Sodium, Divalent Cations, and Guanine Nucleotides Regulate the Affinity of the Rat Mesenteric Artery Angiotensin II Receptor

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SUMMARY. To study the regulation of interactions between angiotensin II and its vascular smooth muscle receptor, we have investigated the effects of mono- and divalent cations and guanine nucleotides on 125I-angiotensin II binding to a particulate fraction of rat mesenteric artery. Relative to the control state (no added cations or nucleotides), radioligand binding was stimulated by divalent cations (Mn2+ > Mg2+ > Ca2+) to a maximum of 350% of control (20 mM Mn2+). Binding was also increased to 125-150% of control by Na+ (50-150 mM), whereas binding was inhibited by other monovalent cations (Li+ ≈ K+ = NH4+). The stimulatory effects of Na+ (50 mM) and Mn2+ (<20 mM) were additive, but only to the same maximal level attained in the presence of 20 mM Mn2+ (350% of control). Under control conditions, GTP and its nonhydrolyzable analogue, Gpp(NH)p, were equipotent in reducing 125I-angiotensin II binding (IC50 = 3 nM) whereas GDP, GMP, and ATP were much less potent. When endogenous divalent cations were chelated by EDTA (2 mM), binding was reduced by 25% and neither GTP nor Gpp(NH)p (1-500 mM) inhibited binding further, suggesting that divalent cations are required for guanine nucleotides to attenuate 125I-angiotensin II binding. The effects of GTP and Gpp(NH)p varied as ambient cation concentrations changed. Scatchard analyses indicated a single class of binding sites with a control state equilibrium dissociation constant (Kd) of 5.5 ± 1.6 (SD) nM. The Mn2+- or Na+-induced increase in binding was due to an increase in receptor affinity which was counteracted by Gpp(NH)p, binding capacity (Bmax) remaining constant. We conclude that cations and guanine nucleotides interact in one or more receptor-associated membrane sites, probably including the guanine nucleotide regulatory protein, to modulate the affinity of the vascular receptor for angiotensin II. This complex interplay of cations and guanine nucleotides to regulate binding may be involved in modulation of physiological smooth muscle responses to angiotensin II. (Circ Res 50: 462-469, 1982)
binding to adrenal cortex (Glossman et al., 1974a). In adrenal cortex, guanine nucleotides reverse the sodium-induced increase in radioligand binding (Glossmann et al., 1974b). Preliminary data indicate guanine nucleotides also convert high affinity sites into low affinity angiotensin II binding sites in liver (Campanile et al., 1981). On the basis of these data, the smooth muscle angiotensin II receptor in resistance-type vessels might be expected to interact with cations and guanine nucleotides, but the specific responses would be hard to predict because of the observed tissue-related heterogeneity of effects on angiotensin II receptors.

As a general approach to understanding the physiology of the vascular angiotensin II receptor, we have used a radioligand-binding assay to characterize the angiotensin II receptor in a particulate fraction prepared from rat mesenteric arterial arcades (Gunther et al., 1980). In the course of characterizing this receptor, we observed that divalent cations increase 125I-angiotensin II binding with a potency order of Mn++ > Mg++ > Ca++. We also noted that Na+ and GTP did not influence binding of the radioligand. However, further studies indicate that the interactions among cations and guanine nucleotides are more complex than we originally reported. To gain further insight into potential interactions of cations and guanine nucleotides in the regulation of the vascular angiotensin II receptor we have reexamined the effects of mono- and divalent cations, guanine triphosphate (GTP) and its nonhydrolyzable analogue, guanylyl-5'-imidophosphate (Gpp(NH)p), on 125I-angiotensin II binding to the mesenteric artery particulate fraction. Our results indicate that cations and guanine nucleotides interact to regulate hormone binding, primarily through modulation of receptor affinity.

### Methods

**Materials**

125I-Angiotensin II (specific activity 1000–1600 Ci/mmol) was obtained from New England Nuclear. The ligand was reconstituted to a concentration of 50 nM. Aliquots were immediately frozen in liquid nitrogen, stored at −20°C, and were used only once after thawing. Purity was greater than 98% by thin layer chromatography (t-butanol:3% NH3, 105:35) on cellulose plates (Eastman 6064). Bovine serum albumin (grade V) was obtained from Miles Laboratories. All other chemicals were obtained from Sigma Chemical Corporation.

Male Sprague-Dawley rats (225–250 g) were obtained from Charles River Breeding Labs and were fed standard laboratory chow and tap water. Rat mesenteric artery arcades were obtained by a modification of the method developed by Wei et al. (1976), as previously described (Gunther et al., 1980). Five to seven arcades were used in each assay. After homogenization of the arteries in a Brinkmann Polytron (setting 8, 2 X 10 seconds) the suspension was centrifuged at 1500 g for 10 minutes at 4°C. The supernatant was centrifuged at 100,000 g for 30 minutes. The pellet was resuspended in buffer (50 mM Tris, pH 7.5 at 25°C) at a protein concentration of approximately 1 mg/ml (measured by the method of Lowry et al. (1951), using bovine serum albumin as the standard).

**Binding Assay**

All cation additives used in the binding assays were in the form of chloride salts. Gpp(NH)p was stored as an aqueous stock solution (0.02 M) at −20°C. All other nucleotides (GTP, GDP, GMP, ATP) were freshly prepared immediately before the assay.

Binding assays were initiated by adding 0.10–ml aliquots of freshly prepared particulate fraction to 0.10 ml of an assay mix. The assay mix was adjusted so that each tube contained a final volume of 0.20 ml with 50 mM Tris (pH 7.5 at 25°C), 0.25% bovine serum albumin, 125I-angiotensin II in an appropriate concentration, and the cation or nucleotide additives being investigated.

Assay tubes then were incubated in a gyratory metabolic shaker at 25°C for 35 minutes, since preliminary studies had shown that binding of 125I-angiotensin II to the mesenteric artery particulate fraction had reached equilibrium by 35 minutes, regardless of the presence or absence of cation or nucleotide additives. After 35 minutes, 3 ml of ice cold 0.9% NaCl were added to each tube. Bound and free radioactivity were separated by filtration through glass-fiber filters (Whatman GF/C) prewetted with a solution of 50 mM Tris (pH 7.5 at 25°C) and 0.25% bovine serum albumin. The tubes and filter wells were rinsed with three more 3-ml portions of ice cold 0.9% NaCl. Radioactivity trapped on the filters was counted in a y-counter (Beckman) at 70% efficiency. Except as noted, each determination was performed in duplicate or triplicate, and experiments were performed two to six times. All data presented represent specific binding, defined as total filter-retained radioactivity from tubes containing no added unlabeled angiotensin II minus filter-retained radioactivity from otherwise identically prepared tubes containing 1 µM angiotensin II during incubation. At 0.3 nM 125I-angiotensin II, specific binding was 92–98% of total binding. All assays except those specifically designed to determine binding capacity (Bmax) and equilibrium dissociation constant (Kd) were performed with 0.3 nM 125I-angiotensin II. The amount of radioligand specifically bound in the presence of cation or nucleotide additives was compared with “control binding,” the amount of 125I-angiotensin II bound in the absence of cation or nucleotide additives. Control binding was determined in each assay of this type.

In those assays designed to determine binding capacity or equilibrium dissociation constant, specific binding after 35 minutes of incubation at 25°C was determined for 125I-angiotensin II concentrations ranging from 0.05 to 5.0 nM. The data obtained from determinations of specific binding for these various concentrations of radioligand were subjected to Scatchard analysis. The line of best fit was determined by least squares linear regression. The effects of cations and nucleotides on the equilibrium dissociation constant were determined from these data. Some day-to-day variability in binding capacity was noted, thus making difficult comparisons of the effects of various assay conditions on this parameter. In order to assess the effects of Mn++ and Gpp(NH)p on Bmax, simultaneous Scatchard plots were derived from assays performed in the same particulate fraction preparation under pairs of contrasting conditions. The simultaneously performed Scatchard plots in this set of experiments were obtained with single observations of total and nonspecific binding, since the increased time required for preparation of the large amounts of...
particulate fraction protein necessary to perform duplicate
determinations for each set of conditions was found to
result occasionally in instability of the $^{125}$I-angiotensin II
binding characteristics.

Statistical comparison of receptor affinity under various
conditions was performed by a Student’s $t$ test, the null
hypothesis being rejected when $P < 0.05$. Data are presented
as mean ± SD (when applicable).

Results

Effect of Monovalent Cations on $^{125}$I-Angiotensin II
Binding to Mesenteric Artery Particulate Fraction

With the addition of sodium, the amount of $^{125}$I-
angiotensin II bound to the particulate fraction in-
creased in a concentration-dependent manner up to
50 mM NaCl, with a plateau at about 125–150% of
control binding between 50 and 150 mM (Fig. 1). In
order to investigate whether this effect of sodium ion
was specific or related to other properties such as
cationic size, similar studies were performed in the
presence of ammonium, potassium, and lithium ions.
Ammonium and potassium ions (100 mM) did not
stimulate but decreased binding slightly (to 80–85%
of control). Lithium ion (100 mM), however, caused a
pronounced decrease in $^{125}$I-angiotensin II binding to
34 ± 1% of control (Fig. 1).

Effect of Divalent Cations on $^{125}$I-Angiotensin II
Binding

Binding of $^{125}$I-angiotensin II (0.3 nM) to the mes-
enteric artery particulate fraction was increased to 198
± 9%, 164 ± 16%, and 158 ± 19% of control by 1 mM
Mn$^{++}$, Mg$^{++}$, and Ca$^{++}$, respectively. Mn$^{++}$ caused a
dose-related increase in binding of up to 354 ± 11%
of control at 20 mM (Fig. 2).

Interactions between Cations to Modulate
$^{125}$I-Angiotensin II Binding

Sodium (50 mM) increased $^{125}$I-angiotensin II bind-
ing in the presence of 1 mM Mn$^{++}$ from 198 ± 9% of
control to 312 ± 8%. However, in the presence of 5,
10, or 20 mM Mn$^{++}$, 50 mM Na$^{+}$ caused progressively
less enhancement of binding with no further stimu-
latory effect being seen in the presence of 20 mM
Mn$^{++}$. At Mn$^{++}$ concentrations between 5 and 20 mM,
Na$^{+}$ stimulated binding to the level found in the
presence of 20 mM Mn$^{++}$ alone (about 350% of con-
trol) (Fig. 2).

Effect of Nucleotides on $^{125}$I-Angiotensin II
Binding

Guanosine triphosphate and its nonhydrolyzable
analogue, Gpp(NH)p, caused a dose-dependent re-
duction in $^{125}$I-angiotensin II binding to the mesenteric
artery particulate fraction under control conditions
with 50% of total inhibition at approximately 3 $\mu$M. In
comparison, GDP, GMP, and ATP were less potent
inhibitors of $^{125}$I-angiotensin II binding (Fig. 3).

The effects of Gpp(NH)p on $^{125}$I-angiotensin II
binding were investigated in the presence of 2 mM
EDTA, a chelator of divalent cations. Binding was
75% of control ($n = 2$) and was not attenuated by
Gpp(NH)p at concentrations as high as 500 $\mu$M, demon-
strating that the effects of guanine nucleotides on
the vascular angiotensin II receptor require the pres-
ence of divalent cations. In the presence of each
monovalent or divalent cation tested, Gpp(NH)p
caused a decrease in $^{125}$I-angiotensin II binding, 50%
of maximal effect occurring at approximately 20 $\mu$M
Gpp(NH)p (Fig. 4).

Interactions between divalent cations and guanine
nucleotides were explored in more detail using Mn++, the divalent cation most potent in stimulating 125I-angiotensin II binding. The concentration of Mn++ was found to affect the sensitivity to guanine nucleotides. Whereas 1 μM Gpp(NH)p had little effect on 125I-angiotensin II binding at a Mn++ concentration of 1 mM (Fig. 5), when 125I-angiotensin II binding was enhanced further by 5 mM Mn++, this increase was completely reversed by 1 μM Gpp(NH)p. At higher concentrations of Gpp(NH)p, the concentration response curve for displacing 125I-angiotensin II was nearly superimposable on the one generated in the presence of 1 mM Mn++. The response to Gpp(NH)p of 125I-angiotensin II binding in the presence of both 1 mM Mn++ and 50 mM Na+ was similar to the response in the presence of 5 mM Mn++ alone, as described above (Fig. 5). That is, 1 μM Gpp(NH)p caused a substantial reduction in 125I-angiotensin II binding to the mesenteric artery particulate fraction, in contrast to the modest attenuation of binding by 1 μM Gpp(NH)p seen in the presence of 1 mM Mn++ alone or 50 mM Na+ alone (Figs. 4 and 5).

Although GTP exerted effects similar to those of Gpp(NH)p under control conditions (Fig. 3), the concentration of GTP inhibiting 125I-angiotensin II binding by 50% increased slightly relative to Gpp(NH)p in the presence of 1 mM Mn++ (Fig. 6A). In the presence of 5 mM Mn++ or Mg++, there was markedly attenuated inhibition of 125I-angiotensin II binding by GTP (Fig. 6B), perhaps due to activation of guanine nucleotide specific or nonspecific nucleotide phosphatases.

**FIGURE 3.** Effect of nucleotides on binding of 125I-angiotensin II (0.3 nM) to rat mesenteric artery particulate fraction. Results are the means of two or three experiments.

**FIGURE 4.** Effect of Gpp(NH)p on binding of 125I-angiotensin II (0.3 nM) in the presence of various divalent or monovalent cations. Gpp(NH)p at the specified concentrations was added in the presence of 1 mM Mn++, Mg++, or Ca++ or in the presence of 50 mM Na+ or Li+. Results for each ion are the means of three to six separate experiments.

**FIGURE 5.** Effect of Gpp(NH)p on binding of 125I-angiotensin II (0.3 nM) in the presence of 1 mM Mn++, 1 mM Mn+ + with 50 mM Na+ (Δ), or 5 mM Mn++ (○). Results are the means of three to six experiments.

**FIGURE 6.** Differential effects of GTP (●) and Gpp(NH)p (○) on binding of 125I-angiotensin II (0.3 nM). A. Incubation with 1 mM Mn++. B. Incubation with 5 mM Mn++. Similar results were obtained with 5 mM Mg++. Results are the means of two or three determinations.
Effect of Cations and Gpp(NH)p on Receptor Affinity and Number

In order to determine whether the changes in 125I-angiotensin II binding to the mesenteric artery particulate fraction were due to changes in receptor affinity or number, we performed Scatchard analyses of radioligand binding (0.05-5.0 nM 125I-angiotensin II) under a variety of conditions. Under control conditions, the equilibrium dissociation constant (Kd) was 5.5 ± 1.6 nM (n = 4). Sodium (100 mM) increased affinity approximately 2-fold to give a Kd of 2.3 ± 0.8 nM (n = 3) (P < 0.05 vs. control). Manganese (1 mM) also increased the affinity of the angiotensin II receptor as manifested by a decrease in Kd to 2.1 ± 0.3 nM (n = 5) (P < 0.05 vs. control). Under all of these assay conditions there was a single class of binding sites (linear regression correlation coefficients being −0.94 to −0.99 for each Scatchard plot).

When we assessed the effects of Mn++ and Gpp(NH)p on Bmax by performing simultaneous saturation curves under contrasting conditions with only single determinations of each data point, linear regression correlation coefficients of the Scatchard analyses were not as close to unity (−0.83 to −0.96). Nevertheless, in all paired experiments, Bmax did not change, either when control conditions were compared to Mn++ (1 mM) or when Mn++ (1 mM) was compared to Mn++ (1 mM) in the presence of Gpp(NH)p (100 μM) (Fig. 7). In each paired experiment, the Kd’s differed (Fig. 7) in a manner consistent with the data presented above.

Discussion

In the present study we have extended our initial characterization of the vascular angiotensin II receptor, as studied in a muscular, resistance-type blood vessel (Gunther et al., 1980). Previously, we had observed that divalent cations enhance 125I-angiotensin II binding to the rat mesenteric artery particulate fraction. Data obtained in the current study indicate that: (1) of the monovalent cations tested, only Na+ increases 125I-angiotensin II binding, by increasing receptor affinity, whereas Li+ reduces binding; (2) Mn++ induces increased affinity of the vascular angiotensin II receptor without changing receptor number; (3) at low Mn++ concentrations, Na+ is additive with Mn++ in stimulating binding; (4) GTP and its nonhydrolyzable analogue, Gpp(NH)p, decrease the affinity of the angiotensin II receptor; and (5) the sensitivity of radioligand binding to inhibition by guanine nucleotides varies with ambient cation concentrations.

The observed specificity of sodium among monovalent cations in enhancing 125I-angiotensin II binding appears to be unique for the mesenteric artery angiotensin II receptor. In rabbit uterus (Bennett and Snyder, 1980) and aorta (Devynck and Meyer, 1976), Na+ does not affect angiotensin II binding. However, in tissues such as bovine adrenal cortex or cerebellum in which Na+ does stimulate binding to angiotensin II receptors, other monovalent cations, such as K+,

![Graph](image_url)
can also stimulate binding (Glossmann et al., 1974a; Bennett and Snyder, 1980). Similarly, the effects of Na⁺ (either stimulatory or inhibitory) on agonist binding to the dopamine (Usdin et al., 1980), opiate (Pert and Snyder, 1974), α-adrenergic (Glossmann and Presek, 1979), histamine (Chang and Snyder, 1980), and muscarinic cholinergic receptors (Rosenberger et al., 1980) are qualitatively similar to the effects of K⁺, Li⁺ or other monovalent cations. The inhibition of Li⁺ of 125I-angiotensin II binding to mesenteric artery particulate fraction is qualitatively similar to that observed in the adrenal cortical angiotensin II receptor (Glossmann et al., 1974b). However, maximal inhibition of radioligand binding by Li⁺ in the adrenal cortex is approximately 25%, whereas binding in the mesenteric artery preparation is reduced by as much as 66%. The physiological implications of these observations are not clear, since the cells are bathed in a high-Na⁺, low-Li⁺ extracellular fluid. However, our data suggest the existence of a membrane monovalent cation site closely related to this vascular smooth muscle angiotensin II receptor. A similar membrane site was proposed originally to explain the effects of Na⁺ on the adrenal cortical angiotensin II receptor (Glossmann et al., 1974a).

Paralleling the heterogeneity of effects of monovalent cations in different tissues, the effects of divalent cations on angiotensin II receptors in various tissues appear to differ markedly. Whereas divalent cations enhance binding to the mesenteric artery receptor, Mg⁺⁺ and Ca⁺⁺ at concentrations less than 10–20 mM have minimal effect in bovine adrenal cortex (Glossmann, 1974a, 1974b) and inhibit binding to angiotensin II receptors in rabbit aorta (Devynck and Meyer, 1976). The increase in 125I-angiotensin II binding to the rat mesenteric artery particulate fraction induced by divalent cations is similar in magnitude to that observed in rat renal glomeruli (Blanc et al., 1978), although the order of the potency in glomeruli (Ca⁺⁺ > Mg⁺⁺ > Mn⁺⁺) is opposite to that observed in mesenteric artery. In other receptor systems in which effects of divalent cations have been studied, Mn⁺⁺, and often Mg⁺⁺ and Ca⁺⁺, increase agonist binding by increasing either receptor number or affinity. In the rat brain dopamine receptor, Mn⁺⁺ increases the total number of binding sites without changing affinity (Usdin et al., 1980). By contrast, 10 mM Mg⁺⁺ induces a 2-fold increase in α2-adrenergic receptor affinity in rat cerebral cortex with little change in number (Glossmann and Presek, 1979). The rat mesenteric artery particulate fraction angiotensin II receptor follows the second pattern, as Mn⁺⁺ increases the affinity for 125I-angiotensin II without changing receptor number.

One mechanism by which divalent cations may modulate receptor affinity has been studied in the opiate receptor of rat brain. Divalent cations stimulate agonist binding by activating phosphatases which hydrolyze GTP, thus reducing the inhibitory effects of endogenous nucleotides (Childers and Snyder, 1980). In the mesenteric artery, the loss of potency of GTP, but not Gpp(NH)p, in the presence of 5 mM Mn⁺⁺ or Mg⁺⁺ suggests that GTPases may be activated by the higher concentrations of divalent cations. Thus it is possible that at least a portion of the divalent cation-induced increase in angiotensin II receptor affinity may be due to degradation of endogenous GTP. To test this hypothesis in a small series of experiments, we preincubated the particulate fraction with 5 mM Mn⁺⁺. Following preincubation, which would have allowed the putative GTPases to degrade endogenous GTP, the membranes were treated with EDTA and washed to remove the Mn⁺⁺ and EDTA. The binding of 125I-angiotensin II to this preincubated preparation was still stimulated by Mn⁺⁺ or Na⁺ to roughly the same extent as in the standard preparation (unpublished observations), suggesting that mechanisms other than the activation of GTPases are involved in the stimulation of binding by cations.

An alternative explanation for the regulatory influence of cations on apparent receptor affinity involves a direct effect on the conformation of the radioligand, since very high concentrations of angiotensin II or analogues have been shown to interact in artificial lipid membranes (Elliott and Goodfriend, 1979; Degani and Lenkinski, 1980). Although it is difficult to test this hypothesis directly in the system used in this study, this possibility seems unlikely. Cation regulation of receptor affinity appears to be a general phenomenon, involving agonists as structurally diverse as peptides, catecholamines, and opiates. Thus, it seems more likely that cations might act through cation-sensitive membrane sites common to many hormone receptors, rather than by a specific physical chemical alteration in the angiotensin II molecule.

Cations not only regulate the affinity of the vascular angiotensin II receptor but also modulate the interaction of guanine nucleotides with receptor-associated nucleotide-sensitive sites. The effect of guanine nucleotides in inhibiting 125I-angiotensin II binding varies with the ambient cation concentrations associated with receptor affinity changes. Under control conditions, 1 μM Gpp(NH)p causes an 18% decrease in binding, or about 32% of maximal effect (Fig. 3). When receptor affinity is increased by 1 mM Mn⁺⁺, 1 μM Gpp(NH)p causes binding to decrease from 200% to 180% of control, only about 12% of maximal Gpp(NH)p effect (Fig. 3). Yet, when receptor affinity increases further, either with 1 mM Mn⁺⁺ plus 50 mM Na⁺ or with 5 mM Mn⁺⁺ alone, 1 μM Gpp(NH)p exerts 50% of its maximal effect (Fig. 5). A possibly related phenomenon occurs in the rat brain α-adrenergic receptor, where Mg⁺⁺ enhances agonist binding and also enhances the potency of Gpp(NH)p (Glossmann and Presek, 1979). Thus, the guanine nucleotide-sensitive site associated with vascular angiotensin II and other receptors is one locus where cation effects may be mediated.

It has been suggested that membrane guanine nucleotide-binding proteins play a pivotal role in the transduction of signals from a variety of surface receptors (Rodbell, 1980). This theory originally was
derived from data on adenylate cyclase-coupled receptors, particularly the β-adrenergic receptor, and has been extended to guanine nucleotide-sensitive receptors which are not coupled to adenylate cyclase (Limbird et al., 1980; Rodbell, 1980). In the case of adenylate cyclase-coupled receptors, it is postulated that an agonist, but not an antagonist, induces the formation of a complex between the receptor and a membrane nucleotide regulatory protein. In the absence of guanine nucleotides, the receptor binds the agonist with high affinity. When GTP binds to a site on the regulatory protein, simultaneous (or sequential) development of a low affinity state for the agonist and coupling of the regulatory unit to the catalytic unit of adenylate cyclase lead to activation, or inhibition, of the enzyme. From our data, we suggest that Na+ and divalent cations facilitate the formation of a receptor-nucleotide regulatory protein complex of high affinity for angiotensin II. When GTP binds to this complex, the angiotensin II receptor affinity decreases. We speculate that, in a manner analogous to that of adenylate cyclase-coupled systems, this step ultimately result in contraction of the smooth muscle cell. Recent evidence suggesting that the liver cell angiotensin II receptor is linked in an inhibitory fashion to adenylate cyclase (Jard et al., 1980) raises the possibility, currently under investigation, that vascular smooth muscle cell angiotensin II receptors may also be coupled to adenylate cyclase.

In a previous report from this laboratory, Na+ and GTP both were reported to exert no effects on [125]I-angiotensin II binding to the mesenteric artery preparation (Gunther et al., 1980). With an increased appreciation of the complexity of interactions among the angiotensin II receptor, cations, and guanine nucleotides, those results can now be understood. The previous experiments were performed in a buffer containing 5 mM Mg2+. At that concentration of divalent cation we have shown that Na+ has little additional stimulatory effect. Similarly, as previously discussed, GTP exerts little influence on binding in the presence of 5 mM Mg2+, possibly due to the breakdown of GTP by divalent cation-activated GTPases.

In conclusion, cations and guanine nucleotides appear to interact in a complex fashion to regulate the affinity of the vascular angiotensin II receptor. These effects imply the existence of receptor associated, cation-sensitive sites as well as a membrane guanine nucleotide regulatory protein. The complex interplay of cations and guanine nucleotides to regulate angiotensin II binding in vascular smooth muscle may be involved in the modulation of physiological responses to angiotensin II and thus may be important in the control of vascular tone.

We would like to thank Catherine Lake for excellent assistance in the preparation of the manuscript. This study was supported by Grant HL20054 from the National Heart, Lung and Blood Institute and NIH Biomedical Research Support Funds.

Dr. Wright was supported in part by U.S. Public Health Service Training Grant ST32 HI20719302 and by a research fellowship from the American Heart Association, Southeast Massachusetts Division (# 1-414-010). Dr. Alexander is a recipient of National Institutes of Health Career Development Award K04 HL 0332. Dr. Gimbrone is an Established Investigator of the American Heart Association.

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Received August 10, 1981; accepted for publication December 28, 1981.

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