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Shear Stress Induction of C-type Natriuretic Peptide (CNP) in Endothelial Cells is Independent of NO Autocrine Signaling

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Abstract—C-type natriuretic peptide (CNP) is secreted by endothelial cells and has vasodilatory and antiproliferative activity against smooth muscle cells. Using defined laminar shear stress exposures of cultured bovine aortic endothelial cells, we investigated the regulation of CNP gene by PhosphorImaging the ratio of CNP mRNA to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. A 6 h exposure to arterial shear stress of 25 dyn/cm^2 caused a marked elevation (10.5 $\pm$6.2-fold; $n=10$, $p<0.001$) of CNP/GAPDH mRNA ratio compared to stationary controls. Arterial shear stress was 2.6 times more potent than a venous level of shear stress of 4 dyn/cm^2 in elevating the CNP/GAPDH mRNA ratio. After 6 h, CNP secretion by shear stressed BAEC was elevated over stationary controls by 3.1-fold ($n=5$, $p<0.001$) to a level of 34 $\pm$7.5 pg/cm^2 BAEC. Shear stress elevated CNP mRNA in the presence of L-NAME (400 μM) indicating that autocrine signaling through shear-induced NO production or guanylate cyclase activation was not involved. Similarly, the tyrosine kinase inhibitor genistein (10 μM), which can also block shear-induced NO production, had no effect on CNP mRNA induction by shear stress in BAEC. The intracellular calcium chelator BAPTA/AM (5 μM) attenuated the shear stress-induced CNP mRNA expression by 71%. Interestingly, dexamethasone (1 μM) potentiated by 2-fold the shear stress enhancement of CNP mRNA. Shear stress was a more potent inducer of CNP than either phorbol myristrate acetate or lipopolysaccharide. Hemodynamic shear stress may be an important physiological regulator of CNP expression with consequent effects on vasodilation and regulation of intimal hyperplasia. © *1999 Biomedical Engineering Society.* [S0090-6964(99)00104-6]

Keywords—Endothelium, Shear stress, Natriuretic peptide, Hemodynamics.

INTRODUCTION

The endothelium is the local source of paracrine mediators of vasomotor control, thrombosis, and intimal hyperplasia. These factors include dilators such as nitric oxide (NO), prostacyclin (PGI_2), and C-type natriuretic peptide (CNP) and constrictors such as endothelin type 1 (ET-1). CNP acts as both a potent vasodilator[39,40] and inhibitor of smooth muscle cell proliferation in vitro.[14,24] In contrast to NO and CNP, endothelin is a SMC mitogen.[3,16] CNP has been shown to reduce intimal hyperplasia in rat and rabbit artery injury models of air-drying endothelial denudation and balloon angioplasty, respectively.[8,30]

CNP, a 22 amino acid peptide, is the third member of the natriuretic peptide family to be identified[32] and is found in plasma.[31] Atrial and brain natriuretic peptides are cardiac hormones while CNP acts as a neuropeptide and as an endothelium-derived vessel wall regulator.[21,35] The natriuretic peptide receptor type B (NP-B) is a guanylate cyclase-linked receptor that is highly expressed in SMC and selectively activated by CNP.[13,34] CNP is secreted from endothelial cells[31,35] and expressed widely in vessels *in vivo*.[15] It is a potent SMC vasorelaxing factor via cGMP-dependent K^+ channel stimulation[39] and may function as an antagonist of the vascular renin-angiotensin system. Interestingly, the aortic relaxing action of CNP is impaired in spontaneously hypertensive rats.[39] Endothelial production of CNP is induced by various growth factors and cytokines, such as TGF-β, TNF-α, lipopolysaccharide (LPS), interleukin-1, basic fibroblast growth factor and thrombin.[21,33,35] Expression of CNP in mouse is tissue specific (high levels of CNP in brain) and gender specific with high expression in mouse ovary and uterus.[11]

Shear stress has been shown to elevate mRNA and protein levels of several endothelial genes, such as endothelial constitutive NOS (eNOS)[20,27,41] and tissue plasminogen activator (tPA)[7] while suppressing expression of endothelin.[29] In a short report, Okahara[23] noted the elevation of CNP mRNA by shear stress in human umbilical endothelial cells but made no measurement of CNP secretion. The mechanotransduction pathways in endothelial cells are multiple (for review, see Ref. 6), as well as strongly dependent on the precise qualitative and quantitative nature of the physical stimuli. These stimuli include hemodynamic shear stress, mechanical strain due to pressure-induced vessel distension, as well as complex

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temporally and spatially dependent combinations of forces due to complicated vascular geometries and the pulsatile nature of blood flow. Considerable evidence suggests that ion transporters,[6] G-proteins,[17,37] phospholipases C and A_2, tyrosine kinases,[37] protein kinase C,[10,37] cGMP-dependent kinases,[22] are involved in mechanotransduction as well as cellular architectures[6] involving the cytoskeleton, cytoskeletal membrane and receptor interactions, and cellular focal adhesion plaques.

Using an *in vitro* perfusion system to expose cultured endothelial cells to steady laminar flow, we have investigated endothelial regulation of the CNP gene and secretion of CNP in response to fluid shear stress exposures. We showed that CNP gene expression can be elevated in a dose-dependent manner by fluid shear stress in bovine aortic endothelial cells and the induction was independent of flow induced NO production, but was calcium dependent. In concert with the large change in CNP mRNA levels, the secretion of CNP was also elevated suggesting a rapid and pronounced coupling between CNP transcript level and protein synthesis/secretion. Dexamethasone did not block induction of CNP indicating that the response to shear stress was not a classical inflammatory type behavior. Hemodynamic fluid shear stress may be an important regulator of the CNP gene under physiological conditions.

MATERIALS AND METHODS

Cell Culture and Shear Stress Exposure

Bovine aortic endothelial cells (BAEC) (Cell systems, Kirkland, WA) were grown to confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated newborn calf serum, 0.30 mg/ml glutamine, and 0.05 mg/ml gentamicin (GIBCO, Grand Island, NY) (10% complete DMEM). Confluent monolayers of BAEC (passages 2–8) were exposed to a steady laminar shear stress of 4 or 25 dyn/cm^2 in individual, parallel plate flow chamber systems with recirculating medium (20 ml) driven by a constant hydrostatic pressure head under sterile conditions as previously described[7,27,41] or maintained in a CO_2 incubator. Venous and arterial shear stresses of 4 and 25 dyn/cm^2, respectively,[7] can be achieved with the parallel-plate flow chamber. The pH and aeration of the perfusion media was controlled with a sterile 5% CO_2 in air mixture. To block NO production and NO-dependent elevation of cGMP, BAEC were preincubated for 30 min with 400 μM N^G-nitro-L-arginine methyl ester (L-NAME). Using the diaminonaphthalene reagent to detect nitrite in the culture medium, we have previously shown[38] that shear induced-NO production is blocked by 87.5% by preincubating BAEC with a level of only 100 μM L-NAME. Ohno *et al.*[22] have previously shown that L-N-monomethylarginine at 10 μM (which is a weaker inhibitor than LNAME and was used at lower dose than in the present study) prevented shear-induced elevation of cGMP in BAEC exposed to 40 dyn/cm^2. To inhibit shear-induced tyrosine kinase activation[37] and subsequent tyrosine kinase-dependent activation of nitric oxide production[5] in BAEC, we used the tyrosine kinase inhibitor, genistein. Cells were preincubated in 10 μM genistein for 30 min with genistein in the circulating media. Corson *et al.*[5] have previously shown that a 1 h incubation with genistein was sufficient to block shear stress induced NO production by BAEC. We have observed over 80% inhibition by 10 μM genistein of shear induced BAEC NO production as indicated by fluorescence assay of nitrite. To chelate intracellular calcium, we preincubated BAEC in 5 μM BAPTA/AM for 30 min following the methods of Hsieh.[10] To inhibit NF-κB-dependent gene expression, we used dexamethasone.[2,25] A level of 0.1 μM dexamethasone (1 h pretreatment) can inhibit NF-κB-dependent expression of a promoter construct in BAEC[2] and 1.0 μM dexamethasone treatment of porcine aortic endothelial cells can inhibit the induction of iNOS.[25] Similarly, a level of 0.1–10 μM dexamethasone was shown to prevent induction of the iNOS (NOS II) promoter construct in cytokine-treated A549/8 cells.[12] Therefore, we preincubated BAEC with dexamethasone at 1 μM for 24 h. We found that this level of dexamethasone attenuated by about 50% the induction of NF-κB binding activity by 25 dyn/cm^2 of shear stress (60 min exposure) as seen in gel shift assay (data not shown).

RNA Isolation and Northern Blot Analysis

Total RNA was extracted from control and shear stress-stimulated endothelial cells as described previously[27,41] using the Chomczynski method.[4] Briefly, endothelial cell monolayers (15 cm^2) were rinsed with sterile PBS, lysed in 1.0 ml of denaturing buffer (4.0 M guanidium isothiocyanate, 0.5% N-lauroylsarcosine, 25 mM sodium citrate, and 0.1 M mercaptoethanol), followed by addition of 0.1 ml of 2.0 M sodium acetate (pH 4.0). The mixture was extracted twice with redistilled buffered phenol and chloroform/isoamyl alcohol (49:1). RNA was precipitated by addition of an equal volume of isopropyl alcohol and overnight incubation at −20 °C, followed by centrifugation at 12,000 g for 30 min. The RNA pellet was rinsed with ethanol, dried, and redissolved in diethylpyrocarbonate-treated water.

The plasmid pSKCNP-1 (obtained from Dr. J. Gordon Porter, Scios Nova, Mountain View, CA) was used as the template to synthesize a CNP cDNA probe. pSKCNP-1 contains the 237 bp coding region of the second exon of human CNP gene as described by Tawaragi *et al.*[36] The cDNA probe was synthesized by the random priming hexanucleotide method with [α-^{32}P]dATP. Total RNA extract was run on each lane

of a 1.0% agarose-formaldehyde gel. Ethidium bromide staining indicated complete integrity of the rRNA bands as well as even lane loading. The gel was blotted overnight to Zeta-Probe GT blotting membranes (Bio-Rad, Hercules, CA), and then the membranes were probed with 10^6 cpm/ml of probe in hybridization buffer (0.5 M Na_2HPO_4, 7% SDS, pH 7.2), followed by high-stringency washes [40 mM Na_2HPO_4, 5% SDS (pH 7.2), washed twice for up to 60 min each; followed by 40 mM Na_2HPO_4, 1% SDS (pH 7.2), washed twice for up to 60 min each]. Glyceraldehyde 3-phosphate dehydogenase (GAPDH) mRNA was used as an internal control for normalization. CNP mRNA is expressed at extremely low levels in control cultures of BAEC in contrast to other mRNA species such as BAEC eNOS mRNA.[27,41] Baseline levels of CNP mRNA were difficult to detect despite the sensitivity of Northern blotting/PhosphorImaging with large quantities of total RNA (20–30 μg) run in each lane. Thus, we pooled the total RNA from two independent BAEC monolayers maintained under identical but independent conditions. The capacity in our laboratory was increased to run four simultaneous flow systems exposing two individual monolayers to 25 dyn/cm^2 and two monolayers to shear stress in the presence of each inhibitor. Experimental values for each CNP/GAPDH mRNA ratio ± SE were the average of two independent monolayers. Northern blotting of BAEC total RNA produced a single band at 1.2 kb for CNP mRNA and at 1.3 kb for GAPDH mRNA. A 425S PhosphorImager system (Molecular Dynamics, Sunnyvale, CA) was used for quantification of Northern blots. Membranes were placed in an exposure cassette (Molecular Dynamics) for 72 h at room temperature. The imaging plates were scanned with a 10 mW helium-neon laser and a speed of 15 μs per 176 μm pixel. Before and after use, remaining images and background noise were erased by exposing imaging plates to bright visible light using 410A Image Eraser (Molecular Dynamics). All scanner operations, data display and analysis were performed using IMAGEQUANT software operating on a Sony CPD1430 microprocessor. The absolute value of the CNP/GAPDH mRNA ratio in each experiment was also dependent on the ^{32}P incorporation yield of each labeling reaction as well as the precise age of each probe at the time of hybridization. Also, the background of the blot can vary and this can influence the magnitude of the CNP/GAPDH ratio. Thus, the CNP/GAPDH control signal serves as an internal control for each individual experiment but does not facilitate comparisons of the CNP baseline from experiment to experiment. To avoid these experiment-to-experiment variations, statistical comparisons were made using the student *t*-test for ratios obtained from RNA samples that had been simultaneously isolated from replicate monolayers, electrophoresed, blotted, probed, and imaged. The lane-to-lane standard deviation in each experiment as determined by the variability of the GAPDH signal was used for the statistical comparisons. The uncertainty in phosphorImaging each band is extremely small (no visible error bars). Rather, the important indicator of the experimental uncertainty in Northern blotting is the loading of RNA into each lane (indicated by the standard deviation of the GAPDH signal from lane to lane in each blot) and is given in the legend of each blot. All standard deviations in lane loading of each gel were less than ±25%.

Detection of CNP Antigen

Secretion of CNP antigen was detected by radioimmunoassay (Penisula laboratories) using a rabbit anti-C-type natriuretic peptide 22 (human, porcine, rat) that crossreacted with bovine CNP. The assay was calibrated between 1 and 128 pg/tube (100 μL sample/tube). In order to detect CNP antigen, 1 ml conditioned media samples were concentrated (to about 120 μL) by vacuum drying at room temperature (Savant Speedvac) to increase the concentration of antigen by a factor of about eight-fold. No precipitate was observed during the procedure. The concentrating factor was determined by weighing each sample before and after water evaporation.

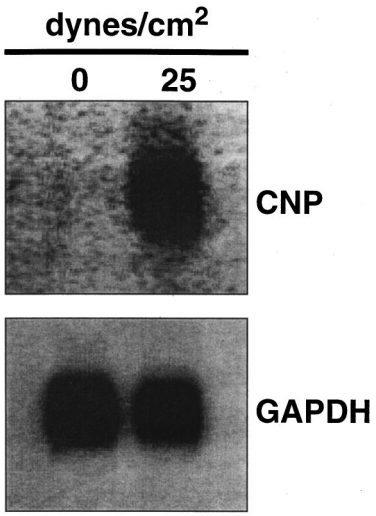

FIGURE 1. Arterial level of laminar shear stress induces CNP mRNA levels in bovine aortic endothelial cells (BAEC). The CNP and GAPDH mRNA levels are shown in BAEC that were exposed to shear stress of 25 dyn/cm^2 or maintained as stationary controls (0 dyn/cm^2) for 6 h. Total RNA (20 μg) was subjected to electrophoresis in each lane, and ethidium bromide staining indicated uniform lane loading. Identical results were seen in eight separate experiments using independent passages of BAEC.

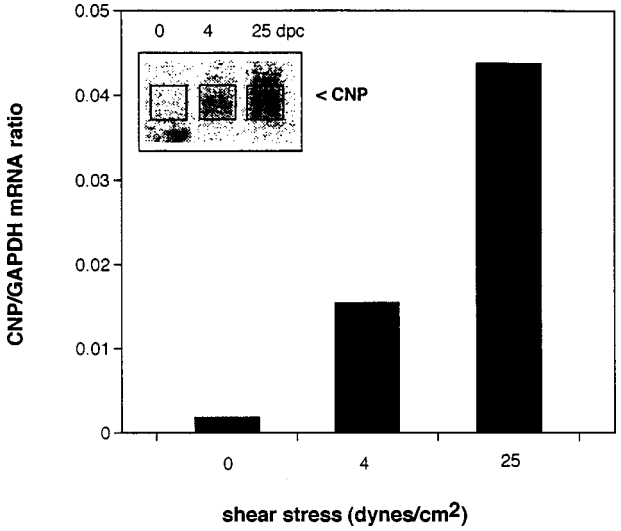

FIGURE 2. Dose-dependent induction by shear stress of CNP mRNA levels in BAEC. PhosphorImaging of CNP mRNA levels (insert) analyzed by Northern blotting of total RNA (27.5 μg/lane) from BAEC that were maintained in stationary culture (0 dyn/cm^2) or exposed to shear stress of 4 or 25 dyn/cm^2 for 6 h. The CNP mRNA signal for each lane was normalized to its GAPDH mRNA signal which had a lane-to-lane standard deviation of ±15.5%. This experiment was representative of two separate experiments.

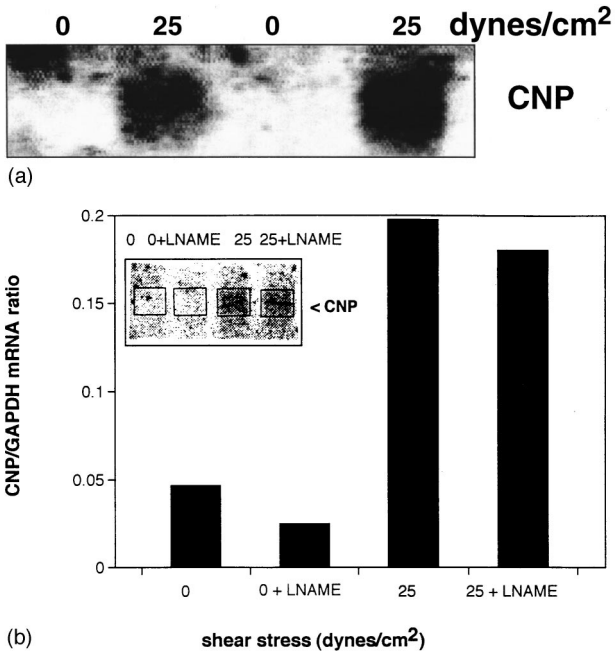

FIGURE 3. Blockade of NO production does not prevent elevation of BAEC CNP mRNA by shear stress. Levels of CNP mRNA in BAEC maintained in stationary culture (0 dyn/cm^2) or exposed to shear stress (25 dyn/cm^2) for 6 h, all in the presence of 400 μM N^G-nitro-L-arginine methyl ester (L-NAME) (a). Two separate experiments were conducted, each with an independent passage of BAEC (first experiment, lanes 1 and 2; second experiment, lanes 3 and 4). In the third experiment (b), the CNP/GAPDH mRNA ratio obtained by PhosphorImaging (23 μg total RNA/lane) indicated that the extent of shear stress induction of CNP mRNA was the same either in the presence or absence of 400 μM L-NAME. The GAPDH mRNA signal had a lane-to-lane standard deviation of ±18.3%.

RESULTS

Total RNA extracted from the cells exposed to shear stress for 6 h or maintained as stationary controls was Northern blotted and quantified with PhosphorImaging. A marked elevation of CNP/GAPDH mRNA ratio of 12.5-fold was observed in BAEC exposed to arterial shear stress of 25 dyn/cm^2 (Fig. 1). CNP mRNA was not detectable in cells maintained in stationary culture under the conditions used in this study. Thus, the CNP mRNA signal for stationary control was set equal to the background signal of the blot to provide the most conservative estimate of the fold induction of CNP mRNA by shear stress. Over all experiments, a 6 h exposure to arterial shear stress of 25 dyn/cm^2 caused a marked elevation (10.5 ± 6.2-fold; $n = 10$, $p < 0.001$) of CNP/GAPDH mRNA ratio compared to stationary controls. We further examined the dose dependency of the shear stress mediated elevation of CNP mRNA by exposing BAEC cells to 4 or 25 dyn/cm^2 for 6 h. The CNP/GAPDH mRNA was 2.6-fold higher ($p < 0.01$) in cells exposed to 25 dyn/cm^2 than in cells exposed to 4 dyn/cm^2 for 6 h (Fig. 2).

Since NO can elevate cGMP in endothelial cells through an autocrine mechanism[22] and CNP can be induced by elevated cGMP,[19] we investigated the role of NO in the elevation of CNP mRNA by shear stress. BAEC cells were pretreated for 30 min with L-NAME (400 μM) to block NO production during the experiment. CNP mRNA was markedly elevated when BAEC were exposed to shear stress in the presence of L-NAME [Fig. 3(a)]. The lack of effect with L-NAME was further investigated by PhosphorImaging of the CNP/GAPDH mRNA ratio in cells exposed to shear stress in the presence or absence of L-NAME [Fig. 3(b)]. As before, L-NAME had no effect on the shear stress elevation of CNP mRNA. In three separate experiments with eight separate monolayers exposed to shear stress in the presence or absence of LNAME, CNP mRNA was dramatically elevated regardless of NO production.

To test whether a calcium-dependent step was required for the elevation of CNP mRNA levels in cells exposed to shear stress, BAEC were preincubated with the cell-permeable intracellular calcium chelator BAPTA/AM (5 μM) for 30 min and then exposed to shear stress of 25 dyn/cm^2 with BAPTA in the perfusion medium. The presence of BAPTA caused a marked 71% reduction ($n = 2$) of shear-induced CNP mRNA as indicated by the CNP/GAPDH mRNA ratio (Fig. 4).

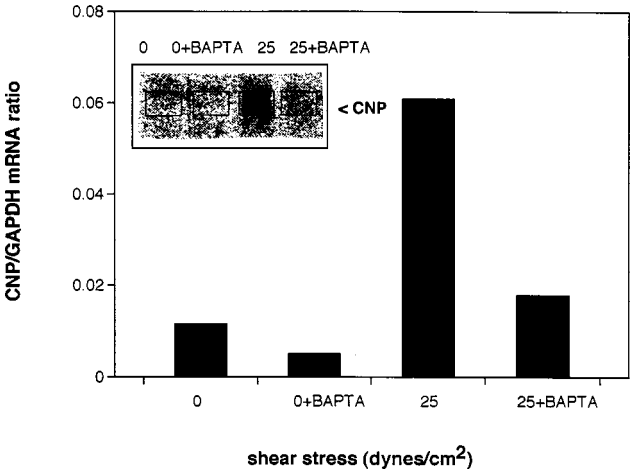

FIGURE 4. BAPTA/AM (5 μM) attenuated the induction of BAEC CNP mRNA levels by shear stress. PhosphorImaging of CNP mRNA levels (insert) are shown for BAEC that were maintained in stationary controls (0 dyn/cm^2) or exposed to laminar shear stress of 25 dyn/cm^2 for 6 h in the presence or absence of BAPTA/AM. The CNP mRNA signal for each lane was normalized to its GAPDH mRNA signal which had a lane-to-lane standard deviation of ±25.6%.

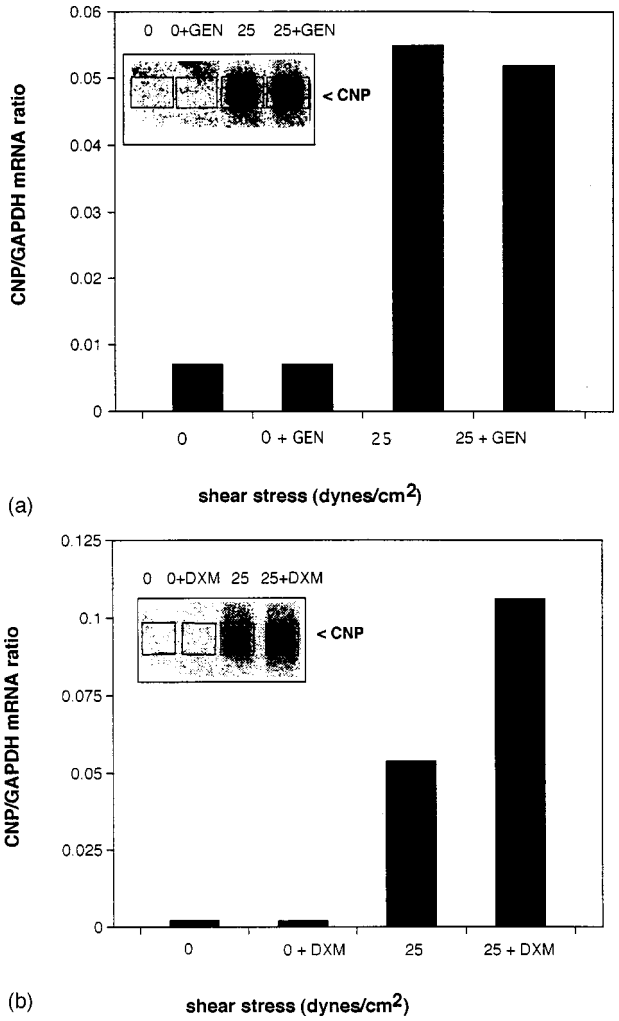

FIGURE 5. Induction of CNP mRNA by shear stress does not require activation of tyrosine kinase or activation of nuclear factor NF-κB. CNP/GAPDH mRNA levels (inserts) are shown in BAEC that were exposed to shear stress of 25 dyn/cm^2 for 6 h or maintained in stationary culture (0 dyn/cm^2) in the presence or absence of: 10 μM genistein (GEN), a tyrosine kinase inhibitor (a); or 1 μM dexamethasone (DXM), an inhibitor of NF-κB function (b). Total RNA per electrophoresis lane was 34.0 (a) and 29.3 μg (b). The CNP mRNA signal for each lane was normalized to its GAPDH mRNA signal which had a lane-to-lane standard deviation of ±18.2% (a) and ±15.2% (b).

We have conducted experiments to explore the potential role for activation of tyrosine kinase or NF-κB in the induction of CNP by shear stress since these mediators are known to be activated during shear stress exposure of endothelium.[1,5,18,37] Recently, it has been shown[1,5] that tyrosine phosphorylation (but not large calcium transients) is required for shear stress-induced NO production in endothelial cells. To test the role of tyrosine kinase activation and associated NO release as mediators of shear stress-induced CNP expression, BAEC cells were preincubated with the tyrosine kinase inhibitor, genistein and then exposed to arterial shear stress for 6 h with genistein in the perfusion medium. No significant changes ($n=2$) were observed in CNP mRNA levels in the presence of genistein [Fig. 5(a)]. Cells were pretreated with dexamethasone (1 μM) for 24 h before the experiment to inhibit the function of NF-κB and then exposed to shear stress of 25 dyn/cm^2 for 6 hr. Dexamethasone had no inhibitory effect on the shear stress elevation of CNP mRNA levels, but caused a nearly 2-fold potentiation of the induction of CNP mRNA levels by shear stress [Fig. 5(b)].

Immunofluorescent staining of BAEC using an antibody against CNP-22 and CNP-53 (Peninsula Laboratories, Belmont, CA) revealed that CNP antigen was predominately perinuclear with some granular staining in the cytoplasm (data not shown). Using quantitative epifluorescence video microscopy,[26,27] we observed no obvious difference in immunofluorescent staining between shear stressed endothelium and stationary controls, although this would be difficult to quantify in cells stained for a secreted protein. This does not necessarily preclude elevated synthesis of CNP protein in shear stressed endothelium since the CNP that is synthesized may be secreted. In fact, we found that the secreted levels of CNP-22 of 34 pg/cm^2-BAEC at 6 h shear stress exposure of 25 dyn/cm were 3.1-fold higher ($n=5$, $p<0.001$) than those detected in conditioned media of stationary cultures (Fig. 6).

Finally, as a measure of the potency with which shear stress can elevate CNP mRNA levels, we incubated BAEC for 6 h with phorbol myristrate acetate (PMA)

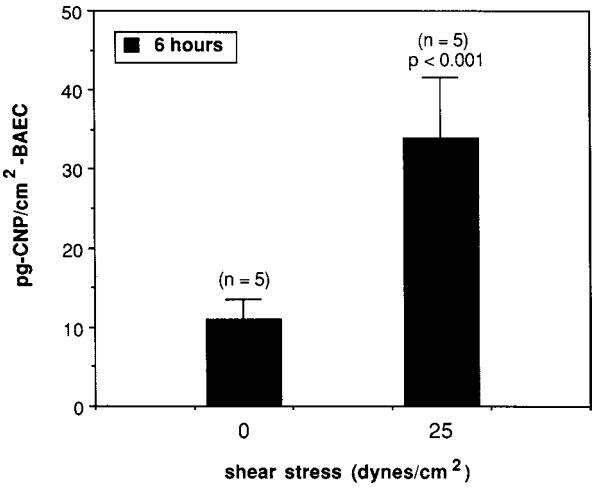

FIGURE 6. Shear stress (25 dyn/cm^2) elevates secretion of CNP by BAEC. Production of CNP by BAEC after 6 h was determined by radioimmunoassay of samples taken from five independent experiments.

(0.5 μg/ml) and lipopolysaccharide endotoxin (LPS, 1.0 μg/ml). At a time of 6 h, LPS caused a small (<25%) elevation of BAEC CNP mRNA levels relative to controls (data not shown), while PMA did not cause an elevation. Thus, shear stress was a particularly potent and rapid activator of CNP mRNA levels in BAEC in contrast to LPS or PMA.

DISCUSSION

We have demonstrated that arterial levels of shear stress caused a large 10-fold elevation in the level of BAEC CNP mRNA and a 3-fold elevation of CNP protein secretion within 6 h after the onset of flow. This is the first demonstration that shear stress induction of arterial endothelial cell CNP mRNA corresponds to an actual elevation in CNP secretion. Consistent with an earlier report,[23] arterial levels of shear stress caused a greater elevation of CNP mRNA than the elevation observed with venous levels of shear stress exposure. The enhancement with shear stress was markedly greater than that observed using a 6 h exposure of BAEC to endotoxin or phorbol ester. The elevation of CNP mRNA was not blocked by L-NAME or genistein suggesting that shear stress-induced NO production (or generation of cGMP through NO-activated soluble guanylate cyclase) was not required for the induction. Chelation of intracellular calcium with BAPTA/AM attenuated the CNP induction by 71%. The attenuation of CNP mRNA induction by BAPTA was similar to other studies of shear stress mediated increases of PDGF B chain mRNA[10] and eNOS mRNA.[41] In contrast, BAPTA has no effect on the activation of MAP kinase by shear stress.[37] The mechanisms of action of BAPTA may be at either the level of rapid secondary signaling or slower modes of cell function since BAPTA may interfere with the importation of proteins into the nucleus[9] or calcium-dependent binding events during transcription initiation.

The biological actions of natriuretic peptides are mediated by two guanylate cyclase-linked natriuretic receptors (NP-A and NP-B receptors). ANP and BNP can stimulate CNP production through a guanylate cyclase receptor on endothelial cells.[19] Our studies showed that the shear stress induction of CNP mRNA did not require shear induced NO since neither L-NAME nor genestein, both inhibitors of NO production, blocked CNP induction by shear stress. Thus, CNP mRNA levels in endothelial cells can be elevated either by cGMP-dependent mechanisms[19] or by NO/cGMP-independent mechanisms as seen in this study. CNP is not expected to autocrinically elevate cGMP due to the low expression of the NP-B receptor in endothelium.

The phosphorylation of paxillin in focal adhesion plaques of shear stressed endothelium[6] indicates that tyrosine kinases are activated during shear stress exposures. Our experiments with genistein showed that tyrosine phosphorylation was not required for the enhancement of CNP mRNA levels. Thus, the dilatory pathway of CNP can be induced in endothelial cells independent of tyrosine phosphorylation events commonly linked to integrin mediated adhesion and remodeling.[28]

We observed that dexamethasone does not prevent the elevation of CNP mRNA by shear stress. Dexamethasone has multiple modes of action in addition to inhibition of NF-kB or AP1 function.[2] Because of this, more experiments are needed to fully clarify the exact roles of NF-kB or dexamethasone during the shear stress induction of the CNP gene. The endothelial cell response to laminar shear stress appears distinct from a classical dexamethasone-inhibited inflammatory response (e.g., iNOS induction). Dexamethasone actually potentiated the induction of CNP mRNA expression by shear stress.

The ability of arterial shear stress to induce CNP suggests a potential protective role for CNP in vessel wall physiology. The rapid increases in NO and PGI_2 production along with elevated expression of eNOS and CNP, with concomitant suppression of endothelin, is a remarkable coordination of multiple metabolisms and genes by the endothelial cell in response to hemodynamic forces. Overall, these studies have pointed toward the shear stressed endothelial cell phenotype as one primed for vasodilation and smooth muscle cell growth inhibition.

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