

Physiological Research Pre-Press Article

Vasorelaxing Action of Vasonatrin Peptide is Associated with Activation of Large-conductance Ca^{2+} -activated Potassium Channels in Vascular Smooth Muscle Cells

Short title: VNP Enhances BK_{Ca} in VSMCs

Jun Yu^{1,*}, Miao Zhang Zhu^{2,*}, Zhaojun Fu^{3,*}, Xiaoxing Zhu⁴, Yufeng Zhao²,
Baoying Chen⁵

* These authors contributed equally.

¹ *Preclinical Experiment Center, Fourth Military Medical University, Xi'an, Shaanxi, China*

² *Department of Physiology, Fourth Military Medical University, Xi'an, Shaanxi, China*

³ *Center of clinical aviation medicine, General Hospital of Air Force, Beijing, China*

⁴ *Department of Pharmacology, Fourth Military Medical University, Xi'an, Shaanxi, China*

⁵ *Department of Radiology, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi, China*

Correspondence to: Dr. Baoying Chen, Department of Radiology, Tangdu Hospital, Fourth Military Medical University, 1 Xinsi Road, Xi'an, Shaanxi, 710038, China. Tel.: 86-29-84777617; E-mail: chenby128@yahoo.com;

Dr. Jun Yu, Department of Preclinical Experiment Center, Fourth Military
Medical University, 169 Changle West Road, 710032 Xi'an, China Tel.:
86-29-84774766; E-mail: pclamper@163.com

Summary

The aim of this study was to test the hypothesis that vasorelaxing action of vasonatrin peptide (VNP) is due to activation of the large-conductance Ca^{2+} -activated potassium channel (BK_{Ca}) via guanylyl cyclase (GC)-coupled natriuretic peptide receptors (NPRs) in vascular smooth muscle cells (VSMCs). Contraction experiments were performed using human radial artery whereas BK_{Ca} current by patch clamp was recorded in cells from rat mesenteric artery. Contractility of rings cut from human radial artery was detected in vitro. As a result, VNP induced a dose-dependent vasorelaxation of human radial artery, which could be mimicked by 8-Br-cGMP, whereas suppressed by TEA, a chemical blocker of BK_{Ca} , HS-142-1, a blocker of GC-coupled NPRs, or Methylene Blue (MB), a selective inhibitor of guanylyl cyclase. Sequentially, whole-cell K^+ currents were recorded using patch clamp techniques. BK_{Ca} current of VSMCs isolated from rat mesentery artery was obtained by subtracting the whole cell currents after applications of 10^{-7} mol/l Iberiotoxin (IBX) from before applications of it. In accordance with the results of arterial tension detection, BK_{Ca} current was significantly magnified by VNP, which could also be mimicked by 8-Br-cGMP, whereas suppressed by HS-142-1, or MB. Taken together, VNP acts as a potent vasodilator, and NPRA/B-cGMP- BK_{Ca} is one possible signaling system involved in VNP induced relaxation.

Key words

Natriuretic peptides, Vasonatrin peptide, Vascular smooth muscle cells, Calcium-activated potassium channels, Cyclic guanosine monophosphate

Introduction

The dysregulation of vascular diastolic and systolic functions is culpable for cardiovascular disease like hypertension. The cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) is the most important signal transduction element in maintaining myogenic tone and triggering cell contraction (Jackson 2000). Membrane potential (E_m) in VSMCs (vascular smooth muscle cells) plays a critical role in regulating $[\text{Ca}^{2+}]_{\text{cyt}}$ by governing the activity of voltage-dependent Ca^{2+} channels (VDCs) (Nelson and Quayle 1995) and by facilitating the production of inositol (1,4,5)-triphosphate [$\text{Ins}(1,4,5)\text{P}_3$], which opens Ca^{2+} -release channels in the sarcoplasmic reticulum and triggers Ca^{2+} release (Ganitkevich and Isenberg 1993). K^+ channels are ion-conductive pathways playing a dominant role in the maintenance of resting E_m of VSMCs (Jackson 2000, Nelson and Quayle 1995). Because of the existing electrochemical gradient for K^+ , opening of K^+ channels leads to diffusion of K^+ out of the cells and membrane hyperpolarization, conversely, closure of K^+ channels leads to depolarization. Membrane depolarization opens VDCs, whereas hyperpolarization closes them (Jackson 2000). The roles of large-conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels in maintaining and regulating E_m and myogenic tone have been studied most widely (Nelson and Quayle 1995). Acute increases in intravascular pressure produce depolarization and constriction of arteries in vitro (Brayden and Nelson 1992, Harder *et al.* 1999, Knot and Nelson 1998) and in vivo (Paternò *et al.* 2000). Several lines of evidence suggest that BK_{Ca} channels in VSMCs participate in the autoregulation of vascular tone

during acute pressure increases. In hypertensive animals, a sustained elevation in blood pressure is associated with loss of BK_{Ca} channels in VSMCs (Cox *et al.* 2001). Overexpression of BK_{Ca} channels has been proposed to be a universal protective mechanism to buffer the increased vasoreactivity and limit active vasoconstriction during hypertension (Brayden and Nelson 1992, Cox *et al.* 2001, Jackson 2000, Liu Y *et al.* 1998, Nelson and Quayle 1995, Paternò *et al.* 1997, Paternò *et al.* 2000, Rusch and Runnells 1994).

The natriuretic peptides (NPs) are a family of structurally similar but genetically distinct peptides including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C type natriuretic peptide (CNP). NPs are known to play important roles in the control of cardiorenal homeostasis. ANP is a 28-amino acid peptide, whereas BNP contains 32 amino acids and CNP contains 22. All of them have a 17-amino acid ring structure which has been identified as essential for their pharmacological activity. Vasonatin peptide (VNP), the man-made novel member of the NPs family, is a chimera of CNP and ANP (Wei *et al.* 1993). This synthetic peptide possesses the 22-amino acid ringed structure of CNP, along with the COOH terminus of ANP. Most functions of NPs appear to be mediated through the elevation of intracellular cyclic 3',5'-guanosine monophosphate (cGMP) after their binding to natriuretic peptide receptors, NPR-A and NPR-B, which are coupled to the particulate guanylyl cyclase and blocked by selective NPR-A/NPR-B antagonist HS-142-1, a polysaccharide isolated from *Aureobasidium* (Morishita *et al.* 1991). ANP and BNP mainly bind to NPR-A, while CNP functions via NPR-B. Recently, the functional receptor subtype for VNP was determined as NPR-A (Woodard *et al.* 2005).

Since invented and reported by Wei in 1993, VNP has been demonstrated both in vitro and in vivo as a potential vasodilator of human intramammary artery (Yu *et al.* 2003), pulmonary artery, abdominal aorta and celiac vein from rat (Feng *et al.* 1999), aorta from Wistar-Kyoto rats and spontaneously hypertensive rats (Wei *et al.* 1994), canine femoral, saphenous, and renal arteries (Wei *et al.* 1993). However, to the best of our knowledge, the signaling pathways underlying the vasorelaxing effects of VNP are not well-understood. Thus, the aim of present study was to highlight possible signaling transduction mechanisms involved in VNP-induced vasorelaxation.

Materials and Methods

Reagents

VNP was synthesized at the Shanghai Institute of Biochemistry of China. HS-142-1 was kindly provided by Pro. Chiming Wei (John Hopkins University, USA). Iberiotoxin (IBX), L-norepinephrine bitartrate (NE), acetylcholine chloride (Ach), 8-bromo-cGMP, Methylene Blue (MB), papain, dithioerythritol and TEA (tetraethylammonium chloride) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The working concentrations of 8-bromo-cGMP (Galvez *et al.* 1990), TEA (Nelson *et al.* 1990), MB (Okamura *et al.* 1997, Peral de Bruno *et al.* 1999), IBX (Galvez *et al.* 1990) and HS-142-1 (Morishita *et al.* 1991) we used in present study have been reported before. All drugs were dissolved in distilled water immediately before study, added directly into the bath and the concentrations given are the calculated final

concentrations in the bath solution.

Arterial Ring Preparation and Vasoreactivity Measurement

Human radial arteries were obtained from patients undergoing coronary artery bypass surgery by an explant technique, with the approval of the Hospital Ethical Committee and signed consent of the patients. Rings cut from human radial arteries were suspended for the measurement of isometric force in organ chambers filled with Krebs solution, maintained at 37 °C and bubbled with a gas mixture of 95 % O₂-5 % CO₂ (Warshaw *et al.* 1979, Zhang *et al.* 2001). The Krebs solution contained (in 10⁻³ mol/l) 115 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 MgCl₂, 1.2 KH₂PO₄, 2.5 CaCl₂, and 10 glucose; pH 7.4. In one-half of the rings, the endothelium was removed by gently rubbing the intimal surface with a cotton swab wet with control solution. For each individual vessel ring, the proper length-tension relationship during repeated exposures to 10⁻¹ mol/l KCl was determined by 100-mg increments until an optimal resting force around 500 mg was identified. All the subsequent pharmacological examinations were conducted at this initial resting force.

Experimental procedure is as follows. After 30 min of equilibration, the presence of functional endothelium was determined at the beginning of the experiment by relaxation to acetylcholine (10⁻⁶ mol/l) during a contraction to norepinephrine (10⁻⁶ mol/l) at optimal length. In order to study the vasorelaxant properties of VNP or 8-Br-cGMP in the RA, the segments were initially contracted with norepinephrine (10⁻⁶ mol/l), which elicited 80 % of maximal contraction. When the contraction reached a steady state, cumulative concentration-response curves to VNP or 8-Br-cGMP were obtained by adding

increasing logarithmic molar concentrations indicated. A subsequent concentration was added to the organ bath after the previous concentration had produced its equilibrium response or after 10 min if no response was obtained. Relaxation produced by each concentration of VNP or 8-Br-cGMP was measured and expressed as a percentage of the maximum possible relaxation (i.e., relaxation back to the baseline tension). The concentration of VNP or 8-Br-cGMP-producing 50% of its own maximum response (EC50) was determined for each curve by using a non-linear least square fitting procedure of the individual experimental data. Experiments followed a multiple curve design.

Therefore, the following protocol was used: 1) contraction to norepinephrine and concentration-response curve to VNP or 8-Br-cGMP, followed by three washes (15 min/wash), addition of selective inhibitors such as HS-142-1, TEA or MB and a 20 min equilibration period; 2) contraction to norepinephrine and the concentration-response curve to VNP.

Electrophysiological Measurement

Electrophysiological measurements were performed as previously described (Jackson *et al.* 1996). Animal experiments were approved by University Ethics committee. The superior mesenteric artery with its branches in the mesentery were removed from Sprague-Dawley (SD) rats anesthetized with ketamine/xylazine (0.1 ml/100 g, IP) and placed in 4°C cold physiological salt solution (PSS). PSS contained (in 10⁻³ mol/l) 137 NaCl, 5.6 KCl, 1 MgCl₂, 0.42 Na₂HPO₄, 0.44 NaH₂PO₄, 4.2 NaHCO₃, and 10 HEPES, bubbled with 95 % O₂-5 % CO₂; pH was adjusted to 7.4 with NaOH. The tissues were then placed in a petri dish with PSS containing 1 mg/ml BSA and 30 μmol/l sodium nitroprusside.

The second- to sixth-order small mesenteric arteries and arterioles (250.6 ± 45.7 μm in diameter) were isolated, dissected free of connective and fat tissues, and then cut into 1- to 2-mm in length. Segments from vessels were digested in separate test tubes with 5 mg/ml papain, 2 mg/ml dithioerythritol, and 1 mg/ml BSA in PSS at 37°C for 20 min. Tissue was then transferred to enzyme-free, BSA-containing PSS, stored for 10 min, and triturated with a flame-polished pipette to disperse VSMCs. The suspension was stored in Ca^{2+} -free PSS at 4°C for use within 6 h.

Whole cell currents were recorded with Axon 200B amplifier (Axon Instruments, Burlingame, CA, USA). Patch pipettes (tip resistance, 2-6 M Ω) were fabricated on an electrode puller (PP830, Narishige Scientific Instrument Lab, Tokyo, Japan) with the use of borosilicate glass tube. The perfusion bath (extracellular) solution contained (in 10^{-3} mol/l) 135 NaCl, 4 KCl, 1 MgCl_2 , 2 CaCl_2 , 10 HEPES, and 10 glucose, bubbled with 95 % O_2 -5 % CO_2 ; pH was adjusted to 7.4 with NaOH. The pipette solution contained (in 10^{-3} mol/l) 143 KCl, 1 MgCl_2 , 0.5 EGTA, and 10 HEPES; pH was adjusted to 7.2 by KOH. Command-voltage protocols and data acquisition were performed using pCLAMP software (version 8.0, Axon Instruments, Burlingame, CA, USA). Step-pulse protocols and data acquisition were performed at room temperature (22-24°C). Currents were filtered at 4 KHz and digitized at 1 KHz. Gigaseal was obtained as first. For measurement of whole cell K^+ currents, the VSMC was perforated by applying a negative suction to the surface of the cell placed onto the bottom of a 2-ml recording chamber. In the whole-cell mode after perforation, cell capacitance and access resistance were estimated from the capacitive current transient evoked by applying a 20-mV pulse for 40 ms from a holding

potential of -60 to -40 mV. I-V relationships were generated in voltage-clamped cells held at an E_m of -70 mV and then stepped in 10-mV increments from -60 to +60 mV. Voltage steps were 1500 ms in duration and 10-s intervals were allowed between steps. Currents were filtered at 1 kHz and sampled at 4 kHz. Currents during the last 400 ms in each step of two or three voltage-clamp trials were sampled and averaged before analysis of the current amplitudes. Currents were normalized to cell capacitance to obtain the current densities.

To separate BK_{Ca} from total currents, the following protocol was adopted. First, whole cell currents were recorded in two trials separated by 2 min, and the currents were then recorded after 3, 5, and 10 min after the perfusion fluid was changed to fluid containing 10^{-7} mol/l Iberitoxin (IBX). IBX blocks BK_{Ca} channels and has not been shown to block any other channel (Galvez *et al.* 1990). By subtracting the average currents after each perfusion changing from that of before, we obtained the BK_{Ca} currents in succession.

The following protocol was performed: 1) Recording of total K^+ currents before and after adding 10^{-6} mol/l VNP or 10^{-5} mol/l 8-Br-cGMP, subtraction BK_{Ca} by IBX, followed by three washes (10 min/wash) 2) Pretreatment with or without 2×10^{-5} mol/l HS-142-1, or 10^{-5} mol/l MB 10 min before adding 10^{-6} mol/l VNP, recording of total K^+ and subtraction of BK_{Ca} by IBX.

Statistical Analyses

Results are expressed as the means \pm SD. One-way analysis of variance (ANOVA) was used to determine the overall differences in K^+ current density and vasoreactivity among different groups. Student's *t*-test was used to

determine the differences between 2 different groups. Statistical significance was determined at $p < 0.05$ calculated by SPSS, version 10.0 (SPSS Science, Chicago, IL, USA).

Results

VNP markedly relax human radial arteries

Before identification of signaling pathways, we first testified the effects of VNP on the contraction of human radial arteries. As shown in Figure 1A, VNP reduced the contractile responses of the arterial ring to 10^{-6} mol/l NE in a dose dependent manner. The equivalent responses of deendothelium and endothelium-intact artery rings to VNP indicated that vasodilating actions of VNP are independent of endothelium, as reported previously (Feng *et al.* 1999).

Activation of BK_{Ca} participated in the vasorelaxing role of VNP

TEA is a chemical blocker of BK_{Ca}. The half-block concentrations (K_i) of TEA for BK_{Ca} channels is 2×10^{-4} mol/l. At concentrations below 10^{-3} mol/l, TEA preferentially blocks BK_{Ca} channels (Nelson *et al.* 1990). Thus, we used 10^{-3} mol/l TEA as blocker of BK_{Ca} in the in vitro perfusion experiments of human radial artery. As shown in Figure 2A, addition of TEA elicited significant reductions of VNP-evoked vasorelaxation, indicating critical role of BK_{Ca} involved.

To further rule out the role of BK_{Ca} in the pharmacological activity of VNP, we performed whole-cell patch clamp to test the status of BK_{Ca} in VSMCs exposed to VNP. As a result, a family of voltage-dependent, outward K⁺ currents

was elicited by depolarizing the cell from a holding potential of -70 mV to a series of command potentials ranging from -60 to +60 mV (Fig. 3A). The total K⁺ currents were enhanced in the presence of VNP (Fig. 3B). Extracellular application of 10⁻⁷ mol/l IBX, a highly potent blocker of BK_{Ca} markedly reduced the amplitude of the K⁺ currents and diminished the current noise associated with higher positive command potentials (Fig. 3C). These properties suggest that the outward currents blocked by IBX were currents through BK_{Ca} channels (Nelson and Quayle 1995).

In Figure 4A, I-V relationships were further expressed in terms of current density, the ratio of total K⁺ current and estimated membrane capacitance. Compared with control group, the BK_{Ca} current density was enhanced by VNP significantly ($p < 0.05$).

VNP induced BK_{Ca} activation and vasorelaxation via NPRA/B coupled with guanylyl cyclase

Due to homologous structure, it is speculated that VNP might act via the same signaling transduction pathway as other members of NPs to increase the intracellular cGMP levels via guanylyl cyclase (GC)-coupled NPRA/B (Misono KS, 2002). The involved receptor was determined by application of HS-142-1, which reduced the vasorelaxation (Fig. 2B), as well as BK_{Ca} activation (Fig. 4 B) by VNP. VNP-evoked vasorelaxation (Fig. 1B) and enhancement of BK_{Ca} (Fig. 4A) could be mimicked by 8-Br-cGMP, a membrane-permeable homologue of cGMP, whereas be suppressed by MB, a selective inhibitor of GC (Fig. 2C,4B), indicating role of cGMP as intracellular secondary messenger downstream of NPR in the signaling transduction of VNP.

Discussion

The current study gives evidences that NPRA/B-cGMP-BK_{Ca} signaling pathway participates in vasorelaxation induced by VNP. This conclusion is based on following findings: First, whole-cell recording mode of patch clamp detected enhancement of BK_{Ca} current after application of VNP. Second, 10⁻³ mol/l TEA, a blocker of BK_{Ca}, reversed vasorelaxing effect of VNP. Third, VNP-induced activation of BK_{Ca} and relaxation of arteries was mimicked by 8-Br-cGMP, but suppressed by MB (inhibitor of guanylyl cyclase). Fourth, diminishing effects of HS-142-1, a blocker of NPR-A/B suggested critical role of NPR-A/B involved.

The reason why we select human radial artery to identify the relaxing effect of VNP is that autogenous radial artery, along with saphenous vein (SV) and internal mammary artery (IMA) are the most frequently used grafts in coronary artery bypass grafting (CABG). Just as evaluating of the anti-proliferation effects by VNP on SV and IMA (Lu SY *et al.* 2004), the current study give evidence that VNP possesses function of relaxation on the human radial artery. It suggests that VNP is favorable for the patients with radial artery spasm after CABG. In the following electrophysiological experiments, we isolated VSMCs from rat mesentery arteries, rather than human radial artery, because clinical samples of human radial artery are not easy to be obtained, and the percentage of VSMCs are relatively less in collagen-rich arteries than in small resistance arteries.

We have previously reported that VNP attenuates the enhancement

of $[Ca^{2+}]_{\text{cyt}}$ in rat cardiac myocytes and fibroblasts (Guo *et al.* 2004, Guo *et al.* 2001). In VSMCs, the regulation of $[Ca^{2+}]_{\text{cyt}}$ by VNP has not been reported. However, findings about other members of NPs, such as ANP and CNP, suggest that NPs are negative regulator of intracellular Ca^{2+} signal in VSMCs (Olivera *et al.* 1994, Shin *et al.* 1989, Ding *et al.* 1999). Although direct evidences of $[Ca^{2+}]_{\text{cyt}}$ decrease by VNP were not shown in this study, we found VNP-induced activation of BK_{Ca} , which has been widely known to decrease $[Ca^{2+}]_{\text{cyt}}$ and contractile tone of VSMCs (Kudlacek *et al.* 2003, Cornwell and Lincoln 1989, Taguchi *et al.* 1997, Lincoln *et al.* 1994). Although our data are encouraging, we emphasize that further work in single-channel recording mode would provide a deeper insight into the regulation of VNP on ion channels. Beside BK_{Ca} , other kinds of channels, such as voltage-gated K^+ channel (K_v) and ATP-sensitive K^+ channel (K_{ATP}) are also critical components regulating $[Ca^{2+}]_{\text{cyt}}$ and vascular contractions. ANP has been reported to be able to modulate the gating of K_{ATP} channels in cultured VSMCs (Kubo *et al.* 1994). The investigation about effects of VNP on K_v and K_{ATP} are underway.

As a man-made member of NPs, VNP was reported to have similar, but different cardiovascular bioactivity compared with natural NPs, such as ANP, BNP and CNP. Feng *et al.* proved VNP to be more potential than ANP or CNP to relax pulmonary and abdominal artery (Feng *et al.* 1999). Similarly, VNP, rather than ANP or CNP, is more effective to inhibit the proliferation of pulmonary VSMCs (Dong *et al.* 2000). Allowing for critical role of BK_{Ca} in the regulation of contraction and proliferation of VSMCs, it is tempting to speculate that activating effects of VNP on BK_{Ca} might be stronger than that of ANP or CNP, which is worthy of investigation and might account for the previously reported differences

between VNP and natural NPs on vasorelaxation and proliferation of VSMCs.

In conclusion, VNP is a potent opener of BK_{Ca}, which opening is one possible signaling for VNP induced vasorelaxation. Other channels, especially TEA sensitive voltage-gated K⁺ channels and K_{ATP} channels may also be involved.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

This work was supported by the Chinese Natural Science Fund (No. 39970327, 30801385 and 30800478).

References

BRAYDEN JE, NELSON MT: Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* **256**: 532-535, 1992.

CORNWELL TL, LINCOLN TM: Regulation of intracellular Ca²⁺ levels in cultured vascular smooth muscle cells. Reduction of Ca²⁺ by atriopeptin and 8-bromo-cyclic GMP is mediated by cyclic GMP-dependent protein kinase. *J Biol Chem* **264**: 1146-1155, 1989.

COX RH, LOZINSKAYA I, DIETZ NJ: Differences in K⁺ current components in mesenteric artery myocytes from WKY and SHR. *Am J Hypertens* **14** :897-907, 2001.

DING KH, ALI N, ABDEL-LATIF AA: Atrial natriuretic peptide provokes a

dramatic increase in cyclic GMP formation and markedly inhibits muscarinic-stimulated Ca^{2+} mobilisation in SV-40 transformed cat iris sphincter smooth muscle (SV-CISM-2) cells. *Cell Signal* **11**:87-94, 1999.

DONG MQ, ZHU MZ, YU J, SHANG LJ, FENG HS: Comparison of inhibitory effects of three natriuretic peptides on the proliferation of pulmonary artery smooth muscle cells of rats. *Sheng Li Xue Bao* **52**: 252-254, 2000.

FENG HS, ZANG YM, ZHU MZ, PEI JM, WANG YM, WANG L, SHI PT: Comparison of vasorelaxing actions of vasonatin peptide, C-type natriuretic peptide and atrial natriuretic peptide. *Sheng Li Xue Bao* **51**:515-520, 1999.

GALVEZ A, GIMENEZ-GALLEGO G, REUBEN JP, ROY-CONTANCIN L, FEIGENBAUM P, KACZOROWSKI GJ, GARCIA ML: Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. *J Biol Chem* **265**: 11083-11090, 1990.

GANITKEVICH VYA, ISENBERG G: Membrane potential modulates inositol 1,4,5-trisphosphate-mediated Ca^{2+} transients in guinea-pig coronary myocytes. *J Physiol* **470**: 35-44, 1993.

GUO HT, ZHU MZ, LU SY, YU J, DONG MQ, GAO Z, SHI PT: Vasonatin peptide attenuates hypoxia-induced increase in $[\text{Ca}^{2+}]_i$ of cultured rat cardiac fibroblasts. *Sheng Li Xue Bao* **53**: 286-290, 2001.

GUO HT, ZHU MZ, ZHANG RH, BI H, ZHANG B, ZHANG HF, YU J, LU SY, PEI JM: Vasonatin peptide attenuates the enhancement of

- electrically-induced intracellular calcium transient by isoproterenol in rat cardiac myocytes. *Sheng Li Xue Bao* **56**: 335-40, 2004.
- HARDER DR, SMEDA J, LOMBARD J: Enhanced myogenic depolarization in hypertensive cerebral arterial muscle. *Circ Res* **57**: 319-322, 1985.
- JACKSON WF, HUEBNER JM, RUSCH NJ: Enzymatic isolation and characterization of single vascular smooth muscle cells from cremasteric arterioles. *Microcirculation* **3**: 313-328, 1996.
- JACKSON WF: Ion channels and vascular tone. *Hypertension* **35**: 173-178, 2000.
- KNOT HJ, NELSON MT: Regulation of arterial diameter and wall $[Ca^{2+}]$ in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol* **508**: 199-209, 1998.
- KUBO M, NAKAYA Y, MATSUOKA S, SAITO K, KURODA Y: Atrial natriuretic factor and isosorbide dinitrate modulate the gating of ATP-sensitive K^+ channels in cultured vascular smooth muscle cells. *Circ Res* **74**: 471-476, 1994.
- KUDLACEK PE, PLUZNICK JL, MA R, PADANILAM B, SANSOM SC: Role of hbeta1 in activation of human mesangial BK channels by cGMP kinase. *Am J Physiol Renal Physiol* **285**: F289-294, 2003.
- LINCOLN TM, KOMALAVILAS P, CORNWELL TL: Pleiotropic regulation of vascular smooth muscle tone by cyclic GMP-dependent protein kinase. *Hypertension* **23**: 1141-1147, 1994.
- LIU Y, HUDETZ AG, KNAUS HG, RUSCH NJ: Increased expression of Ca^{2+} -sensitive K^+ channels in the cerebral microcirculation of genetically hypertensive rats: evidence for their protection against

- cerebral vasospasm. *Circ Res* **82**: 729-737, 1998.
- LU SY, ZHU MZ, WANG DS, CHEN SY, ZHANG WD, DONG H, YU J, GUO HT:
Inhibition of the proliferation of smooth muscle cells from human coronary bypass vessels by vasonatrin peptide. *Physiol Res* **53**: 387-393, 2004.
- MISONO KS: Natriuretic peptide receptor: structure and signaling. *Mol Cell Biochem* **230**: 49-60, 2002.
- MORISHITA Y, SANO T, ANDO K, SAITOH Y, KASE H, YAMADA K, MATSUDA Y:
Microbial polysaccharide, HS-142-1, competitively and selectively inhibits ANP binding to its guanylyl cyclase-containing receptor. *Biochem Biophys Res Commun* **176**: 949-957, 1991.
- NELSON MT, PATLAK JB, WORLEY JF, STANDEN NB: Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* **259**: C3-C18, 1990.
- NELSON MT, QUAYLE JM: Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* **268**: C799-C822, 1995.
- OKAMURA T, AYAJIKI K, KANGAWA K, TODA N: Mechanism of adrenomedullin-induced relaxation in isolated canine retinal arteries. *Invest Ophthalmol Vis Sci* **38**: 56-61, 1997.
- OLIVERA A, CAMELO C, ARRIBA G, LAMAS S, RODRIGUEZ-PUYOL D, SCHRIER RW, RODRIGUEZ-BARBERO A, LÓPEZ-NOVOA JM: Effect of atrial natriuretic peptide and calcium antagonists on platelet-activating factor-induced contraction and intracellular calcium mobilization in rat mesangial cells. *J Cardiovasc Pharmacol* **24**:

388-393, 1994.

PATERNÒ R, HEISTAD DD, FARACI FM: Functional activity of Ca²⁺-dependent K⁺ channels is increased in basilar artery during chronic hypertension.

Am J Physiol **272**: H1287-H1291, 1997.

PATERNÒ R, HEISTAD DD, FARACI FM: Potassium channels modulate cerebral autoregulation during acute hypertension. *Am J Physiol Heart*

Circ Physiol **278**: H2003-H2007, 2000.

PERAL DE BRUNO M, ROMANO L, PROTO M, COVIELLO A: Atrial natriuretic peptide relaxes arterial basal tone induced by coarctation hypertension.

Peptides **20**: 485-491, 1999.

RUSCH NJ, RUNNELLS AM: Remission of high blood pressure reverses arterial potassium channel alterations. *Hypertension* **23**: 941-945, 1994.

SHIN WS, TOYO-OKA T, NAITOH T, DAN Y, OKAI Y, IIZUKA M, SUGIMOTO T: Response of intracellular Ca²⁺ transients in cultured vascular smooth muscle cells to angiotensin II, vasopressin, acetylcholine and atrionatriuretic peptide. *Jpn Heart J* **30**: 77-83, 1989.

TAGUCHI K, UEDA M, KUBO T: Effects of cAMP and cGMP on L-type calcium channel currents in rat mesenteric artery cells. *Jpn J Pharmacol* **74**:

179-186, 1997.

WARSHAW DM, MULVANY MJ, HALPERN W: Mechanical and morphological properties of arterial resistance vessels in young and old spontaneously hypertensive rats. *Circ Res* **45**: 250-259, 1979.

WEI CM, KIM CH, KHRAIBI AA, MILLER VM, BURNETT JC JR: Atrial natriuretic peptide and C-type natriuretic peptide in spontaneously hypertensive rats and their vasorelaxing actions in vitro. *Hypertension*

23: 903-907, 1994.

WEI CM, KIM CH, MILLER VM, BURNETT JC JR: Vasonatin peptide: a unique synthetic natriuretic and vasorelaxing peptide. *J Clin Invest* **92**: 2048-2052, 1993.

WOODARD GE, LI X, BROWN J, ROSADO JA: Receptor subtypes for vasonatin peptide in renal glomeruli and arteries. *Regul Pept* **129**: 183-189, 2005.

YU J, ZHU MZ, WEI GZ, CHEN BY, LU SY, KANG YF, GUO HT, MA H: Vasorelaxing role of vasonatin peptide in human intramammary artery in vitro. *Sheng Li Xue Bao* **55**: 187-190, 2003.

ZHANG LN, ZHANG LF, MA J: Simulated microgravity enhances vasoconstrictor responsiveness of rat basilar artery. *J Appl Physiol* **90**: 2296-2305, 2001.

Figure Captions

Fig. 1. Relaxant responses of human radial artery to VNP and 8-Br-cGMP. Endothelium-intact rings cut from human radial arteries from 5 patients ($n=5$) undergoing coronary artery bypass surgery were suspended in organ chambers. In one-half of the rings, the endothelium was removed by gently rubbing the intimal surface with a cotton swab. After setting resting force to about 500 mg weight, artery rings were contracted with 10^{-6} mol/l extracellular norepinephrine and relaxed with cumulatively increasing concentration of VNP (A) or 8-Br-cGMP (B), respectively. Data are the reduced (minus) percentages of maximal contractile amplitudes to 10^{-6} mol/l norepinephrine, and presented as mean \pm SD. Symbols obscure some of the smaller error bars.

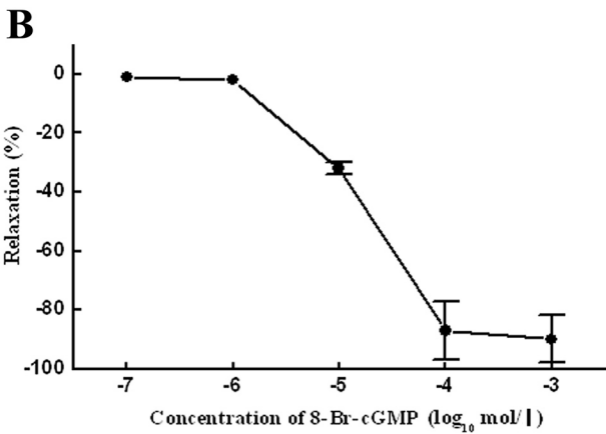
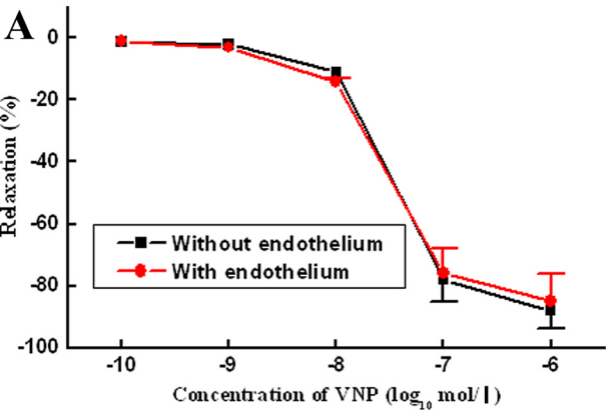
Fig. 2. Inhibitory effects of TEA, HS-142-1, and MB on relaxant responses of human radial artery to VNP. Endothelium-intact rings cut from human radial arteries from 5 patients ($n=5$) undergoing coronary artery bypass surgery were suspended in organ chambers. After setting resting force to about 500 mg weight, artery rings were contracted with 10^{-6} mol/l extracellular norepinephrine and then pretreated with or without 10^{-3} mol/l TEA (A), 2×10^{-5} mol/l HS-142-1 (B) or 10^{-5} mol/l MB (C), respectively, 20 min before relaxed with cumulatively increasing concentration of VNP. Data are the reduced (minus) percentages of maximal contractile amplitudes to 10^{-6} mol/l norepinephrine, and presented as mean \pm SD. Symbols obscure some of the smaller error bars. ** $p < 0.01$ versus respective response of artery rings to VNP in the absence of HS-142-1, TEA and

MB.

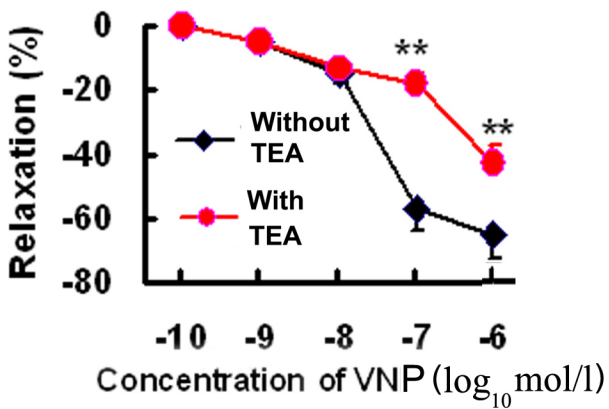
Fig. 3. Representative trace of K⁺ currents of single vascular smooth muscle cell (VSMC) isolated from mesenteric artery of rat. In whole-cell recording mode, the holding potential was -70 mV, and pulse generated from -60 to +60 mV with 10-mV step increment. Voltage steps were 1500 ms in duration and 10-s intervals were allowed between steps. Compared with control (A), whole-cell K⁺ currents were enhanced in the presence of 10⁻⁶ mol/l VNP, whereas suppressed after adding 10⁻⁷ mol/l IBX (C).

Fig. 4. Enhancement of BK_{Ca} by VNP was mimicked by 8-Br-cGMP, whereas inhibited in the presence of HS-142-1 or MB. The component of BK_{Ca} was obtained by subtracting the average currents after exposure to its selective blocker 10⁻⁷ mol/l IBX from those in the absence of IBX. For comparison, BK_{Ca} currents were normalized against the cellular capacitance, and expressed in the term of current density. (A) From -10 to 60 mV, 10⁻⁶ mol/l VNP-treated cells demonstrated significant increased BK_{Ca} current density. Similarly, evoking action of 8-Br-cGMP was shown from -10 to 60 mV. * $p < 0.05$ versus control. (B) From -10 to 60 mV, pretreatment with either 2 × 10⁻⁵ mol/l HS-142-1 or 10⁻⁵ mol/l MB, 10 min before adding 10⁻⁶ mol/l VNP, reversed VNP-activated BK_{Ca} current density. * $p < 0.05$ versus cells exposed to VNP, but in the absence of HS-142-1 and MB. Data are from 6 independent experiments ($n=6$) and presented as mean ± SD. Symbols obscure some of the smaller error bars.

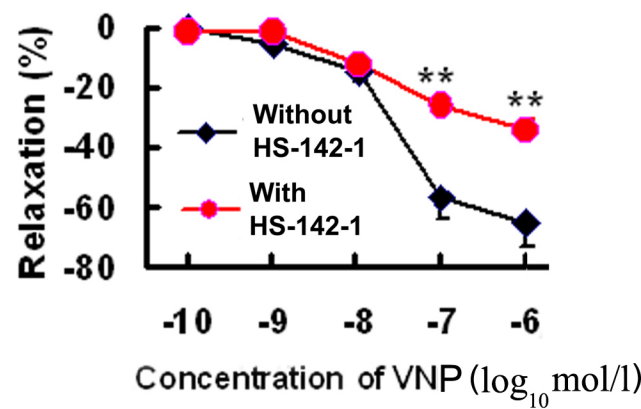
Yu_fig1



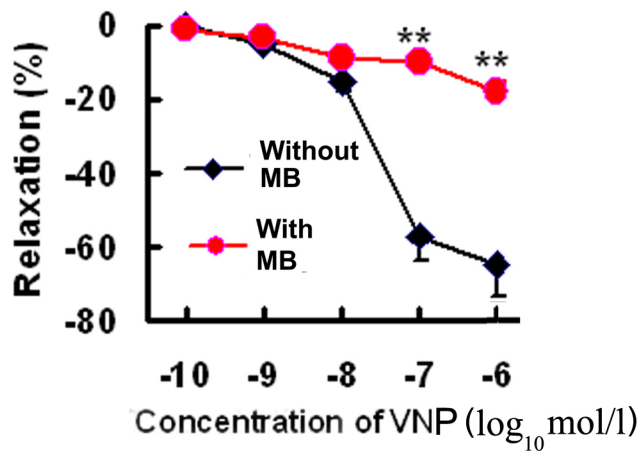
A

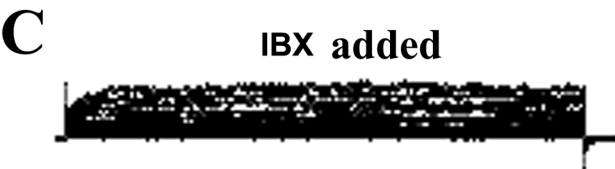
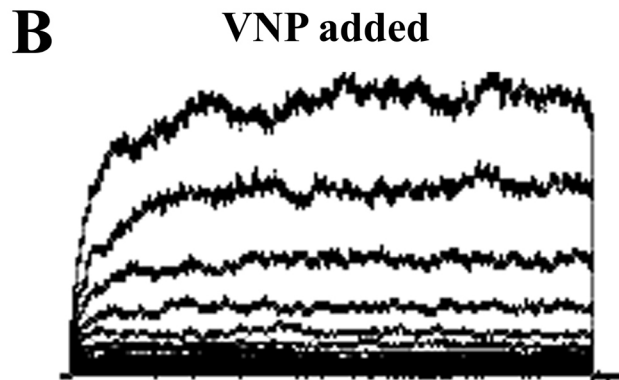
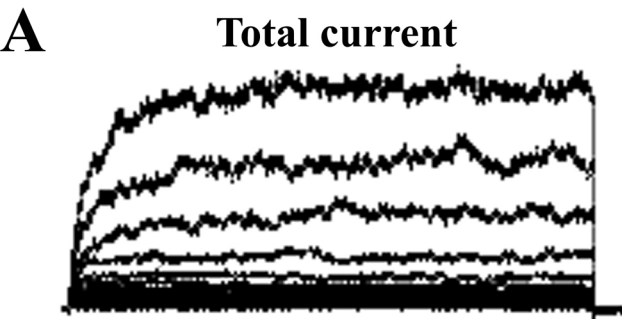


B



C



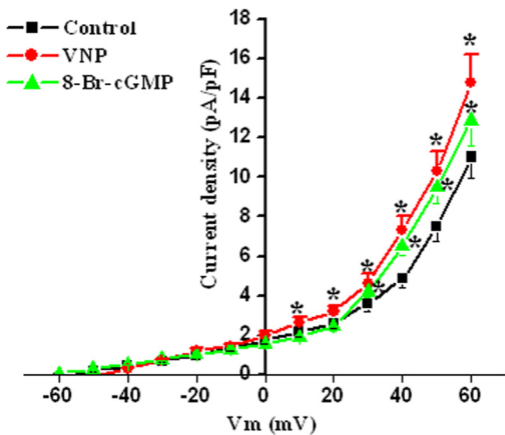


500 pA
500 ms

60 mV
1500 ms
-70 mV -60 mV

The diagram shows a voltage protocol consisting of a series of horizontal steps. The top step is at 60 mV, and the bottom step is at -60 mV. The duration of the protocol is 1500 ms. The baseline voltage is -70 mV.

A



B

