with and without the endothelium were 69 ± 3% and 49 ± 6% dilated (p < 0.05 v.s. with intact endothelium), respectively.

**Effects of indomethacin, L-NAME, or sulfaphenazol on NT-702–induced dilation**

Table 2 shows the summarized data for the effects of indomethacin (10^{-5} M), L-NAME (3 × 10^{-5} M), or sulfaphenazol (10^{-5} M) on changes in the diameter of isolated rabbit spinal arterioles with endothelium. L-NAME significantly constricted the spinal arterioles with endothelium, but neither indomethacin nor sulfaphenazol affected the diameter of isolated rabbit spinal arterioles with endothelium, suggesting that isolated rabbit spinal arterioles with endothelium produced endogenous NO under the present experimental conditions.

There were no significant differences of NT-702–induced dilation between the absence and presence of 10^{-5} M indomethacin, 3 × 10^{-5} M L-NAME, or 10^{-5} M sulfaphenazol.

**Effects of catalase on NT-702–induced dilation**

Table 2 shows the summarized data for the effects of catalase (1,250 U/ml) on changes in the diameter of isolated rabbit spinal arterioles with endothelium. Catalase did not affect the diameter of isolated rabbit spinal arterioles with endothelium.

Figure 3b shows the summarized data for the effects of NT-702 on the spinal arterioles with endothelium in the absence (open circles) or presence (closed circles) of 1,250 U/ml catalase. Pretreatment with catalase significantly reduced the higher concentrations (10^{-7} and 10^{-6} M) of NT-702–induced vasodilation of the spinal arterioles with endothelium; at 10^{-7} M NT-702, the spinal arterioles with endothelium in the absence or presence of catalase were 46 ± 3% (n = 5) and 34 ± 3% dilated (n = 5, p < 0.05 v.s. with an absence of catalase), respectively.

**Effects of KT5720 on NT-702–induced dilation**

Table 2 shows the summarized data for the effects of KT5720 (3 × 10^{-7} M) on changes in the diameter of isolated rabbit spinal arterioles with endothelium. KT5720 did not affect the diameter of isolated rabbit spinal arterioles with endothelium.

Figure 4a shows the summarized data for the effects of NT-702 on the spinal arterioles with endothelium in the absence (open circles) or presence (closed circles) of 3 × 10^{-7} M KT5720. Pretreatment with KT5720 significantly reduced the NT-702–induced vasodilation of the spinal arterioles with endothelium; at 10^{-6} M NT-702, the spinal arterioles with endothelium in the absence or presence of KT5720 were 58 ± 5% dilated (n = 5, p < 0.05 v.s. absence of KT5720), respectively.

**Effects of TEA, iberiotoxin or glibenclamide on NT-702–induced dilation**

Table 2 shows the summarized data for the effects of
Table 1. PD₂ value, –ED₁₀, and Eₘₐₓ of NT-702, beraprost sodium (BPS), limaprost, and sodium nitroprusside (SNP) in isolated rabbit spinal arterioles with endothelium.

|                | NT-702 | BPS  | Limaprost | SNP  |
|----------------|--------|------|-----------|------|
| n              | 8      | 5    | 5         | 5    |
| PD₂ value      | 7.21 ± 0.06 | 6.74 ± 0.02 | 7.35 ± 0.04* | 7.18 ± 0.04 |
| –ED₁₀          | 9.05 ± 0.06* | 8.33 ± 0.06 | 8.74 ± 0.03  | 8.65 ± 0.04 |
| Eₘₐₓ (%)       | 77.71 ± 0.47 | 82.40 ± 1.37 | 83.00 ± 1.08 | 85.40 ± 0.59 |

* indicates significant difference (p < 0.05) from BPS.

Fig. 3. a: Effects of disruption of endothelium by CHAPS (0.6%) on the NT-702–induced vasodilation of the spinal arterioles. Open [EC(+), n = 4] and closed [EC(−), n = 4] circles indicate the spinal arterioles with and without endothelium, respectively. * denotes a significant difference (p < 0.05) from the spinal arterioles with endothelium. b: Effects of 1,250 U/ml catalase (n = 5) on the NT-702–induced vasodilation of the spinal arterioles with endothelium. Open and closed circles indicate the spinal arterioles in the absence and presence of catalase, respectively. * denotes a significant difference (p < 0.05) from the absence of catalase.

Table 2. Effects of indomethacin (10⁻⁵ M), L-NAME (3 x 10⁻⁵ M), sulfaphenazol (10⁻⁵ M), catalase (1,250 U/ml), KT5720 (3 x 10⁻⁷ M), TEA (3 x 10⁻³ M), IBTx (10⁻⁷ M), or glibenclamide (10⁻⁵ M) on changes in the diameter of isolated rabbit spinal arterioles with endothelium.

|                | Before (µm) | After (µm) |
|----------------|-------------|------------|
| Indomethacin (10⁻⁵ M) | 49 ± 7 | 47 ± 7 ns |
| L-NAME (3 x 10⁻⁵ M) | 59 ± 5 | 54 ± 5 *  |
| Sulfaphenazol (10⁻⁵ M) | 52 ± 3 | 52 ± 3 ns |
| Catalase (1,250 U/ml) | 44 ± 3 | 42 ± 4 ns |
| KT5720 (3 x 10⁻⁷ M) | 55 ± 4 | 57 ± 4 ns |
| TEA (3 x 10⁻³ M) | 55 ± 5 | 47 ± 4 ns |
| IBTx (10⁻⁷ M) | 54 ± 3 | 56 ± 3 ns |
| Glibenclamide (10⁻⁵ M) | 41 ± 5 | 41 ± 5 ns |

* and ns indicate significant (p < 0.05) and not significant difference from before, respectively.
TEA ($3 \times 10^{-3}$ M), iberiotoxin ($10^{-7}$ M), or glibenclamide ($10^{-5}$ M) on changes in the diameter of isolated rabbit spinal arterioles with endothelium. TEA, iberiotoxin, or glibenclamide did not affect the diameter of isolated rabbit spinal arterioles with endothelium.

Figure 4b shows the summarized data for the effects of NT-702 on the spinal arterioles with endothelium in the absence (open circles) or presence (closed circles) of $3 \times 10^{-3}$ M TEA ($n = 4$). Pretreatment with TEA significantly reduced the NT-702–induced vasodilation of the spinal arterioles with endothelium; at $10^{-7}$ M NT-702, the spinal arterioles with endothelium in the absence or presence of TEA were $61 \pm 4\%$ ($n = 4$) and $11 \pm 4\%$ dilated ($n = 4$, $p < 0.05$ v.s. absence of TEA), respectively. On the other hand, there was no significant effect of $10^{-7}$ M iberiotoxin or $10^{-5}$ M glibenclamide on the NT-702–induced vasodilation of the arterioles with endothelium.

**Effects of TEA on hydrogen peroxide–induced dilation**

Figure 5 shows the summarized data for the effects of hydrogen peroxide on the spinal arterioles with endothelium in the absence (open circles) or presence (closed circles) of $3 \times 10^{-3}$ M TEA. Hydrogen peroxide ($10^{-8}$ to $10^{-4}$ M) caused a dose-dependent vasodilation of the spinal arterioles with endothelium that was significantly reduced...
by the pretreatment with TEA (3 × 10⁻³ M). At 10⁻⁴ M hydrogen peroxide, the spinal arterioles with endothelium in the absence and presence of TEA were 21 ± 2% (n = 4) and 3 ± 2% dilated (n = 4, p < 0.05 v.s. absence of TEA), respectively.

**DISCUSSION**

**NT-702–mediated vasodilation of isolated rabbit spinal arterioles**

In the present study, we accepted BPS (an analogue of PGI₂), limaprost (an analogue of PGE₉), and SNP (NO donor), which have been known to increase intracellular concentration of cAMP or cGMP, to compare the vasodilator effectiveness of NT-702. A selective inhibitor of PDE 3, NT-702 caused a dose-dependent vasodilation of isolated rabbit spinal arterioles with endothelium as well as BPS, limaprost, or SNP. The PD₂ value of NT-702 was quite similar to that of limaprost and SNP. There were no significant differences in E_max between NT-702, BPS, limaprost, and SNP. The –ED₅₀ of NT-702 (9.05 ± 0.06) was higher than that of BPS (8.33 ± 0.06), limaprost (8.74 ± 0.03), or SNP (8.65 ± 0.04). These data suggest that NT-702 elicits a significant vasodilation of isolated rabbit spinal arterioles with endothelium at lower concentrations.

Previously, we reported that cilostazol, a selective PDE 3 inhibitor, caused a significant dilation of arterioles isolated from rabbit spinal cords (PD₃₀: 4.7 ± 0.1) [6] and brains (PD₃₀: 5.6 ± 0.1) [19]. Ishiwata et al. [13] demonstrated that NT-702 and cilostazol relaxed precontracted rat aortic rings and that the NT-702–mediated relaxation was 42 times more potent than that of cilostazol. It is therefore possible that NT-702 could produce dilation of spinal arterioles at lower concentrations than cilostazol and cause a significant increase in the blood flow of organs and peripheral tissues in vivo.

PDE inhibitors have become useful drugs for the treatment of cardiovascular disease because of their antithrombotic and dilatory effectiveness. Cilostazol has been clinically used for the treatment of chronic arterial occlusive diseases such as intermittent claudication [23–27]. Recently it has been demonstrated that NT-702 therapy improved the exercise performance of patients with peripheral arterial disease [14] and reduction of the walking distance in a rat experimental intermittent claudication model [13]. However, there have been no reports studying the effect of NT-702 on the vasoreactivity of spinal and cerebral resistance vessels. Furthermore, it has become clear that protection of the spinal cord circulation should be considered during thoracic and abdominal aortic surgery with aortic cross clamping [28, 29], and that PGE₉ is an effective drug for protection against ischemia of the spinal cord [30]. The present study is the first to show that NT-702 is a potent vasodilator of the resistance vessels in the central nervous system in vitro and to suggest that NT-702 could be used to treat cerebral and spinal vascular diseases as well as peripheral circulatory disorders. The present study is limited by a focus on spinal arterioles. Thus further comprehensive studies on other resistance vessels will be necessary in the future.

**Involvement of the endothelium in NT-702–mediated vasodilation**

At higher concentrations of NT-702 (10⁻⁴ to 10⁻⁶ M), the NT-702–induced vasodilation of the spinal arterioles was significantly reduced by disruption of the endothelium. These findings suggest that the higher concentrations of NT-702 cause a mixed vasodilation resulting from endothelium-dependent and endothelium-independent mechanisms, and that most of the response is attributable to the endothelium-independent mechanism. We further examined the effects of enzyme inhibitors, including NO synthase (L-NAME), cyclooxygenase (indomethacin), and cytochrome P-450 monoxygenase (sulfaphenazol) on the NT-702–induced vasodilation of the spinal arterioles with intact endothelium. The concentration of each inhibitor was effective to significantly reduce the activity of the respective enzyme [5–7, 20]. Treatment with L-NAME, indomethacin, or sulfaphenazol did not significantly affect NT-702–induced vasodilation, suggesting that endothelial cells of the spinal arterioles do not produce endogenous NO, PGs, or cytochrome P-450–dependent EDHF. Kawai et al. [3] reported that histamine H₂-mediated endothelium-dependent relaxation of canine spinal artery was independent on PGs, lipoxygenase, and NO, suggesting that EDHF may be involved.

It has become well known that in response to blood flow or chemical agonists, endothelial cells produce hydrogen peroxide (as one of the EDHFs) in coronary arteries and arterioles [21, 31, 32], mesenteric arteries [33], and pial arterioles [34] in human and animals [35]. In these experiments, treatment with catalase, a scavenger of hydrogen peroxide, significantly reduced the endothelium-derived hydrogen peroxide–mediated vasodilation. These results may be compatible with our present study that the NT-702–induced vasodilation of the spinal arterioles with endothelium was significantly reduced by pretreatment with catalase. Furthermore, hydrogen peroxide significantly dilated the isolated spinal arterioles with intact endothelium. However, we have not confirmed that hydrogen peroxide does not affect the endothelium-independent vasodilation of the spinal arterioles because of experimental difficulties. Further investigation, therefore, is necessary to evaluate the production of hydrogen peroxide from the endothelium of spinal arterioles because endothelium-dependent hydrogen peroxide–mediated vasodilation was observed only at the higher concentrations of NT-702. Moreover, further investigation will be needed to examine whether the vasodilation is a PDE 3 inhibitor-specific phenomenon, or an NT-702–specific
Roles of PKA inhibitors in NT-702–mediated vasodilation

It is well known that the substrate specificity of PDE 3 is cAMP > cGMP; therefore, PDE 3 inhibitors will increase the intracellular cAMP concentration [9–11]. Downstream effector proteins of cAMP and cGMP include PKA, PKG, cyclic nucleotide-gated ion channels, and cAMP-regulated guanine nucleotide exchanger factors [11]. H89, an inhibitor of PKA, did not affect cilostazol-induced vasodilation of isolated cerebral penetrating arterioles, suggesting that the PDE 3 inhibitor cilostazol diluted the cerebral penetrating arterioles via a cAMP-PKA independent pathway [19]. In the present study, we demonstrated that KT5720, a specific PKA inhibitor, significantly reduced the NT-702–induced vasodilation of the spinal arterioles with endothelium, although the reduction was not complete. These findings suggest that NT-702–mediated vasodilation of the spinal arterioles in the present study may be partially dependent on PKA. Since NT-702 has an IC50 of 87 nM on PDE 5 [13], it is possible that the NT-702–induced vasodilation at higher concentrations of 10−3 to 10−6 M may be related partially to PDE 5 inhibition. Further investigation will be also needed to evaluate the crucial roles of PKA inhibitors in the NT-702–induced vasodilation by using other more endothelium-specific PDE inhibitors, such as PDE 4 or PDE 5 inhibitors.

Roles of K+ channels blockers in NT-702–mediated vasodilation

In cerebral arterioles, it has been clear that K+ channels greatly contribute to the regulation of the arteriolar vasoreactivity [36]. Voltage-dependent and inward-rectifier, but not ATP-sensitive K+ channels, are active under basal conditions and maintain vasomotor reactivity in isolated rat cerebral and brain stem arterioles [37, 38]. Regional heterogeneities of Ca2+-activated K+ channels exist between cerebral and brain stem arterioles [37]. Moreover, extracellular acidosis dilates isolated rat cerebral and brain stem arterioles through the relaxation of arteriolar smooth muscles via ATP-sensitive K+ channel–dependent mechanisms [39]. In contrast to the cerebral vessels, there have been no studies of the involvement of K+ channels in the regulation of spinal arterioles. The present study indicated that TEA (nonselective blocker of K+ channels) significantly reduced NT-702–induced vasodilation of the spinal arterioles with endothelium, whereas the presence of iberiotoxin (a selective blocker of large conductance of Ca2+-activated K+ channels) or glibenclamide (a selective blocker of ATP-sensitive K+ channels) did not affect the NT-702–induced vasodilation of the spinal arterioles with endothelium. TEA is a nonspecific K+ channel blocker that modulates voltage-gated and Ca2+-activated K+ channels [40]. These findings suggest that NT-702–mediated vasodilation of isolated rabbit spinal arterioles may be controlled by the opening of voltage-gated K+ channels. Hydrogen peroxide caused a significant vasodilation of isolated spinal arterioles with endothelium in the present study. In the presence of TEA, hydrogen peroxide-mediated vasodilation of the spinal arterioles with endothelium was significantly reduced. Matoba et al. [21] reported that hydrogen peroxide–mediated relaxation of isolated murine mesenteric arteries without endothelium was significantly reduced by treatment with tetrabutylammonium (a nonspecific inhibitor of Ca2+-activated K+ channels), but not apamin (an inhibitor of small conductance Ca2+-activated K+ channels) plus charybdotoxin (an inhibitor of large and intermediate conductance Ca2+-activated K+ channels). Regarding endothelium-independent mechanisms in the NT-702–induced vasodilation, there was a significant difference in the extent of the inhibitory effect between TEA and KT5720, suggesting an involvement of other intracellular mechanisms to open K+ channels, such as cyclic nucleotide-gated ion channels other than PKA. Further investigation is necessary to evaluate the specific K+ channels in the NT-702– and hydrogen peroxide–mediated vasodilations of spinal arterioles with intact endothelium.

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