Maturational differences between vascular and bladder smooth muscle during ovine development

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Arens, Yvonne H. J. M., Charles R. Rosenfeld, and Kristine E. Kamm. Maturational differences between vascular and bladder smooth muscle during ovine development. Am J Physiol Regulatory Integrative Comp Physiol 278: R1305–R1313, 2000.—Maturation rates of vascular and visceral smooth muscle (SM) during ovine development were compared by quantifying contractile protein, myosin heavy chain (MHC) isoform contents, and contractile properties of aortas and bladders from female fetal (n = 19) and postnatal (n = 21) sheep. Actin, myosin, and protein contents rose progressively throughout development in both tissues (P < 0.003); however, expression patterns differed. During the last trimester, i.e., 101–145 days (term = 145 days), bladder actin and MHC contents were approximately twofold greater (P < 0.04) than those in the aorta. Although the fractional content of 204-kDa SM1 MHC in the bladder decreased from 74 ± 3% at midgestation to 48 ± 2% 3 mo postnatal, the aorta exhibited an increase from 30 ± 2% to 65 ± 2%. Bladder MHC (MHC-B) migrating at 200 kDa contained only SM2 throughout development. In contrast, 200-kDa MHC in the aorta was predominantly nonmuscle MHC-B at midgestation, which was gradually replaced by SM2 as development progressed. Along with its early expression of SM2, bladder muscle obtained maximal stress generating capacity (1.7 × 10⁵ N/m²) by term gestation, whereas the aorta exhibited no contractions until after birth. We conclude that whereas aortic SM maturation is delayed until after birth, bladder SM matures biochemically and functionally during prenatal development, thus supporting early requirements for micturition.

actin; myosin; aorta

RAPID GROWTH AND MATURATION of the smooth muscle (SM)—containing organ systems are hallmarks of fetal development. Internal organs are formed in the mid to late stages of embryogenesis with differentiation-specific SM protein markers seen first in the vascular outflow tract, followed by the bronchial buds, gut, peripheral vasculature, and bladder (27). Subsequent increases in contractile protein content, as well as muscle-specific composition, during fetal and postnatal development should accompany the need for increasing contractile capacity during maturation brought about by growing demands for organ function. In support of this, we found that, within the vasculature of near-term fetal sheep, umbilical arteries were both biochemically and functionally comparable to systemic vessels from adults, whereas fetal systemic vessels exhibited an immature phenotype (4) consistent with the relatively minor contribution of the systemic circulation compared with the umbilical circulation to alterations in fetal vascular resistance (12, 20). Likewise, in a preliminary study comparing protein composition of visceral and vascular SMs from late fetal gestation and neonatal male sheep (11), we noted that the bladder expressed only SM isoforms of myosin heavy chain (MHC), whereas the aorta expressed both smooth and nonmuscle isoforms, suggesting that among organ systems there may also be differential rates of SM maturation.

SM differentiation and maturation are distinguished by the gradual and sequential induction of tissue-specific protein markers (30). Smooth muscle myosin heavy chain (SM-MHC) is a key marker of the differentiated phenotype in that it appears exclusively in the SM cell lineage during development (27). Mature, “contractile” SMs express SM and, to a minor extent, nonmuscle isoforms of MHC (30, 36). The four SM-MHC isoforms arise from a single gene through alternative splicing (22). SM1 (204 kDa) and SM2 (200 kDa) differ in their carboxy terminal tail region, and additional splice variants of these forms contain a small insert in the amino terminal head region. Nonmuscle MHC isoforms MHC-A (196 kDa) and MHC-B (200 kDa) are the products of two distinct genes (35). They are expressed in all cell types and implicated in actin-based motile functions such as cytokinesis and cellular locomotion (40). Developmental patterns of MHC isoform expression in SM are largely understood from studies of blood vessels and exemplified by the presence of SM1 and nonmuscle MHC in the late gestation fetus and neonate, with a gradual onset of SM2 expression after birth accompanied by reciprocal declines in nonmuscle MHC (30). Expression patterns of MHC markers are evaluated as qualitative assessments of differentiation; however, functionally consequential quantitative increases in content of these with maturation have received limited attention.

In the present study, we further investigate the proposal that SM maturation parallels requirements for organ function. Amounts and patterns of actin and MHC isoform expression as well as stress-generating capacity were compared between the bladder, a visceral tissue known to be active in fetal sheep (37, 39), and the aorta, a vascular SM tissue extensively described in
other species (1, 9, 14–16, 24) and known to be less reactive in fetal than adult sheep (4, 20). The sheep offers certain advantages as a model for studying aspects of fetal physiology, owing to the experimental ability to modify the hormonal milieu while studying physiological responses in vivo (19, 29, 41). Moreover, significant quantities of SM tissues can be obtained from fetal and neonatal sheep to perform protein analysis and contractile measures, thus providing a model system from fetal and neonatal sheep to perform protein analysis.

METHODS

Tissue preparation. Female fetal (72-140 days of gestation, n = 19; term ~145 days) and postnatal (1–120 days, n = 25) sheep were euthanized by a bolus infusion of pentobarbital sodium via the external jugular vein (50 mg/kg) of the mother in the case of the fetus or the newborn. Segments of the abdominal aorta and the entire bladder were quickly removed and placed into iced PBS (in mM: 137.0 NaCl, 2.7 KCl, 10 Na2HPO4, and 1.76 KH2PO4, 0.1% diethyl pyrocarbonate, pH 7.4), which was bubbled with oxygen. Tissues for contraction measures were transported within 30 min to another laboratory and transferred to oxygenated physiological salt solution (PSS) (3) for dissection as described below. Endothelium and adventitia were removed from the aorta with a soft cotton swab and blunt dissection, respectively, and the epithelium was removed from the bladder by sharp dissection. Strips of tissue were cut, blotted dry to remove excess fluid and capillary blood, frozen in liquid nitrogen, and stored at −60°C until studied. Some strips were reserved unfrozen for measurements of isometric force. Protocols were approved by the Institutional Animal Care Research Advisory Committee.

Protein analysis and content. Samples of frozen tissue (10–20 mg) were homogenized in 40 volumes of SDS buffer containing 2% SDS, 20% sucrose, and 0.4 M Tris (pH 6.8) (3, 11). The homogenates were divided into two aliquots. The first was used to determine the total homogenate protein content. The second was subjected to centrifugation at 10,000 g for 2 min, and the supernatant was removed to determine the soluble or cellular protein in each sample. Densitometric analysis of the supernatant and pellet fractions subjected to SDS-PAGE showed that >90% of myosin and >85% of actin were recovered in the supernatant. Aliquots were analyzed for protein content by bichinonic acid reagent (Pierce). Added to the remaining homogenate and supernatant samples were 2-mercaptoethanol and bromophenol blue to achieve final concentrations of 5% and 0.04%, respectively. Samples of soluble protein (20–40 μg on the basis of loading curves determined for each tissue type (3)) were then subjected to PAGE in 3–20% and 4% polyacrylamide gels to determine the contents of actin and MHC and the relative amounts of MHC isoforms, respectively. Gels contained molecular mass standards used to confirm relative mobility (Bio-Rad Laboratories). Positive identification of the positions of MHC and actin bands was confirmed by Western blotting with antibodies generated in this laboratory (SM-MHC) and purchased from Sigma (α-actin). The fractions of Coomassie blue-stained protein accounted for by actin and MHC in 3–20% gels and MHC isoforms in 4% gels were estimated by scanning laser densitometry to obtain a profile of peaks followed by area integration of the absorbency signal for each peak (model 2202/2220, LKB Instruments). Lanes were scanned in duplicate. The fraction of protein accounted for by actin and MHC was converted to micrograms on the basis of known amounts of protein loaded. Values are expressed as micrograms per milligram wet weight.

Western blot analysis. Tissue extracts were subjected to electrophoresis in 4% PAGE, and proteins were electrophoretically transferred to nitrocellulose paper at 80 mA overnight. Blots were incubated overnight with antisera. Antisera to SM2 (1:4,000) or MHC-B (1:20,000) were raised in this laboratory against synthetic peptides specific to each form and characterized as described previously (11). Antisera against MHC-A (1:20,000) were generously provided by Drs. N. Murakami and J. R. Sellers. Purified bovine brain MHC-B was the gift of Dr. Barbara Barylko. Antibodies to myosin light chain kinase (MLCK) (1:3,000) were raised in this laboratory against purified bovine tracheal SM MLCK. After 2 h incubation with goat anti-rabbit IgG conjugated with horseradish peroxidase (1:15,000), immunoreactive protein was visualized by chemiluminescence (ECL Amersham).

Contractile measurements. Muscle strips (1.4-mm wide) from the aorta and bladder were prepared using a double-bladed cutting tool (3, 18). Strips of aorta were open rings with the adventitia removed by gently rolling a moist cotton swab over the luminal surface. Bladder strips were cut from muscle in the longitudinal orientation and dissected clean as described above. Strips were mounted in organ baths for measurement of isometric force no more than 2 h after being removed from the animals. Baths contained oxygenated (95% O2, 5% CO2) PSS (in mM: 120.5 NaCl, 4.8 KCl, 1.2 MgCl2, 1.6 CaCl2, 1.2 NaH2PO4, 20.4 NaHCO3, 10 dextrose, and 1 pyruvate, pH 7.4, 37°C) (3). Length-force relations were determined for each tissue type at each gestational age. Stresses in response to phenylephrine (10-6 M) or PSS containing 65 mM KCl (replacing NaCl) were determined in strips stretched to the optimal length (l0) for maximal force. Tissue cross-sectional area was calculated based on weight, density, and length of the tissue at l0. Stress (N/m2) was calculated by dividing active force at l0 by the cross-sectional area (2, 17).

Myosin light chain phosphorylation. The ability of contractile proteins to be activated by myosin light chain phosphorylation was assessed by comparing values in strips relaxed in calcium-free buffer with those maximally activated. Strips of aorta and bladder mounted for measurement of isometric force were quick frozen with tongs precooled in liquid nitrogen either relaxed or at the time of maximum contraction, using 10-3 M phenylphorine for the aorta or 10-3 M carbocoll for the bladder. The frozen muscle was weighed, placed in a frozen slurry of TCA (10% wt/vol) in acetone containing 1,4-dithiothreitol (DTT) (10 mM), and allowed to thaw. After 10 min of incubation, the liquid was decanted and the strip was homogenized in 60 volumes of TCA (10% wt/vol) and DTT (10 mM). Precipitated protein was washed with diethyl ether and then suspended in urea-glycerol buffer for electrophoretic analysis of regulatory light chain (RLC) phosphorylation by immunoblotting with antisera raised against light chain purified from bovine tracheal SM (31). Separated proteins were transferred to nitrocellulose paper, and light chain was detected by immunoblotting with specific antibodies using the ECL detection system (Amersham). Relative amounts of nonphosphorylated and phosphorylated light chain were quantified by scanning laser densitometry.

Statistics. Data were analyzed using polynomial regression analysis to obtain estimates for significance of fit to functions, indicating a non-zero slope (Sigma Stat 1.0). The variables used were protein contents (y-axis) versus gestational age in days.
**RESULTS**

Protein contents. In both the aorta and the bladder, total and soluble protein contents increased during development. Regression analysis in aortic SM demonstrated gradual increases from <100 days gestation to ≥3 mo after birth ($r = 0.40$ and $0.48$; $P = 0.007$ and $0.001$ respectively; first order). Because of the gradual nature of these increases, significant differences between age groups were detected only at 3 mo after birth (Table 1). In contrast, bladder SM total and soluble protein increased around the time of birth, as evidenced by highly significant polynomial regressions ($r = 0.62$ and $0.83$; $P < 0.001$ each; second order), and pronounced differences occurring after 130 days gestation (total) or 7 days postnatal (soluble; Table 1). Additional but modest increases in soluble protein occurred after week 1 postnatal (Table 1).

Differences between tissues were also observed. Before 100 days gestation, aortic total protein exceeded that of the bladder ($P < 0.02$, ANOVA), whereas after that age, values did not differ. In contrast, soluble protein contents were similar at <100 days gestation, but after that, bladder contents always exceeded the aorta ($P < 0.03$, ANOVA).

Actin and MHC contents in the aortic SM also increased during development (Fig. 1). Actin contents rose abruptly after 100 days gestation (Fig. 1), increasing ~75%, and then rose gradually, resulting in a nearly fourfold increase by 3 mo postnatal. In contrast, MHC contents rose gradually, levels doubling during development. The pattern in bladder...
SM was quite different. Actin contents rose rapidly in the last third of gestation, increasing about fourfold by term, and remained stable thereafter (Fig. 1). The rise in MHC contents paralleled that of actin, increasing twofold by term and 3.3-fold by 30 days postnatal.

The most striking differences between tissues were seen for MHC contents. At <100 days gestation, the aorta MHC contents were similar to the bladder. Beyond 100 days gestation, values in the bladder were approximately twofold greater (P < 0.004) than those in the aorta. This difference continued throughout the remainder of the development. Differences between tissues for actin contents were more variable. Nonetheless, between 131 days gestation and 21 days postnatal, bladder actin contents exceeded those in the aorta (P < 0.04).

MHC isoforms. MHCs separated by electrophoresis in gels containing 4% polyacrylamide yielded two protein species at relative mobilities 204 and 200 kDa. No species of lower relative mass (196 kDa) were observed for any tissue studied. The relative amounts of the 204 and 200 kDa bands were quantified by densitometry and expressed as percent of total. Changes in species content during development were determined as the product of the measured total myosin content and the percent mass of each species. Subsequently, identification of MHC isoforms within the 200-kDa species was performed by Western analysis.

The predominant MHC species in the aortas from midgestation fetal sheep (<100 days) was the 200-kDa form that composed 70 ± 3% of the total MHC (Fig. 2). The percentage of 200-kDa species declined rapidly thereafter (r = 0.61, P < 0.001; second order) to 35 ± 2% at >3 mo. The relative amount of the 204-kDa isoform rose reciprocally (r = 0.64, P < 0.001; second order) over the period studied from 30 ± 2% to 65 ± 2%, respectively. Significant differences in the amount of each species were detected between fetuses at <100 days gestation and animals >1 wk postnatal (P < 0.001, ANOVA). In striking contrast to the aorta, the predominant MHC species in the bladder muscle from midgestation fetuses was the 204-kDa form that composed 74 ± 3% of total MHC (Fig. 2). This gradually declined (r = 0.84, P < 0.001; first order) to 48 ± 2% at

![Fig. 2. Relative and absolute changes in 204-kDa and 200-kDa MHC isoforms in aorta (A) and bladder (B) during ovine development. A and B: values were derived by calculating percent of total staining MHC (204 + 200 kDa) comprised of each form. Polynomial regression curves are shown as solid lines through data to distinguish 2 groups. C-F: absolute changes in contents of 204-kDa and 200-kDa MHC isoforms (µg/mg wet wt) in aorta (C and E) and bladder (D and F) during ovine development. Graph properties as described in Fig. 1. Significant polynomial regression analyses for 204-kDa protein were obtained for aorta (r = 0.68, P < 0.001; first order) and bladder (r = 0.58, P < 0.001; second order) as well as for 200-kDa protein in bladder (r = 0.65, P < 0.001; first order). The 200-kDa isoform content of aorta was unchanged, averaging 1.4 ± 0.1 µg/mg wet wt.](http://ajpregu.physiology.org/10.1152/ajpregu.00301.2004)
3 mo, whereas the 200-kDa species increased ($r = 0.84, P < 0.001; \text{first order}$) from 26 ± 3% to 51 ± 2%.

Values at <100 days gestation significantly differed ($P < 0.003, \text{ANOVA}$) from those at >21 days postnatal. Calculation of the tissue content of these MHC species (µg/mg wet wt) resulted in a pattern distinct from that seen for the relative amounts (Fig. 2). In the aorta, the 204-kDa isoform content increased 4.3-fold by 3 mo postnatal ($P < 0.01, \text{ANOVA}$). The 200-kDa isoform content, however, was unchanged throughout development (Fig. 2). In contrast, amounts of both 204- and 200-kDa species in the bladder increased rapidly and in parallel with increases of 2.7- and 6-fold, respectively ($P < 0.02$ and $< 0.002, \text{ANOVA}$).

The 200-kDa MHC species may contain SM2 and/or MHC-B. The developmental dependence of expression of each was determined by immunoblotting with antibodies specific for SM2 and MHC-B (7). This analysis was used to qualitatively assess patterns of isoform expression (Fig. 3). Each blot was loaded with three standards: sheep platelet lysates (Pit), MHC-B purified from bovine brain (BB), and adult myometrial homogenate (SM2). Although we never detected a protein species at 196 kDa, it is possible that ovine MHC-A may migrate at a different position. We were not able to rule out the presence of MHC-A in these samples because antibodies against human platelet MHC-A peptide did not cross-react with sheep platelet protein, and polyclonal antibodies against human platelet MHC-A showed cross-reactivity with MHC-B. The specificities of anti-MHC-B and anti-SM2 are illustrated on immunoblots in Fig. 3. Although there was no change in the aortic total 200-kDa MHC content during development (Fig. 2), the isoform distribution changed dramatically (Fig. 3). MHC-B was abundant prenatally and declined rapidly after birth, whereas SM2 was barely detected at early time points prenatally and increased most dramatically after birth. In striking contrast to the aorta, the bladder muscle showed no detectable expression of MHC-B, even at midgestation, whereas SM2 was abundant throughout development and increased before and after birth (Fig. 3).

Isometric force development. We compared contractile responses of the aorta and the bladder SM strips from 100 days gestation and near-term fetal sheep with 2- to 4-wk neonates and adults. Strips of the fetal aorta showed no contractile response (Fig. 4) to any agent.
tested (KCl, phenylephrine, histamine, serotonin, PGF$_{2\alpha}$, ATP, carbachol, ANG II, or endothelin), whereas strips of the bladder muscle gave robust contractions with KCl depolarization and carbachol. All strips of the adult aorta and bladder contracted in response to KCl and other agents. Maximal active stress was determined from the results of the length-force relations. Strips were stretched in calcium-containing PSS, stimulated with 65 mM KCl PSS, then relaxed in calcium-free PSS containing 2 mM EGTA. Active force was calculated as the difference between peak force and the resting (passive) force after relaxation in calcium-free PSS. Bladder muscle exhibited significant tone after stretch, which was removed after incubation in calcium-free PSS (Fig. 4). Values of maximal active stress are shown in Fig. 5. Contraction responses in 100 days and term fetal aortic strips were markedly attenuated compared with vessels from neonates and adults. Although the responses of the aortas from fetal animals were unmeasurable, aortic stress increased to near-adult values soon after birth. In contrast, the bladder muscle generated significant stress at 100 days gestation, and values increased sevenfold during the late fetal period (Fig. 5). This was followed by a progressive decline postnatally.

Myosin light chain phosphorylation. To determine whether the failure of aortic SM to contract in the prenatal period arises from a deficit in the excitation process, we measured RLC phosphorylation in response to high concentration of agonist as an index of activation. In the passive state achieved with calcium-free conditions, no phosphorylation was observed in the aorta or bladder strips collected at any age. Consistent with the results of contraction experiments, all muscles that contracted exhibited ~0.5 mol phosphate/mol RLC at the time of maximal stress development in response to high concentrations of agonist (Fig. 6). These included neonatal and adult aortas stimulated with 10$^{-3}$ M phenylephrine as well as the bladder from any developmental stage stimulated with 10$^{-3}$ M carbachol.

In the fetal aortic SM strips, however, no RLC phosphorylation was observed in response to 10$^{-3}$ M phenylephrine, consistent with a failure to contract.

To test the possibility that a deficiency in MLCK may account for a lack of phosphorylation, we performed immunoblotting analysis on tissues. Aortic and bladder SMs expressed MLCK throughout development. Relative amounts of MLCK were determined by densitometry followed by normalization to adult values as 100%. MLCK increased in the aorta from 79 ± 10% at 100 days gestation, peaked at 2 wk postnatal (136 ± 13%), and then declined to 100% in adult (P = 0.04, ANOVA). In contrast, no significant differences in MLCK amount.
were found with ANOVA among bladder tissues from the different developmental stages.

**DISCUSSION**

Fetal and postnatal development are marked by rapid growth accompanied by an orderly sequence of maturational changes. The maturation of SM cells in the walls of hollow organs are expected in parallel growth and requirements for organ function. Results of the present study reveal that in a large mammal, maturation of vascular and visceral SM tissues, as assessed by increases in contractile protein content, occurs continuously from early midgestation through the first month of life. Nevertheless, distinct differences are seen between the tissues with regard to the absolute contents of contractile proteins, the interconversion of MHC isoforms, and the early onset of contractile function, leading to the conclusion that bladder SM matures far in advance of the vascular SM of the aorta.

Increases in MHC and, particularly, actin contents exceed those of total or soluble protein for both tissues, demonstrating specific upregulation of the contractile component of SM after midgestation and providing evidence of cellular maturation. Comparison between tissues reveals that the bladder contents of soluble protein, actin, and MHC exceed those in the aorta after 100 days gestation, leading to the conclusion that bladder muscle growth and maturation are initiated early in development and proceed rapidly thereafter. We previously measured actin and MHC contents in the aorta and the bladder from male animals over a more limited developmental period (119 days gestation to 33 days postnatal) (11). Values for actin, MHC, and soluble protein are similar in respective tissues from female and male animals during this period; thus sex per se does not appear to exert significant effects on processes regulating normal maturation of the aorta or bladder muscle. Previous studies (6, 11, 33, 40) of quantitative increases in contractile protein content during SM maturation focused on late fetal or postnatal periods. By extending our observations to much earlier times in gestation (72 days), we identified the time around 100 days as an important transition point for SM growth and, particularly, for increases in contractile protein content.

MHC isoform expression clearly illustrates the differences in maturational patterns between the bladder and the SM of the aorta. For example, the ratio of 204/200-kDa MHC species increases between <100 days gestation and 3 mo after birth in the aorta, but it decreases in the bladder over the same period. Although plots of percent MHC species show dramatic relative changes, they provide a limited picture of alterations occurring throughout development because the total amount of MHC varies and because the composition of isoforms within a band of given molecular mass may be heterogeneous. Therefore, we analyzed the total contents of each MHC species and the changes in isoform expression within the 200-kDa species. Amounts of SM1 (204-kDa MHC) increase throughout the development in both tissues, and in the bladder, values exceed those of the aorta by three- to fivefold at all times. SM1 is an exclusive marker of SM differentiation (27), and its presence in both tissues confirms that each contains differentiated SM cells at midgestation. The succeeding increases in SM1 content illustrate an important quantitative attribute of SM maturation not previously described. Immunohistochemical studies are required to determine whether maturation of all cells within a tissue occurs gradually and parallel or whether individual cells mature more rapidly but in gradual succession. Distinct populations of cells are shown to exist within different tissues (16, 25, 26); thus the latter cannot be ruled out. Abundant SM-MHC, as well as actin and other contractile proteins, may arise from transcriptional, translational, or posttranslational regulatory processes that constitute a significant facet of the mature SM phenotype. Mechanisms regulating contractile protein quantities require future investigation.

Qualitative analysis of isoforms within the 200-kDa MHC species demonstrates further dramatic differences between the aorta and the bladder. Whereas MHC-B predominates before birth in the aorta, no MHC-B was detected in the bladder at any stage. In the ovine aorta, MHC-B was gradually replaced by SM2 after birth. These results are consistent with those of others in that postnatal increases in SM2 fraction lead to adult values in human (1, 15), rabbit (9, 24), and mouse (14) aortas, as well as in swine carotid arteries (13), accompanied by reciprocal declines in MHC-B (1, 24). The replacement of nonmuscle isoforms of cytoskeletal proteins, such as α-caldesmon, α-tropomyosin-2b, and MHC-B, with SM isoforms constitutes a maturational transition in SM (21, 23). Striking in the present study was the observation that no MHC-B was expressed in the bladder after 90 days gestation, supporting the conclusion that maturational transitions are accelerated in the bladder SM. Postnatal alterations in the rat and mouse bladder 204- and 200-kDa proteins are modest and resemble those seen in postnatal sheep (13, 14). Although the specific contributions of MHC isoforms to muscle function are not fully defined, SM1 and SM2 are associated with a contractile phenotype, whereas MHC-B is associated with growth conditions either during development, disease, or cell culture (10, 23, 34, 35). The present finding that little or no MHC-B is expressed in the bladder muscle during fetal maturation suggests that this muscle has a fully expanded population of mature SM cells at an early stage compared with the aorta.

Maturation of contractile protein content and phenotype should be reflected in an increased contractile capacity during development. This tenet is well illustrated in the bladder muscle where active stress increases some sevenfold between 100 and 140 days gestation, reflecting significant increases in actomyosin content over the same period. Moreover, the increase in stress generation does not appear to result from changing responsiveness to stimulation during fetal growth because both MLCK content and the fraction of myosin phosphorylated in response to maximal agonist were...
similar at 100 and 140 days gestation. Stress generation is also reported to increase with fetal development in pig tracheal SM (8). In contrast, aortic muscle from fetal sheep was unresponsive to a host of agonists, and failure to contract was associated with a lack of myosin phosphorylation, despite significant expression of MLCK. We previously found contraction and myosin phosphorylation to be inhibited in near-term femoral arteries (4). These results, in combination with generally lesser sensitivity of the fetal ovine systemic vasculature to vasoconstrictors compared with the placent al circulation, support the notion that some components of systemic vascular SM are functionally immature up to the time of birth (20, 40). This immaturity is reflected in the expression of the nonmuscle MHC-B in these vessels, which decreases after birth. However, the lack of contractile responsiveness in these vascular muscles appears less likely to result from immaturity of the contractile apparatus than from deficits in the excitation-contraction pathway. This tentative conclusion is on the basis of the following: 1) agonists do not elicit myosin phosphorylation, indicating failure to elevate intracellular calcium required to activate MLCK; 2) vascular muscle contains amounts of SM-MHC and actin before birth that are comparable to amounts found in the contractile bladder muscle at 100 days gestation; and 3) contractile responsiveness is observed within 1 wk after birth at which time changes in the contractile protein profile are minimal. Further investigations are required to determine whether maturation associated with the onset of contractility involves the induction of receptor expression, coupling pathways, ion channels, or other regulatory proteins.

After birth, aortic stress increased to near-adult values. A similar pattern was seen in second generation pulmonary arteries from sheep (6); however, others have noted increases in the maximum contractile response between neonatal and adult aortas from rats (33), gastric myocytes from rabbits (38), and carotid arteries from sheep (7). More surprising in the present study was the decline in stress generation observed in bladder muscle with postnatal maturation. This could not be attributed to differences in actomyosin contents nor to levels of activation as assessed by myosin phosphorylation. No references to postnatal changes in bladder stress were found in the literature. Declines in stress generation might be accounted for by age-dependent changes in cellular density or orientation in the bladder wall.

The advanced maturation and growth of the bladder SM compared with that from the aorta supports the notion that demands for organ function may influence the developmental course. The aorta is a conduit vessel, thus the need for rapid maturational changes before birth may be minimal. In contrast, the bladder is functional early in the midtrimester, excreting urine at a rate of 7–10 ml·kg⁻¹·h⁻¹ which increases to 30–40 ml·kg⁻¹·h⁻¹ at term (37, 39). It is established that the differentiation of SM in the bladder is dependent on interactions with the epithelium (5). It remains to be determined whether the accelerated maturation of the bladder is also dependent on epithelial signals that may be influenced by bladder filling and/or distension. In addition, bladder SM cells may be independently influenced by these mechanical forces. Contractile responses of the fetal aortic and bladder SM tissues were consistent with the above notion in that only the bladder muscle exhibits robust force generation in response to stimulation.

Perspectives

The period of fetal development supports growth and maturation of organ systems essential for survival to birth and independent life thereafter. Owing to the relatively large size of the fetus, the ovine model allows analysis of functional properties of SM tissues during development. These and our previous results (4, 11) demonstrate a relation between early onset of organ function and accelerated maturation of its SM component during ovine fetal development. Whether maturation is brought about by neuronal, hormonal, paracrine, and/or mechanical factors remains to be investigated. SM differentiation is a complex process that involves the orderly activation of genes encoding SM-specific proteins such as SM1. In quantifying SM1 protein contents (as well as those of total MHC and actin) at multiple points throughout development, this study distinctively illustrates that maturation after differentiation includes increases in contractile protein content over the last third of ovine gestation and through the first month postnatally, laying the foundation for increasing contractile capacity (28, 33, 38). Further studies are required to determine threshold conditions leading to contractile function as well as specific queues promoting maturation during development.

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