Regional Differentiation of Desmin, Connexin43, and Connexin45 Expression Patterns in Rat Aortic Smooth Muscle

Yu-Shien Ko, Steven R. Coppen, Emmanuel Dupont, Stephen Rothery, Nicholas J. Severs

Abstract—The gap-junctional protein, connexin43, is differentially expressed in vascular smooth muscle cells (SMCs) according to phenotype. Previous studies suggest that desmin-negative SMCs are characterized by high levels of connexin43, whereas desmin-positive SMCs (of a more contractile phenotype) typically have low connexin43 levels. In this study, we examine systematically the inverse relationship between connexin43 and desmin in SMCs of defined regions of the rat aortic media and determine whether additional connexin isotypes are expressed and contribute to this relationship. Immunoconfocal microscopy demonstrated that (1) the inverse relationship between connexin43 and desmin expression holds true for the media of sequential aortic zones, with 1 exception, the ascending aorta, and (2) an additional vascular connexin, connexin45, is expressed by aortic SMCs. Examination of connexin43, connexin45, and desmin expression in sequential aortic zones reveals 3 SMC subpopulations. The first, predominating in the aortic arch and thoracic aorta, is desmin negative and contains high connexin43 levels; the second, predominating in the abdominal aorta and iliac artery, is desmin positive and contains low connexin43 levels; and the third, which is restricted to the ascending aorta, is desmin positive and expresses high connexin43 levels. Connexin45 levels are high in the ascending aorta but low in the other aortic segments. In para-aortic veins, a fourth SMC subpopulation appears, one that is desmin positive and contains connexin45 but not connexin43. These results demonstrate that a diversity of connexin expression patterns characterizes distinctive subpopulations of medial SMCs in situ with a potential to contribute to regional differentiation of vascular function. (Arterioscler Thromb Vasc Biol. 2001;21:355-364.)

Key Words: connexin43 ■ connexin45 ■ desmin ■ smooth muscle ■ aorta
gesting a role related to synthesis and/or maintenance of extracellular matrix. However, differential Cx43 expression is not confined to phenotypic extremes but is also apparent between mature medial SMCs of more subtle phenotypic distinction. Cx43 is highly expressed in SMCs of the media of elastic arteries but occurs only at low or immunocytochemically undetectable levels in muscular arteries and arterioles; in the human internal mammary artery, the SMCs expressing high Cx43 levels in the elastic medial regions are mainly desmin negative, whereas those with low Cx43 levels in the muscular medial regions are mainly desmin positive. Both SMC subpopulations are mature and express late differentiation markers, such as myosin heavy chain isoforms SM1 and SM2. Such an inverse relationship between Cx43 and desmin expression is also apparent in human coronary arteries and in cultured porcine aortic SMCs.

From this background, we set out to examine systematically the consistency of the proposed inverse relationship between connexin43 and desmin and to identify whether other “cardiovascular” connexins are expressed in the SMCs of defined regions of the rat aorta. Previous studies in the rat suggest higher levels of Cx43 gap junctions in the proximal than in the distal aorta and more desmin-positive SMCs in the distal than in the proximal aorta, but the existing data are fragmentary. First, only Cx43 but no other major cardiovascular connexins were examined; second, the initial part of the aorta, the ascending aorta, has not previously been investigated; and third, because these earlier studies were performed separately by different groups, no direct comparison between Cx43 and desmin profiles within the same study has previously been undertaken.

Methods

Tissue Preparation

Samples of ascending aorta, aortic arch, thoracic aorta, abdominal aorta, and iliac arteries were obtained by rapid dissection from adult male Sprague-Dawley rats (300 to 450 g) killed by dislocation of the neck or CO2 asphyxiation. After they were rinsed with PBS containing heparin (10 U/mL), the samples were quickly cut into segments, frozen immediately in isopentane or in Cryo-M-Bed embedding compound (Bright) cooled with liquid nitrogen, and stored in liquid nitrogen or in a −70°C freezer. Ten-micrometer-thick cryosections of the vascular segments were cut transversely, mounted on poly-L-lysine–coated slides, and kept overnight in a −20°C freezer before immunolabeling. Animal work was conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.

Antibodies for Gap-Junctional Connexins, SMC Differentiation Markers, and Endothelial Cell Markers

We investigated by immunocytochemistry the 4 connexin types now known to be the principal connexins expressed by cardiovascular cells: Cx37, Cx40, Cx43, and Cx45. For this purpose, we prepared a panel of polyclonal antibodies against these connexins, of which the following were used in the present study: for Cx37, Y16Y/R4 (dilution, 1:30) raised in rabbit; for Cx40, V15K/GP319 (dilution, 1:500) donkey anti-rabbit immunoglobulin conjugated to FITC (Chemicon, dilution 1:50), donkey anti-chicken immunoglobulin conjugated to Cy3 (Jackson, dilution 1:250). For immunoelectron microscopy, 10-nm gold/rabbit anti-chicken complexes (BioCell, 1:50) were used. Primary antibodies and secondary antibody/detection systems were diluted in 1% BSA in PBS (pH 7.4) before use.

Characterization of Anti-Cx43 (S10C/CK84) Antibody

The specificity of the new anti-Cx43 (S10C/CK84) polyclonal antibody raised in chicken was tested by immunofluorescence labeling of HeLa cell transfectants (kindly provided by Prof Dr Klaus Willecke, Institute für Genetik, Bonn, Germany) and by immunogold thin-section electron microscopy of rat myocardium. Wild-type and cx37-, cx40-, cx45-, and cx43-transfected HeLa cells were immersed in methanol at −20°C for 5 minutes and then in 1% BSA/PBS at room temperature for 1 hour, incubated in the anti-Cx43 antibody followed by donkey anti-chicken Cy3 at room temperature (1 hour each), and examined by confocal microscopy. For immunoelectron microscopy, specimens of rat ventricle were fixated with 2% formaldehyde (freshly prepared from paraformaldehyde) for 15 minutes and embedded in Lowicryl K4M. Thin sections were labeled with the anti-Cx43 antibody followed by 10-nm gold/rabbit anti-chicken complexes and examined with a Philips EM301 electron microscope. In addition, the use of the immunofluorescence technique for confirmation of antibody specificity, in nonmatching secondary antibodies. All secondary antibodies were affinity-purified, characterized, and demonstrated to be isotype specific by Western blot and immunofluorescence analysis of cells transfected to express different connexin types and by immunogold labeling at the electron-microscopic level.

For Cx43 single labeling, a mouse monoclonal anti-Cx43 antibody of established specificity was used (Chemicon, dilution 1:1000). In addition, to permit multiple labeling studies in which Cx43 could be visualized simultaneously with other proteins that were detected with the use of mouse and rabbit secondary antibodies, a new anti-Cx43 polyclonal antibody was raised in chicken. This antibody, designated S10C/CK84, was produced against a peptide sequence corresponding to residues 314 to 322 of rat Cx43 (with an additional C-terminal cysteine to facilitate coupling to the carrier protein) and affinity-purified. This sequence has previously been reported for the production of specific Cx43 antibodies in rabbit.

Commercially available mouse monoclonal antibodies were used to detect the following proteins as SMC differentiation markers: desmin (N. D1033, Sigma; 1:100), smooth muscle α-actin (No. A-2547, Sigma; 1:1600), calponin (No. C-2687, Sigma; 1:10000), and smooth muscle myosin heavy chain isoforms (SM2, No. 7601, Seikagaku; I:400). Anti–von Willebrand factor (VWF) polyclonal antibody (Dako, 1:2500) raised in rabbit was used as a cell-specific marker for vascular endothelium in double labeling.

The secondary antibody/detection systems used for immunofluorescence labeling were donkey anti-rabbit immunoglobulin conjugated to Cy3 (Jackson, dilution 1:500), donkey anti-mouse immunoglobulin conjugated to Cy5 (Chemicon, dilution 1:1500), donkey anti-rabbit immunoglobulin conjugated to Cy2 (Jackson, dilution 1:500). For immunoelectron microscopy, 10-nm gold/rabbit anti-chicken complexes (BioCell, 1:50) were used. Primary antibodies and secondary antibody/detection systems were diluted in 1% BSA in PBS (pH 7.4) before use.

Immunofluorescence Labeling of Connexins and Differentiation/Cell-Type Markers

For immunofluorescence labeling of aortic tissues, cryosections were fixated in methanol at −20°C for 5 minutes, washed with PBS, blocked with PBS/BSA at room temperature for 60 minutes, and then incubated at room temperature in the selected primary antibody or antibodies for 1 hour. After a washing with PBS, incubation in the secondary antibody (or antibodies) followed (1 hour, room temperature). The sections were then washed with PBS and mounted. For single-labeling studies, guinea pig anti-Cx45 was detected with donkey anti-mouse immunoglobulin conjugated to Cy3; for all mouse monoclonal primary antibodies, detection was with donkey anti-rabbit immunoglobulin conjugated to Cy3. For double- and triple-labeling studies, sections were treated sequentially with mixtures of primary antibodies from different species and then with their corresponding secondary antibodies conjugated to different fluorochromes (except for the anti-Cx37 antibody, for which an overnight incubation was used before application of other primary antibodies). Negative controls included (1) omission of the primary antibody and (2) for multiple labeling, using each primary antibody with matching and nonmatching secondary antibodies. All secondary antibodies...
were confirmed to be species specific to their individual primary antibody.

**Confocal Microscopy and Quantification**

Confocal laser scanning microscopy of immunolabeled sections was carried out with use of a Leica TCS 4D with an argon/krypton laser (single-labeling studies) and a Leica TCS SP with argon, krypton, and He/Ne lasers (multiple-labeling studies), both equipped for the detection of FITC, Cy3, and Cy5 fluorescence. The images were recorded by single- or sequential dual/triple-channel scanning. Single optical sections and projection views (for which sets of consecutive optical sections were used) were examined.

To compare Cx43, Cx45, and desmin expression in different arterial regions, the immunofluorescent microscopic images of Cx43, Cx45, desmin, and ethidium bromide (20 μg/mL PBS for 1 to 5 minutes) labeling from 5 rats were used. For each immunolabeled component, 3 single optical images were acquired, with use of the ×63 oil lens (with a theoretical focal depth of 0.5 μm; from 3 individual sections for each arterial region of each rat. From each image, a 900-μm² sample area in the corresponding medial layer was randomly selected for quantification. The images were quantified by use of Scion Image analysis software (Scion Corp) as described previously. The number and individual areas of immunolabeled Cx43 and Cx45 gap-junctional spots, the total area of desmin signal, and the number of ethidium bromide–stained nuclei in each corresponding sample area were determined. These data provided indices of the mean area of gap-junctional spots, the numerical density of gap-junctional spots per cell, the mean area of desmin signal per cell, and the cellular density of each sample. It should be emphasized that the values given are not absolute values per cell but are comparative measures determined from a sample thickness of 0.5 μm; hence, they are referred to throughout as indices of the various parameters. The data from different arterial regions were presented as mean ± SD and were compared statistically by the Wilcoxon rank sum test with statistical significance defined as *P* < 0.05.

**Results**

**Characterization of Anti-Cx43 (S10C/CK84) Antibody**

Immunofluorescence labeling of HeLa cell transfectants and immunoelectron microscopy of rat myocardium confirmed the specificity of the new affinity-purified anti-Cx43 (S10C/CK84) polyclonal antibody (Figure 1). Whereas the wild-type and Cx37-, Cx40-, and Cx45-transfected HeLa cells showed no positive signal for this antibody (Figures 1A through 1D), prominent punctate labeling was apparent at the cell borders between the Cx43-transfected HeLa cells (Figure 1E). This labeling was inhibited by the corresponding S10C peptide (Figure 1F). In immunoelectron microscopy of ventricular myocardium, gap junctions showing the typical pentalaminar morphology in rat ventricular myocardium (G). Bar=55 μm (A through F) and 200 nm (G).

**Expression of Smooth Muscle Differentiation Markers, Cx43, and Cx45 in the Rat Aorta and Iliac Artery**

Of the SMC differentiation markers examined, whereas smooth muscle α-actin, calponin, and myosin heavy chain isoform SM2 were positively labeled in the majority of vascular SMCs in all arterial regions studied, the labeling of desmin was distinctly heterogeneous. Figure 2 compares the labeling patterns of SM2 and desmin in the different regions of the aorta and iliac arteries. For myosin heavy chain isoform SM2, no detectable difference of the labeling intensity was observed between different arterial segments (Figures 2A, 2C, 2E, 2G, and 2I). In marked contrast, the labeling pattern of desmin showed distinct differences according to location along the aorta and the iliac artery (Figures 2B, 2D, 2F, 2H, and 2J). In the ascending aorta (Figure 2B), abdominal aorta (Figure 2H), and iliac artery (Figure 2J), the majority of the SMCs were positively labeled with anti-desmin antibody, but in the aortic arch (Figure 2D) and thoracic aorta (Figure 2F), some of the cells were strongly labeled, whereas others showed negative desmin labeling. The distinction between desmin-positive and desmin-negative regions was clear-cut and without intermediate grades. In the aortic arch (Figure 2D), both cell groups were scattered across the medial layers, without major segregation. The desmin-positive cells were present at the luminal, middle, or adventitial side of the media, without major preference. In the thoracic aorta (Figure 2F), however, desmin-positive cells were consistently more abundant at the luminal side of the vessel than at the adventitial side.

Cx43 and Cx45, but not Cx37 or Cx40, were detected in the medial SMCs (Figure 3). The distribution patterns of Cx43 and Cx45 punctate labeling in the medial layer varied according to the arterial zone. The levels of Cx43 signals were high in the ascending aorta (Figure 3A), aortic arch (Figure 3C), and thoracic aorta (Figure 3E) but low in the abdominal aorta (Figure 3G) and iliac artery (Figure 3I). In the ascending aorta (Figure 3A) and thoracic aorta (Figure 3E), Cx43 labeling was more abundant at the adventitial side of the vessel than at the luminal side, but such a zonal difference was not apparent in the aortic arch (Figure 3C), abdominal aorta (Figure 3G), or iliac artery (Figure 3I).

For Cx45, the immunofluorescent labeling showed distinct punctate signals in the medial regions of all vascular segments examined (Figures 3B, 3D, 3F, 3H, and 3J). However,
the sizes and amounts of labeled Cx45 gap junctions varied in different arterial segments. Cx45 spots were much larger and more abundant in the ascending aorta (Figure 3B) than in the other arterial segments. No Cx45 signal was observed in the endothelium.

Quantification of the number of vascular SMCs by counting the cell nuclei (after ethidium bromide staining) showed that the cell densities in the medial layer of the different arterial zones were similar (Figure 4A). Comparing the indices of the desmin signal area per cell (Figure 4B) and the numerical density (Figure 4C) and mean area (Figure 4D) of Cx43 gap-junctional spots per cell showed that the relationship between Cx43 and desmin expression varied according to arterial zone. An inverse relationship between Cx43 and desmin expression was consistently apparent from the aortic arch down to the iliac artery, with high Cx43 and low desmin levels at the aortic arch and low Cx43 and high desmin levels at the iliac artery. An exception was the ascending aorta, in which both proteins were highly expressed. Quantification of the Cx45 signal showed that the numerical densities of Cx45 gap junctions per cell were significantly higher in the ascending aorta and decreased conspicuously in the aortic arch and in the remainder of the distal artery ($P<0.0001$) (Figure 4E). The mean area of Cx45 gap junctions declined progressively from proximal to distal arterial segments (Figure 4F).

Quantification demonstrated distinctive patterns of desmin, Cx43, and Cx45 expression across the media in the different arterial regions (Figures 4G through 4I). In the ascending aorta, the area of desmin signal per cell (Figure 4G) and the numerical density of Cx43 and Cx45 gap-junctional spots per cell (Figures 4H and 4I) were both significantly lower on the luminal side than on the adventitial side ($P<0.0001$ for Cx43, and $P<0.0005$ for Cx45). In the thoracic aorta, the numerical density of Cx43 gap-junctional spots was lower on the luminal side ($P<0.0001$), but the area of desmin signal per cell was higher ($P<0.0001$). In the abdominal aorta, both sides showed a similar tendency for low Cx43 and high desmin levels ($P>0.05$). For Cx45, no variation across the wall was apparent in the thoracic and abdominal aorta ($P>0.05$).

The relationships between Cx43, Cx45, and desmin were further explored in multiple-labeling studies (Figure 5). In the aortic arch (Figure 5A) and thoracic aorta (Figure 5B), the inverse expression pattern of Cx43 and desmin was found to hold true down to the level of the individual cell, as demonstrated by double labeling with the new anti-Cx43 antibody. Although Cx43 labeling was abundant and prominent between desmin-negative cells, it was much less frequent, and the spots were smaller in size between desmin-positive cells (Figure 5). For Cx45, the punctate labeling in the ascending aorta was clearly seen between desmin-positive cells (Figures 6A and 6B), and much of this signal was colocalized with that for Cx43 (Figures 6C through 6F).

**Expression of Connexins in Small to Medium-Sized Veins**

Apart from its presence in the aorta and iliac artery, Cx45 was also expressed abundantly in the small- to medium-sized
Figure 3. Immunoconfocal images of Cx43 and Cx45 labeling (arrows) in different zones of rat aorta and iliac artery. Cx43 punctate labeling is prominent in the ascending aorta (A), aortic arch (C), and thoracic aorta (E) but is scarce with smaller spots in the abdominal aorta (G) and iliac artery (I). In the ascending aorta (A) and thoracic aorta (E), the labeling tends to be more abundant on the adventitial (AD) side of the vessel than on the luminal (L) side. The labeling of Cx45 is prominent in the medial layers of the ascending aorta (B) but is scarce in the more distal arterial segments (D, F, H, and J). In the ascending aorta (B), the labeling is more abundant on the adventitial side than on the luminal side. Bar=30 μm.
veins along the para-aortic regions, from the aortic root to
distal iliac arteries (Figure 6G). Because internal elastic
laminae in veins of these sizes were not as obvious as those
in large-sized veins and arteries, the distinction between
endothelium and medial layers was difficult to define in the
presence of elastic autofluorescence, and the cellular local-
ization of Cx45 cannot be determined with certainty by single
labeling. To overcome this problem, Cx45 was colabeled
with desmin and VWF for identifying SMCs and endothelial
cells, respectively. In the veins, the majority of medial SMCs
were desmin positive, and it became apparent by triple
labeling that Cx45 in the para-aortic veins was localized to

Figure 4. Histogram showing results of quantification of SMC density, desmin area per cell, Cx43 and Cx45 gap junction mean area,
and number per cell. Medial cellular densities are similar in all arterial segments studied (A). Desmin level is high in the ascending aorta
and low in the aortic arch and progressively increases downward along the course of the aorta (B). Numerical density of Cx43 gap
junctions per cell (C) and mean area of Cx43 spots (D) are high in the ascending aorta, aortic arch, and thoracic aorta but low in the
abdominal aorta and iliac artery. For Cx45, although number per cell (E) and mean area (F) of immunolabeled Cx45 gap junctions are
high in the ascending aorta, the former variable is reduced conspicuously, and the latter declines progressively in distal arterial seg-
ments. Further statistical analysis comparing zones within the media of thoracic aorta shows that desmin levels are higher at the lumina-
lar (L) side than at the adventitial (A) side (G), and Cx43 gap junction densities show an inverse relationship in each case (H). In the
ascending aorta, the “sidedness” of desmin abundance is opposite that of the thoracic aorta, whereas that of gap junctions is the
same. Desmin and Cx43 levels do not show significant differences between the 2 medial sides of the abdominal aorta. For Cx45 gap
junctions, the number per cell on the adventitial side is significantly higher than that on the luminal side in the ascending aorta but not
in the thoracic and abdominal aorta (I). Division between the luminal side and adventitial side was set halfway across the vessel wall.
Analysis carried out by using a section depth of 0.5 μm. **P<0.005; ***P<0.0001.
SMCs in the medial layers and not to the endothelium (Figure 6H). Multiple-labeling studies for Cx40 (Figure 6I), Cx37 (Figure 6J), Cx43 (Figure 6K), and desmin with or without VWF demonstrated that these 3 connexins were all expressed exclusively in the endothelium but were never detected among the venous SMCs.

Discussion

The present study demonstrates 2 key novel findings. First, the inverse relationship between Cx43 and desmin expression in medial SMCs holds true for sequential regions of the aorta down to the iliac artery, with 1 exception—the ascending aorta. Second, we show that in addition to Cx43, vascular SMCs in situ express a second connexin, Cx45. In the vascular system, although Cx45 transcript has previously been detected in a rat aortic SMC line (A7r5 cells), no other reports have previously demonstrated the presence of Cx45 gap junctions in vessel walls or in cultured vascular cells.

Systematic examination of Cx43, Cx45, and desmin expression in the different zones of the rat aorta down to the iliac artery reveals 3 SMC subpopulations. The first subpopulation, predominating in the aortic arch and thoracic aorta, is desmin negative and contains high Cx43 levels, whereas the second subpopulation, predominating in abdominal aorta and iliac artery, is desmin positive and contains low Cx43 levels. The previously identified inverse relationship between Cx43 and desmin expression is thus maintained in these subpopulations. The third subpopulation, which is restricted to the ascending aorta, is different from those identified previously in that it is desmin positive and also expresses high Cx43 levels. This subpopulation also expresses high levels of Cx45, in contrast to the other regions, which have lower levels of Cx45. The labeling for other SMC differentiation makers, such as smooth muscle α-actin (early-stage marker), calponin (midstage marker), and myosin heavy chain isoform SM2 (late-stage marker) was strongly positive in the medial layer of all arterial zones examined, with no detectable regional difference. In the para-aortic small to medium-sized veins, however, a fourth vascular SMC subpopulation, which is desmin positive and contains only Cx45 gap junctions, is present.

Figure 5. Immunoconfocal images of triple labeling for Cx43, VWF, and desmin in rat aortic arch (A) and thoracic aorta (B). In both aortic segments, the Cx43 gap junctions are more abundant and prominent in desmin-negative areas (thick arrows) but sparse and smaller between desmin-positive SMCs (arrowheads), demonstrating the inverse Cx43-desmin relationship down to the individual cell level. In the aortic arch (A), some medium- to large-sized Cx43 gap junctions are noted between desmin-positive and desmin-negative cells (thin arrows). Bar=23 μm.
present. No other connexins are expressed at detectable levels in any of the vascular SMC subpopulations; however, Cx37, Cx40, and Cx43 are all expressed in the venous endothelium.

The present results are in accord with the idea developed from our previous studies, ie, that Cx43 is expressed differentially in phenotypically distinct mature vascular SMCs and that desmin is the key differentiation marker to show any relationship with Cx43 expression.5,6 However, it is now apparent that the expression of an additional connexin, Cx45, has to be considered within this framework. The preferential

Figure 6. Cx45 expression in the rat aortic and venous tissues. Panels B and D through F are enlarged views of the box areas indicated in panels A and C, respectively. Panel E shows the FITC and Cy3 channels of panel D, and panel F shows the FITC and Cy5 channels. In the ascending aorta (panels A through F), Cx45 gap junctions are abundantly present between desmin-positive SMCs (arrows in panel B). Whereas most of the Cx45 signal is colocalized with Cx43 (arrowheads in panels D through F), some gap junctions show only Cx43, not Cx45 (arrows in panels D and F). In the rat para-aortic small- to medium-sized veins, Cx45 gap junctions are abundantly expressed between the desmin-positive medial SMCs (arrows, panels G and H) but not in the endothelium, where Cx40 (arrows, panel I), Cx37 (arrows, panel J), and Cx43 (arrows, panel K) are expressed. L indicates lumen. Bar=20 μm (A and G), 6 μm (B, D through F, and I through K), 40 μm (G), and 10 μm (H).
distribution of 4 phenotypically distinct medial SMC subpopulations in different arterial and venous segments suggests that the regulation of Cx43, Cx45, and desmin expression is closely linked with regional diversity of the vascular system.

From previous studies, we suggested that high Cx43 levels in desmin-negative SMCs of elastic medial regions may be related to extracellular matrix production/maintenance, with low Cx43 levels in desmin-positive cells of muscular medial regions reflecting local regional control of blood flow. The present data extend this concept to the elastic medial regions of different anatomic locations within the aorta. In the rat, the SMCs of the abdominal aorta have higher contractile performance and express lower elastin levels than do those in the thoracic aorta. The general picture to emerge is that in the proximal elastic great arteries (aortic arch and thoracic aorta), the main SMC type consists of desmin-negative cells with high levels of Cx43, whereas in small- to medium-sized muscular arteries, the main cell type is desmin-positive cells with low levels of Cx43, with the abdominal aorta and iliac artery being of an intermediate transitional form with elastic media and desmin-positive/low-Cx43-expressing cells.

The occurrence of phenotypically distinct medial SMCs in different locations may have its origin in embryonic development. During development, SMCs of different embryonic origin are distributed to defined anatomic locations. The distribution boundary of SMCs of different embryonic origin in the aorta appears to correspond to that demonstrated in the present study between desmin-negative/high-Cx43 and desmin-positive/low-Cx43 SMCs. In the avian abdominal aorta and small muscular arteries, the SMCs are known to be of mesodermal origin, whereas those of the aortic arch and thoracic aorta are mainly derived from the ectodermal cardiac neural crest. Functionally, although the participation of SMCs of ectodermal origin is essential in the formation and organization of elastic laminae in the great vessels, the SMCs of mesodermal origin possess a significantly greater capacity for contraction. In contrast to other proximal elastic great arteries, the proximal end of the ascending aorta, the coronary sinuses, does not (at least in the chicken embryo) contain neural crest cells. A recent report suggests that in the rat, the medial SMCs of the proximal ascending aorta may be transdifferentiated from embryonic cardiomyocytes of the left ventricular outflow tract, a cell lineage in which Cx43 expression is high and positively correlated with desmin expression. From the 12th embryonic day, the cardiomyocytes in the distal truncal part of the ventricular outflow tract, which is distal to the emerging locations of the developing aortic arch, similarly express high levels of Cx43. Gap junction channels composed of Cx45 have distinct biophysical properties compared with those made from Cx43 (eg, lower unitary conductance, stronger voltage dependence, and a lower anion-to-cation permeability ratio), potentially permitting distinctive intercellular communication properties according to the type of connexin expressed. Whether low quantities of Cx43 or Cx45 SMC gap junctions in the distal arteries are geared to the needs of delicate regional regulation of vasomotor tone and whether large quantities of Cx45 gap junctions in the venous desmin-positive SMCs are geared to the coordination of more extensive venous contraction (and hence, diastolic return) are yet to be established.

In conclusion, the identification of up to 4 SMC subpopulations in the rat aorta and para-aortic veins that differentially express Cx43, Cx45, and desmin provides a comprehensive picture of regional differentiation of connexin expression in phenotypically distinct medial SMCs of the vessel wall. Further studies are needed to explore the relationship of embryonic origin to vascular SMC phenotype and to determine the roles of SMC Cx43 and Cx45 gap-junctional intercellular communication in different regions of the vascular tree.

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References

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