Contractility of Intrarenal Arteries in Goldblatt Hypertensive Rabbits

Neil D. McElroy and Ben G. Zimmerman

The present investigation examined contractile responses of microdissected intrarenal arcuate arteries from sham-operated rabbits and two-kidney, one-clip Goldblatt hypertensive rabbits at 2 and 12 weeks after procedure. Arcuate arteries from both kidneys of the sham-operated rabbits and stenotic and nonstenotic kidneys of the Goldblatt hypertensive rabbits were studied. Mean arterial blood pressures of the sham-operated and Goldblatt hypertensive rabbits were 72±2 and 85±2, and 130±3 and 125±4 mm Hg, at 2 weeks and 12 weeks, respectively. In vitro isometric contractile force measurements were made with a small-artery myograph. Responses to graded concentrations of norepinephrine were evoked in the arcuate arteries, and the maximum active force was developed and -log EC50 was determined. At 2 weeks after procedure, the maximum responses of the vessels from the left kidney of the sham-operated rabbits and those from the stenotic left kidney of the Goldblatt hypertensive rabbits did not differ. The responses of the vessels from the right kidney of the sham-operated rabbits did not differ from those of the nonstenotic right kidney of the hypertensive rabbits. A markedly depressed maximum response of the vessels from the nonstenotic kidney of the hypertensive as compared with the right kidney of the sham-operated rabbits was found at 12 weeks after procedure, whereas the vessels from the stenotic kidney of the hypertensive and the left kidney of the sham-operated rabbits exhibited almost identical maximal responses. Responses to U 46619 were similarly affected in the two groups of rabbits. Cold-induced contractile responses of the arcuate arteries from the nonstenotic kidney of the hypertensive rabbits did not differ from those of the sham-operated rabbits at the 12-week interval. Norepinephrine-stimulated phosphoinositide hydrolysis in the intrarenal arterial network isolated from the kidneys of the sham-operated and hypertensive rabbits paralleled the changes in contractility. It is speculated that the depressed reactivity of the arcuate arteries reflecting a defect in the preglomerular resistance vessels could explain the inability of the nonstenotic kidney to normally autoregulate blood flow in certain models of hypertension. (Hypertension 1990;15:753–760)

Because increased peripheral vascular resistance is the common denominator in human essential hypertension and in experimental hypertensive animals, its cause has been the subject of intense investigation. Increased vascular resistance has been attributed to, at least in part, greater responsiveness of the hypertensives' blood vessels based on either structural changes,1 for example, medial thickening, functional changes,2 that is, increased vascular smooth muscle sensitivity to agonist agents, or both. Increased vascular responsiveness in hypertension is demonstrable in isolated conduit arteries such as the aorta3,4 and femoral artery5,6 as well as in vessels with a narrower diameter considered to be resistance vessels.7 Although this increase in responsiveness is in part caused by an increase in vascular smooth muscle sensitivity, recent evidence points to the importance of an increased number (hyperplasia) or size of smooth muscle cells (hypertrophy) in arterial contractile function.7–10 Because of our observation that the renal vascular reactivity to norepinephrine was increased in the conscious instrumented Goldblatt hypertensive dog,11 we examined in the rabbit intrarenal vascular α-adrenergic receptor properties in this model of hypertension. α1-Adrenergic receptor affinity but not receptor number was increased early in the hypertension (2 to 4–6 weeks after clipping). It was of interest to know whether this α-adrenergic receptor change was related to the increase in renal vascular reactivity seen in our conscious dog model.11 The present investigation examined the contractile responses to several agonists in isolated arcuate

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arteries of two-kidney, one clip Goldblatt hypertensive (GBH) rabbits. These particular renal arteries were selected because they were considered to be resistance vessels and are the smallest renal arteries capable of being studied in a myograph designed in this laboratory. Inositol phosphate generation in an intrarenal arterial preparation was also contrasted between GBH and sham-operated rabbits.

Methods

New Zealand White rabbits approximately 4-6 weeks old and weighing 0.6-0.9 kg were randomly assigned to hypertensive or sham-operated groups. Young rabbits were used to facilitate hypertension development by allowing the renal artery to grow into the Goldblatt clip. A retroperitoneal flank incision was made to expose the left renal artery while the rabbits were anesthetized with sodium pentobarbital (15 mg/kg). A rigid clip, 2.0 mm in width and 5.0 mm in length, made of silver ribbon, and with a fixed gap of 0.22 mm was placed on the left renal artery. Surgical implantation was performed aseptically, and the renal innervation was carefully avoided. A similar procedure was performed on the sham-operated rabbits; however, the clip was placed in the region near but not on the exposed artery. All rabbits were maintained on standard rabbit chow (Purina Mills, St. Louis, Missouri) and tap water ad libitum. Animals were studied 2 and 12 weeks after the procedure.

Arterial pressures were determined in the conscious state by a femoral artery catheter implanted at the time of clipping or sham operation. Measurement of mean arterial blood pressure was made once daily beginning the first day after the Goldblatt or sham procedure and continued for at least 5 days after the blood pressure readings were consistent. The arterial catheter was connected to a Statham pressure transducer (P23AA, Gould-Statham, Oxnard, California), and pressure was measured between 9:00 AM and 12:00 noon. This catheter was also used to withdraw a blood sample for radioimmunoassay of plasma renin activity (PRA).12

Contractility Experiments

Both kidneys were rapidly removed from either the sham-operated rabbits or hypertensive rabbits at the time they were killed and immediately placed in ice-cold Krebs-bicarbonate solution (pH 7.5 at 37°C). Composition of the buffer was (mM) NaCl 118, NaHCO3 25, KCl 3.5, MgSO4 1.4, CaNa2-EDTA 0.026, CaCl2 2.5, KH2PO4 1.6, and glucose 11. Cocaine (10^-6 M) and propranolol (10^-5 M) were included in the buffer to block adrenergic uptake and β-adrenergic receptors, respectively. After the renal capsule was removed, kidneys were dissected under a stereomicroscope, an arcuate artery was exposed, and a segment of artery (0.3-0.5 mm i.d.) approximately 5 mm in length was excised.

Two lengths of suture (Vicryl Polyglactin 910 braided 6-0 urologic suture, Ethicon-Johnson and Johnson, Somerville, New Jersey) were passed through the lumen of the artery with the aid of a short loop of stainless-steel wire (200 μm in diameter). This procedure at the same time removes the endothelium. Each suture was threaded through an upper and lower bridge of a myograph used to anchor the arterial segment for isometric recording. Sufficient tension was applied to the sutures that hold the vessel so that they would not yield during contraction. The myograph was secured in a 150-ml tissue bath, and the upper bridge was attached to a force transducer (Statham model 652, Gould-Statham). Krebs medium in the bath is gassed with 95% O2 and 5% CO2 and maintained at 37°C. In preliminary experiments, optimal resting tension was found to be 50 mg and was similar in arteries from sham-operated and hypertensive rabbits. Thus, 50 mg tension was applied at the onset and maintained at that level in all experiments.

Norepinephrine bitartrate (as base) was added to the bath in eight incremental concentrations ranging from 10^-9 to 10^-3 M, allowing each response to reach a maximum level. Concentration-response curves were repeated at least three times until two successive reproducible curves were obtained. Contractile responses to U 46619, a thromboxane-mimetic, in five cumulative increments ranging from 10^-9 to 10^-3 M were also studied.

After the agonist-induced responses were obtained, three exchanges of bathing medium and a rest period of at least 20 minutes were allowed, and an experimental protocol for eliciting contractile responses to acute cooling was used. Cooling was achieved by circulating ice water through the jackets surrounding the organ baths. Temperature was lowered in 3-5°C decrements from 37°C to 5°C, and the effect on basal tension was determined after an approximate 3-minute stabilization period at each temperature level.

Isometric force recordings were made on a Grass polygraph (model 7D, Grass Instr. Co., Quincy, Massachusetts). Exact length measurements of the unstretched vessels were made before mounting in each experiment by using a micrometer. Solutions of agents used in each experiment were added directly to the bath in amounts not exceeding 0.5% of the volume of the bath (150 ml). Active force development was defined as that isometric force produced that was greater than the 50 mg basal tension.

Phosphoinositide Metabolism

After an arcuate artery was removed, the kidney was placed in ice-cold Krebs buffer, and the entire intrarenal arterial vasculature was isolated and removed by microdissection as previously reported.13 The renal artery and its major proximal branches were removed, and the remaining arterial network, devoid of glomeruli and postglomerular vessels, was divided into at least two sections of equal weight of at least 100 mg. Each section included similar arterial segments. The labeling of membrane phospholipids and stimulation of the production of [3H]inositol phosphates were modifications of the methods of
Berridge et al.14 Incubation of the intrarenal arteries in buffer with myo-2-[3H]inositol (0.66 μM, 10 μCi/ml) was for 120 minutes after a previous equilibration period of 10 minutes. Next, incubations in buffer with unlabeled myoinositol (10 mM) for 10 minutes and with LiCl (10 mM) and myoinositol (30 μM) for another 10 minutes were performed. To stimulate phosphoinositide turnover, an incubation was performed for 30 minutes with $10^{-5}$ M norepinephrine. For determination of the control basal levels of inositol phosphates, the final incubation was conducted for 30 minutes without norepinephrine.

The last incubation was terminated by adding 2 ml extraction solution (CHCl$_3$, CH$_3$OH, and 0.01N HC1) in a ratio of 0.5:1:0.4 at −70°C. Tissue was homogenized in this solution, and the supernatant was added to 2 ml of a 1:1 mixture of CHCl$_3$ and 0.01N HC1. The aqueous phase was removed and dried in vacuo (model RH300-13, Savant Instrs., Inc., Farmingdale, New York). Separation of the reconstituted extract was accomplished by using anion-exchange columns, and the column eluates were counted in a Beckman LS8502 liquid scintillation counter (Beckman Instrs., Inc., Palo Alto, California).

Values are presented in the text, tables, and figures as mean±SEM. Statistical analysis was by one-way analysis of variance or Student's $t$ test for paired or group data.

Materials

Myo-2-[3H]inositol was from New England Nuclear (Boston, Massachusetts), norepinephrine bitartrate was from Sigma Chemical Co. (St. Louis, Missouri), and U 46619 (9,11-dideoxy-11a,9α-epoxymethano prostaglandin F$_2$α) was from Cayman Chemical Co., Inc. (Ann Arbor, Michigan).

Results

Six sham-operated and six GBH rabbits were studied at the 2-week interval, and nine sham-operated and nine GBH rabbits were studied at the 12-week interval after procedure. The mean arterial pressure and PRA were 72±2 and 2.1±1.5 versus 130±3 mm Hg and 13.2±4.7 ng/ml/hr at 2 weeks and 85±2 and 2.6±1.3 versus 125±4 mm Hg and 2.5±1.2 ng/ml/hr at 12 weeks in the sham-operated and GBH rabbits, respectively. The blood pressure difference between the sham-operated and GBH rabbits was statistically different ($p<0.05$) at 2 and 12 weeks, and the difference in PRA was statistically significant at 2 weeks ($p<0.05$).

Contractile Responses of Arcuate Arteries to Adrenergic and Other Agonists

Norepinephrine, $10^{-5}$ to $10^{-3}$ M and in the presence of cocaine and propranolol, contracted the arcuate arteries in a concentration-dependent manner. At the 2-week interval after procedure, the maximal response in arcuate arteries taken from the left kidneys (stenotic) of GBH rabbits did not differ from that of the left kidneys of sham-operated rabbits (Figure 1, panel B). Similarly, the maximal responses to norepinephrine of vessels from the nonstenotic right kidney of the GBH rabbits and right kidney of the sham-operated rabbits were not statistically different (Figure 1, panel A). At the 12-week interval after procedure, the maximum response in the vessels from the nonstenotic kidney of the GBH rabbits showed substantial differences from their nortenseven counterparts. These vessels, which were from the kidney subjected to the elevated blood pressure, displayed a dramatic reduction in maximum active force development as compared with vessels of the right kidney of the sham-operated group of rabbits (Figure 1, panel D). The stenotic left kidney compared well with its sham-operated counterpart in that the norepinephrine concentration–response curves were almost superimposable between the two groups (Figure 1, panel C). The vascular sensitivity of the vessels, −log $EC_{50}$, was similar in all cases, except in the nonstenotic kidney of the 12-week GBH rabbits, which showed an increase as compared with the sham-operated counterpart (5.6±0.14 vs. 6.3±0.06, $p<0.05$). A maximum response obtained at 12 weeks, larger than that obtained at 2 weeks in arteries from the sham-operated rabbits' kidneys and the stenotic kidneys of the GBH rabbits, is probably attributable to age difference.

To determine whether the depressed contractility to norepinephrine was specific for the catecholamine caused by an overall decrease in vascular reactivity, it was important to examine the responses to another vasoconstrictor substance. Comparison of responses to U 46619 in both 2-week and 12-week sham-operated and GBH rabbits is summarized in Table 1. At the 2-week time interval, sham-operated and GBH rabbit vessels were not significantly different in terms of maximum responsiveness or sensitivity to U 46619. As with norepinephrine, the maximum response to U 46619 was markedly depressed in arcuate arteries from the nonstenotic kidney of the 12-week GBH rabbits, whereas the $EC_{50}$s were unchanged. Arteries from the stenotic left kidney compared well with its sham-operated counterpart in terms of sensitivity and maximum response. Although the data are not presented, results qualitatively similar to those with norepinephrine were obtained with caffeine-induced and acetylcholine-induced contractions in these two groups of rabbits at both time intervals after procedure.

Contractile Responses to Cooling

Cooling from 37°C to 5°C resulted in a significant increase in active force generation in a temperature-dependent manner in all arcuate arteries studied. Contractile responses to cooling in 2-week sham-operated rabbits were similar in both left and right kidneys (Figure 2, panels A and B), and the same held true for the 12-week sham-operated rabbits (Figure 2, panels C and D). Although both the 2-week and 12-week sham-operated groups of rabbits appeared equally sensitive to the effects of
cooling (half maximal response at approximately 20°C), for an unknown reason the maximal response was significantly reduced in the latter group (Figure 2, panels C and D).

In the 2-week GBH rabbits, arteries from the nonstenotic left kidney exhibited a maximal response and sensitivity similar to that of the sham-operated rabbits (Figure 2, panels A and B). Those of the right (nonstenotic) kidney, however, had a significantly depressed maximum response (*p<0.05). Arcuate arteries from the 12-week GBH rabbits responded somewhat differently from those of the 2-week rabbits. The maximal response in stenotic kidney was significantly increased as compared with that in the sham-operated rabbits’ left kidney (Figure 2, panels C and D) (*p<0.05); however, the responses of the vessels from the nonstenotic kidney and the vessels from the sham-operated rabbits did not differ. Thus, at 12 weeks, it appears that there is no depression of reactivity to cold in the nonstenotic kidney but that the responses are potentiated in the stenotic kidney. The cause of this difference in response of the arteries from the stenotic kidney is unknown.

**Phosphoinositide Metabolism**

Values of basal and norepinephrine-stimulated intrarenal arterial inositol phosphate content are presented in Figures 3 and 4. Thirty-minute exposure to norepinephrine (10^-5 M) significantly increased (*p<0.05) the content of [3H]glycerolphosphoinositol.

<table>
<thead>
<tr>
<th>Week</th>
<th>Maximum force (mg)</th>
<th>-Log EC50 [M]</th>
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<tr>
<td></td>
<td>Sham</td>
<td>Hypertensive</td>
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<td></td>
<td>LK</td>
<td>RK</td>
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<tr>
<td>2</td>
<td>580±53</td>
<td>560±45</td>
</tr>
<tr>
<td>12</td>
<td>625±95</td>
<td>650±83</td>
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Values represent mean±SEM. Units of force are milligrams of active force per 5 mm; n=6-9. EC50, effective concentration for 50% of maximal response; Sham, sham-operated rabbits; Hypertensive, Goldblatt hypertensive rabbits; LK, left kidney; RK, right kidney.

*p<0.05 vs. corresponding kidney of sham-operated rabbits, at the same weekly interval.
Depressed Vascular Reactivity

FIGURE 2. Plottings showing active force development of intrarenal arcuate arteries caused by temperature decrease (from 37° to 5°C). (n=6 for sham-operated [sham] and Goldblatt hypertensive [GBH] rabbits at 2 weeks, and n=9 for sham-operated and hypertensive GBH rabbits at 12 weeks). Left and right kidneys were from the same rabbits. Responses of arcuate arteries from stenotic left kidney of GBH rabbits are compared with those from left kidney of sham-operated rabbits (panel A), and responses of arteries from nonstenotic right kidney of GBH rabbits are compared with those from right kidney of sham-operated rabbits (panel B) at 2-week interval after procedure. Responses of arcuate arteries from stenotic left kidney of GBH rabbits are compared with those from left kidney of sham-operated rabbits (panel C), and responses of arteries from nonstenotic right kidney of GBH rabbits are compared with those of right kidney of sham-operated rabbits (panel D) at 12-week interval after procedure. *p<0.05, nonstenotic kidney of hypertensive rabbit group vs. right kidney of sham-operated rabbit group in panel B, and stenotic kidney of GBH rabbit group vs. left kidney of sham-operated rabbit group in panel C.

tide ([H]GPI), [H]inositol 1-phosphate ([H]IP1), and [H]inositol 1,4-bisphosphate ([H]IP2) in kidneys of sham-operated rabbits at both the 2-week and 12-week intervals after procedure (Figure 3, panel A, and Figure 4, panel A). The increase in [H]inositol 1,4,5-trisphosphate ([H]IP3) did not reach statistical significance. At the 2-week interval in the GBH rabbits, the basal and stimulated contents of [H]GPI and [H]IP3 were similar to controls (Figure 3, panel B). Both basal and norepinephrine-stimulated [H]IP1 and [H]IP3, however, were significantly depressed in the nonstenotic right kidneys of the GBH rabbits as compared with the stenotic kidneys and sham-operated rabbits' kidneys (Figure 3, panels A and B). Norepinephrine-stimulated [H]IP2, [H]IP3, and [H]GPI were greatly depressed in the nonstenotic kidneys of 12-week GBH rabbits as compared with the stenotic kidneys and kidneys from sham-operated rabbits, respectively (Figure 4, panels A and B).

Discussion

Arcuate arteries are representative preglomerular resistance vessels, and as shown in the present study, they generate a pronounced contractile response to the α1-adrenergic receptor agonist norepinephrine. Concentration-force curves obtained at the 2-week interval after procedure were almost superimposable for arteries from sham-operated and GBH rabbits' stenotic kidneys. There was a shift to the right in the curve obtained for the nonstenotic kidney at 2 weeks; however, the shift was not statistically significant. The most striking finding in this investigation was the pronounced depression of the concentration-force curve for norepinephrine obtained for the nonstenotic kidney at the 12-week interval. These arcuate arteries that were from the kidney exposed to an elevation in blood pressure over the entire course of the hypertension were almost totally unresponsive to norepinephrine. In contrast to these findings, the arcuate arteries from the stenotic kidney, even at the 12-week interval, exhibited concentration-force curves no different from those of arteries from sham-operated rabbits.

This depressed vascular responsiveness of the 12-week GBH rabbits' arcuate arteries was not caused by an α1-adrenergic receptor defect. As shown in a previous study, α1- and α2-adrenergic receptor binding characteristics did not differ between the GBH and sham-operated rabbits' intrarenal arteries at the 10–12-week interval after procedure.13 To obtain an adequate amount of arterial tissue in the receptor study, the entire intrarenal arterial network had to be
used, and thus it is assumed that the arcuate arteries and the total intrarenal arterial network, including interlobar, arcuate, interlobular arteries, and afferent arterioles, have equivalent α1-adrenergic receptors. In agreement with the lack of specificity of this defect, there was a similar depression of vascular reactivity of the arcuate artery to U 46619, acetylcholine, and caffeine. A structural change in the arterial wall or a postreceptor functional change, possibly a G-protein defect, would appear to explain these results. Because this contractility defect develops over a long period of time (2–12 weeks) and is seen in the nonstenotic kidney only, it would appear to be related to the effect of pressure on the arterial smooth muscle. Vessels from the kidney exposed to the elevation in blood pressure showed the effect, whereas those protected from the pressure elevation by the Goldblatt clamp did not. There have been reports of an attenuated maximal contractile response to vasoconstrictor agonists in both conduit arteries5,16 and in smaller resistance vessels,10,17 and this observation has been attributed to some to a change in composition of the smooth muscle protein.10,17 We considered the possibility that the contractile machinery of the vascular smooth muscle was incapable of generating active force. Two approaches using postreceptor mechanisms were taken to evoke contraction of the smooth muscle. Caffeine, which triggers smooth muscle contraction through calcium release and blockade of reuptake by the sarcoplasmic reticulum, evoked only weak contractions of the arcuate arteries in the 12-week nonstenotic kidney. Cold, which acts by inducing calcium-stimulated release of intracellular calcium,18 evoked the strongest contractions of the arcuate arteries of the nonstenotic kidneys of 12-week GBH rabbits. Contractions induced by cold were similar in arcuate arteries from the GBH rabbits and sham-operated rabbits at the 2-week and 12-week intervals. There was an unexplained increase in cold-contraction of the vessels from the stenotic kidney; however, the important point is that the arteries from the nonstenotic kidney did not show a contractility defect to cold. Thus, the contractile mechanism of these vessels had the capacity to function, albeit at a relatively low level of activity triggered by a postreceptor mechanism.

Stimulation of phosphoinositide hydrolysis and formation of inositol phosphates was evoked by norepinephrine in the intrarenal arterial network of 2-week GBH rabbits and sham-operated rabbits, and this was of a similar magnitude in the stenotic
Figure 4. Bar graphs showing basal content and norepinephrine-stimulated accumulation of [3H]inositol phosphates in the rabbit intrarenal arterial vascular network. Panel A: Content in left and right renal arterial network of sham-operated rabbits, 12-week interval after procedure. Panel B: Content in arterial vasculature of left (stenotic) and right (nonstenotic) kidneys from Goldblatt hypertensive (GBH) rabbits, 12-week interval after procedure. (n=6 for sham-operated and GBH rabbits.) *p<0.05 vs. contralateral value for comparisons of basal and stimulated content within sham-operated rabbits or GBH rabbits. †p<0.05, ipsilateral value of basal and stimulated content between sham-operated rabbits and GBH rabbits. GPI, [3H]glycerolphosphoinositide; IP1, [3H]inositol 1-phosphate; IP2, [3H]inositol 1,4-bisphosphate; IP3, [3H]inositol 1,4,5-trisphosphate.

There was, however, a significantly decreased formation of [3H]IP1 and [3H]IP2 in the arteries from the nonstenotic kidney. This early change in stimulated phosphoinositide turnover might mark the beginning of signal transduction failure in the preglomerular arteries of the nonstenotic kidney. At 12 weeks, as with the contractile response to vasoconstrictor agonists, there was a complete loss of norepinephrine-stimulated phosphoinositide turnover in the nonstenotic but not in the stenotic kidney. Although these results do demonstrate that the phosphoinositide signal pathway is impaired in arteries of long-term nonstenotic kidneys of GBH rabbits, they do not totally explain the depressed vascular reactivity of the arcuate arteries. It is unlikely that depression of caffeine-induced contractions can be attributed to a failure of phosphoinositide hydrolysis. Thus, there would appear to be an impairment in more than one process affecting contractility in these vessels.

There are several important implications that can be drawn from the results of this investigation. The first is that a sustained increase in arterial blood pressure can, through some biochemical lesion or structural change, markedly suppress vascular reactivity to agonist agents. Investigation into how pressure can induce such a change offers an intriguing avenue for future work. Secondly, if preglomerular vascular reactivity in general is markedly suppressed, as would be suggested by the depression of phosphoinositide metabolism in the entire total preglomerular intrarenal arterial network, renal vascular autoregulation might be lost. Impaired autoregulation of the afferent arteriole has, in fact, been reported for the nonstenotic kidney of the GBH rat. If these results can be equated with the findings in post-salt and Dahl salt-sensitive rats, the lack of “autoregulatory protection” of the glomerulus could lead to nephrosclerosis as seen in these hypertensive rat models.

References

**KEY WORDS** • kidney • hypertrophy • receptors, adrenergic • vascular smooth muscle • Goldblatt hypertension • rabbit studies
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