AJH 1989; 2:32–39

# Atrial Natriuretic Peptide: Binding and Cyclic GMP Response in Cultured Vascular Smooth Muscle Cells From Spontaneously Hypertensive Rats

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**Atrial natriuretic peptide (ANP) is vasodilatory and natriuretic, but whereas increased plasma ANP levels occur in spontaneously hypertensive rats, their elevated vascular resistance suggests inappropriate target tissue responsiveness to ANP. This study examines ANP-receptor binding properties (at 25°C and 4°C) in cultured vascular aortic smooth muscle cells from spontaneously hypertensive (SHR) and control Wistar-Kyoto (WKY) rats. [$I^{125}$]-human ANP saturation (0.0625–12.0 nmol) profiles were analyzed using nonlinear regression (LIGAND). Vascular smooth muscle cells from WKY possessed both high affinity ($K_{D1}$ 0.3 nmol; $R_1$ 33 fmol/$10^5$ cells) and low affinity ($K_{D2}$ 15 nmol; $R_2$ 400 fmol/$10^5$ cells) binding sites for ANP. In contrast, for smooth muscle cells from SHR, two receptor forms could not be resolved using identical analytical protocols. Parameter estimates at 25°C and 4°C were not different for either SHR or WKY. The number of receptors for SHR ($B_{max}$ ~ 100 fmol/$10^5$ cells) was lower than the total number of receptors for WKY (high plus low affinity ~ 430 fmol/$10^5$ cells). The intermediary $K_D$ value (~1.0 nmol) for ANP binding in SHR suggests an ANP-receptor interconversion from high affinity to low affinity in smooth muscle cells from SHR. Competition-binding experiments also revealed a decreased affinity for ANP in SHR-derived smooth muscle cells. The cyclic GMP response (intracellular accumulation and extracellular levels) was decreased in SHR smooth muscle cells compared to WKY, although this difference was evident only after prolonged (one hour) stimulation with ANP. Our data indicate a reduced sustained vascular responsiveness to ANP in hypertension. Am J Hypertens 1989; 2:32–39**

KEY WORDS: **Atrial natriuretic peptide, receptors, cyclic GMP, cultured vascular smooth muscle cells, spontaneous hypertension.**

Atrial natriuretic peptide (ANP) has potent natriuretic, aldosterone-renin secretion-inhibitory, and vasorelaxing properties that may play a central role in the control of blood volume, sodium excretion, and blood pressure,[1–3] (for reviews). Receptors for this peptide in vascular tissues have been demonstrated for aorta,[4] mesenteric and renal arteries,[5] and for cultured endothelial and smooth muscle cells.[6–9] Studies on the postreceptor actions of ANP have shown that ANP activities particulate guanylate cyclase and increases both intracellular and extracellular cyclic GMP accumulation.[7–12]

Elevated plasma ANP concentrations have been demonstrated in disease states associated with an increased cardiopulmonary volume including congestive heart failure,[13,14] essential[15,16] and spontaneous hyper-

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This study was supported by the Swiss National Foundation No. 3.924.083.

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0895-7061/89/$3.50

tension.[17-20] However, in these conditions there is both an inappropriate natriuresis and an elevated peripheral vascular resistance, to which decreased target tissue responsiveness to ANP at receptor or postreceptor sites may contribute. Some[21-23] but not all[24] whole animal studies have reported that ANP (bolus administration) is more effective and/or potent in eliciting physiological responses in spontaneously hypertensive rats (SHR) than in Wistar-Kyoto animals (WKY). Similarly in humans, natriuretic[25] and vasodilatory[26] responsiveness to ANP is greater in patients with essential hypertension (EHT) than in normotensive subjects (NT). On the other hand, ANP produced a less sustained fall in blood pressure in EHT than in NT,[25] whereas sustained infusions of ANP indicated a lower sensitivity in SHR and WKY.[27] In contrast to whole body studies, investigations using isolated thoracic vessels clearly demonstrate reduced vasorelaxant responsiveness to ANP in SHR as compared to WKY.[28-30] Reasons for the general opposite nature of hypertensive/normotensive sensitivity differences between isolated vessel and whole body study models are unclear. However, proposals have been made that exaggerated physiological responses to ANP in hypertensives (for whole body models) may relate to their hypertensive arterial[25] and/or renal[31] perfusion pressures.

This study compares the properties of ANP-receptors in cultured thoracic aorta smooth muscle cells from SHR and WKY under conditions in which in vivo effects relating to differential pressure and circulating levels of ANP may be excluded. We have also studied the cyclic GMP production response to ANP in these cells.

## MATERIALS AND METHODS

**Materials** With the exception of fetal calf serum (Fakola AG, Basel, Switzerland) all tissue culture material and chemicals were purchased from Gibco AG, Basel, Switzerland. Human $\alpha$-ANP (99-126) (h-ANP) was from Nova Biochem AG, Laufelfingen, Switzerland, and [3-($I^{125}$)$Tyr^{126}$] human $\alpha$-ANP (99-126), 2000 Ci/mmol was from Anawa Laboratories AG, Wangen, Switzerland. [$^3$H]cyclic GMP RIA kit was from Amersham International, Amersham, England. All other chemicals were of analytical grade and were purchased from Merck, Darmstadt, Germany, Fluka AG, Buchs, Switzerland or from Sigma Chemical Co., St. Louis, MO, USA.

**Animals** SHR and WKY rats were obtained from Madoerin AG (Füllinsdorf, Switzerland). Systolic blood pressure (mean ± SEM) was measured by the tail cuff method and was 215 ± 6 mm Hg for SHR (n = 6), 130 ± 5 mm Hg for WKY (n = 6). All animals were male, aged ~20 weeks and weighed 200 g to 250 g.

**Isolation and Culture of Smooth Muscle Cells** Procedures for vascular smooth muscle cell (VSMC) isolation were as described previously by Jones et al[32] and Chamley-Campbell et al[33] using matched (SHR *v* WKY) thoracic aorta sections. Primary cultures were routinely passaged for use in experimental regimes, and phenotypic characterization was performed exactly as described by Jones et al.[32] Typically, smooth muscle cells from third to sixth passages were used in this study. Cell cultures were maintained in minimal essential medium (MEM) containing Earles salts, 20 mmol glutamine, 20 mmol TES-NaOH, 20 mmol HEPES-NaOH (pH 7.3) 15% fetal calf serum, 100 U/mL penicillin and 100 U/mL streptomycin and were incubated at 37°C in a 5% $CO_2$ humidified incubator. For every separate experiment, cell numbers were determined following trypsin-dissociation and suspension in Isoton.

**Binding Experiments** Binding assays were performed in 24-well dishes using procedures described previously.[7] For kinetic binding studies VSMC were incubated at 25°C or at 4°C in the presence of 1.0 nmol [$I^{125}$]h-ANP for various time periods; parallel series of dishes also contained 0.5 $\mu$M unlabeled h-ANP for determination of nonspecific binding that was routinely less than 10%. Surface bound and internalized [$I^{125}$]h-ANP were determined as described by Haigler et al.[34] Specific binding is defined as total binding (surface or internalized) minus nonspecific binding. Saturation binding experiments were conducted for one hour at 25°C and 4°C in the presence of various concentrations (0.0625-12.0 nmol, final) of [$I^{125}$]h-ANP without or with 0.5 $\mu$M unlabeled h-ANP for nonspecific binding. Competition experiments were performed in the presence of 50 pmol [$I^{125}$]h-ANP without or with various concentrations ($10^{-12}$-$10^{-6}$ mol/L final) of unlabeled h-ANP.

**Measurement of Cylic GMP** Intracellular cyclic nucleotide levels are controlled not only by cyclase and phosphodiesterase activities, but also by egression, such that intracellular accumulation represents only a fraction of that synthesized from the onset of stimulation.[35] Sustained ANP-induced cyclic GMP egression has been demonstrated both in vivo[12] and in VSMC cultures.[10] For VSMC from SHR and WKY linearity of cyclic GMP intracellular accumulation and extracellular egression in response to ANP occurred for up to 15 minutes and 120 minutes, respectively (data not shown). We have therefore measured intra/extracellular cyclic GMP after ten-minute and 60-minute treatment with h-ANP. Vascular smooth muscle cells (in 12-well dishes) were incubated at 37°C in 500 $\mu$l PBS containing 0.5 mmol isobutyl-1-methylxanthine in the absence or presence of varying concentrations ($10^{-9}$-$10^{-5}$ mol/L) h-ANP. Before preparation of intracellular cyclic GMP extracts[7] aliquots of buffer overlay were withdrawn for measurement of extracellular cyclic GMP. Cyclic GMP was measured after acetylation[36] by radioimmunoassay.

**Data Analysis** Saturation binding profiles were individually processed by the computer program "EBDA"[37] and then tested for single- and two-site binding models using the nonlinear curve fitting program "LIGAND".[38] Application of the LIGAND curve fitting program either using experimentally determined nonspecific binding (N) or by allowing N to float during the fitting process[38] yielded essentially identical parameter estimates. Sigmoidal log dose-response and competition-binding curves for each separate experiment were analyzed using a weighted nonlinear regression computer program. $ED_{50}$ is defined as the concentration of ANP required to elicit a half-maximal response. Statistical analysis was performed using Student's *t* test for unpaired data.

## RESULTS

The kinetics of [$I^{125}$]h-ANP binding to VSMC is presented in Figure 1. For VSMC from both SHR and WKY equilibrium binding was reached after a 30- to 60-minute incubation at either 25°C or 4°C. Within each VSMC type surface bound [$I^{125}$]h-ANP at equilibrium was not significantly different between experiments performed at 25°C and 4°C. At 25°C, but not at 4°C, both SHR- and WKY-derived cells internalized 7% of total ligand bound.

Binding of [$I^{125}$]h-ANP, at 25°C, to VSMC was competitive (Figure 2). The concentration of h-ANP required to half-maximally displace [$I^{125}$]h-ANP was ~six-fold greater ($P < .01$) in SHR-derived cells (1.72 ± 0.4 nmol, n = 4) than that in WKY-derived cells (0.27 ± 0.08, n = 4). Similar differences were evident for experiments performed at 4°C and competition pro-

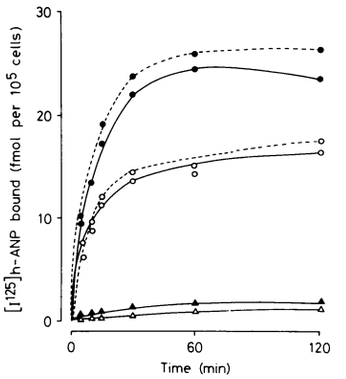


**FIGURE 1.** *[$I^{125}$]h-ANP binding kinetics in cultured aortic smooth muscle cells. VSMC from SHR* (closed symbols) *and WKY* (open symbols) *were incubated with 1.0 nmol [$I^{125}$]h-ANP for the indicated times at 25°C* (solid lines) *and 4°C* (dashed lines). *Specifically surface-bound* (○,●) *and internalized* (△,▲) *ligand were determined as detailed in the Materials and Methods section. Data (mean of triplicate determinations) are from a single typical comparative experiment. Kinetic profiles were similar in two other experiments.*

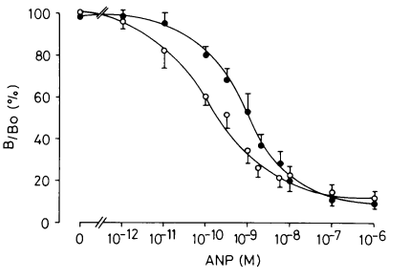


**FIGURE 2.** *Competition binding of [$I^{125}$]h-ANP to cultured aortic smooth muscle cells. VSMC from SHR (●) and WKY (○) were incubated for one hour at 25°C with 50 pmol [$I^{125}$]h-ANP and varying concentrations of unlabeled h-ANP as described in the Materials and Methods section. Bound ligand (B) is expressed as the percentage of that bound ($B_o$) in the absence of unlabeled h-ANP ($B_o$ taken as 100%). Values are mean ± SEM from seven separate experiments.*

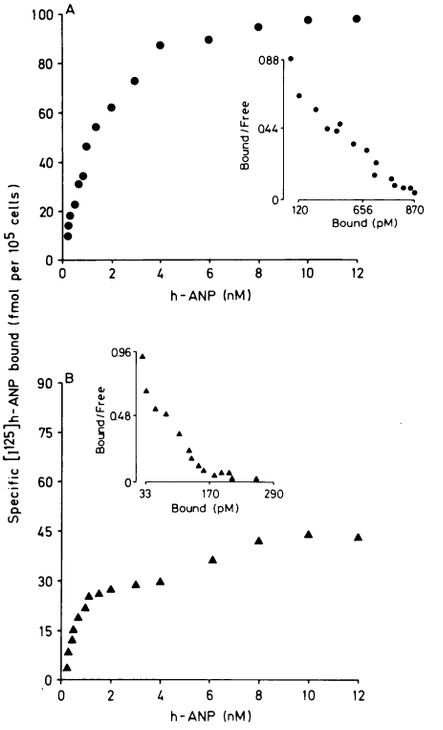


**FIGURE 3.** *Saturable binding of [$I^{125}$]h-ANP to culture aortic smooth muscle cells. Saturation binding was performed at 4°C on VSMC from SHR (panel A, ●) and WKY (panel B, ▲) as described in the Materials and Methods section. Scatchard plots for presented binding data are inset. Data (specific binding) are representative of single typical experiments for SHR and WKY, and each point is the mean of duplicate determinations. Cell numbers of SHR and WKY were 7.5 × $10^5$ and 5.5 × $10^5$, respectively. Final LIGAND parameter estimates for all experiments at 25°C and 4°C are presented in Table 1.*

TABLE 1. PARAMETER ESTIMATES FOR [$I^{125}$]h-ANP BINDING TO VASCULAR AORTIC SMOOTH MUSCLE CELLS FROM SHR AND WKY

| Animal | Binding (°C) | One-Site Modeling | |
|---|---|---|---|
| | | $K_D$ (nmol) | $B_{max}$ (fmol/$10^5$ cells) |
| WKY (n = 7) | 4° | 0.30 ± 0.10 | 38.0 ± 9.8 |
| | 25° | 0.38 ± 0.08 | 36.8 ± 10.2 |
| SHR (n = 7) | 4° | 1.01 ± 0.29* | 102.5 ± 23.4* |
| | 25° | 0.92 ± 0.12* | 101.4 ± 20.0* |

| | Two-Site Modeling | | | |
|---|---|---|---|---|
| | $K_{D1}$ (nmol) | $K_{D2}$ | $R_1$ (fmol/$10^5$ cells) | $R_2$ |
| WKY (n = 14) | 0.33 ± 0.08 | 14.8 ± 2.1 | 32.9 ± 6.6 | 403.2 ± 36.1 |

*Binding parameters were obtained from "LIGAND" data analysis.*

*For one-site modeling $K_D$ and $B_{max}$ represent the dissociation constant and maximum number of binding sites respectively.*

** indicates significant differences (P < .01) between SHR and WKY for binding at 4°C or 25°C.*

*For two-site modelling $K_{D1}$ and $K_{D2}$ are dissociation constants and $R_1$ and $R_2$ are numbers of sites with high and low affinity respectively.*

*Parameter estimates from 25°C and 4°C binding experiments were similar, thus combined values are presented.*

*All values are given as mean ± SEM where n = number of separate experiments.*

files using rat-ANP were essentially identical to those obtained with h-ANP (data not shown).

Equilibrium binding of [$I^{125}$]h-ANP to SHR and WKY VSMC was saturable at both 4°C (Figure 3) and 25°C (profiles not shown and not significantly different from data obtained at 4°C). For VSMC from WKY, saturation profiles demonstrated deviation from a simple bimolecular binding isotherm (Figure 3B). Scatchard-transformed data for WKY was curvilinear (Figure 3B, inset) and Hill analysis yielded a coefficient significantly less than unity (0.59 ± 0.07, n = 7). In contrast, for SHR-derived VSMC (Figure 3A, inset) Scatchard transformation yielded an apparently linear plot, whereas the Hill coefficient approximated unity (0.94 ± 0.02, n = 7).

In the LIGAND analysis for a single site model both the maximum number of binding sites ($B_{max}$) and the dissociation constant ($K_D$) were greater ($P < .01$) for VSMC from SHR than VSMC from WKY (Table 1). These differences were evident at both 25°C and 4°C. However, LIGAND analysis for a two-site model improved the goodness of fit ($P$ at least .05) for VSMC from WKY and enabled resolution of high ($K_{D1} \sim 10^{-10}$ mol) and low ($K_{D2} \sim 10^{-8}$ mol) affinity binding sites could be resolved, and the number of high affinity sites (Table 1). For VSMC from SHR, in only two out of the fourteen experiments (25°C and 4°C) was a significantly improved data-fit obtained (data not shown). The total number of binding sites ($R_1$ plus $R_2$, from two-site analysis) for VSMC from WKY (428 ± 29.3 fmol/$10^5$ cells, 25°C and 4°C data pooled (n = 14)) was greater ($P < .01$) than the number of sites (101.9 ± 21.2 fmol/$10^5$ cells; $B_{max}$ from one-site analysis, 25°C and 4°C data pooled (n = 10) fro VSMC from SHR.

Spontaneously hypertensive rat- and WKY-derived VSMC were examined for their physiological responsiveness to h-ANP in terms of both intra- and extracellular cyclic GMP accumulation (Figures 4 and 5). Basal levels of intracellular cyclic GMP were not significantly different between SHR and WKY. After a ten-minute incubation with h-ANP, neither levels of intracellular cyclic GMP accumulated (Figure 4A) nor sensitivity ($ED_{50}$) to h-ANP (Table 2) differed between SHR and

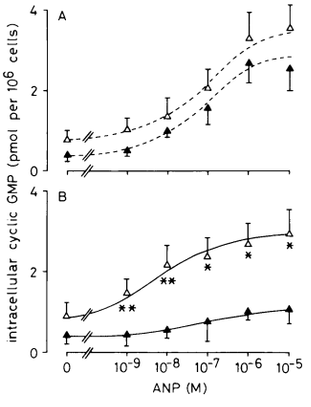


**FIGURE 4.** *Intracellular cyclic GMP accumulation in response to h-ANP. VSMC from WKY (△) and SHR (▲) were treated at 37°C with varying concentrations of h-ANP (in the presence of 0.5 mmol isobutylmethylxanthine) for either 10 minutes (panel A, n = 11) or 60 min (panel B, n = 5). Intracellular cyclic GMP was quantitated as described in the Materials and Methods section. Data points are mean ± SD and $ED_{50}$ values are given in Table 2. Asterisks indicate significant differences between SHR and WKY; *P < .05, **P < .01.*

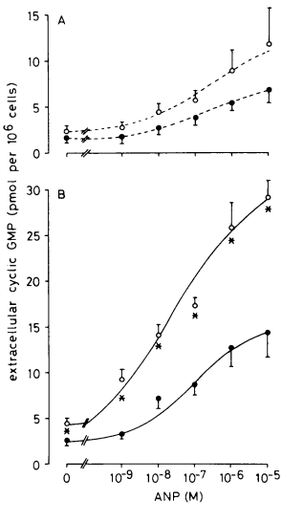


**FIGURE 5.** *Extracellular cyclic GMP accumulation in response to h-ANP. Cells from WKY (○) and SHR (●) were treated at 37°C with h-ANP (in the presence of isobutylmethylxanthine) for 10 minutes (panel A) or 60 minutes (panel B). Cyclic GMP in the extracellular medium overlay was determined as described in the Materials and Methods section. Data are mean ± SD where n = 5 for both time periods. $ED_{50}$ values are given in Table 2. Asterisks indicate significant (P < .01) differences between SHR and WKY.*

WKY. After a 60-minute exposure to h-ANP, levels of intracellular cyclic GMP were significantly higher (Figure 4B) and the $ED_{50}$ for h-ANP significantly lower (Table 1) in WKY than in SHR. While stimulated cyclic GMP levels in WKY were sustained with prolonged hormone treatment, the apparent accumulation of intracellular cyclic GMP by SHR was markedly lower after 60 minutes relative to that at ten minutes (Figure 4).

After a ten-minute incubation in the absence or presence of h-ANP, extracellular cyclic GMP levels were not significantly different between WKY- and SHR-derived cells, although there was a trend for greater egress of this cyclic nucleotide in WKY (Figure 5A). However, after a 60-minute incubation, both basal and h-ANP stimulated levels of extracellular cyclic GMP were significantly greater for WKY-derived cells than SHR (Figure 5B). The $ED_{50}$ for h-ANP associated cyclic GMP egress was comparable between SHR and WKY in short-term (ten minute) incubations, but was significantly lower for WKY-derived cells than SHR when calculated after long-term (60 minute) incubations (Table 2).

## DISCUSSION

This study demonstrates differences in both the number and affinity of ANP-receptors on cultured thoracic aorta VSMC from SHR and WKY. In addition, the cyclic GMP response to ANP is decreased in SHR-derived VSMC in terms of their ability to increase either intracellular or extracellular cyclic GMP levels.

**TABLE 2. CYCLIC GMP RESPONSE TO h-ANP IN SMOOTH MUSCLE CELLS FROM SHR AND WKY**

| Animal | Incubation (min) | $ED_{50}$ (nmol) for ANP: Intracellular cyclic GMP | $ED_{50}$ (nmol) for ANP: Extracellular cyclic GMP |
|---|---|---|---|
| WKY | 10 | 90 ± 15 (11) | 100 ± 20 (5) |
| SHR | 10 | 85 ± 20 (11)$^{NS}$ | 90 ± 10 (5)$^{NS}$ |
| WKY | 60 | 29 ± 19 (5) | 35 ± 17 (5) |
| SHR | 60 | 80 ± 17 (5)† | 73 ± 20 (5)* |

*$ED_{50}$ values (mean ± SD) were obtained from experiments presented in Figure 5.*

*Number in parentheses indicates the number of separate experiments performed.*

* P < .05.

† P < .01 *and indicates significance between SHR and WKY (60-min incubation).*

*Abbreviation: NS, no significant difference between SHR and WKY (10-min incubation).*

Analysis of our data for [$I^{125}$]h-ANP binding according to a single-site model yielded parameter estimates ($K_D$ 0.3–1.0 nmol, $B_{max}$ 30–100 fmol/$10^5$ cells) that are comparable to those previously reported ($K_D$ 0.6–2.1 nmol, $B_{max}$ 18–80 fmol/$10^5$ cells) for VSMC.[7-9] Such analysis indicated that in VSMC from SHR, the affinity for ANP was decreased and the $B_{max}$ was increased relative to WKY. These findings agree with two previous reports comparing ANP-binding on cultured aortic VSMC from SHR and WKY.[19,39] However, other studies using similar binding techniques have reported a decreased $B_{max}$ in aortic smooth muscle tissue[20] and kidney,[40,41] for SHR with unaltered[20] or increased[41] affinities for ANP. Using autoradiographic techniques $B_{max}$ and affinity for ANP were decreased in brain from SHR,[42,43] whereas the $B_{max}$ was unaltered in adrenals and kidney.[43] The reasons for these discrepant observations are unclear, but may relate to variations in tissue selection, age, and methodologies.

Our observation of two ANP-receptor classes on VSMC ($K_{D1}$ ~ 0.3 nmol, $K_{D2}$ ~ 15 nmol) has also been made for bovine adrenal zona glomerulosa ($K_{D1}$ ~ 0.04 nmol, $K_{D2}$ ~ 6 nmol).[44] Because binding parameter estimates obtained at 25°C and 4°C were not significantly different within either SHR- or WKY-derived VSMC and both cell strains internalized [$I^{125}$]h-ANP to the same extent, our binding parameters are a measure of cell-surface bound [$I^{125}$]h-ANP. We can thus exclude the possibility that differences between WKY (two-site model) and SHR (one-site model) are due to an additional internal component in WKY only. The lack of demonstration of two ANP-receptor classes (using saturation-binding methodology) in previous studies on smooth muscle cells may be due to insufficient data representation at higher (~4 nmol) ANP concentrations or to inadequate curve-fitting analysis.

Our inability to reproducibly resolve two receptor classes on SHR-VSMC may be due to their very low proportion of high affinity receptors (~3% as assessed from the two experiments in which a significant improvement of fit for two-site LIGAND analysis was obtained). This, together with their apparent decreased affinity for ANP suggests that in SHR, ANP-receptors are predominantly in the low affinity form. Since their $K_D$(~1.0 nmol) is one order of magnitude lower than the expected value (~15 nmol) if all receptors were to exist in the low affinity form, the total conversion of receptors from the high to the low affinity type does not apparently occur in SHR. Moreover, whereas single site analysis indicated a greater receptor number in SHR than WKY, the $B_{max}$ for SHR (~100 fmol/$10^5$ cells) was lower than the total receptor number obtained for WKY ($R_1$ + $R_2$ ~ 400 fmol/$10^5$ cells) with more appropriate two-site analysis. This may be a further reflection of incomplete receptor-interconversion, in which case $B_{max}$ values from one-site Scatchard analysis, albeit the best fit for SHR, represent an underestimation of the true total receptor number.

Interpretation of our binding data in term of receptor-interconversion remains speculative however, and is not the only possible explanation for our findings. Down-regulation of ANP-receptors on VSMC[45] requires prolonged exposure to hormone and is reversible.[46,47] Thus, although plasma ANP levels are elevated in SHR,[17-20] ANP is not present in culture media (unpublished observation) and culturing conditions thus favor reversibility of any prior in vivo down-regulation. Alternatively, receptor desensitization through covalent receptor modification, for example by cyclic AMP-dependent protein kinase or protein kinase C[47,48] may also be considered because in hypertension, abnormalities in both cyclic AMP and phosphoinositide metabolism occur. Whatever the factors/mechanisms responsible for alterations of ANP-receptor properties in SHR-VSMC, we can assume they are inherent, because our observations were made in passaged cell cultures.

In order to assess the physiological relevance of alterations in ANP-receptor binding properties, we examined the cyclic GMP response of VSMC from SHR and WKY. A striking observation for both VSMC strains, and one that has been previously made in a variety of tissues including smooth muscle[5,7-9] is that the $ED_{50}$ values for intracellular and extracellular cyclic GMP responses to ANP are at least one order of magnitude greater than receptor $K_D$ values. This problem of dissociation between ANP-receptor binding and concomitant cyclic GMP response remains to be resolved. Undoubtedly particulate guanylate cyclase activation/cyclic GMP formation are involved in ANF-elicited vasodilatation,[8-11] but changes in cyclic GMP need not necessarily reflect the primary/sole cellular manifestation of ANP-receptor-mediated action. It is conceivable that cofactors may be required for the cyclic GMP response and that alternative/synergistic second-messenger (receptor-coupled) pathways for ANP exist.[7]

Previous comparative studies (SHR *v* WKY) on intracellular cyclic GMP accumulation in response to ANP have reported either decreased levels in VSMC from SHR,[39] or increased levels in smooth muscle and adrenal capsular cell suspensions.[20] In this study, differences in the cyclic GMP response to ANP between SHR- and WKY-derived VSMC emerged only after prolonged exposure to ANP, whereby WKY exhibited a greater ability to persistently accumulate intracellular cyclic GMP than SHR. This may account for the leftward shift (decreased apparent $ED_{50}$) in WKY dose profiles after prolonged treatment. These data may be relevant to observations of less sustained physiological responsiveness to ANP in EHT[25] and in SHR.[27]

That extracellular cyclic GMP levels were lower for SHR-derived cells than those from WKY probably reflects the attenuation of sustained intracellular cyclic GMP accumulation in SHR. In addition the capacity for cyclic GMP extrusion may be decreased in SHR as evidenced by their significantly lower extracellular cyclic GMP levels (relative to WKY at 60 minutes) even in the absence of stimulation by ANP. The biological consequences of cyclic GMP egression, and the relevance to hypertension of alterations in this process remain to be determined.

In physiological terms, the findings reported herein may be interpreted to suggest that aortic VSMC from SHR exhibit decreased responsiveness to ANP, and/or the biological effectiveness of ANP is lowered in SHR. While we ourselves have no direct physiological data to support these conclusions, thoracic aorta[29,30] and mesenteric resistance[28] vessels from SHR have been demonstrated to be significantly less sensitive to the relaxing actions of ANP. Therefore, altered ANP binding and transduction processes thereof in target tissues may be relevant to hypertension.

## ACKNOWLEDGMENTS

We thank Maria Bürgin, Amanda de Sola Pinto, and Bernadette Libsig for their skilled assistance.

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