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Developmental Regulation of the α_{1B}-Adrenoceptor in the Sheep Kidney

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ABSTRACT. The expression of renal α_{1B}-adrenoceptor (α_{1B}-AR) mRNA was studied and contrasted with the expression of renal renin mRNA in fetal and newborn sheep. Fetal sheep between 90 and 91, 116 and 118, and 139 and 141 d gestation (term is 145 d gestation) as well as newborn lambs between 1 and 2 d old and 8 and 10 d old were studied (n = 3 for each age range). The role of the renal nerves in regulating changes in α_{1B}-AR gene expression was also investigated by measuring renal cortical α_{1B}-AR mRNA levels and receptor k_d and maximum number of binding sites in 24-h-old lambs that were either denervated (n = 6) or sham-operated (n = 5) 3 d before birth. During development, renal α_{1B}-AR mRNA levels show a marked increase in term fetuses; this increase persists into the first 2 d of life and is distinct from the developmental pattern seen for renal renin mRNA levels. Denervation of term fetuses does not alter the expression of renal α_{1B}-AR mRNA in newborn lambs when compared with sham-operated controls but decreases significantly the expression of the renin gene ($p < 0.05$). These results suggest that the α_{1B}-AR gene is developmentally regulated in the kidney in a pattern distinct from that seen for renin. Furthermore, the renal nerves, which play an important role in the regulation of renin gene expression at the time of birth, do not appear to be an important factor in the regulation of the α_{1B}-AR during the maturation from fetal to newborn life. (*Pediatr Res* 34: 124–128, 1993)

Abbreviations

α_{1B}-AR, α_{1B}-adrenoceptor
GAPD, glyceraldehyde-3-phosphate dehydrogenase
B_{max}, maximum number of binding sites

Previous studies (1) have suggested that stimulation of α-adrenoceptors modulates renal hemodynamics and fluid and electrolyte homeostasis during development. For example, the renal and vasoconstrictor response to intrarenal infusion of phenylephrine (2), an α_1-adrenoceptor agonist, or to low-frequency renal nerve stimulation (3, 4) is greater in the near-term sheep fetus than in the newborn lamb. Similarly, fetal and newborn sheep show a greater antinatriuretic response to renal α_1-adrenergic stimulation than do older animals (5). It has also been shown that renal nerves modulate the natriuretic response to volume expansion in fetal and newborn animals (6) and that renal denervation before birth increases renal sodium excretion in the immediate postnatal period (7). Furthermore, studies in sheep (8) have demonstrated that renal α_1-adrenoceptor density (B_{max}) and affinity (k_d) are greater in near-term sheep fetuses than in 1-wk-old lambs and older sheep, suggesting that the changing responses to renal nerve stimulation and α_1-adrenoceptor stimulation observed during renal maturation may be due to changes in α_1-adrenoceptors.

In an effort to elucidate further the mechanisms regulating the developmental changes in renal α_1-adrenoceptors, the present study was designed to determine the ontogenic changes in α_{1B}-AR mRNA expression in the sheep, because the α_{1B}-AR is the predominant α_1-adrenoceptor in the sheep renal cortex (8). To achieve this aim, we characterized the expression of renal α_{1B}-AR mRNA at different times during gestation and postnatally and compared these changes with those seen for renal renin gene expression. In addition, we investigated the possibility that changes in baseline renal sympathetic nerve activity that occur during development could influence renal α_{1B}-AR gene expression.

MATERIALS AND METHODS

Animal preparation and surgical procedures. Lambs of Dorset and Suffolk mixed breeding were studied. Gestational ages were based on the induced ovulation technique as described previously (9).

In a first series of studies, designed to characterize ontogenic changes in α_{1B}-AR gene expression, five groups of animals were used. Groups 1, 2, and 3 consisted of fetal sheep between 90 and 91, 116 and 118, and 139 and 141 d gestation, respectively, term being 145 d. Groups 4 and 5 consisted of newborn lambs, between 1 and 2 and 8 and 10 d old, respectively, delivered vaginally. Three animals were studied in each group. The fetuses were anesthetized by administration of a mixture of 1% halothane, 33% O_2, and 66% N_2O to the ewe by endotracheal tube. Newborn lambs were anesthetized with the same mixture administered by endotracheal tube. The fetuses' and lambs' abdominal cavities were opened, and both kidneys were removed, snap-frozen in liquid nitrogen, and stored at −70°C.

In a second series of studies, designed to determine the role of renal nerves on α_{1B}-AR gene expression at the time of birth, two groups of fetuses between 140 and 142 d of gestation were prepared. One group of fetuses (n = 6) had renal denervation, whereas the other group (n = 5) had sham surgery. Anesthesia of the ewe and surgery of the fetus were performed as described previously (10). Briefly, the pregnant ewes were anesthetized using a mixture of 1% halothane, 33% O_2, and 66% N_2O. The uterus was opened near the fetal hindlimbs, and polyethylene catheters, previously impregnated with dimethylpolysiloxane (Acumetric, Elizabethtown, CT) to reduce clotting, were inserted

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in both femoral veins and arteries. Fetal skin incisions were sutured, and an additional catheter was secured to the fetal skin for later measurement of intraamniotic pressure.

Bilateral flank incisions were made, and the renal nerves were severed and stripped bilaterally from along the aorta, renal arteries, and veins and ureters in six fetuses. In these fetuses, this was followed by careful application of 10% phenol in absolute alcohol to the renal plexus and the surrounding area, as previously described (11, 12). Five sham-operated fetuses were submitted to the same surgical procedure, except that the renal nerves were left intact and no phenol solution was applied. The flank incisions were closed, and the fetuses were returned to the uterus.

Uterine and maternal abdominal muscle and skin were closed in separate layers. All catheters were exteriorized through an s.c. tunnel and placed in a cloth pouch on the ewe's flank. At the end of surgery, ampicillin was infused directly into the amniotic cavity (1 g) and administered intramuscularly (1 g) to the ewe.

After surgery, the ewes were kept in a restricted area, fed a standard diet, and allowed a 72-h recovery period. Dexamethasone (5 mg) was given intramuscularly 12 h before starting the experiment to prevent respiratory distress after delivery by cesarean section, as previously described (13). Before delivery, each fetus received a continuous i.v. infusion of 5% dextrose in water at a rate of 0.1 mL/min. After allowing a 60-min equilibration period, fetal hemodynamic monitoring studies were done over 30 min and, during this 30-min period, 5 mL of fetal arterial blood were sampled. To avoid any hemodynamic effect of sampling, fetal blood samples were replaced by infusing an equal amount of maternal blood i.v. into the fetus.

After the fetal monitoring period, the pregnant ewe was brought back to the surgical suite and the fetus was delivered by cesarean section performed under spinal-epidural anesthesia using 10 mL of 1% lidocaine, as described previously (13). Immediately after delivery, the newborn lamb was weighed and placed under a standard overhead infant radiant warmer, to keep body temperature between 38 and 39°C, and dried. Thereafter, the newborn lamb was placed in a specially designed harness to be supported in a standing position. The lamb was then brought to the laboratory, and experiments were resumed 1 h postnatally. During the postnatal period, a continuous infusion of a solution of 10% dextrose, 0.2% NaCl, and 0.03 M KCl was administered i.v. at a rate of 100 mL/kg/24 h. Arterial blood pressure and heart rate were monitored continuously during the first 24 h of life. Five mL of arterial blood were collected at 1, 4, 8, and 24 h after birth. Newborn blood samples were replaced with blood collected from the fetal side of the placental circulation after the umbilical cord was cut at the time of cesarian section, as described previously (13).

After 24 h of postnatal life, newborn lambs were anesthetized with a mixture of 1% halothane, 33% O_2, and 66% N_2O administered by endotracheal tube. The lambs' abdominal cavities were opened, and both the kidneys were removed, snap-frozen in liquid nitrogen, and stored at −70°C. All procedures were approved by the University of Iowa Committee for the Care and Use of Animals.

Analytical procedures. Arterial blood for pH, P_{CO_2}, and P_{O_2} was collected anaerobically in heparinized syringes, and measurements were made immediately with appropriate pH, P_{CO_2}, and P_{O_2} electrodes at 39°C using an IL-1303 pH blood gas analyzer (Laboratory Instruments, Schaumburg, IL). Plasma Na^+ and K^+ concentrations were determined by flame photometry (model 430, Corning, Medfield, MA). Hematocrit was determined in duplicate using a micrometer caliper. Plasma and kidney tissue catecholamine levels were assayed by HPLC with electrochemical detection (Bioanalytical Systems, West Lafayette, IN).

Isolation of RNA and preparation of probes. Total renal cortical cellular RNA was isolated using a modified isothiocyanate-CsCl method as previously described (14). RNA was quantified spectrophotometrically by absorbance at 260 nm. RNA samples were stored as an ethanol precipitate at −70°C until further analysis.

The α_{1B}-AR gene from the rat brain has recently been sequenced and cloned (15). A full-length α_{1B}-AR cDNA was kindly provided by Dr. H. Chin (National Institutes of Health), and from this, a 458-bp *DraI/PstI* 5′ fragment was subcloned into a pGEM-7Zf(+) vector (Promega, Madison, WI). The sequence and orientation of the subcloned fragment were confirmed by the dideoxynucleoside sequencing technique of Sanger (16). This subcloned fragment corresponds to the amino terminus and first three of the seven membrane-spanning portions of the α_{1B}-AR and yields antisense RNA when transcribed from the T7 promoter after linearization with the restriction endonuclease *MluI*.

For comparison, we made a probe for renin mRNA, as plasma renin activity is known to be developmentally regulated. A ^{32}P-uridine triphosphate–labeled renin probe was constructed from a 0.7-kb subcloned fragment of a full-length 1.43-kb rat renin cDNA kindly provided by Dr. K. R. Lynch of the University of Virginia. This cDNA yields antisense RNA when transcribed from the T7 promoter after linearization with *EcoRI*. A human fetal liver GAPD cDNA was obtained from the American Type Culture Collection (ATCC no. 57090) (17). After digestion with *PstI* and *XbaI*, a 780-bp fragment was gel-purified for use as a DNA probe. This GAPD fragment contains sequences encoding the first 250 amino acids as well as a portion of the 5′ noncoding region of the gene.

Radiolabeled RNA probes were generated by the method of Melton *et al.* (18) using [α-^{32}P]uridine triphosphate (Amersham, Arlington Heights, IL). Radiolabeled DNA probes were generated by the random primer extension method (19) using [α-^{32}P] deoxycytidine triphosphate (Amersham).

Northern blot hybridization. Aliquots of 5 μg of RNA, as measured by absorbance at 260 nm, were fractionated by 1% formaldehyde agarose gel electrophoresis (20). After electrophoresis, RNA was transferred to a 0.45-mm Nytran filter (Schleicher and Schuell, Inc., Keene, NH). Hybridization conditions were as previously published (21). Wash temperatures ranged between 65 and 74°C. DNA probes were hybridized overnight in 10% dextran sulfate; 1 M NaCl; 1% SDS; 50 mM Tris, pH 7.5; and 200 mg/L sheared salmon-sperm DNA. Filters were sequentially washed in 2 × SSC at room temperature; 2 × SSC, 1% SDS at 65°C; and 0.1 × SSC at room temperature. (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate.) The washed filters exposed Kodak XAR film at −70°C. The autoradiographs were quantified using a Soft Laser Scanning densitometer, model SLA-2D10 (Biomed Instruments, Inc., Fullerton, CA).

In the second series of studies, filters used for the determination of α_{1B}-AR mRNA expression were also used to probe for renin and GAPD mRNA expression. Before rehybridizing with a second RNA probe, the first RNA probe was stripped from the filters by washing in a solution of 50% formamide and 6 × sodium chloride, sodium phosphate, EDTA at 70°C for 30 min and by rinsing in 2 × sodium chloride, sodium phosphate, EDTA at 24°C.

Radioligand binding studies. The effect of renal denervation on renal α_{1B}-AR density and affinity was studied using radioligand binding. Kidneys used for these studies were immediately frozen at −70° in sucrose buffer (0.25 M sucrose, 10 mM $MgCl_2$, 5 mM Tris·HCl, pH 7.5) after being harvested. Previous studies in our laboratory have shown that receptor density and affinity are not affected by freezing (8, 22).

Renal cortical membranes were prepared by differential centrifugation as previously described (8, 23). Briefly, after isolating cortical tissue, it was weighed, minced to a fine paste, and homogenized in 20 vol of sucrose buffer. Thereafter, the homogenate was centrifuged at 500 × *g* for 15 min and the supernatant at 30 000 × *g* for 25 min. The resulting pellet was resuspended, washed once in incubation buffer (50 mM Tris·HCl, pH 7.4), recentrifuged, and then resuspended to a protein concentration of 1 to 2 g/L in incubation buffer.

Radioligand binding studies were performed in triplicate as previously described (8, 23) using [^{3}H]prazosin (an α_1-adrenergic antagonist) as the radioligand. Specific binding was defined as the difference between the amount of radioligand bound in the absence (total binding) and the presence (nonspecific binding) of 10 μM phentolamine. Both the k_d and the B_{max} were determined by incubating the renal cortical membranes with 11 different concentrations of [^{3}H]prazosin (0.001 to 1.5 mM). The k_d and B_{max} were calculated from plots of the data according to Scatchard, analyzed by nonlinear regression using the Lundon-1 program (Lundon Software, Cleveland, OH). Protein concentrations of the renal cortical homogenates were measured by the method of Lowry *et al.* (24).

Data analysis. For quantitation of mRNA abundance, all samples were analyzed together on a single Northern blot hybridization to control for day-to-day variation in hybridization efficiency. Northern blots were done in duplicate. Abundance of the specific mRNA is expressed in densitometric units.

Comparisons among the groups were performed by nonpaired *t* tests. Statistical significance is defined as $p < 0.05$, and the results are presented as mean ± SEM.

RESULTS

Expression of renal α_{1B}-AR and renin mRNA during development. Changes in renal α_{1B}-mRNA levels during fetal and postnatal development are shown in Figure 1. The expression of α_{1B}-AR mRNA is low during early fetal life (groups 1 and 2) and increases in near-term fetuses (group 3). The increase is present immediately after birth (group 4), and α_{1B}-AR mRNA levels remain elevated 1 wk postnatally (group 5). Densitometric analysis of the different autoradiograms shows that α_{1B}-AR mRNA levels, expressed in densitometric units, are 370 ± 57 in group 1, 293 ± 22 in group 2, 975 ± 241 in group 3, 966 ± 273 in group 4, and 668 ± 132 in group 5.

Contrary to changes in α_{1B}-AR mRNA expression, renin mRNA levels do not increase significantly before birth (Fig. 2). An important rise in renin mRNA levels is observed after birth with levels decreasing significantly after 1 wk of life. The renin mRNA levels are 236.3 ± 67.3 in group 1, 288.0 ± 69.7 in group 2, 334.3 ± 49.2 in group 3, 1040.7 ± 150.8 in group 4, and 309.3 ± 79.5 in group 5.

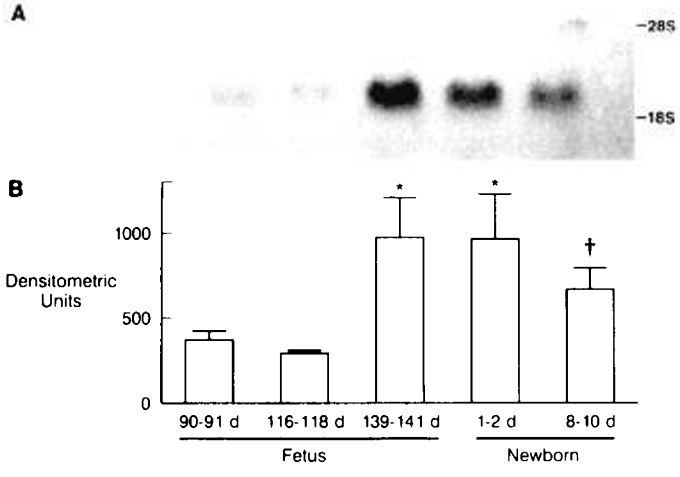

Fig. 1. *A*, Representative autoradiogram of a Northern blot of total renal cortical RNA hybridized with a rat α_{1B}-AR RNA probe labeled with ^{32}P. Total RNA was extracted from fetal and newborn sheep, one from each of the five age groups. From left to right, results from 90 to 91 d gestation (group 1), 116 to 118 d gestation (group 2), 139 to 141 d gestation (group 3), 1- to 2-d-old (group 4), and 8- to 10-d old newborn lambs (group 5) are presented. *B*, Densitometric analysis of the expression of renal cortical α_{1B}-AR mRNA in the five age groups; $n = 3$ in each group. Values are mean ± SEM.

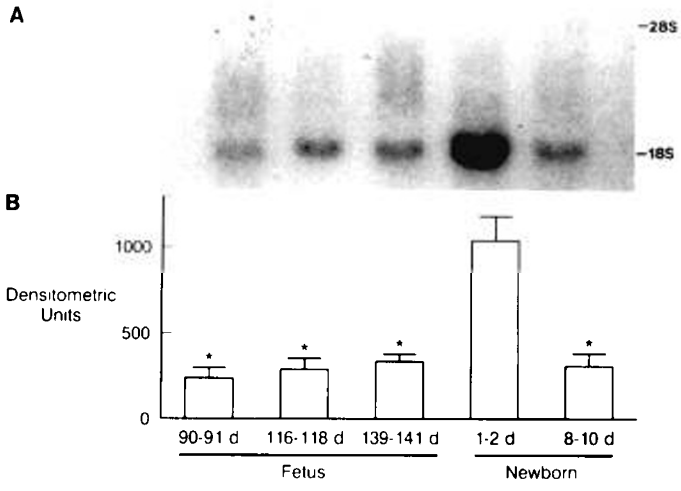

Fig. 2. *A*, Representative autoradiogram of a Northern blot of total renal cortical RNA hybridized with a rat renin probe labeled with ^{32}P (ages left to right are the same as in Fig. 1), one from each of the five age groups. *B*, Densitometric analysis of the expression of renal cortical renin mRNA in the five age groups; $n = 3$ in each group. Values are mean ± SEM.

Effects of renal denervation on expression of α_{1B}-AR mRNA during transition from fetal to newborn life. To determine whether an increase in renal sympathetic nerve activity at the time of birth could attenuate the rise in α_{1B}-AR mRNA levels at birth, we studied the effect of fetal renal denervation on both α_{1B}-AR mRNA levels and α_1-adrenoceptor density and affinity in 24-h-old newborn lambs after cesarean delivery.

Renal denervation has no significant effect on mean arterial pressure, heart rate, arterial pH, P_{O_2}, plasma electrolyte concentrations, or hematocrit at 24 h of age (Table 1). A significant but small rise in P_{CO_2} is seen in denervated newborn lambs (Table 1). We have previously shown that renal denervation produces a significant decline in renal norepinephrine content (21), a trend seen in this study as well; renal denervation produces a significant decline ($p < 0.005$) in renal norepinephrine content in those animals we assayed. Innervated animals ($n = 3$) have 510 ± 191 pmol/g of kidney-weight renal norepinephrine content, whereas denervated animals ($n = 6$) have 21.7 ± 19.7 pmol/g of kidney weight. The effect of renal denervation on the expression of α_{1B}-AR and renin mRNA is presented in Figures 3 and 4. Renal denervation has no significant effect on α_{1B}-AR gene expression. (Fig. 3) but significantly decreases renin mRNA levels (Fig. 4). Densitometric analysis of the autoradiograms shows that mean α_{1B}-AR mRNA levels are 978 ± 155 in intact kidneys and 1018 ± 103 in denervated kidneys, whereas mean renin mRNA levels are 1269 ± 108 and 954 ± 84, respectively. No significant changes in α_{1B}-AR density or affinity are observed between innervated ($k_d = 0.64 \pm 0.24$, $B_{max} = 21.0 \pm 4.42$) and denervated animals ($k_d = 1.07 \pm 0.20$, $B_{max} = 33.5 \pm 13.5$).

To confirm that renal denervation does not affect α_{1B}-AR gene expression but decreases renin mRNA levels, the same filters were rehybridized with a probe for GAPD mRNA. No changes in GAPD gene expression are observed between innervated and denervated animals. Densitometric analysis of the autoradiograms shows that mean GAPD mRNA levels are 242 ± 31 in innervated kidneys and 236 ± 11 in denervated kidneys. The ratio of α_{1B}-AR to GAPD mRNA levels are similar when innervated (4.58 ± 1.4) and denervated kidneys (4.43 ± 0.59) are compared. However, the ratio of renin to GAPD mRNA levels differ significantly between innervated (5.7 ± 0.9) and denervated (4.1 ± 0.2) kidneys.

DISCUSSION

The present results demonstrate that the expression of renal α_{1B}-AR mRNA is developmentally regulated in sheep. There is

Table 1. *Arterial blood values, arterial blood pressure, and heart rate in innervated and denervated newborn lambs**

	pH	P_{CO_2} (kPa)	P_{O_2} (kPa)	Na (mmol/L)	K (mmol/L)	Hct	MABP (mm Hg)	HR (bpm)
Innervated	7.392 ± 0.022	5.25 ± 0.19	11.0 ± 1.32	147 ± 0.88	4.6 ± 0.19	0.372 ± 0.026	65 ± 3.4	135 ± 7.4
n	5	5	5	3	3	5	5	5
Denervated	7.364 ± 0.014	6.13 ± 0.35†	9.70 ± 1.2	149 ± 1.2	4.7 ± 0.08	0.387 ± 0.021	60 ± 2.6	132 ± 10
n	6	6	6	6	6	6	6	6

* Hct, hematocrit; MABP, mean arterial blood pressure; HR, heart rate; *n* = number of animals. Values are mean ± SEM.
† $p < 0.05$ compared with innervated lambs.

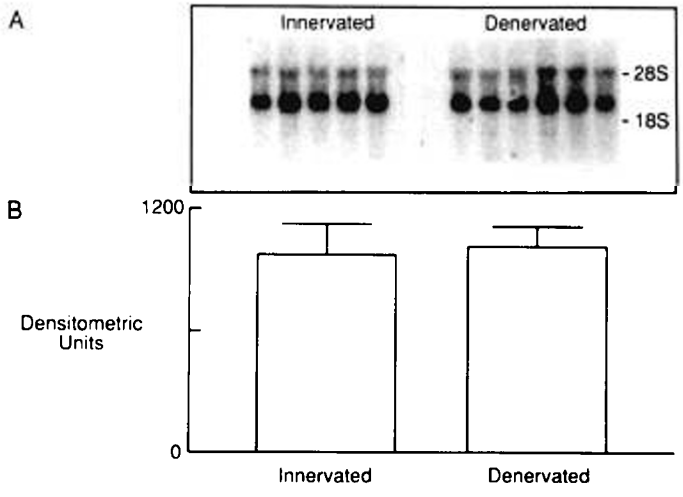

Fig. 3. *A*, Autoradiogram of a Northern blot of total renal cortical RNA hybridized with a rat α_{1B}-AR RNA labeled with ^{32}P. Total renal cortical RNA was extracted from denervated ($n = 6$) and intact ($n = 5$) kidneys of newborn lambs at 24 h of age. *B*, Densitometric analysis of the autoradiogram. Values are mean ± SEM.

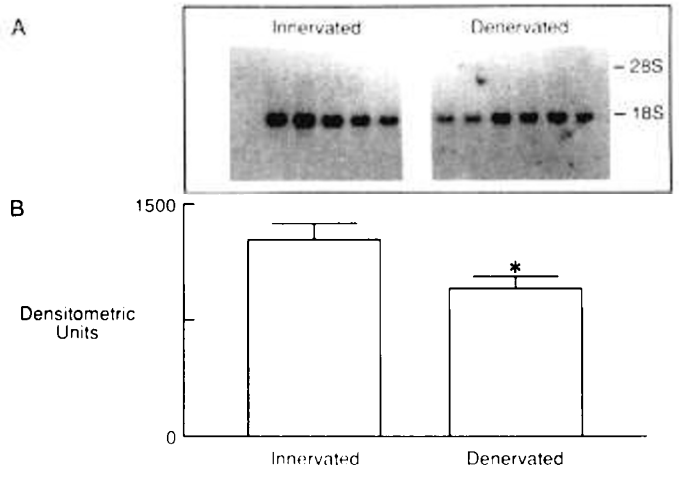

Fig. 4. *A*, Autoradiogram of a Northern blot of total renal cortical RNA hybridized with a rat renin probe labeled with ^{32}P. Total renal cortical RNA was extracted from denervated ($n = 6$) and intact ($n = 5$) kidneys from newborn lambs at 24 h of age. *B*, Densitometric analysis of the autoradiogram. Values are mean ± SEM.

little change in α_{1B}-AR mRNA levels between the second and third trimesters of gestation but a dramatic increase is seen in term fetuses. This increase tends to persist during the first week after birth. In contrast to this trend for the α_{1B}-AR, the expression of renal renin mRNA, which has been shown to be developmentally regulated (25, 26), follows a different pattern in the sheep, with a greater increase immediately after birth, suggesting that α_{1B}-AR and renin genes are regulated in an independent manner. In the rat, increased renal renin mRNA levels are seen in the late gestation fetus (25). The different pattern we have found in the sheep fetus may be explained on the basis of species differences; the late-gestation sheep fetus has completed nephrogenesis, whereas the late gestation rat has not. It is therefore not surprising that developmental patterns of mRNA expression might differ.

It is likely that multiple factors are involved in the regulation of these two genes; clearly, the renal nerves regulate renal renin mRNA expression during parturition (21). Such a relationship between renal sympathetic nerve activity and α_{1B}-AR mRNA expression is not obvious from our data. Down-regulation of adrenoceptor density and affinity is known to occur after exposure to catecholamines and up-regulation after withdrawal from catecholamines (27–32). Postnatal decreases in α_1-adrenoceptor density in the lung involve homologous (*i.e.* catecholamine-induced) down-regulation coincident with increased catecholamine release to postsynaptic sites (29).

It is possible, however, that factors independent of renal sympathetic nerve activity and circulating catecholamines modulate the rise in α_{1B}-AR gene expression around the time of birth. One may speculate that such factors could continue to be functional postnatally and attenuate potential down-regulation of the α_{1B}-AR gene that would occur through postnatal increases in circulating catecholamines and renal sympathetic nerve activity. Glucocorticoids could be such factors increasing α_{1B}-AR mRNA levels in the term fetus and maintaining high levels of expression of the α_{1B}-AR gene after birth. Glucocorticoid levels are known to increase in the several d before birth and they decline rapidly within a d after birth (33). In support of this hypothesis, it has been suggested that a glucocorticoid regulatory element may be present in the promoter region of the α_{1B}-AR gene (34). Glucocorticoids are known to up-regulate adrenergic receptor gene expression (35). Administration of dexamethasone increases α_{1B}-AR gene expression in cultured hamster smooth-muscle cells (34). The surge in cortisol that occurs in the near-term fetus roughly coincides with the observed peak in α_{1B}-AR mRNA levels and could well be the factor inciting gene up-regulation.

We postulated that the increase in renal sympathetic nerve activity that occurs at birth dampens the rise in expression of α_{1B}-AR mRNA that has begun shortly before birth. To investigate this possibility, we measured levels of renal α_{1B}-AR density, affinity, and mRNA levels in 24-h-old lambs that had undergone renal denervation before birth. Renal denervation had no effect on either α_{1B}-AR density, affinity, or mRNA levels. In contrast to the situation for the α_{1B}-AR gene, renal denervation produced a decrease in renin mRNA levels. Therefore, the influence of renal sympathetic nerve activity in regulating the α_{1B}-AR at the time of birth seems to be minimal and may be obscured by stronger influences from humoral factors such as glucocorticoids. The single prenatal dose of dexamethasone given in this series of animals is unlikely to have influenced the effect of denervation or the pattern of gene expression mediated by endogenous glucocorticoids. Regulation of the renal α_{1B}-AR gene is clearly different from that which is seen with the renal renin gene; in the case of the latter, the regulatory influence from the nerves is obvious.

In summary, the term sheep fetus shows an increase in renal α_{1B}-AR mRNA levels when compared with earlier gestational

ages. This increase persists into the first 2 d of life, abates by 1 wk, and is distinct from the increase seen in renal renin mRNA levels in sheep, which does not occur until after birth. Renal sympathetic nerve activity, which is an important regulator of renal renin mRNA levels, does not appear to play as important a role in the regulation of the renal α_{1B}-AR gene at the time of birth.

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