Influence of Vascular Smooth Muscle Heterogeneity on Angiotensin Converting Enzyme Activity in Chicken Embryonic Aorta and in Endothelial Cells in Culture

Stavros Topouzis, John D. Catravas, James W. Ryan, and Thomas H. Rosenquist

The smooth muscle of the abdominal region of the chicken aorta derives from locally recruited mesenchyme (mesenchymal smooth muscle), whereas that of the thoracic region derives from the neural crest (ectomesenchymal smooth muscle). We hypothesized that this smooth muscle heterogeneity might affect important enzymatic functions of the vessel wall. Therefore, we measured angiotensin converting enzyme (ACE) activity in homogenates of chicken thoracic and abdominal aorta at different embryonic stages (days 10, 14, and 18 of gestation). ACE activity increased in both regions over the time of gestation ($p<0.001$ in both cases); the increase was steeper and ACE activity was higher in thoracic than in abdominal segments ($p<0.001$). $K_m$ values were similar ($=7 \mu M$) at all times and between the two segments, whereas changes in $V_{max}$ values closely paralleled those in enzyme activity, indicating gestation-dependent increases in the amount of enzyme. Neural crest ablation at an early developmental stage resulted in an increase of ACE activity in thoracic homogenates ($p<0.001$), predictably leaving that in abdominal homogenates unaffected. Bovine pulmonary artery endothelial cell monolayers exposed to media conditioned with cultured mesenchymal or ectomesenchymal smooth muscle cells exhibited elevated ACE activity (46% and 83%, respectively, relative to control medium, with $p<0.01$ in both cases; $p=0.05$ between the two media). Increases in endothelial cell ACE activity corresponded to proportional increases in ACE protein determined by enzyme-linked immunosorbent assay ($r=0.99$) and were interpreted as indicative of enhanced enzyme synthesis subsequent to exposure of endothelial cells to smooth muscle–conditioned media. We conclude that, in addition to mechanical and humoral factors, smooth muscle ontogeny may differentially influence vascular ACE activity. (*Circulation Research* 1992;71:923–931)

**KEY WORDS**  angiotensin converting enzyme  •  aorta  •  embryo  •  smooth muscle  •  endothelium

It is now recognized that the smooth muscle of the proximal great vessels, including the arch of the aorta, the common carotids, the common pulmonary trunk, and the brachiocephalic artery, is derived from the neural crest (ectomesenchymal smooth muscle). More distal vessels, including the abdominal aorta and the right and left carotid arteries, are derived from mesenchyme. At the interfaces of vessels where the smooth muscle derives from the neural crest and of those where the muscle derives from mesenchyme, there are regions with smooth muscle of mixed origin, both mesenchymal and mesenchymal.1 In mammals, there is indirect evidence that the neural crest plays a critical role in the development of the vascular system. When the neural crest is ablated surgically in chicken embryos, virtually all of the survivors show cyanotic congenital heart defects, especially persistent truncus arteriosus.2 The same kinds of defects in heart development occur in the Splotch mutant mouse, whose neural crest migration is congenitally impaired.3 Birds ablated of neural crest, mutant mice, and human babies with cyanotic congenital heart disease share many cardiovascular and other neural crest–related defects, further supporting a common role for neural crest cells in cardiovascular development.2,4–8 Furthermore, an “elastogenic defect” in the great vessels of chickens after ablation of the neural crest9,10 is similar to a defect that occurs in humans with cyanotic congenital heart disease.9,11,12 Based on these data, a common role for the neural crest is implied in the higher vertebrates, including humans.

This role is still only partially defined. It is known from studies using avian models that neural crest–derived smooth muscle cells in the aortic arch arteries show a pattern of differentiation that is profoundly different from the development of the mesenchyme–derived vessels.9,13,14 These differences may have significant consequences: the smooth muscle cells play a
prominent role in the vascular remodeling observed in diseases such as hypertension and atherosclerosis and after graft injury. On the basis of these observations, we have hypothesized that the embryonic source of the smooth muscle is one of the factors that influence remodeling in a particular vascular region; presumably, this must occur by modification of regulatory systems that are implicated in myocyte proliferation and/or migration, such as the renin-angiotensin system.

Angiotensin converting enzyme (ACE) inhibitors effectively reduce the myointimal proliferation in rat vessels after focal vascular injury in vivo, whereas exogenous angiotensin II induces smooth muscle proliferation and hypertrophy in the normal and injured rat arterial wall; furthermore, angiotensin II can differentially influence the ability of smooth muscle and of endothelial cells to migrate in vitro. Since the major components of the renin-angiotensin system, including ACE, are present locally in the vasculature (References 21–23 and references therein), the activity of any of the components (e.g., ACE) may influence local vascular homeostasis. According to our hypothesis, the degree of activity of such components is subject to regulation by intrinsic factors that vary with the anatomic location of the vessel, reflecting heterogeneity of the constituent smooth muscle cells; these intrinsic factors are independent of extrinsic factors such as mechanical forces or hormonal influence.

The present experiments were designed to test the hypothesis that smooth muscle heterogeneity can affect ACE function in the vascular wall. To this purpose, we determined ACE activity of the chicken aorta (thoracic versus abdominal segments) at different stages of embryonic development in normal embryos as well as in embryos with surgically ablated neural crests. We also studied the influence of mesenchymal versus neural crest–derived smooth muscle cell–conditioned media on endothelial ACE activity and ACE protein in culture.

Materials and Methods

Fertilized Arbor Acre chicken eggs (Seaboard Hatchery, Athens, Ga.) were incubated in a forced-draft incubator at 99% humidity and 38°C. In an experimental group of embryos, cardiac neural crest (somites 1–3) was ablated at Hamburger-Hamilton24 stage 8 or 9 by using a microcutting unit devised by the Department of Bioengineering at the Medical College of Georgia. The successful ablation of the neural crest was morphologically assessed during the removal of the aorta in experimental embryos by ascertaining that the heart outflow formed a persistent truncus arteriosus. Control and experimental eggs were then placed into an incubator with 70% humidity at 37°C. Embryos were collected at days 10, 14, and 18 of incubation, at the day of hatching (day 21), and at various times after hatching, and the aorta was carefully excised. The segments of the aorta considered “thoracic” and “abdominal” are depicted in Figure 1. In some experiments, the lungs were removed and processed in the same way as were the vessels. The survival rate of operated embryos at day 18 was approximately 5%.

![Figure 1. Schematic diagram of the segments of the chicken aorta.](image-url)

Tissue ACE Activity

Vessel segments were removed, quickly blotted on filter paper to remove excess fluid, weighed, and transferred into glass tubes containing 100 μl buffer (0.1 M HEPES and 0.15 M NaCl, pH 7.4) per milligram wet weight. An equal volume of the buffer, containing Triton X-100 (0.1%), was then added; the tissue was homogenized; and the tubes were capped and allowed to stand overnight at 4°C. Subsequently, they were centrifuged at 3,000 rpm for 30 minutes (4°C), and the supernatant was transferred into another glass tube. Preparations thus obtained were kept at 4°C and were assayed within a few days. ACE activity in these samples remained unchanged for 2.5 months. Enzyme activity in these preparations was determined under first-order or mixed-order kinetics, as described before, with [³H]benzoyl-Phe-Ala-Pro (BPAP, 25 Ci/mmol [final activity in the reaction volume, 0.1 μCi/ml], Amersham, Arlington Heights, Ill.) as the substrate. Enzyme activity was calculated using the integrated first-order equation:

\[
V_{max}/K_m = \frac{\ln(S_o/S)}{t}
\]

where \(V_{max}/K_m\) is the first-order rate constant, \(S_o\) is the initial concentration of the substrate, \(S\) is the remaining concentration of the substrate at time \(t\), and \(t\) is the time of incubation. The first-order rate constant thus calculated was subsequently adjusted for the enzyme dilution and normalized to 1 mg wet tissue weight.

The remaining initial enzyme preparations were combined in one tube per category (i.e., day-10 thoracic segments, day-10 abdominal segments, etc.) and were used in the calculations of individual \(V_{max}\) and \(K_m\) values. In this series of experiments, the reaction volume also contained nonradioactive substrate (0.5–20 μM). \(K_m\) and \(V_{max}\) values for thoracic and abdominal
segments at different stages of embryonic development were determined in three different sets of experiments, in which all the tissues had been harvested and processed in parallel, i.e., in three different series of pooled enzyme preparations. All assays were done in triplicate.

**Endothelial and Vascular Smooth Muscle Cell Cultures**

Bovine pulmonary artery endothelial cells. Bovine pulmonary artery endothelial (BPAE) cells were obtained by mechanical removal of the endothelial monolayer from tissues obtained at the local abattoir. The detached cell sheets were washed in sterile conical test tubes containing Medium 199 and 10% fetal calf serum. The cell suspension was centrifuged at 800 rpm for 10 minutes. The supernatant was discarded, and the pellet was suspended in Medium 199 containing 10% fetal calf serum and antibiotics. The suspension was evenly distributed into 100-mm-diameter dishes. Cells obtained in this way exhibited a typical cobblestone morphology and contact inhibition of growth; they were also characterized by accumulation of 1,1-dioctadecyl-3,3,3,3-tetramethyl-indocarbocyanine-perchlorate acetylated low density lipoproteins (Dil-Ac-LDLs), immunofluorescence to the von Willebrand factor, and expression of substantial ACE activity. For the present study, endothelial cells at passages 7 and 8 were used.

Chicken vascular smooth muscle cells. Chicken embryos at day 14 of gestation were used as a source of pure mesenchymal smooth muscle cells from the abdominal aorta and eotmesenchymal smooth muscle cells from the fourth aortic arch. The vessel segments were collected in sterile conditions and trimmed of any attached connective tissue under a dissecting microscope. The tissues were transferred into 15-ml centrifuge tubes containing 5 mg collagenase and 1.25 mg elastase in 10 ml serum-free Medium 199 (30 minutes, 37°C). Tissues underwent four such digestions. The supernatant from the first digestion was discarded. The supernatant from the subsequent three digestions were collected and centrifuged at 300g for 4 minutes, and the pellet was transferred into 2 ml fetal bovine serum to stop the digestion. Pellet-containing sera from the three digestions were pooled and centrifuged as before, and the pellet was washed twice with 5 ml complete medium (Medium 199 containing 10% fetal bovine serum and antibiotics). Finally, the pellet was resuspended in complete medium and filtered through a nucleopore membrane (10 µm), and the cells were seeded into 100-mm-diameter dishes. Cells grew within 36–48 hours to confluence and presented the typical morphological characteristics of vascular smooth muscle cells. The vast majority (>90%) of cells obtained and grown in this way present positive staining to vascular smooth muscle-specific α-actin antibody (data not shown). Cells from the two vascular regions presented similar patterns of growth and overall morphology typical of vascular smooth muscle. Smooth muscle cells were used at passages 1 and 2.

**ACE Assay in Cells in Culture**

At 2 days after confluence, the medium of the endothelial cells was removed and immediately replaced with the appropriate conditioned media obtained as follows: when bovine endothelial or chicken embryonic vascular smooth muscle cells reached confluence, their medium was replaced with fresh medium (Medium 199 containing 10% fetal calf serum). The medium was then left in contact with the three different cell types (endothelial, abdominal aorta smooth muscle, and fourth arch smooth muscle) for 48 hours. Media were then removed, pH-checked, centrifuged to discard any cells in suspension, and used as “conditioned media” by immediately transferring them to 2-day postconfluent endothelial cell monolayers. In parallel, freshly prepared medium (unconditioned medium) was treated in the same way.

After 48 hours in contact with the endothelial cells, media were removed, and cells were washed twice with freshly prepared Earle’s balanced salt solution (EBSS, pH 7.4 at 37°C). The third time, EBSS was removed and replaced in each well with 0.6 ml EBSS containing 0.5 μCi/ml of the ACE substrate [3H]BAPAP. The plate was then placed back in the incubator for 45 minutes. At the end of this period, three 0.1-ml aliquots were removed from each well and added to 7-ml plastic scintillation vials containing 2.9 ml of 0.12N HCl to stop the reaction. The rest of the assay was performed as with the tissue homogenates.

**Determination of ACE Protein by Enzyme-Linked Immunosorbent Assay**

ACE protein was determined using the two-plate enzyme-linked immunosorbent assay (ELISA) of Dasarathy et al. BPAE cells grown in 60-mm dishes were exposed to media conditioned by endothelial, mesenchymal smooth muscle, or eotmesenchymal smooth muscle cells, as described above. Forty-eight hours later, the dishes were washed with HEPES buffer (pH 7.4), and BPAE cells were scraped and collected in HEPES. Subsequently, they were briefly sonicated in ice, and the sonicate was treated with 1% CHAPS (Sigma Chemical Co., St. Louis, Mo.) for 30 minutes at 37°C. The extract was then centrifuged at 3,000 rpm for 30 minutes at 4°C. The resulting supernatants were used for the ELISA. We also determined ACE activity in the supernatants by using the method described for the tissue homogenates; the activity was normalized per milliliter extract.

For the ELISA, the wells of a 96-well plate (Costar Corp., Cambridge, Mass.) (plate 1) were coated with 20 ng purified ACE per well. The ACE antigen was derived from rat lung. In a second plate (plate 2), wells were filled with different amounts of rat lung ACE (10–300 ng per well, for the standard curve) or with serially diluted ACE extracted from BPAE cells and with a standard dilution (1:3,000) of a monoclonal antibody. The monoclonal antibody used in this study was raised against rabbit lung ACE and strongly cross-reacts with both rat lung and bovine plasma ACE. For a second antibody, we used goat anti-mouse immunoglobulin G conjugated with horseradish peroxidase at 1:1,000 dilution (Bio-Rad Laboratories, Richmond, Calif.). The color that developed after addition of the substrate-containing buffer (citrate-phosphate buffer, pH 5, containing 0.42 mM 3,3',5,5'-tetramethylbenzidine and 15 µl of 3% H2O2 per 10 ml; Sigma) was quantified at 630 nm using an ELISA plate reader (model MR700, Dynatech Laboratories, Inc., Chantilly, Va.). Appropriate blanks were included by omitting the antibody, the
Tukey's or Dunnett's test, as appropriate. Calculations were performed with the aid of a Zenith microcomputer equipped with STATPACK (Northwest Analytical, Oregon).

Results

ACE Activity in Tissue Homogenates

ACE activity could be detected in the chicken embryonic aortas of control (nonoperated) animals as early as day 6 (results not shown) and throughout the time of gestation, as well as for several days after hatching. ACE activity increased during this period. As can be seen in Figure 2A, this pattern of change is qualitatively similar in both thoracic and abdominal segments.

In thoracic segments of aortas from control embryos, ACE activity, expressed as the first-order rate constant $V_{\text{max}}/K_m$, increased by approximately 6.3-fold between days 10 and 14 of gestation and a further 1.9-fold between days 14 and 18 ($p<0.001$ in both cases). In abdominal segments, ACE activity also increased by approximately threefold and 4.3-fold between day 10 and days 14 and 18 of gestation, respectively ($p<0.001$ in both cases, Figure 2). One-way ANOVA showed a significant difference in ACE activity between the two vessel regions ($p<0.001$, Table 1). This difference was more clear at days 14 and 18 of incubation, when the ACE activity associated with the thoracic segments was higher than that associated with the abdominal aorta by approximately 40% ($p<0.05$) and 80% ($p<0.01$), respectively (Figure 2A). Two-way ANOVA revealed that the pattern of the observed changes in ACE activity between the two vascular regions over this period was also significantly different ($p<0.001$, Table 1).

All the control (unoperated) embryos showed a normal pattern of heart septation and aortic arch arterial anatomy. Therefore, the survival of the control group to hatching (21 days of incubation) was very high, approximately 90%.

On the other hand, essentially 100% of the experimental embryos showed an abnormal pattern of heart septation and an abnormal pattern of the branching of the aortic arches. In every case, the pattern was a

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ANOVA, analysis of variance; ACE, angiotensin converting enzyme; NS, not significant.
variation of truncus arteriosus, caused by the failure of aorticopulmonary septation in the absence of neural crest. No two of the several dozens of experimental embryos showed an identical pattern of development; this high variability is always the case in the absence of neural crest. Because of the profound impact of truncus arteriosus on embryo viability, significantly fewer of the experimental embryos survived to harvest compared with the control embryos. By day 18, only 5% of the embryos without neural crest were alive for harvest. Thus, to be sure of a harvest of eight vessels for a given assay for day 18, it was necessary to operate on at least 160 embryos. The vessels used in this study were not evaluated histologically, since previous studies have shown that 100% of the embryos without neural crest show a characteristic disruption of the spatial order of the extracellular matrix in the regions that ordinarily contain neural crest–derived smooth muscle, including a loss of lamination. On the other hand, 100% of the control embryos show a highly ordered and well-laminated vessel wall. This difference is not found in the abdominal aorta, which is the same in control and experimental embryos.

The same overall pattern of changes in ACE activity was observed in aortas from operated (neural crest–ablated) animals (Figure 2B). In this case, ACE activity in thoracic segments at days 14 and 18 of incubation was 6.3- and 9.3-fold higher than the activity measured at day 10 (p<0.001). In the abdominal region, the first-order rate constant at days 14 and 18 was 1.3-fold (p=NS) and 2.3-fold (p<0.01) higher, respectively, than that at day 10 of gestation. Again, the activity in thoracic segments was significantly more pronounced than that in abdominal segments at days 14 and 18 (2.3- and 1.9-fold higher, p<0.001 and p<0.05, respectively). One-way ANOVA showed that, as in aortas from control embryos, ACE activity in thoracic versus abdominal segments was different (p<0.001, Table 1). Two-way ANOVA also revealed a significant difference (p<0.001, Table 1) in the pattern of activity changes between thoracic and abdominal segments over this period of time.

Comparison of the Vmax/Km values in the thoracic segments of aortas excised from nonoperated versus operated embryos by one-way ANOVA showed a significant difference (p<0.001), the latter expressing higher ACE activity. However, no significant difference in activity was found between abdominal sections of control and those of operated embryos (Table 1).

In control (unoperated) animals, the differences in ACE activity between the thoracic and the abdominal regions were maintained up to the sixth day after hatching (p<0.01, n=7–10, Figure 3).

In aortas collected from control embryos, the increase in enzyme activity was associated with an increase in Vmax rather than with a reduction in Km. As seen in Table 2 and Figure 4, Km values, obtained through the Lineweaver-Burk transformation of data, did not change over the days of gestation, and there were no differences detected between values determined in thoracic and abdominal segments of control embryos. Km values thus determined were approximately 7–8 μM (Table 2). Similar values were obtained through transformation of data according to Scatchard analysis (results not shown). In contrast to unchanging Km values, Vmax values increased over the course of the gestation period in both thoracic and abdominal segments. In the abdominal segments, the difference was significant only between vessel segments harvested at days 10 and 18 (Table 2). This increase in Vmax values was of greater magnitude in the thoracic parts, in which there was a fourfold augmentation between days 10 and 14 (p<0.001) and a further 2.2-fold (p<0.001) increase between days 14 and 18 (Table 2). Vmax values determined in thoracic segments were significantly lower than those determined in abdominal segments (p<0.05) at day 10 of gestation but increased to comparable levels at day 14 and to significantly higher levels at day 18 of gestation (p<0.001, Table 2). Changes in the Vmax/Km ratio correlated well with the increase in Vmax over the period of gestation in both thoracic (r=0.99) and abdominal (r=0.98) segments.

To assess the influence of gestation on vascular ACE activity in a different tissue, we measured activity in lungs. In lungs, ACE is localized on the endothelial luminal surface, and because of the huge surface area of the vascular network in this tissue, the majority of ACE is of endothelial origin. In crude lung preparations, ACE activity (as reflected in the first-order rate constant) between tissues obtained at day 10 and those obtained at day 14 increased by a modest 60% (p<0.05); however, a 6.7-fold increase was observed between values determined in lungs harvested at days 14 and 18 (p<0.001, Figure 5). Km values remained unchanged throughout the period studied (=7 μM), whereas there was a twofold increase in the Vmax values between days 10 and 14 and a further sixfold increase between days 14 and 18 of gestation (Figure 5).

ACE Activity and ACE Protein in Cultured Endothelial Cells

The smooth muscle cells of the abdominal aorta derive exclusively from locally recruited mesenchyme. In the part of the aorta that we term “thoracic,” there is a minor population of smooth muscle cells of nonectomesenchymal origin, whereas the vast majority of them derive from differentiated neural crest cells. However,
to ensure a cell population as pure as possible, for the cell isolation we used the fourth arch region as a source of pure ectomesenchymal cells. This tissue is composed of neural crest–derived smooth muscle only.

The mean $\frac{V_{\text{max}}}{K_m}$ value determined in 10 wells of endothelial cells after exposure to endothelial cell–conditioned medium for 48 hours (0.038±0.003 min$^{-1}$ per well) was taken as baseline (100%). Results are thus expressed as a percentage of this value. The pH values of all conditioned media measured immediately after their removal from the respective cells were identical (pH 7.4). Similarly, there was no difference in the number of smooth muscle cells per dish during the conditioning of the media. Exposure of endothelial cell monolayers to fresh medium for 48 hours did not have any significant effect on ACE activity (n=11 wells, Figure 6). Exposure of cells to conditioned medium from cultures of vascular smooth muscle cells from either the fourth arch (ectomesenchymal smooth muscle) or the abdominal aorta (mesenchymal smooth muscle) resulted in an enhancement of ACE activity in the endothelial monolayer by 82.8±14.5% and 45.5±5.1%, respectively (n=11 in each case, $p<0.01$). The increase in ACE activity induced by the condi-
toned medium of the vascular smooth muscle cells from the arc region was significantly higher than that induced by the conditioned medium from cells derived from abdominal aortic segments \((p<0.05)\).

When conditioned media were removed from the smooth muscle cells in culture and, after routine treatment, were kept in the sterile incubator for 7 days, their capacity to increase ACE activity of endothelial cells was diminished. Conditioned medium from mesenchymal (abdominal aortic) smooth muscle cells after such treatment did not affect ACE activity significantly \((28.1\pm8.2\% \text{ above baseline, } n=6, p>0.05)\), whereas medium from ectomesenchymal (aortic arch) smooth muscle cells still increased ACE activity by \(54.6\pm13.9\% \text{ above baseline (} p<0.05, n=6)\).

In a separate series of experiments, performed with different isolates of endothelial and smooth muscle cells, we extracted ACE from endothelial cells exposed for 48 hours to conditioned media, as described above. As before, the exposure of endothelial cells to the different conditioned media did not affect the endothelial cell number or protein \(\text{(data not shown).}\)

In a parallel series of experiments, ACE activity was determined not in extracts but in endothelial monolayers after treatment with conditioned media as before. This time, the \(V_{\text{max}}/K_{\text{m}}\) values in the monolayers \(\text{(normalized per milligram protein)}\) were \(0.14\pm0.02, 0.22\pm0.01, \text{ and } 0.93\pm0.17\) in cells exposed to media conditioned with endothelial, mesenchymal smooth muscle, and ectomesenchymal smooth muscle cells, respectively \((n=4)\).

In the endothelial extracts, the first-order rate constant values for \(V_{\text{max}}/K_{\text{m}}\), normalized per milliliter extract, were \(0.202\pm0.009, 0.395\pm0.029, \text{ and } 1.32\pm0.082 \text{ min}^{-1} \) \((n=3)\) in extracts from cells exposed to media conditioned with endothelial, mesenchymal smooth muscle, and ectomesenchymal smooth muscle cells, respectively.

(Figure 7). Comparison of ACE activity in the extracts with that determined in parallel in cell monolayers demonstrated a linear \((r=0.99)\) relation. Therefore, in this series of experiments too, ACE activity was enhanced by exposure of the endothelial cells to the smooth muscle conditioned media \((p<0.01 \text{ in both cases})\). Again, the effect of medium conditioned with ectomesenchymal (neural crest-derived) smooth muscle cells was higher \((p<0.01)\) than that of the medium conditioned with mesenchymal smooth muscle cells. ACE protein was determined to be equivalent to 90, 180, and 360 ng rat lung ACE per ELISA well in these same extracts from cells exposed to endothelial-, mesenchymal smooth muscle–, and ectomesenchymal smooth muscle–conditioned media, respectively. ACE protein was thus directly proportional to the \(V_{\text{max}}/K_{\text{m}}\) values \(\text{(Figure 7).}\) Results identical to those depicted in Figure 7 were obtained in a replica experiment as well.

**Discussion**

ACE activity is present in the embryonic aorta and lungs of the chicken. This activity increases throughout development. The vessel segment with smooth muscle originating primarily from the neural crest expresses higher ACE activity, and the expression of this activity increases during development more rapidly than in the segment with smooth muscle of mesenchymal origin. The present results also show that vascular smooth muscle cells can regulate ACE activity of endothelial cells in vitro differently, depending on their embryonic origin.

Unchanging \(K_{\text{m}}\) and increasing \(V_{\text{max}}\) values suggest that the increase in ACE activity over the time of gestation is not caused by an increase in the enzyme-substrate affinity but rather from increases in the amount of the metabolically active enzyme. A possible
cause of such differences may be altered enzyme expression and synthesis.

On the basis of our data obtained with control embryonic aortas, we hypothesized that the differences in ACE activity between the two vascular regions are linked to the embryonic origin of the smooth muscle. Predictably, there was no difference in the ACE activity of the abdominal aortas between control and neural crest–ablated embryos. In contrast, there was a significant difference in enzyme activity of the thoracic aortas between control and neural crest–ablated embryos.

The persistent difference in ACE activity between thoracic and abdominal segments found in operated embryos suggests that 1) the new mesenchymal cells that take the place of the neural crest–derived cells express different intrinsic ACE-modulatory properties (in fact, vessels derived from “normal” mesenchyme and those derived from mesenchyme replacing the absent neural crest exhibit other differences, such as elastogenesis and collagen expression),9,10,28 and/or 2) there are other extrinsic factors (e.g., shear stress) that contribute to the regulation of the expression of ACE activity.

An increase in tissue ACE activity during embryonic development was also observed in lung homogenates; again, this increase was probably due to an augmentation in the amount of the tissue enzyme. Since most of ACE in lungs is associated with the vasculature,29 the present results suggest that the observed increase in ACE activity during gestation applies to the pulmonary vasculature. ACE activity reached a plateau at hatching day, as did the ACE activity ratio of the thoracic to the abdominal segments; the latter was maintained for at least 6 days after hatching.

The hypothesis that the differences in ACE activity between aortic segments might be related to smooth muscle ontogeny was further tested and corroborated by studies using chicken embryonic smooth muscle cells in culture. There is now ample evidence demonstrating that the endothelium can influence vascular smooth muscle function through the production and release of a large variety of substances (e.g., see References 30–32). However, it is less clear how vascular smooth muscle can influence activity of the adjacent endothelial cells. Recent evidence indicates that smooth muscle cell–conditioned medium is capable of enhancing ACE activity of endothelial cells in culture in a time- and concentration-dependent manner.33 Also, medium from vascular smooth muscle cells modulates the secretion of endothelin-1 by endothelial cells.34 Our in vitro data show that media conditioned with vascular smooth muscle cells in culture can indeed influence endothelial ACE activity in vitro. An additional important finding with respect to our initial hypothesis is that medium conditioned with ectomesenchymal (fourth arch) vascular smooth muscle cells induced a significantly greater increase in ACE activity in endothelial monolayers than medium conditioned with mesenchymal (abdominal arterial) vascular smooth muscle cells. It is pertinent to our hypothesis that this difference is qualitatively similar to that found in tissue homogenates, in which higher ACE activity was detected in the vascular region populated principally by ectomesenchymal (neural crest–derived) smooth muscle cells. The increase in enzyme activity in the cultured endothelial monolayers was not due to alterations in the pH of the media or to a difference in the number of smooth muscle cells used to condition the respective media. Consequently, it indicates the presence of a soluble factor secreted in higher quantities by the ectomesenchymal than the mesenchymal cells. The enhanced endothelial ACE activity was accompanied by an analogous increase in the ACE protein, strongly suggesting enhancement of enzyme synthesis.

Altered vascular ACE activity would affect local concentrations of angiotensin II, which in turn might affect angiotensin II receptor density on the smooth muscle cells, as has been shown to occur in vitro,35 and thus modulate, in an autologous way, the action of the peptide on the vasculature. In addition, angiotensin II exerts a wide range of indirect effects on vascular smooth muscle cells by interference with the expression and production of other mitogenic products by these cells36–38 or by modulating the composition of the extracellular matrix synthesized by these cells in vitro.39

Fine tuning of angiotensin II levels, which are partly regulated by the intrinsic properties of the vessel wall, may result in altered vascular homeostasis and reactivity to a variety of stimuli. Intrinsically defined different properties of the smooth muscle cells might have important consequences: in the best-studied avian model of atherosclerosis, the White Carneau pigeon, the neural crest–derived vessels are the least susceptible to develop the disease, the mesenchyme-derived vessels are more susceptible, and the most susceptible regions coincide with sites identified as neural crest–mesenchyme interfaces.40–42

In summary, we have demonstrated that the ACE-modulatory properties of smooth muscle vary with its ontogeny. Origin–independent factors related to the anatomic location of the vessel, e.g., shear stress,33 also influence tissue ACE activity directly, suggesting that the regulation of vascular ACE activity in vivo may result from a combination of intrinsic and extrinsic factors.

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