Endothelium-Derived Nitric Oxide Inhibits the Relaxation of the Porcine Coronary Artery to Natriuretic Peptides by Desensitizing Big Conductance Calcium-Activated Potassium Channels of Vascular Smooth Muscle

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Received January 28, 2010; accepted March 22, 2010

ABSTRACT

The present experiments investigated whether endothelium-derived mediators modulate the effect of natriuretic peptides in porcine coronary arteries. Rings with and without endothelium were suspended in organ chambers for isometric tension recording. Concentration–relaxation curves to C-type natriuretic peptide (CNP) and atrial natriuretic peptide (ANP) were obtained during contractions to endothelin-1. Removal of the endothelium potentiated relaxations to both CNP and ANP. N-nitro-L-arginine methyl ester potentiated relaxations to natriuretic peptides only in arteries with endothelium. Sodium nitroprusside (SNP) inhibited the response to the natriuretic peptides only in the absence of the endothelium. In rings with endothelium, 1H-[1,2,4]oxadiazolo[4,3-a]quinolin-1-one (ODQ) and 4H-8-bromo-1,2,4-oxadiazolo[3,4-d]benz[b][1,4]oxazin-1-one (NS2028) potentiated CNP-mediated relaxations. Iberiotoxin (IBTX) reduced the relaxation response only in rings without endothelium. Glybenclamide inhibited the relaxations in both the presence and absence of endothelium. CNP-induced relaxations were reduced by 8-bromo-guanosine 3’5’-cGMP (8-bromo-cGMP) to the same extent in rings with and without endothelium. There was no significant difference between the increased cGMP content caused by CNP in porcine coronary arteries with or without endothelium. In patch-clamp studies in porcine coronary arterial smooth muscle cells, the natriuretic peptide–mediated enhancement of the IBTX-sensitive big conductance calcium-activated potassium channel (BKca) amplitude was reversed by SNP and 8-bromo-cGMP. These findings demonstrate that, in the porcine coronary artery, the opening of BKca and ATP-dependent calcium channels of the vascular smooth muscle contributes to CNP-mediated relaxations. Endothelium-derived and exogenous NO inhibit the direct relaxing effect of natriuretic peptides by desensitizing the response of the BKca of the vascular smooth muscle to the generation of cGMP.

Nitrergic NO (NO) and natriuretic peptides contribute to the regulation of vascular tone (Moncada et al., 1991, Woodard and Rosado, 2008). The mammalian natriuretic peptide family includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin (Levin et al., 1998). ANP and BNP are produced in the atria and ventricles of the heart (Woodard and Rosado, 2008). CNP is produced by endothelial cells (Stingo et al., 1992).

Three subtypes of natriuretic peptide receptors (NPRs) have been cloned and characterized: NPR-A, NPR-B, and NPR-C. NPR-A binds ANP and BNP with equal affinity; NPR-B binds CNP and urodilatin; and NPR-C promotes the release of NO and the production of cGMP in target tissues. CNP has been shown to stimulate phosphodiesterase activity and to inhibit the opening of BKca, a calcium-activated potassium channel (Côté et al., 1994; Robichaud et al., 1994). The BKca channel consists of a large alpha subunit, BKα, and a small regulatory subunit, BKβ, which are encoded by separate genes (Nahorski et al., 1992). The alpha subunit contains five transmembrane segments and a large intracellular domain with a potassium-conducting pore, located at the C-terminus of the large subunit. The BKβ subunit binds to the alpha subunit and modulates channel properties. The BKβ subunit is responsible for the large conductance of 140 μS at 37°C in the absence of membrane potential (Nahorski et al., 1991).

NPR-A and NPR-B are coupled to Gq/11, and these receptors couple with Gαq to the phospholipase C (PLC) and calcium/cyclic nucleotide phosphodiesterase systems (Nahorski et al., 1995; Kadowaki et al., 1995). The signaling pathways downstream of CNP and ANP involve the activation of phospholipase C and the mobilization of intracellular calcium. CNP and ANP both inhibit the opening of BKca and stimulate phosphorylation of the alpha subunit (see Yoshimoto et al., 1998). The BKca channel plays a role in the regulation of vascular tone and in the maintenance of vascular smooth muscle tone (see Lipton et al., 1997). Among other effects, cGMP inhibits the opening of BKca and stimulates phosphorylation of the alpha subunit.

Supplemental material to this article can be found at: http://jpet.aspetjournals.org/content/suppl/2010/03/31/jpet.110.166652.DC1

doi:10.1124/jpet.110.166652.
however, CNP seems to be the endogenous ligand for NPR-B (Woodard and Rosado, 2008). Both NPR-A and NPR-B are coupled to particulate guanylyl cyclase, which stimulates the production of the intracellular second messenger cGMP (Koller et al., 1991). In contrast to NPR-A/NPR-B, NPR-C functions as a clearance receptor for ANP, BNP, and CNP, although it may also contribute to CNP-induced relaxation (Sandow and Tare, 2007).

NO is one of the main endothelium-derived relaxing factors (Palmer et al., 1987; Moncada et al., 1991) and causes relaxation in arteries in part by stimulating soluble guanylyl cyclase and thus inducing the synthesis of cGMP (Ignarro et al., 1987). Prostaglandins and NO contribute to the relaxation of renal afferent arterioles to CNP (Amin et al., 1996). Relaxations to CNP are larger in rings of canine coronary arteries without endothelium than those with endothelium (Wright et al., 1996). In the aorta of eNOS knockout mouse, CNP causes larger relaxations than in the aorta of wild-type controls (Madhani et al., 2003). These earlier findings suggest an interaction between CNP and NO. The common link between these two vasodilators is that both stimulate the production of cGMP (Ignarro et al., 1987; Koller et al., 1991; Moncada et al., 1991; Woodard and Rosado, 2008).

In addition to activating the production of cGMP, CNP can cause hyperpolarization of vascular smooth muscle cells in the porcine coronary artery, an effect that may involve the opening of potassium channels (Barber et al., 1998). Actually, the natriuretic peptide has been proposed as an endothelium-derived hyperpolarizing factor (EDHF) (Chauhan et al., 2004). It is unknown whether EDHF-mediated responses (Félotou and Vanhoutte, 2006; Ko et al., 2008) affect the relaxing effect of natriuretic peptides. Likewise, it is unknown whether in intact blood vessels the release of endothelium-derived contracting factors (Lüscher and Vanhoutte, 1986) attenuates the relaxations to the natriuretic peptides.

The present experiments were designed to determine how NO and other endothelium-derived mediators affect relaxations to natriuretic peptides in the porcine coronary artery and how potassium channels are involved in the dilator response of that artery to these peptides.

**Materials and Methods**

**Tissue Preparation.** The experiments were performed on isolated coronary arteries, which were dissected free from porcine hearts obtained at the local slaughterhouse. In brief, the arteries were placed in ice-cold Krebs-Ringer-bicarbonate buffer: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\)Cl, 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25 mM NaHCO\(_3\), and 11.1 mM glucose (control solution). The adherent fat and connective tissue were removed, and the arteries were cut into rings (approximately 3–4 mm in length). In certain preparations, the endothelium was removed by the infusion of 0.5% Triton X-100 at a rate of 1 ml/min for 30 s before the arteries were cut into rings. Removal of endothelium was confirmed by the loss of relaxation in response to 10\(^{-6}\) M bradykinin [7-methoxytocoumarin-4-acetyl [Ala7-2,4-dinitrophenyl]Lys9]-bradykinin trifluoroacetate salt].

**Isometric Tension.** The rings were suspended in conventional organ chambers (5 ml), filled with control solution aerated with 95% O\(_2\) and 5% CO\(_2\), and maintained at 37°C. The rings were subjected to 5 g optimal tension for rings of porcine right coronaries obtained from the same source. Isometric tension was measured by means of force transducers (ADInstruments Pty Ltd., Castle Hill, Australia) coupled to an amplifier and a personal computer (PowerLab, ADInstruments Pty Ltd.) for data collection. The rings were exposed to 60 mM potassium chloride twice before the actual experiment.

Certain rings were incubated for 40 min with the following agents alone or in combination: N\(^*-\)nitro-l-arginine methyl ester (l-NAME; NO synthase inhibitor; 10\(^{-4}\) M) (Tang et al., 2005); indomethacin (nonselective cyclooxygenase inhibitor; 10\(^{-5}\) M) (Tang et al., 2008); sodium 3-([6R]-6-[(4-chlorophenyl)sulfonylamido]-2-methyl-5,6,7,8-tetrahydroanaphthalen-1-yl)[propanoate (S18886; TP receptor antagonist; 10\(^{-7}\) M) (Tang et al., 2008); ibiotoxin (N-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonyl-N-\(^*\)-cyclohexylurea, 5-chloro-N-[4-cyclohexylureidosulfonfyl]phenethyl]-2-methoxybenzamide (glybenclamide); IBTX; selective inhibitor of big conductance calcium-activated potassium channels (BK\(_{ca}\); 10\(^{-7}\) M) (Leuranguer et al., 2008); charybdoxin (nonselective IK\(_{ca}\) and BK\(_{ca}\) inhibitor; 5 \times 10\(^{-7}\) M) (Leuranguer et al., 2008); 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole (TRAM-34) (IK\(_{ca}\) inhibitor; 5 \times 10\(^{-7}\) M) (Leuranguer et al., 2008); UCL-1684 (nonpeptide blocker of SK\(_{ca}\); 5 \times 10\(^{-7}\) M) (Leuranguer et al., 2008); apamin (selective SK\(_{ca}\) inhibitor; 10\(^{-6}\) M) (Keung et al., 2005); sodium nitroprusside (SNP; NO donor; 10\(^{-3}\) M); oxadiazoloquinazoline [1H-1,2,4]oxadiazolo [3,4-e][quinazolin-1-one; ODQ (selective inhibitor of soluble guanylyl cyclase; 10\(^{-6}\) M) (Tang et al., 2005); 4H-8-bromo-1,2,4-oxadiazolo[3,4-d][benz][1,4]oxazin-1-one (NS29028, specific inhibitor of soluble guanylyl cyclase; 10\(^{-6}\) M) (Yang et al., 2004); 8-bromoguanosine 3',5'-cGMP (8-bromo-cGMP) (cell-permeable analog of cGMP; 10\(^{-5}\) M) (Leuranguer et al., 2008); and glibenclamide (blocker of ATP-sensitive K\(^+\) channels; 10\(^{-6}\) M) (Hedayati et al., 2009). After the incubation period, sustained contractions were obtained with the ED\(_{50}\) concentration of either prostaglandin P\(_2\); [1-(hydroxy-2-isopropanol)ethyl]benzenes-1,2-diol (isoproterenol), endothelin-1, [Z]-7-[(1R, 2R,3R,5S)-3,5-dihydroxy-2-[(E,3S)-3-hydroxoyct-1-enyl][cyclopenyl]hept-5-enoic acid; 10\(^{-6}\) M] or endothelin-1 (10\(^{-9}\) M). There were no statistically significant differences in precontraction levels between the different experimental groups. The rings were then exposed to progressively increasing concentrations of CNP (10\(^{-10}\) to 10\(^{-6}\) M), ANP (10\(^{-10}\) to 10\(^{-7}\) M), isoproterenol (10\(^{-10}\) to 10\(^{-6}\) M) or 1,3-dihydroxy-2-[2-(trifluoroacetyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one (NS1619; selective BK\(_{ca}\) activator, 10\(^{-10}\) to 10\(^{-6}\) M) (Edwards et al., 1994). Decreases in tension are expressed as percentage of the maximal reference relaxation to isoproterenol (10\(^{-6}\) M) or sodium nitroprusside (10\(^{-4}\) M) (O’Rourke et al., 2003) obtained at the end of the experiment.

**cGMP.** Rings with or without endothelium, studied in the organ chambers, were incubated for 45 min with the nonselective phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX; 10\(^{-5}\) M) before exposing them to endothelin-1 (10\(^{-7}\) M), followed by a single, maximal concentration of CNP (10\(^{-6}\) M). The rings were flash-frozen in liquid nitrogen either immediately before (time 0) or 5 min after the addition of CNP. The frozen rings were homogenized in 1 ml of 6% trichloroacetic acid, and the homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was extracted four times with four volumes of water-saturated ether and lyophilized. The levels of cGMP were determined with a radioimmunoassay cGMP EIA kit (Cayman Chemical, Ann Arbor, MI) and normalized to the protein content determined by using a protein assay reagent.

**Patch-Clamp Experiments.** Pig left anterior descending coronary artery smooth muscle cells were dissociated enzymatically as reported previously (Shan Lu et al., 2003) for single-cell, patch-clamp electrophysiology experiments. Whole-cell, membrane-rapture recording of the macroscopic ibiotoxin-sensitive, Ca\(^{2+}\)-activated K\(^+\) (BK\(_{ca}\)) channel gating of single coronary artery smooth muscle cells were recorded (Shan Lu et al., 2003). The external physiological solutions for recording the BK\(_{ca}\) amplitude contained 130 mM NaCl, 5 mM KCl, 1.2 mM MgCl\(_2\), 1.5 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). The internal pipette solution had the following composition: 10 mM NaCl, 110 mM...
KCl, 5 mM MgCl₂, 2 mM CaCl₂, 10 mM methylene glycol-bis [β-aminoethylether]N,N',N'-tetraacetic acid, 5 mM K₃ATP, and 10 mM HEPES (pH 7.2 with KOH). For a free Ca²⁺ concentration of approximately 444 nM (estimated by using the computer program Maxcholator, Stanford University, Stanford, CA), the pipette solution contained 7 mM instead of 2 mM CaCl₂, and 4 mM instead of 5 mM MgCl₂, as described previously (Seto et al., 2007). To allow for an equilibration of the pipette solution with the cell interior, all recordings were started 5 min after the establishment of the whole-cell configuration. Most experiments were performed within 15 min of gaining access, during which time the macroscopic BKCa current amplitude remained stable.

To measure the effects of drugs on BKCa, the BKCa current was elicited with a test potential to +80 mV (500-ms duration) from a holding potential of −60 mV and stimulated at 0.1 Hz. Effects of drugs (e.g., ANP, CNP) on BKCa amplitude were examined/compared after a stable BKCa amplitude was achieved (i.e., control). Cell membrane capacitance was estimated, as described previously (Shan Au et al., 2003), and averaged 19.2 ± 2.3 pF (n = 30). The amplitude of the BKCa current was recorded with an Axopatch 200A patch-clamp amplifier (Molecular Devices, Sunnyvale, CA) before treatment (control), during treatment, and after washout. Only one concentration of a particular drug was tested in each cell. The external solution was delivered through gravity and controlled by solenoid valves coupled to a four-channel valve driver (General Valve, Brookshire, TX). A solution change (approximately 5 ml, corresponding to 10 times the volume of the recording chamber) could be completed in 15 to 20 s. Drugs (dissolved in the external recording solution) were applied to the external cell surface.

Drugs. CNP, ANP, prostaglandin F₂α, 1-NAME, 2-[1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid (indomethacin), sodium pentacyanonsitrosylferrate(III) (sodium nitroprusside), ODQ, NS2028, charybdotoxin, TRAM-34, UCL-1684, apamin, IBTX, IBMX, bradykinin, and NS1619 were purchased from Sigma-Aldrich (St. Louis, MO). 8-Bromo-cGMP was obtained from Biolog (Bremen, Germany). S18886 (Terutroban) was a kind gift from the Institut de Recherches Servier (Suresnes, France). Triton X-100 was purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Stock solutions of indomethacin, ODQ, and NS2028 were prepared in 1 mM sodium bicarbonate. NS1619 was dissolved in pure ethanol. All other compounds were prepared in deionized water.

Data Analysis. The results are shown as means ± S.E.M. with n being the number of individual observations on rings from different pigs. Data were analyzed by using the statistical program Prism version 5 (GraphPad Software Inc., San Diego, CA). For the sake of clarity, most of the results are represented as area under the concentration-relaxation curve, using Prism version 5. Student’s t test for paired observations was used for comparison of two groups. One-way analysis of variance with repeated measures followed by the Bonferroni post hoc test was carried out for multiple comparisons. A difference was accepted as statistically significant when the P value was less than 0.05. In the whole-cell, patch-clamp electrophysiology experiments, n refers to the number of single vascular smooth muscle cells used. The results are expressed as means ± S.E.M.

Results

Relaxations

Endothelium Removal. CNP evoked concentration-dependent relaxations in rings, with or without endothelium, during contractions to prostaglandin F₂α, (10⁻⁶ M) (Supplemental Fig. 1) and endothelin-1 (10⁻⁵ M; Fig. 1). The relaxations were significantly larger in rings without endothelium than in those with endothelium (Fig. 2, top left). ANP had a comparable effect to that of CNP in rings with and without endothelium (Fig. 2, top right).

Endothelium-Derived Vasoactive Factors. In rings with endothelium, the concentration of prostaglandin F₂α, (Supplemental Fig. 2) or endothelin-1 (Fig. 1) or endothelin-1 (10⁻⁵ M; Fig. 1) significantly potentiated the relaxations to CNP. The degree of relaxation observed after incubation with 1-NAME was comparable with that achieved by removal of the endothelium. 1-NAME potentiated the relaxations of rings with endothelium to ANP to a similar extent as those exposed to CNP (Fig. 2, bottom right). Indomethacin (10⁻⁵ M) and S18886 (10⁻⁷ M) did not significantly affect the relaxations to CNP in arteries with and without endothelium (Supplemental Figs. 3 and 4).

Interaction with NO. In rings with endothelium, ODQ (10⁻⁶ M) and NS2028 (10⁻⁶ M) significantly potentiated the relaxations to CNP (Fig. 3). Previous incubation with sodium nitroprusside (10⁻⁵ M) did not significantly affect the re-

Fig. 1. Isometric tension recordings in rings with (top) and without (bottom) endothelium of the same porcine coronary artery. Concentration-relaxation curves to cumulative concentrations of CNP during contractions to endothelin-1 are shown. At the end of the experiment full relaxation is obtained with isoproterenol. M, mol/liter.
response, whereas that with 8-bromo-cGMP (10^{-5} M) slightly, but significantly, inhibited it (Fig. 3).

In rings without endothelium, L-NAME did not significantly alter the relaxations to CNP (Fig. 4) and ANP (Supplemental Fig. 5) during contractions to endothelin-1 (Fig. 4). Incubation with sodium nitroprusside (10^{-5} M) significantly inhibited the relaxations to CNP (Fig. 4) and ANP (Fig. 5, left). By contrast, sodium nitroprusside did not alter significantly the relaxations to isoproterenol (Fig. 5, right). Incubation with ODQ or NS2028 did not significantly affect relaxations to CNP in rings without endothelium, whereas 8-bromo-cGMP significantly reduced them (Fig. 4).

The relaxations to increasing concentrations of sodium nitroprusside and 8-bromo-cGMP were significantly smaller in rings with endothelium than in those without endothelium (Fig. 6).

**Potassium Channel Inhibitors.** In rings with endothelium, TRAM-34, UCL-1684, apamin, charybdotoxin, and iberiotoxin did not significantly affect the relaxations to CNP, whereas glybenclamide significantly reduced them (Fig. 7).

In rings without endothelium, apamin, TRAM-34, or UCL-1684 did not significantly affect the CNP-induced relaxations (Fig. 8). By contrast, charybdotoxin (5 \times 10^{-7} M), iberiotoxin (10^{-7} M), and glybenclamide (10^{-6} M) significantly reduced the response to the natriuretic peptide (Fig. 8). The inhibitory effects of iberiotoxin and sodium nitroprusside were not additive (Fig. 8). Combined incubation with sodium nitroprusside, iberiotoxin, and glybenclamide nearly abolished the response (Fig. 8). NS1619-mediated relaxation was potentiated in the absence of endothelium (Fig. 9).

**cGMP Levels.** In the presence of IBMX (10^{-4} M), the level of cGMP was significantly larger in rings with endothelium than in those without endothelium (with endothelium, 1745.25 ± 32.07 pmol/ml/g wet weight; without endothelium, 232.25 ± 20.87 pmol/ml/g wet weight). CNP (10^{-6} M) significantly increased the level of cGMP in both rings with and without endothelium (Fig. 10). The absolute increases in cGMP level in response to CNP were not different in the presence and absence of endothelium (Fig. 10). ODQ and NS2028 did not significantly affect the increase in cGMP caused by CNP in the absence of endothelium (Fig. 10).
BK<sub>Ca</sub> Gating. ANP (10<sup>-8</sup> and 10<sup>-7</sup> M) and CNP (10<sup>-7</sup> and 10<sup>-6</sup> M) elicited a concentration-dependent enhancement of iberiotoxin (10<sup>-7</sup> M)-sensitive BK<sub>Ca</sub> amplitude (Figs. 11A and 12A). Incubation (for 5–10 min before the recording of BK<sub>Ca</sub> amplitude) with sodium nitroprusside (10<sup>-9</sup> M) and 8-bromo-cGMP (10<sup>-5</sup> M) augmented the basal noise level of the current recordings. In the presence of sodium nitroprussi-
versus control.

Fig. 9. relaxations to NS1619 during contractions to endothelin-1 (10⁻⁹ M) in isolated porcine coronary arteries with or without endothelium. Data are expressed as percentage of the maximal relaxation to isoproterenol (10⁻⁵ M) obtained at the end of the experiments. n = 5; *, P < 0.05 versus control.

Fig. 10. Effect of 5 min of exposure to CNP (10⁻⁶ M) on the accumulation of cGMP in porcine coronary arteries with (+EC) and without (-EC) endothelium, and after incubation with ODQ (10⁻⁶ M) or NS2028 (10⁻⁶ M) in rings of coronary arteries without endothelium, studied in the presence of IBMX (10⁻⁴ M). Data are shown in absolute values. n = 6.

Discussion

The present data confirm that CNP and ANP relax the porcine coronary artery with or without endothelium during contractions to endothelin-1 (Kediia et al., 2006) and prostaglandin F₂α (Barber et al., 1998), demonstrating the nonselectivity of this inhibitory effect. They also confirm that accumulation of cGMP is involved in the response to CNP (Barber et al., 1998). The present observations further confirm that, under control conditions, the relaxations to the natriuretic peptides are attenuated by the presence of endothelial cells (Wright et al., 1996). In the present study, incubation with the nonselective cyclooxygenase inhibitor indomethacin did not affect the blunting by the presence of the endothelium of the relaxation to CNP, ruling out a contribution of vasodilator endothelium-derived prostaglandins, in particular prostacyclin (Moncada and Vane, 1978). The similar lack of effect of TRAM-34 plus UCL-1684 permits us to rule out a contribution to, or an interference with, the effect of CNP by EDHF-mediated responses (Félétou and Vanhoutte, 2006). Likewise, the absence of effect of the cyclooxygenase inhibitor and the TP receptor antagonist S18886 excludes a confounding role for endothelium-derived vasoconstrictor prostaglandins activating TP receptors on the vascular smooth muscle (Vanhoutte and Tang, 2008). Thus, it seems logical to conclude that the inhibitory effect of the presence of endothelial cells is caused solely by endothelium-derived NO. This conclusion is supported by the experiments showing the potentiation effect of the inhibitor of NO synthase L-NAME on CNP- and ANP-mediated relaxations in arteries with endothelium. These present findings are in agreement with the results obtained in the aorta of eNOS knockout mice in which the CNP-mediated relaxation is greater than in the aorta of wild-type animals (Madhani et al., 2003).

The potentiating effect of L-NAME on the relaxation to CNP and ANP was not observed in the absence of endothelial cells, implying that indeed it is endothelium-derived NO, produced by eNOS, that exerts an inhibitory effect on the response to the peptides. This conclusion is supported by the experiments with the NO donor sodium nitroprusside, which inhibited the CNP-induced relaxations in the coronary artery, as it does in the aorta of eNOS knockout mice (Madhani et al., 2003). Moreover, the potentiating effect of the two inhibitors of soluble guanylyl cyclase tested, ODQ and NS2028, on the relaxations to CNP in the presence of endothelium is consistent with the effect of L-NAME. Also in line with the inhibitory effect of endothelium-derived NO on the response to the natriuretic peptides, the cell-permeable analog of cGMP inhibited the effect of CNP in rings without endothelial cells, an effect comparable with that obtained with the NO donor sodium nitroprusside. Taken in conjunction, the present findings thus suggest that endothelium-derived NO does not affect the relaxations to CNP by interfering with the binding to or the activation of NPR-Bs (Woodard and Rosado, 2008), a conclusion supported by the comparable increases in cGMP evoked by CNP in rings with and without endothelium. The basal levels of cGMP were higher in rings with endothelium than in those without endothelium, illustrating the basal release of NO and the subsequent activation of soluble guanylyl cyclase. This basal production of cGMP in preparations with endothelium must result in a desensitization of the cellular response to the relatively modest amounts of cyclic nucleotide generated by the natriuretic peptides, presumably resulting from activation of the cGMP-degrading phosphodiesterase 5 (Friebe and Koelsing, 2003; Yang et al., 2004). This interpretation is consistent with earlier findings that the inhibitory effect of the endothelium on the relaxations to CNP of the canine coronary artery is no longer observed in preparations treated with a selective inhibitor of particulate guanylyl cyclase (Wright et al., 1996). It is also consistent with the present findings that the inhibitory effect of the presence of the endothelium is also observed for relaxations to both a NO donor (sodium nitroprusside) and a cell-permeable analog of...
**Fig. 11.** Effects of ANP on the BK<sub>KCa</sub> amplitude of single porcine coronary artery smooth muscle cells. Top, the extent of block of BK<sub>KCa</sub> amplitude by ANP (10<sup>-8</sup> and 10<sup>-7</sup> M) (with and without SNP/8-Br-cGMP pretreatment) was evaluated by using train-pulse protocols (holding potential = −60 mV; stimulating test potential = 80 mV for 500 ms, stimulated at 0.033 Hz). Insets illustrate the representative steady-state BK<sub>KCa</sub> traces recorded in controls, ANP/CNP, and ANP/CNP plus iberiotoxin (100 nM) (with and without SNP/8-bromo-cGMP pretreatment). Bottom, summaries of the steady-state maximum effects of drugs on the macroscopic BK<sub>KCa</sub> amplitude recorded (expressed as pA/pF). Results are expressed as mean ± S.E.M., and the number of experiments is indicated in parentheses. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

**Fig. 12.** Effects of CNP on the BK<sub>KCa</sub> amplitude of single porcine coronary artery smooth muscle cells. Top, the extent of block of BK<sub>KCa</sub> amplitude by CNP (10<sup>-7</sup> and 10<sup>-6</sup> M) (with and without SNP/8-bromo-cGMP pretreatment) was evaluated by using train-pulse protocols (holding potential = −60 mV; stimulating test potential = 80 mV for 500 ms, stimulated at 0.033 Hz). Insets illustrate the representative steady-state BK<sub>KCa</sub> traces recorded in controls, CNP, and CNP plus iberiotoxin (100 nM) (with and without SNP/8-bromo-cGMP pretreatment). Bottom, summaries of the steady-state maximum effects of drugs on the macroscopic BK<sub>KCa</sub> amplitude recorded (expressed as pA/pF). Results are expressed as mean ± S.E.M., and the number of experiments is indicated in parentheses. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
cGMP (8-bromo-cGMP), but not for those to isoproterenol, which are mediated by an increase in cAMP (Ehlert et al., 1997). Earlier work has demonstrated that the presence of endothelial cells actually enhances relaxations to the β-adrenergic agonist in coronary arteries (Rubanyi and Vanhoutte, 1985).

The opening of potassium channels contributes to the direct relaxations of vascular smooth muscle to natriuretic peptides (Barber et al., 1998). In the present study, TRAM-34 and UCL-1684 did not affect the relaxations to CNP in rings with or without endothelium, thus ruling out a contribution of SKCa and IKCa (Félétou and Vanhoutte, 2006) to the response. By contrast, both charybdotoxin and iberiotoxin, known inhibitors of BKCa (Cox, 2005; Félétou and Vanhoutte, 2006) inhibited the relaxations to CNP in preparations without endothelium. In the vascular wall, BKCa-8 are located mainly in vascular smooth muscle cells (Cox, 2005), and the present results support their involvement in the endothelium-independent relaxing effect of CNP. The fact that the effects of sodium nitroprusside and iberiotoxin are not additive suggests that the desensitization caused by endothelium-derived NO involves a reduced opening of BKCa-8. This interpretation is supported by the observation that the inhibitory effect of charybdotoxin and iberiotoxin on the response to CNP is not observed in rings with endothelium. Furthermore, the potentiated NS1619-mediated relaxation in the absence of the endothelium is strong evidence that NO causes desensitization of BKCa-8. These conclusions are supported fully by the patch-clamp experiments. Indeed, ANP and CNP caused a concentration-dependent enhancement in BKCa gating, which could be eradicated by iberiotoxin. In line with the organ chamber experiments, this enhancement was abolished or attenuated after incubation with sodium nitroprusside or 8-bromo-cGMP, which strongly suggests a desensitization process. The latter desensitization presumably explains why the prolonged incubation with either sodium nitroprusside or 8-bromo-cGMP did not result in an enhancement of the BKCa current as this effect is transient (Miyoshi and Nakaya, 1994). Alternatively, the present experiments may have used a threshold concentration of sodium nitroprusside and 8-bromo-cGMP that was insufficient to cause overt activation of the channel.

In addition, ATP-dependent potassium channels contribute to the direct relaxing effect of CNP as demonstrated by the inhibition of the response by glybenclamide. However, the ATP-dependent potassium channels seem not to be involved in the inhibitory effect of NO. This conclusion is based on the observations that glybenclamide inhibits the relaxations to CNP in rings with endothelium and that in rings without endothelium the combination of glybenclamide with iberiotoxin and sodium nitroprusside caused a further inhibition of the response to CNP. The opening of such ATP-dependent potassium channels presumably explains the remaining relaxations caused by CNP in the presence of an inhibitor of particulate guanylyl cyclase (Wright et al., 1996).

The present study does not permit us to conclude whether CNP opens potassium channels directly or indirectly as a result of changes in cGMP. NO can activate calcium-activated potassium channels dependently of cGMP in arteries of pig (Miyoshi and Nakaya, 1994), rabbit (Robertson et al., 1993), and rat (Peral de Bruno et al., 1999). Activation of protein kinases by cGMP can result in phosphorylation of potassium channels (Robertson et al., 1993). However, NO can also activate calcium-activated potassium channels directly in the arteries of rabbit (Botolina et al., 1994) and rat (Mistry and Gerland, 1998). To judge from the increased noise level observed in the patch-clamp experiments of the present study, low concentrations of sodium nitroprusside (and 8-bromo-cGMP) may activate calcium-activated potassium channels slightly in porcine coronary arterial smooth muscle, an interpretation that is in line with previous findings (Miyoshi and Nakaya, 1994.). Possibly, this transient effect leads to desensitization of calcium-activated potassium channels, which in turn affects the relaxation to CNP.

The present findings confirm that CNP and ANP are endothelium-independent vasodilators in the porcine coronary artery. Accumulation of cGMP and opening of BKCa-8 and ATP-dependent potassium channels contribute to the direct effect of the natriuretic peptides. Basally released endothelium-derived NO inhibits the response of the vascular smooth muscle of that artery to the natriuretic peptides, at least partially by desensitizing the response of the BKCa-8 of the vascular smooth muscle to the generation of cGMP.

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