Regional Variation of Postjunctional α-Adrenoceptor Responses in the Developing Renal Vascular Bed of Sheep

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ABSTRACT. Postjunctional α_1- and α_2-adrenoceptor vasoconstrictor responses were evaluated in isolated segments of main renal artery, segmental renal artery, and renal vein from fetal (130–138 d of gestation; term 145 d), newborn (3–15 d age), and nonpregnant adult sheep. Vascular rings were mounted at their optimal resting tension and responses to phenylephrine (α_1-adrenoceptor agonist) and guanabenz and UK14304 (both α_2-adrenoceptor agonists) were determined. Optimal resting tension increases with development in the main renal artery, segmental renal artery, and renal vein of sheep. Arterial vessels develop more isometric tension to α_1-adrenoceptor stimulation than to α_2-adrenoceptor stimulation, whereas venous segments develop similar isometric tension to α_1- and α_2-adrenoceptor stimulation. The segmental renal artery develops more isometric tension to α_2-adrenoceptor stimulation than the main renal artery. No large developmental differences exist among vessels in the sensitivity (concentration required for half maximal response, ED_{50}) to α-adrenoceptor stimulation except for the renal vein with α_2-adrenoceptor stimulation. Maximum isometric tension corrected for vessel cross-sectional area decreases with age for all vessels with both α_1- and α_2-adrenoceptor stimulation. These findings may reflect developmental differences in receptor number and affinity or differences in vascular smooth muscle function. In addition, these data suggest that whereas both α_1- and α_2-adrenoceptors mediate vasoconstriction in the renal circulation, they may do so at different sites. (*Pediatr Res* 25:461–465, 1989)

Abbreviations

MRA, main renal artery
SRA, segmental renal artery
RV, renal vein
PE, phenylephrine
Gb, guanabenz
UK, UK14304
ED_{50}, concentration that produces half maximal response

Recent studies in this laboratory have demonstrated that renal vascular α_1- and α_2-adrenoceptors are functional and can produce renal vasoconstriction in fetal sheep and newborn lambs (1). Moreover, the renal hemodynamic response to α_1- and α_2-adrenoceptor stimulation changes during maturation with fetal and newborn α_2-adrenoceptor responses less than those observed in adult sheep, whereas the α_1-adrenoceptor responses were similar (1).

Contrary to our results (1), recent studies by Tayo *et al.* (2) using isolated vascular rings demonstrated that the α_2-adrenoceptor-mediated contractile response is greater in 4- to 8-wk-old rabbits than in adult animals, suggesting that there may be a loss of α_2-adrenoceptor-mediated contraction with maturation. Differences between the study by Tayo *et al.* (2) and our previous results (1) are difficult to explain. However, some of the differences between the two studies may be due to comparing *in vivo* experiments, where the overall hemodynamic response is studied, with *in vitro* experiments, where specific vascular segments are studied.

Therefore, the present study was designed to examine possible regional developmental differences in α_1- and α_2-adrenoceptor-mediated vasoconstrictor responses in the developing renal vascular bed. Isolated segments of MRA, SRA, and RV from fetal, newborn, and nonpregnant adult sheep were studied.

MATERIALS AND METHODS

Animals. Vessels from seven fetal sheep (130–138 d of gestation; term 145 d), seven newborn lambs (3–15 d of age), and seven nonpregnant adult sheep were evaluated using standard *in vitro* techniques (3). Gestational ages were determined as previously described (4).

Vessel preparation. Newborn and adult animals were killed with a lethal dose of intravenous pentobarbital. Pregnant ewes were anesthetized with a mixture of 1% halothane and 66% nitrous oxide and fetuses killed with pentobarbital injected into the umbilical vein. Segments of MRA, SRA, and RV were removed from each animal and immediately placed in cold Krebs solution (composition in mM) NaCl 118, NaHCO$_3$ 25, glucose 11, KCl 4.7, MgSO$_4$ 1.2, CaCl$_2$ 2.2, and disodium EDTA 0.025 (5). Vessels were cleared of adhering fat and connective tissue under a dissecting microscope with care taken to avoid stretching. Transverse rings were cut at 3- to 4-mm width for the MRA, 2- to 3-mm width for the SRA, and 3- to 4-mm width for the RV. Segments of MRA were taken at least 3 mm distal to the origin of the MRA from the aorta and before the MRA branched outside the kidney. The SRA was taken from segments after the first intrarenal branching in the kidney which ran between renal papillae. RV segments were taken from the main renal vein distal to branching outside the kidney and 2–3 mm proximal to the junction with the inferior vena cava.

Rings were mounted by passing two fine, stainless steel wires through the vessel lumen. One side was anchored to a stationary rod and the other to a Grass FT.03 force-displacement transducer (Grass Instrument Co., Quincy, MA) connected to a Gould recorder (Gould Inc., Cleveland, OH) for measurement of isometric force (3). The vascular rings were bathed in an organ bath filled with 25 mL Krebs solution at 39°C and gassed with 95% O_2-5% CO_2. After equilibration for 60 min with solution changed every 15 min, each vascular ring was stretched to the optimal point on its length-tension curve as determined by KCl depolarization using 30–60 mM KCl for the arterial vessels and 60–100 mM KCl for the venous vessels (6, 7).

All experiments were performed in the presence of desipramine (10^{-5} M) and hydrocortisone (10^{-5} M) to block neuronal and extraneuronal uptake of norepinephrine, respectively (5, 8). In addition, propranolol (10^{-5} M) was used to inhibit β-adrenergic receptors (8).

Experimental studies. Concentration-response curves to the α_1-adrenoceptor agonist PE, and the α_2-adrenoceptor agonists Gb and UK were performed by cumulative increases in organ chamber concentration from 10^{-8} M to 10^{-4} M with 100 μL additions of agonist to the bath. The concentration of drug was increased when the previous concentration had produced its maximal response. Prazosin (10^{-6} M), an α_1-adrenoceptor antagonist, was added to the organ baths of vessels evaluated with α_2-adrenoceptor agonists (Gb or UK) to exclude the potential for partial α_1-adrenoceptor agonist activity (2, 9).

After each experiment, vessels were cut open and the circumference (c) and blotted wt (bw) were measured for calculation of cross-sectional area as determined by the equation:

$$A = \frac{2 \times bw}{1.06 \times c}$$

where A is the vessel cross-sectional area and 1.06 is the estimated tissue density in g/cm^3 (10). Normalizing isometric force for cross sectional area allows comparisons of responses from vessels of different sizes (7, 11, 12).

Drugs. The following drugs were used: Gb (Wyeth Laboratories, Philadelphia, PA), UK (Pfizer, Sandwich, UK), prazosin (Pfizer, New York, NY), and L-PE, disodium EDTA, desipramine HCL, hydrocortisone, propranolol (all from Sigma Chemical Co., St. Louis, MO). Drugs were prepared daily in distilled water.

Calculations and statistical treatment. Baseline optimal resting tension was compared across ages and regions with Student's unpaired *t* test and Bonferroni correction. Maximal tensions developed in mN/mm^2 were compared as follows: responses from MRA and SRA were compared across age groups and to each other for each agonist with 2-way ANOVA with Neuman Keuls multiple comparisons when differences were detected. RV responses were compared across age groups with 1-way ANOVA with Neuman Keuls multiple comparison when differences were detected. Maximal tension developed was compared between PE and Gb with Student's paired *t* test and Bonferroni correction.

Mean values of ED_{50} (agonist concentration evoking half-

maximal response) were calculated and statistically compared using the ALLFIT program (13–15). ALLFIT is a nonlinear least-square multiple-curve fitting program that allows simultaneous curve fittings of families of dose response curves based on the four parameter logistic equation:

$$y = \frac{a - d}{1 + (x/c)^b} + d$$

where y is the response; x, the arithmetic dose; a, the response when x = 0; d, the response for "infinite" dose; c, the ED_{50}; and b, the slope factor that determines the steepness of the curve. Goodness of fit of individual dose-response curves was evaluated by: *1*) F ratio test, which represented the ratio of residual variance for one curve to the overall residual variance of the other curves, and *2*) run test, which evaluated the randomness of distribution of data points around and along the fitted curve. Significant F ratio tests ($p < 0.05$) or run tests ($p < 0.05$) suggested poor fits according to the logistic model. Consequences of forcing parameters to be equal were also evaluated by an F ratio test. A significant p value (<0.05) indicated that data supported the hypothesis that shared parameters were in fact different from each other (13). A significance level of $p < 0.05$ was used for all statistical tests.

RESULTS

Baseline values. Optimal resting tension (Table 1) increased postnatally in all vessels ($p < 0.05$, unpaired *t* test). Arterial vessels had greater optimal resting tension than veins at all ages and the MRA had greater optimal resting tension than the SRA in fetus and newborns ($p < 0.05$, unpaired *t* test).

ALLFIT analysis. Concentration response curves for every age, every vessel, and every agonist evaluated were appropriately fitted as evidenced by nonsignificant goodness of fit F ratios and nonsignificant runs test using ALLFIT analysis (all F ratio and runs tests $p > 0.05$).

Effect of α_1-adrenoceptor agonist PE on vascular contraction. Maximum isometric tension developed to PE showed an age-dependent decrease for both MRA and SRA (Table 2) and RV (Table 3) ($p < 0.05$, ANOVA). In addition, the maximum response was greater in the MRA compared with the SRA at all ages ($p < 0.05$, ANOVA). When ED_{50} values were compared, fetal vessels were slightly though significantly more sensitive to PE than newborn vessels (Table 4) ($p < 0.05$, ALLFIT analysis).

Effect of α_2-adrenoceptor agonist Gb on vascular contraction. Maximum isometric tension developed to Gb (in the presence of prazosin 10^{-6} M) also showed an age-dependent decrease for both arterial segments (Table 2) and RV (Table 3) ($p < 0.05$, ANOVA). However, the maximum response for Gb was greater in the SRA compared with the MRA ($p < 0.05$, ANOVA). The Gb ED_{50} values for arterial vessels were similar across age groups (Table 4) ($p > 0.05$, ALLFIT analysis). The RV, by contrast, showed an age-dependent decrease in sensitivity to Gb-mediated α_2-adrenoceptor stimulation ($p < 0.05$, ALLFIT analysis).

Effect of α_2-adrenoceptor agonist UK on vascular contraction. Maximum responses to UK (in the presence of prazosin 10^{-6} M)

Table 1. *Optimal resting tension (expressed in g)**

	Fetus	Newborn	Adult	Comparison by age
MRA	0.95 ± 0.04 (14)	1.34 ± 0.09 (13)	1.26 ± 0.03 (14)	F < N = A
SRA	0.61 ± 0.03 (14)	0.82 ± 0.02 (13)	1.19 ± 0.04 (14)	F < N < A
RV	0.14 ± 0.01 (12)	0.27 ± 0.04 (12)	0.21 ± 0.01 (13)	F < N = A
Comparison by vessel	RV < SRA < MRA	RV < SRA < MRA	RV < SRA = MRA	

* Values are means ± SEM. Numbers in parentheses are the number of observations. <, for difference $p < 0.05$ (unpaired *t* test and Bonferroni correction).

Table 2. *Maximum contractile response of renal arteries to PE, Gb, and UK (mN/mm²)**

	MRA†			MRA age comparison	SRA			SRA age comparison
	Fetus	Newborn	Adult		Fetus	Newborn	Adult	
PE	13.1 ± 2.5‡	9.8 ± 2.1‡	3.8 ± 0.6‡	F > N > A	11.0 ± 2.2‡	4.7 ± 1.2‡	2.0 ± 0.2	F > N > A
Gb	2.4 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	F > N = A	3.1 ± 0.5	1.5 ± 0.8	1.6 ± 0.2	F > N = A
UK	1.0 ± 0.1	0.3 ± 0.2	0.2 ± 0.04	F > N = A	1.9 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	F > N = A

* Values are means $\pm$ SEM; all $n = 7$. >, difference when $p < 0.05$ (2-way ANOVA and Newman-Keuls comparison).
† All MRA different from SRA at $p < 0.05$ (2-way ANOVA and Newman-Keuls comparison).
‡ PE different from Gb at $p < 0.05$ (paired t test).

Table 3. *Maximum contractile response of renal veins to PE, Gb, and UK (mN/mm²)**

	Fetus	Newborn	Adult	Age comparison
				N
PE	1.6 ± 0.3 (7)	1.2 ± 0.2 (7)	0.3 ± 0.1 (7)	F = N > A
Gb	1.6 ± 0.3 (7)	0.6 ± 0.1 (7)	0.1 ± 0.03 (7)	F > N = A
UK	1.9 ± 0.2 (5)	0.8 ± 0.2 (6)	0.1 ± 0.1 (6)	F > N > A

* Values are means $\pm$ SEM. Numbers in parentheses are number of observations. >, difference when $p < 0.05$ (1-way ANOVA and Newman-Keuls comparison). PE responses not different from Gb responses $p > 0.05$ (paired t test).

Table 4. *ED_{50} (molar)**

	PE	Gb	UK
MRA			
Fetus	$2.2 \pm 0.2 \times 10^{-5}$	$2.5 \pm 0.4 \times 10^{-5}$	$0.9 \pm 0.2 \times 10^{-6}$
Newborn	$3.6 \pm 0.3 \times 10^{-5}$	$2.1 \pm 0.4 \times 10^{-5}$	$6.2 \pm 1.9 \times 10^{-6}$
Adult	$3.3 \pm 0.3 \times 10^{-5}$	$2.0 \pm 0.4 \times 10^{-5}$	$7.2 \pm 1.3 \times 10^{-6}$
	F < N = A	F = N = A	F < N = A
SRA			
Fetus	$1.5 \pm 0.2 \times 10^{-5}$	$0.7 \pm 0.1 \times 10^{-5}$	$0.6 \pm 0.1 \times 10^{-6}$
Newborn	$3.1 \pm 0.3 \times 10^{-5}$	$1.2 \pm 0.3 \times 10^{-5}$	$0.8 \pm 0.1 \times 10^{-6}$
Adult	$2.3 \pm 0.4 \times 10^{-5}$	$1.3 \pm 0.3 \times 10^{-5}$	$1.5 \pm 0.2 \times 10^{-6}$
	F < N, N = A, F = A	F = N = A	F = N < A
RV			
Fetus	$1.1 \pm 0.1 \times 10^{-5}$	$5.0 \pm 0.8 \times 10^{-8}$	$0.9 \pm 0.1 \times 10^{-7}$
Newborn	$1.9 \pm 0.2 \times 10^{-5}$	$2.9 \pm 0.7 \times 10^{-7}$	$3.4 \pm 0.3 \times 10^{-7}$
Adult	$1.2 \pm 0.1 \times 10^{-5}$	$4.6 \pm 1.4 \times 10^{-6}$	$1.9 \pm 0.2 \times 10^{-7}$
	F = A < N	F < N < A	F < A < N

* Values are means $\pm$ SEM, number of observations for each vessel, age, and agonist combination may be found in Tables 2 and 3. <, difference when $p < 0.05$ (ALLFIT analysis).

were similar to Gb responses, with age-related decreases in both arterial (Table 2) and venous segments (Table 3) ($p < 0.05$, ANOVA). The maximum response to UK was also greater in the SRA compared with the MRA ($p < 0.05$, ANOVA). The ED_{50} values for UK show small age-dependent decreases in sensitivity for both arterial and venous segments (Table 4) ($p < 0.05$, ALLFIT analysis).

Comparison of α_1- and α_2-adrenoceptor-mediated contractions. The maximum response to PE was greater than the maximum response to Gb in the MRA for all ages (Table 2) ($p < 0.05$, paired t test). In the SRA, the PE response was greater than the Gb response in the fetus and newborn (Table 2) ($p < 0.05$, paired t test). A typical experimental tracing from a fetus (Fig. 1*A*) demonstrates the greater magnitude of the PE response compared with the Gb response in arterial vessels. In the RV, by contrast, the maximum response to PE was not different from the maximum response to Gb (Table 3) ($p > 0.05$, paired t test) and is illustrated by a typical experimental tracing in Figure 1*B*.
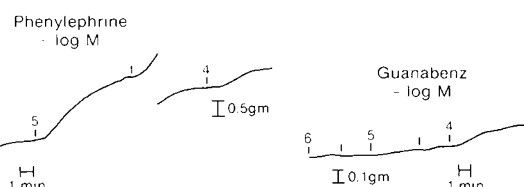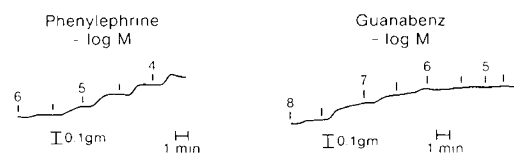

Fig. 1. Isometric tension recordings from segments of MRA in *A* and in segments of RV in *B* from a fetal sheep. Responses of the MRA to phenylephrine are much greater than the response to Gb (*A*), whereas PE and Gb responses are similar in the RV (*B*).

DISCUSSION

The isolated vessel technique used in the present study is a suitable method for examining responses of different regions within a vascular bed and has several distinct advantages. The confounding effects of circulating vasoactive substances, central neural control, and local metabolic control present *in vivo* are all avoided. As such, intrinsic receptor and smooth muscle characteristics of a particular vascular segment can be studied. However, the microcirculation cannot be easily evaluated.

We found age-dependent increases in optimal resting tension (Table 1), supporting observations of Pagani *et al.* in sheep aortas (16) and Cox *et al.* in canine arteries (17), who found that arterial stiffness increases with age. However, it contrasts with the findings of Seidel *et al.* (7), who found no developmental changes in optimal resting tension in canine arteries and veins. Most of the differences we observed, however, were between prenatal and postnatal vessels which were not compared in the study by Seidel *et al.* (7). Changes in optimal resting tensions with age may reflect alterations in physical characteristics of the vessels (18); however, these properties were not the focus of the present investigation.

The finding that arterial vessels develop more isometric tension to α_1-adrenoceptor stimulation than to α_2-adrenoceptor stimulation (Table 2) has been previously reported (19–22), although no studies have examined pre- and postnatal differences. One possible explanation for smaller α_2-adrenoceptor mediated vasoconstriction may be that the receptors are located on small "resistance" vessels rather than larger vessels (20, 23, 24). Our finding that the maximum response to Gb and UK in the SRA was greater than the response in the MRA suggests an increased ability to respond to α_2-adrenoceptor stimulation in smaller arterial vessels. This finding is consistent with results from Muntz *et al.* (24) and Faber (23) who found increased α_2-adrenoceptor

receptors and function on arterioles. Our results, however, provide only supportive evidence for the hypothesis of increased α_2-adrenoceptors on smaller arterial vessels since receptor numbers and location were not determined. Another theory, offered by Ruffolo (22), is that α_1- and α_2-adrenoceptors have different "receptor-effector coupling" and α_2-adrenoceptors may become "uncoupled" from their effector mechanisms when removed for *in vitro* studies. However, the present study was not designed to study this aspect.

In contrast, the maximum responses for PE and Gb were similar to each other in the RV (Table 3). The present results support previous data that α_2-adrenoceptor responses are prominent in the venous circulation, consistent with the hypothesis that postsynaptic vascular α_2-adrenoceptors may play a more important functional role in the venous relative to arterial circulation (19, 22, 25). Thus, one may speculate that during fetal life, renal venous circulation may be controlled, in part, by postsynaptic α_2-adrenoceptor vasoconstrictor tone. The role of venous tone in modifying overall renal hemodynamics is an intriguing concept that awaits further investigation.

Earlier results comparing sensitivity of the vasculature to α-adrenergic stimulation during development have been conflicting (7, 11, 18, 26, 27). Gray (27) found that neonatal canine aortic strips had higher ED_{50} values for PE and norepinephrine than mature dogs, while Cohen and Berkowitz (11) found that young rat aortas had a lower ED_{50} for norepinephrine than older rats. Seidel *et al.* (7) found no change in the ED_{50} for PE in most vessels in the developing dog. Our findings (Table 4) show a small, although at times statistically significant, age-related decrease in sensitivity to all agonists. It is important to note, however, that the physiologic significance of these small differences in ED_{50} may be questioned since most differences were much less than one half log unit. An exception was the renal-vein response to Gb which showed large age-related decreases in sensitivity. The reasons our findings are different from previous studies are not clear although species differences and regional variations could explain the dissimilarities.

For all ages, vessels, and agonists studied, we found age-dependent decreases in maximum isometric tension developed after correction for vessel cross-sectional area (Tables 2 and 3). Similar results were found by Park and Sheridan (28), using phenylephrine to contract guinea pig and rabbit aortas, and by Toda *et al.* (12), using norepinephrine to contract canine cerebral vessels. Gray (27), on the other hand, found age-dependent increases in maximum tension developed to norepinephrine and PE in aortic strips from dogs but the data were not normalized for cross-sectional area. Finally Seidel *et al.* (7) showed increasing maximal tension to PE with maturation using a variety of canine vessels including the renal artery, suggesting that vessels from sheep compared to dogs appear to have different developmental characteristics.

The findings of developmental decreases in maximal isometric tension *in vitro* contrast our *in vivo* data (1), which showed developmental increases in α_2-adrenoceptor vasocontriction and similar α_1 responses at low levels of stimulation. Differences between the *in vivo* and *in vitro* data may relate to differences in the integrative response of the intact renal vascular bed compared with the isolated vessel response. In addition, the contribution of arterioles and venules in control of renal hemodynamics *in vivo* may be significant.

Reasons for the decrease in maximal isometric tension with age were not investigated in our study. Pearce and Ashwal (29) found results similar to ours in the carotid artery using KCl depolarization and attributed the decrease in maximum tension developed to the thicker arterial wall found in the mature animal. The thicker wall is thought to be due to more increases in collagen and elastin than contractile proteins (18, 30). Whether our results are secondary to similar structural changes, differences in receptor characteristics or number, or differences in smooth muscle

characteristics such as actin and myosin content and calcium utilization, remains to be defined.

In summary, the present study demonstrates that: *1*) optimal resting tension increases postnatally in the renal vascular bed of sheep; *2*) renal arterial vessels develop more isometric tension to α_1-adrenoceptor stimulation than to α_2-adrenoceptor stimulation; *3*) renal venous segments develop similar isometric tension to α_1- and α_2-adrenoceptor stimulation; *4*) the SRA develops more isometric tension to α_2-adrenoceptor stimulation than the MRA; *5*) no large developmental differences were found in the sensitivity of the vessels to α-adrenoceptor agonists except for the RV which became less sensitive to Gb during maturation; *6*) maximum isometric tension corrected for vessel cross-sectional area decreases with age for all vessels and all agonists. These findings may reflect developmental differences in receptor number and affinity or may reflect developmental changes in vascular smooth muscle function. In addition, the data suggest that distribution of postsynaptic α-adrenoceptor subtypes that mediate renal vasoconstriction vary within the renal vascular bed during development. One may speculate that α_1- and α_2-adrenoceptor agonists may both produce decreases in renal blood flow, but may be acting predominantly at different sites within the renal circulation.

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