Stimulation of Vascular Glicosaminoglycan Synthesis by Subpressor Angiotensin II in Rats

Geza Simon, Gyorgy Abraham, Stephen Altman

Abstract The vascular trophic effects of angiotensin II (Ang II) in small doses may precede its hypertension-producing effect, and de novo synthesis of components of extracellular matrix may be a requirement for Ang II-stimulated growth. In the present study, therefore, the incorporation of ^35SO_4 into glycosaminoglycans (synthesis) of aorta and bladder wall of young adult, male Sprague-Dawley rats was measured ex vivo after 48 hours of Ang II administration at two dose levels, 100 and 200 ng · kg⁻¹ · min⁻¹ IP. Vehicle-infused rats served as controls. Compared with controls, systolic blood pressure was unchanged in rats receiving 100 ng · kg⁻¹ · min⁻¹ Ang II and rose by 13 mm Hg (P<.05) in rats receiving the 200-ng-kg⁻¹-min⁻¹ dose. In Ang II-treated rats, glycosaminoglycan synthesis of the aorta was increased by 20% (P<.05) and 52% (P<.005) at the two dose levels, respectively. Glicosaminoglycan synthesis of bladder smooth muscle was also increased in Ang II–treated rats (P<.01), but the response was not dose dependent. By 7 to 10 days of Ang II administration (200 ng · kg⁻¹ · min⁻¹), glycosaminoglycan synthesis of aorta returned toward baseline (P<.10, >.05). The rate of synthesis of subtypes of glycosaminoglycans in the aorta was proportionately increased by Ang II. The early occurrence, magnitude, and arterial pressure independence of Ang II–induced glycosaminoglycan synthesis suggest that restructuring of extracellular matrix may play an important role in both the trophic and hypertension-producing action of Ang II. (Hypertension. 1994;23[suppl I]:I-148-I-151.)

Key Words • proteoglycans • extracellular matrix • arteries • muscle, smooth • hypertension, renovascular • angiotensin II

It has been postulated that angiotensin II (Ang II) plays a pathogenetic role in several forms of human hypertension. Circulating levels of Ang II are increased in renovascular hypertension and in about one third of patients with borderline essential hypertension. In the so-called normal-renin and low-renin forms of hypertension, increased production of Ang II in vascular tissue and myocardium may contribute to the development of the disease.

Interest in the pathogenetic role of Ang II in hypertension has been stimulated by evidence that the agonist is a trophic factor of vascular muscle. A trophic effect of Ang II may help to explain the structural changes that accompany the development of hypertension or sometimes precede it. The evidence has been derived predominately from in vitro studies using tissue culture techniques. Depending on experimental conditions, Ang II has been shown to stimulate either hypertrophy or hyperplasia of vascular muscle. Ang II also stimulates the de novo synthesis of components of the extracellular matrix, including glycosaminoglycans (GAGs), that regulate cell growth. In contrast to these in vitro studies, the in vivo evidence for the vascular trophic action of Ang II is scanty and often indirect or unrelated to the hypertensive process. On the arterial side of the circulation, the separation of the trophic from the pressor effect of Ang II has been difficult. For the past several years, we have administered Ang II to rats by constant infusion in initially subpressor doses. Within 24 hours, we found stimulation of vascular Na-K pump activity, a requirement for growth, and of protein synthesis not only in aortic muscle but also in bladder smooth muscle, which is not exposed directly to arterial pressure. In the present experiments, we have extended these investigations to the extracellular matrix of the aorta and bladder by measuring the incorporation of radioactive sulfate (^35SO_4) into proteoglycans in general and GAGs in particular.

Methods

Preparation of Rats

Young adult, male Sprague-Dawley rats weighing 350 to 400 g were used. Adult rats were chosen so that the effects of Ang II on fully differentiated (contractile) vascular muscle could be studied. Male rats were studied to eliminate changes in the renin-Ang II system that accompany the estrous cycle in female rats. Synthetic Ang II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe; Sigma Chemical Co, St Louis, Mo) was infused into rats intraperitoneally with osmotic minipump (Alza Corp, Palo Alto, Calif) for 24 or 48 hours (model 1003D minipump) or for 7 to 10 days (model 2002). To fill the minipumps, Ang II was dissolved in saline at 10 or 5 mg/mL. Acetic acid (final concentration, 0.01 mol/L) was added to maintain the stability of Ang II. The 10- and 5- and mg/mL concentrations of Ang II in the minipumps resulted in the delivery of a mean of 200 or 100 ng · kg⁻¹ · min⁻¹ IP. The higher dose of Ang II delivered subcutaneously by osmotic minipump in rats results in a threefold or fourfold increase of plasma Ang II concentration.) Control rats were fitted with a minipump containing saline and 0.01 mol/L acetic acid. The rats had free access to tap water and received standard chow (Ralston Purina [St Louis, Mo], No 5002; sodium, 115 mmol/kg; potassium, 250 mmol/kg). Systolic blood pressure (SBP) was measured in restrained, awake rats by the tail-cuff method (Narco Biosystems, Inc, Houston, Tex) in the afternoon of the day before the experiments.

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**Fractionation of 35SO4-Labeled GAGs**

The relative incorporation of 35SO4 into specific GAG types (chondroitin sulfate [CS], dermatan sulfate [DS], and heparan sulfate [HS]) was determined by differential digestions. The rates of incorporation into CS and DS were determined by digestion with chondroitinase ABC and AC (Sigma). To 250 μL of dialyzed sample, 50 μL of 500 mmol/L tri(hydroxymethyl)aminomethane acetate (pH 8.0), 100 μL of 300 mmol/L sodium acetate, and 50 μL of 1 g/mL bovine serum albumin (BSA) were added for 15 hours at 37°C with either 50 μL (50 μU) chondroitinase AC or 50 μL (50 μU) chondroitinase ABC. To the cooled mixture (4°C), 100 μL of 50% TCA was added. After 1 hour the mixture was centrifuged, and the supernatant was dialyzed against tap water and then against deionized water. Radioactivity in 100 μL retentate was counted.

Complete lysis of HS was achieved with heparinase II and III (Sigma). To 250 μL of sample, 100 μL of 50 mmol/L (Na) PO4 (pH 7.0) and 50 μL of 1 g/mL BSA were added. To 100 μL of sample, 10 μL of 50 mmol/L NaCl, 0.5 mL of 2% cetylpyridinium HC1, 50 μL of 250 mmol/L cysteine, 50 μL of 2% sodium acetate, and 50 μL of 50 mmol/L (Na) PO4 (pH 7.0) and 50 μL of 1 g/mL BSA were added. The mixture was incubated for 15 hours at 25°C with 50 μL (1.0 μU) heparinase II and 50 μL (1.0 μU) heparinase III and then cooled and precipitated with TCA (see above). Radioactivity in 100 μL retentate was counted. Ten percent to 15% of 35SO4-labeled GAGs were not identified by enzyme digestion.

These procedures were in accordance with institutional guidelines, and the research protocol was approved by the Animal Study Subcommittee of the Minneapolis VA Medical Center.

**Statistical Analysis**

Results are expressed as mean±SEM. Intergroup comparisons were first made with one-way analysis of variance, followed by Student-Newman-Keuls range test for individual group comparisons. Single parameters in Ang II–treated and control rats were compared by unpaired Student’s t test. A value of P<.05 was considered statistically significant.

**Results**

The SBP of sham-infused control rats was 125±2 mm Hg (n=23) 36 hours after the insertion of the minipump. (The SBP of two control rats was not measured.) Compared with controls, SBP of rats treated with 100 and 200 ng·kg−1·min−1 angiotensin (Ang) II IP for 48 hours (c) and of three control rats (e).

**Fig 1.** Graph shows time course of 35SO4 incorporation into glycosaminoglycans of aortic smooth muscle (mean±SEM) of three rats treated with 100 ng·kg−1·min−1 angiotensin (Ang) II IP for 48 hours (c) and of three control rats (e).
Discussion

In the present study, aortic and bladder smooth muscle GAG synthesis (defined as the net incorporation of $^{35}S$O$_4$ into GAGs) was measured at 48 hours of Ang II administration because growth factor–stimulated restructuring of extracellular matrix appears to peak at this time. At 48 hours, the 200 ng $\cdot$ kg$^{-1} \cdot$ min$^{-1}$ dose of Ang II resulted in a 52% increase of GAG synthesis of aorta. The magnitude of this response was significantly greater than the 14% increase of de novo protein synthesis that we have previously detected in similarly prepared rats. That stimulation of aortic muscle GAG synthesis was in part independent of arterial pressure is suggested by two findings. GAG synthesis was also stimulated by Ang II in bladder smooth muscle that is not directly exposed to arterial pressure. We have previously suggested that the in vivo trophic effect of Ang II may be smooth muscle specific. By 7 to 10 days of Ang II administration, GAG synthesis returned toward baseline. The same observation was made when we investigated the in vivo effect of Ang II on aortic protein synthesis. Finally, the synthesis of subtypes of sulfated GAGs was proportionately stimulated by Ang II. A proportional increase in sulfated GAG synthesis was also detected by investigators who have measured proteoglycan synthesis in spontaneously hypertensive rats and in rats with coarctation hypertension. The findings of this study should be interpreted with some caution. Although defined as GAG synthesis, the net increase in $^{35}S$O$_4$ incorporation into GAGs in Ang II–treated rats cannot be equated with an accelerated rate of synthesis without measurements of rates of degradation and enzymatic activities in the synthetic pathways. These measurements will be the object of future experimentation. Also, the findings of this study have been based on ex vivo measurements, in tissue culture medium, without added Ang II. The increased $^{35}S$O$_4$ incorporation into tissue derived from Ang II–treated rats, therefore, was not due to the immediate action of Ang II but rather to in vivo–induced changes in synthetic or degrading enzyme activities or in both. Although suggestive, ex vivo measurements do not guarantee that the changes observed ex vivo also exist in vivo. Additional in vivo measurements will be needed to fully establish the pathophysiological significance of the findings of this study.

Stimulation of vascular GAG synthesis occurs early in the course of experimental renal hypertension, although much of the evidence is indirect. The negatively charged moieties of GAG side chains attract and bind sodium ions avidly. The sodium content of blood vessels is an indirect measure of the cation-binding capacity of proteoglycans. The increase in the total sodium content of arteries in renal hypertensive rats is detectable as early as 24 hours after the application of the renal artery clip. At the same time, there is accumulation of excess sodium in veins that are not exposed directly to arterial pressure. The sodium content of veins is also increased in rats with coarctation hypertension, a form of renal hypertension. We have detected an increase in the total sodium content of the aorta of rats treated with subpressor Ang II for 24 hours without a change in intracellular sodium content, indicating that the excess sodium was extracellularly located. More direct evidence for the accumulation of cation-binding GAGs in arteries was provided by Friedman and Friedman. The authors detected elevations in the paracellularly bound sodium fraction of tail arteries as early as 2 to 4 days after unilateral renal artery constriction of rats. The present report provides direct evidence that increased circulating Ang II levels may stimulate vascular GAG synthesis before the onset of hypertension. The early occurrence and magnitude of Ang II–stimulated vascular GAG synthesis suggest that elaboration of extracellular matrix components may play an
important role in the trophic action of the agonist and in the pathogenesis of the hypertension that results. However, the precise role of GAGs in these processes, and for that matter in the biology of vascular tissue in general, remains to be defined. GAGs have been implicated in a large variety of biological functions in connective tissue matrices and in basement membranes and also in cell-related functions such as cell-to-cell interaction, cell-to-matrix binding, growth factor attachment, and cell growth regulation. In the context of hypertension, we have suggested that by binding and attracting sodium ions, the paracellular accumulation of GAGs may increase the transmembrane sodium gradient and thereby potentiate the vasoconstrictor action of agonists whose mode of action involves the rapid influx of sodium. Ang II is one such agonist. Indeed, we have shown that by 7 to 10 days of subpressor Ang II administration, the agonist potentiates its own vasoconstrictor and pressor action. Autopotentiation of pressor responses by Ang II has been previously reported by others. It may play an important role in the amplification of pressor responses and the vicious cycle that leads to chronic hypertension.

In summary, the continuous administration of Ang II to rats in initially subpressor doses stimulates aortic wall GAG synthesis as defined in this study. GAG synthesis is also stimulated by Ang II in bladder smooth muscle, which is not directly exposed to arterial pressure. The rate of synthesis of subtypes of GAGs is proportionately increased by Ang II. The early occurrence and magnitude of stimulation of GAG synthesis suggest that restructuring of extracellular matrix may play an important role in the development of Ang II-induced hypertension.

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