Atrial natriuretic peptide (ANP) treatment of rat aortic smooth muscle cells suppressed both $^{125}$I-ANP binding and ANP-dependent cGMP accumulation, suggesting reductions in the type C (NPR-C) and type A (NPR-A) natriuretic peptide receptor populations, respectively. NPR-A, but not NPR-C, mRNA levels were reduced in a dose-dependent fashion by ANP. The latter effect appeared to be due, at least in part, to suppression of NPR-A gene promoter activity. ANP effected a dose- and time-dependent reduction in a transiently transfected NPR-A luciferase reporter ($-1575$LU). Analysis of 5′ deletion mutants of the NPR-A promoter demonstrated that the ANP-dependent sequence lies between $-1575$ and $-1290$ relative to the transcription start site. Inhibition of the ANP promoter was also effected by brain natriuretic peptide, type C natriuretic peptide, and 8-bromo-cGMP, but not by the NPR-C-selective ligand cANF. In the case of 8-bromo-cGMP, the responsive element(s) was localized to the same 285-base pair region linked to the ANP effect above. These findings indicate that ANP auto-regulates its own receptors in these cells and, at least in the case of NPR-A, it does so through suppression of receptor gene expression and receptor synthesis. This suppression may operate through a cGMP-dependent element located more than a kilobase upstream from the transcription start site.

Atrial natriuretic peptide (ANP) is a cardiac hormone which has profound effects in the kidney, vasculature, and nervous system (1). It has vasorelaxant activity in vascular smooth muscle (2), and it promotes urinary excretion of sodium and water by the kidney (3). It also has antimitogenic effects in renal mesangial cells (4), vascular smooth muscle (5) and endothelial cells (6), glial cells of the central nervous system (7), and fibroblasts of the cardiac interstitium (8). Thus, it possesses hemodynamic and antigrowth properties which oppose those of vasoconstrictors like the $\alpha$-adrenergic agonists, endothelin, and angiotensin.

ANP is one member of a family of natriuretic peptides which also includes brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). ANP and BNP are both made in and secreted from the heart, circulate in plasma, and share many of the same biological activities. CNP, on the other hand, is made primarily in non-cardiac tissues, does not circulate at appreciable levels in plasma, and has a different activity profile at the level of target tissues (9). At a molecular level these ligands interact with one or more of three different receptor subtypes. The type A natriuretic peptide receptor (NPR-A) has an extracellular ligand-binding domain which recognizes both ANP and BNP. This domain is linked through a trans-membrane region with a carboxy-terminal guanylyl cyclase domain which serves as the effector portion of the receptor. The type B receptor (NPR-B) shares a similar structural topology with NPR-A but binds selectively to CNP. The C-type receptor (NPR-C) is structurally distinct from the guanylyl cyclase receptors. It contains a large extracellular ligand-binding domain followed by a short trans-membrane segment; however, its intracellular domain contains only 38 amino acids and lacks guanylyl cyclase activity. It is the predominant receptor on most vascular cells in culture (10). This, together with its limited intracellular structure, led investigators early on to speculate that it functioned predominantly in a clearance mode, responsible for removing natriuretic peptides from the circulating plasma and processing them to inactive forms. Direct experimental testing supports this hypothesis (11, 12). Additional evidence suggests that NPR-C may also have a signaling function. cANF, an analogue of ANP which associates relatively selectively with NPR-C, has been shown to inhibit adenylyl cyclase activity in a number of different whole cell membrane preparations (13, 14). It also at least partially mimics the actions of ANP in suppressing $[^{3}H]$thymidine incorporation (as an index of mitogenic activity) in vascular smooth muscle cells (5), glial cells (7), and cardiac fibroblasts (8). Such studies raise the possibility that NPR-C may possess important regulatory activity operating either independently or in parallel with that of the guanylyl cyclase-linked receptors (15).

ANP receptor levels and ANP receptor activity (assessed predominantly through ANP-dependent guanylyl cyclase activity) are regulated by a number of factors, including hormones (16–20), growth factors (21–25), neurotransmitters (26), physiological (27, 28) and pathophysiological perturbations (29, 30), and changes in extracellular ion composition (31). ANP, itself, is an important regulator of NPR activity. Physiological changes in the intact animal, which are known to raise circulating ANP levels, have been shown to decrease levels of $^{125}$I-ANP binding in the kidney (27, 28). In vitro, ANP pretreatment has been shown to diminish NPR-C density in vascular endothelial (32) and smooth muscle cells (33–35) and to abrogate ANP-dependent cGMP synthesis in the latter (33, 36, 37). Studies carried out with cells stably transfected with the NPR-A receptor suggest that homologous desensitization of guanylyl cyclase activity by ANP is linked to dephosphorylation of key
serine and threonine residues in the receptor protein (22, 38). The present study demonstrates that the fall in cyclase activity is also associated with a reduction in steady-state levels of NPR-A mRNA and transcriptional activity of the NPR-A gene. This implies that ANP exerts both transcriptional and post-transcriptional effects to regulate the activity of its receptors in target cells.

**EXPERIMENTAL PROCEDURES**

**Materials—**ANP (rANF, 28 amino acids), brain natriuretic peptide (porcine, BNPs–12), C-type natriuretic peptide (porcine, CNPs–22), and cAMP (rat, dog, and Glu–8Ser–9Gly). [3H]NBD-cGMP, [3H]cGMP, [3H]cAMP, [3H]radioiodinated rANP, [125I]cGMP, and [125I]cAMP were purchased from Peninsula Laboratories, Inc. (Belmont, CA). 3′-I-subsituted 1-methylxanthine (IBMX, a nonselective phosphodiesterase (PDE) inhibitor), forskolin, 8-bromo-cAMP, 8-bromo-cGMP, and reagents for cGMP radioimmunooassay (cGMP standard and antibody) were purchased from Sigma. M&B 22948 (a selective inhibitor of the cGMP PDE) was generously provided by the Dagenham Research Center of Rhone-Poulenc, Ltd. (Dagenham, United Kingdom). Fetal bovine serum was purchased from Gemini Bioproducts, Inc. (Calabasas, CA). 125I-cAMP, [α-32P]dCTP, and [3H]acetyl coenzyme A were purchased from DuPont NEN. The bovine NPR-C DNA and rat NPR-A DNA were generously provided by G. Porter and D. Garbers, respectively. The luciferase assay kit was purchased from Promega (Madison, WI). Restriction enzymes were purchased from BclII M. German. For the sake of convenience, constructs have been defined by the length of the rat NPR-A upstream sequence (5′ FS) (46) linked to luciferase coding sequence (e.g., −1575LUC). Six of the deletion mutants were generated through the use of convenient restriction sites. In each case the 3′ linkage employed a NarI site (position +978 in luciferase coding sequence) of the pOXLUC vector. The 5′ linkage employed unique restriction sites (i.e., BglII for −1575LUC, NdeII for −1200LUC, BclI for −716LUC, HindIII for −387LUC, SstII for −273LUC, and DraII for −77LUC) for placement in the polyplymer of the parent vector. The shortest construct, −9LUC, contains 9 base pairs of 5′-flanking sequence from the rat NPR-A gene. It was generated from −77LUC by polymerase chain reaction (PCR), using an upstream sense digonucleotide primer and a GAT CCT CTCAGT sequence for DNA sequencing was purchased from U.S. Biochemical Corp. Other reagents were obtained from standard commercial suppliers.

**Cell Culture—**Embryonic rat aortic smooth muscle (RASM) cells were obtained by passage 19 from H. Ives at University of California San Francisco. Cells were cultured at 37 °C in a 5% CO2 humidified incubator in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2% (v/v) broth, tryptose phosphate. Cells were obtained at passage 19 from H. Ives at University of California San Francisco. Cells were cultured at 37 °C in a 5% CO2 humidified incubator in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2% (v/v) broth, tryptose phosphate. Cells were used after reaching confluency at passage 20–30.

**Receptor Binding Assay—**ANP was labeled with Na125I using the chloramine-T method and purified as described previously (39). Cells were grown to confluence in 24-well plates (1 × 105/well) and preincubated with 1 nM ANP in a random mixture of media (20% serum-free DMEM, 80% serum-free DMEM, and 2% (v/v) broth, tryptose phosphate) at 37 °C for 30 min. The media were aspirated, and 0.4 ml/well ice-cold 10% trichloroacetic acid (TCA) was added to each well. After 30-min incubation at 37 °C, the cells were washed (four times) with acidified DMEM (pH 5.0) prior to extraction of media and addition of 0.4 ml/well ice-cold 10% trichloroacetic acid. The reaction was terminated by aspiration of media and addition of 0.4 ml/well ice-cold 10% trichloroacetic acid. The extraction was continued for 30 min at 4 °C. Cell samples were centrifuged to remove precipitated proteins, and the supernatant fractions were extracted (four times) with 0.5 ml of water-saturated ether. Aliquots were lyophilized and resuspended in 50 mM sodium borate (pH 9.4). Cyclic GMP levels were determined by radioimmunoassay, after acetylation of the samples and standards, using a commercial antisera and 125I-cGMP.

**RNA Isolation and Northern Blot Analysis—**Cells were plated in 10-cm dishes and grown to confluence. They were washed (three times) with 10 ml of serum-free DMEM, then incubated at 37 °C in serum-free DMEM containing different concentrations of ANP for 48 h. Total RNA was isolated from cells by the guanidinium thiocyanate-phenol-chloroform method (40). 30 μg of RNA was size fractionated on a 2.2% formaldehyde, 1% agarose gel, transferred to a nitrocellulose filter, and hybridized either with a full-length (2.1 kilobase pairs) bovine NPR-C cDNA (41) isolated as a HindIII-EcoRI fragment or a 1.2-kilobase pair EcoRI fragment of the rat NPR-A cDNA (42). Blots were subsequently washed and reprobed with a 1.3-kilobase pair Psfl fragment of rat glyceraldehyde-3-phosphate dehydrogenase cDNA (43) to permit normalization of blots for differences in RNA loading and/or transfer to the filter. Each probe was labeled with [α-32P]dCTP using the random primer method (44). Autoradiography was performed with an intensifying screen at −70 °C for 5–10 days. Autoradiographic signals were quantified by laser densitometry.

**Plasmid Construction—**All rat NPR-A LUC constructs were based on a pFXOLUC vector. This vector was generated by substitution of luciferase coding sequence in a pFXCAT vector and provided to us by M. German. For the sake of convenience, constructs have been defined by the length of the rat NPR-A upstream sequence (5′ FS) (46) linked to luciferase coding sequence (e.g., −1575LUC). Six of the deletion mutants were generated through the use of convenient restriction sites. In each case the 3′ linkage employed a NarI site (position +978 in luciferase coding sequence) of the pOXLUC vector. The 5′ linkage employed unique restriction sites (i.e., BglII for −1575LUC, NdeII for −1200LUC, BclI for −716LUC, HindIII for −387LUC, SstII for −273LUC, and DraII for −77LUC) for placement in the polyplymer of the parent vector. The shortest construct, −9LUC, contains 9 base pairs of 5′-flanking sequence from the rat NPR-A gene. It was generated from −77LUC by polymerase chain reaction (PCR), using an upstream sense digonucleotide primer and a GAT CCT CTCAGT sequence for DNA sequencing was purchased from U.S. Biochemical Corp. All of the deletion constructs were sequenced across the fusion junctions to confirm the predicted structure.

**Transfection and Luciferase Assay—**Cells were transfected with 20 μg of one of the rat NPR-A deletion mutants and 5 μg of pRSVCAT, which was employed as an internal control for transfection efficiency. The total amount of transfected DNA was adjusted with pUC18. Cells were transfected by electroporation (GenePulsar; Bio-Rad) using 250 V at 960 microfarads, optimal conditions which were derived empirically. After transfection, cells were plated in six-well plates at a density of 1 × 104 cells/well in 10% FBS/DMEM. Medium was changed at 24 h with serum-free DMEM containing different concentrations of ANP or other reagents, and the incubations were continued for varying periods of time. Cells were then harvested and lysed in 60 μl of 250 mM Tris, 0.1% Triton X-100. Protein concentration of each cell extract was measured using the Coomassie protein reagent. Cell lysates were processed for measurement of both luciferase and CAT activity; the luciferase data were standardized using an equal amount of soluble protein for each reaction and then normalized to an equivalent level of CAT expression. A mock-reaction containing no protein was included in both assays to establish a base-line activity which, in turn, was subtracted from each experimental value. To ensure reproducibility, experiments were repeated three to six times, using at least three different plasmid DNA preparations. Transfection efficiency, assessed from measurements of pRSVCAT activity, varied by less than 15% within a given experiment. Statistical Analysis—All stated otherwise, statistical differences were evaluated by one way analysis of variance with the Newman-Keuls test for significance.

**RESULTS**

Pretreatment with ANP led to a significant decrease in 125I-ANP binding in cultured RASM cells. As shown in Fig. 1, ANP pretreatment effected a dose- and time-dependent reduction in binding which was maximal between 10 and 100 nM ANP after 24 h of exposure. Since the majority of ANP receptors in RASM (85–95%) are of the NPR-C or clearance receptor class, by definition this group of receptors is suppressed by ANP. This inhibition was reproduced in cultures subjected to the acid wash (without wash: 12.3 ± 1.5% of control binding; with wash:
ANP treatment (Fig. 5). Based on the deletion profile presented in this figure, it is clear that NPR-A gene transcription is positively regulated by at least three regions in the promoter positioned between \(-1575\) and \(-1290\), \(-716\) and \(-387\), and \(-273\) and \(-77\) relative to the transcription start site. ANP exerted a significant negative effect on the \(-1575\)LUC reporter, as described above; however, truncation of the reporter to \(-1290\) and smaller derivatives resulted in a loss of the ANP-dependent inhibition. By inference this suggests that the element responsible for suppressing transcriptional activity from the NPR-A gene promoter is located between \(-1290\) and \(-1575\) relative to the transcription start site.

The suppression of NPR-A promoter activity was not limited to ANP. Both BNP and CNP, at comparable concentrations of ligand, effected similar reductions in \(-1575\)LUC activity (Fig. 6). Each of these ligands is capable of increasing cGMP levels in these cells (Table I). ANF, an ANP homolog which lacks the guanylyl cyclase-activating function and binds predominantly to the type C or clearance receptor, failed to suppress NPR-A promoter activity. This suggests that the reduction in transcription requires occupancy of a receptor system capable of triggering the activation of guanylyl cyclase and production of cGMP (i.e. activation of NPR-A by ANP or BNP or activation of NPR-B by CNP). To explore this further we pretreated \(-1575\)LUC-transfected cells with one of two different phosphodiesterase inhibitors (PDEI) in the presence or absence of ANP and assessed the effect on reporter activity (Fig. 7). Both the nonselective PDEI, IBMX, and the type V (cGMP-selective) PDEI, M&B 22948, would be predicted to slow the degradation rate of cGMP in these cells (confirmed in Table I) and, therefore, amplify the effects of agonists (e.g. ANP) operating through a guanylyl cyclase-dependent mechanism. Each of these agents independently reduced NPR-A promoter activity and, furthermore, each amplified the effect of simultaneous ANP treatment. These findings are compatible with a requirement for ANP-dependent cGMP generation in initiating and/or maintaining the inhibitory effect. Next, we examined the effect of 8-bromo-cGMP, a membrane-permeable analogue of the cGMP second messenger, on NPR-A promoter activity (Fig. 8). 8-Bromo-cGMP effected a dose- and time-dependent reduction in \(-1575\)LUC reporter activity similar to that seen with ANP. The inhibition was maximal at \(10^{-4}\) M and required between 6 and 48 h to appear. To determine whether this effect operated through the same region of the NPR-A gene promoter targeted...
We examined a number of the same promoter deletion mutants described in Fig. 5 for suppressibility by 8-bromo-cGMP. As shown in Fig. 9, the dose-dependent inhibition of NPR-A promoter activity was observed only with the 21575LUC construct and was not seen with any construct containing 1290 or fewer base pairs of NPR-A gene 5′ FS. This finding further supports the hypothesis that the inhibitory activity of the natriuretic peptides is cGMP-dependent and that the genomic target for this activity is contained within 285 base pairs of promoter sequence lying between 21575 and 21290 relative to the transcription start site.

Recent studies (47) have suggested considerable crossover in the signaling mechanisms employed by cAMP and cGMP in VSMC. In fact, at least some VSMC cultures seem to harbor little, if any, cGMP-dependent protein kinase. Most cGMP-dependent activity, in this latter case, appears to be mediated through the cAMP-dependent protein kinase. To explore the potential crossover of these pathways at the level of the NPR-A gene promoter, we transfected cells with the same chimeric reporters described above, then treated them with 8-bromo-cAMP or forskolin for 48 h. As shown in Fig. 10, neither of these agents had an effect on NPR-A promoter activity, arguing that the inhibition is selective for cGMP.

Fig. 4. Effect of ANP pretreatment on −1575LUC activity. Cultured RASM cells were co-transfected with 20 μg of −1575LUC and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and treated with serum-free DMEM containing indicated concentrations of ANP for different periods of time. All values are expressed as a percentage of luciferase activity in control (untreated) group. Values represent mean ± S.D. from six different experiments. *, p < 0.01 versus control; †, p < 0.05; ‡, p < 0.01 versus corresponding point in 6-h group.

Fig. 5. Effect of ANP pretreatment on 5′ deletion mutants. Cells were transfected with 20 μg of each of the receptor constructs indicated and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and exposed to serum-free DMEM containing 10 nM ANP for 48 h. All values are expressed as a percentage of luciferase activity in control group. Values represent mean ± S.D. from six different experiments. *, p < 0.01 versus control (untreated) group.
DISCUSSION

The results presented above convincingly demonstrate that prior exposure to ANP results in down-regulation of 125I-ANP binding on the surface of rat VSMC and desensitization of the particulate guanylyl cyclase to subsequent challenge with the ligand. ANP-dependent suppression of binding in these cells has been controversial. While the reduction in binding activity is not disputed, several investigators have argued that it results from prior receptor occupancy with the ligand (i.e. persistent occupancy of receptor sites by unlabeled ligand employed in the pretreatment phase of the experiment) rather than a true decrease in receptor density on the cell surface (19, 48). However, other investigators, by employing an acid wash of the pretreated cells to remove associated ligand, have shown that the decrease in surface 125I-ANP binding represents a true reduction in receptor density (26, 33). Our own findings support this conclusion in that conditions predicted to remove more than 95% of surface-associated ligand (26) failed to eliminate the reduction in ligand binding. The reduction in ANP-coupled guanylyl cyclase activity following prolonged exposure to the ligand, such as we have noted here, is less controversial (22, 30, 33, 37, 38), although a number of investigators have

| Table 1: Effect of natriuretic peptides on cyclic GMP production in cultured RASM cells |
|-----------------------------------------|-------------------|
|                                       | Control | S.D. |
| Control                                | 100     | 23.8 |
| IBMX                                   | 220     | 18.4 |
| M&B                                    | 135     | 21.1 |
| ANP                                    | 403     | 30.4 |
| ANP + IBMX                             | 5078    | 327.3|
| ANP + M&B                              | 2258    | 185.3|
| BNP                                    | 306     | 22.3 |
| BNP + IBMX                             | 3545    | 148.0|
| CNP                                    | 283     | 33.0 |
| CNP + IBMX                             | 2370    | 91.6 |

FIG. 6. Effect of different natriuretic peptides on NPR-A promoter activity. Cells were transfected with 20 μg of p1575LUC and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and treated with serum-free DMEM containing the indicated concentrations of natriuretic peptide for 48 h. All values are expressed as a percentage of luciferase activity in control group. Values represent mean ± S.D. from six different experiments. *, p < 0.01 versus control group.

FIG. 7. Effect of PDE inhibition on NPR-A promoter activity. Cells were transfected with 20 μg of p1575LUC and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and exposed to serum-free DMEM containing 0.1 mM IBMX or 0.1 mM M&B 22948 for 48 h. All values are expressed as a percentage of luciferase activity in control group. Values represent mean ± S.D. from four different experiments. *, p < 0.01 versus control group without PDE inhibition; **, p < 0.01 versus groups with ANP treatment alone; †, p < 0.05 versus corresponding groups without ANP treatment.

FIG. 8. Effect of 8-bromo-cGMP on NPR-A promoter activity. Cells were transfected with 20 μg of p1575LUC and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and treated with serum-free DMEM containing increasing concentrations of 8-bromo-cGMP for the indicated periods of time. All values are expressed as a percentage of luciferase activity in control group. Values represent mean ± S.D. from four different experiments. *, p < 0.01 versus control group.

FIG. 9. Effect of 8-bromo-cGMP on NPR-A 5' deletion mutants. Cultured RASM cells were transfected with 20 μg of each of the 5' deletion mutants and 5 μg of pRSVCAT. After 24-h incubation in 10% FBS/DMEM, cells were washed and exposed to serum-free DMEM containing increasing concentrations of 8-bromo-cGMP for 48 h. All values are expressed as a percentage of luciferase activity in control group. Values represent mean ± S.D. from six different experiments. *, p < 0.01 versus control group.
ANP Suppresses NPR-A Gene Transcription

failed to observe the effect (32, 35, 49). This discrepancy remains unexplained, although it may reflect differences in passage number, ANP pretreatment, or the conditions of cell culture.

The reduction in ANP-dependent cGMP synthesis is not likely to be a consequence of the reduction in NPR-C binding activity. In instances where it has been examined closely, reduction in NPR-C levels on the cell surface have more commonly been associated with an amplification of ANP-dependent cGMP generation (26, 50), perhaps reflecting the increased availability of ligand as clearance of ANP decreases in parallel with NPR-C density.

VSMC are known to harbor both NPR-A and NPR-B receptors. The relative proportion of these two populations is tied to the growth status of the cells with NPR-B becoming predominant as the cells are passaged in culture (51). Since ANP is the primary ligand for NPR-A, it is likely that the reduction in ANP-linked guanylyl cyclase activity is, in large part, related to a decrease in this receptor population. The associated fall in NPR-A mRNA levels implies that the fall in NPR-A activity results, at least in part, from a reduction in new receptor synthesis. However, this is unlikely to be the sole mechanism for desensitization of this receptor. Potter and Garbers (22, 38) recently showed that ANP effects a dephosphorylation of NPR-A in parallel with desensitization of guanylyl cyclase activity. Taken together with the findings presented here, this indicates that ANP operates through both transcriptional and post-transcriptional loci in regulating NPR-A activity. The decrease in NPR-C levels, on the other hand, was not accompanied by a decrease in NPR-C mRNA levels, a finding which has been reported by others (47), implying that the ligand effect in this instance is confined to a post-synthetic locus (i.e. at the level of post-transcriptional activity).

The decrease in NPR-A mRNA levels can be explained, at least partially, by a reduction in the transcriptional activity of the NPR-A gene promoter (see Figs. 4 and 5). This inhibition is shared by all three natriuretic peptide ligands as well as 8-bromo-cGMP, and is amplified by PDE inhibition, but it is not shared by the NPR-C-selective ligand cANF. These findings argue that the reduction in promoter activity is mediated by elevations in cellular cGMP which are signaled by either NPR-A or NPR-B in these cells and that NPR-C is not directly involved. Participation of other non-guanylyl cyclase-signaled events from the NPR-A/B receptors cannot be formally excluded; however, co-localization of the ANP-sensitive and cGMP-sensitive region of the NPR-A promoter to the same 285 base pairs of DNA suggests that the inhibition is predominantly cGMP-signaled. Precise localization of the ANP/cGMP-sensitive element remains undefined; however, there are few regions with homology to known transcriptional regulatory elements in this segment of DNA and nothing bearing even remote homology to the cAMP-regulatory element (CRE), an important consideration given the potential for cross-activation of protein kinase A by cGMP (47) in these cells of interest, treatment of cells transfected with −1575LUC with either ANP or 8-bromo-cGMP reduced reporter activity to that of −1290LUC. This implies that the positive regulatory element(s) located in this region can be completely neutralized by elevations in cellular cGMP and may provide a clue as to the mechanism underlying the inhibitory effect. In any event this regulatory element is likely to be of interest since, in general, we know very little of the mechanism(s) underlying cGMP effects on eukaryotic gene promoters.

Given the reduction in both NPR-C and NPR-A activity in these cells, one is left with the question as to the predicted outcome at the level of the intact cell following prolonged exposure to agonist. A priori, one would anticipate that there would be a reduction in ANP-dependent activity given the impairment in the guanylyl cyclase effector mechanism; however, the reduction in NPR-C with presumed decrease in NP clearance would be expected to increase the availability of ligand in the microenvironment surrounding the target cell. Thus, it is possible that under some circumstances (e.g. greater reduction in NPR-C versus NPR-A activity) the combined effect would result in little effect, or perhaps a modest increase, in guanylyl cyclase-linked activity. Identification of those conditions (e.g. based on the concentration or kinetics of exposure to the natriuretic peptides) favoring one or the other of these phenomena could provide interesting insights into the physiology of the natriuretic peptides in the vascular wall and, by inference, identify situations where malfunction could lead to pathological consequences.

Acknowledgments—We are grateful to Drs. David Garbers and Stephanie Schulz for providing the rat NPR-A cDNA and genomic clones, Gordon Porter for the bovine NPR-C cDNA clone, and Mike German for the pFOXLUC vector.

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J. Biol. Chem. 1995, 270:24891-24897.
doi: 10.1074/jbc.270.42.24891

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