Connexin45 Cannot Replace the Function of Connexin40 in Conducting Endothelium-Dependent Dilations Along Arterioles

Stephanie E. Wölfle,* Volker J. Schmidt,* Bernd Hoepfl, Andreas Gebert, Sébastien Alcoléa, Daniel Gros, Cor de Wit

Abstract—Intercellular communication through gap junctions coordinates vascular tone by the conduction of vasomotor responses along the vessel wall. Gap junctions in arterioles are composed of different connexins (Cx) (Cx40, Cx37, Cx45, Cx43), but it is unknown whether Cxs are interchangeable. We used mice with a targeted replacement of Cx40 by Cx45 (Cx40KI45) to explore whether Cx45 can functionally replace Cx40 in arterioles. Arterioles were locally stimulated using acetylcholine, bradykinin, adenosine, and K⁺ in the cremaster of Cx40KI45, Cx40-deficient (Cx40ko), and wild-type mice, and diameter changes were assessed by intravital microscopy. Additionally, arterial pressure was measured by telemetry and Cx expression verified by immunofluorescence. Acetylcholine initiated a local dilation of a similar amplitude in all genotypes (~50%), which was rapidly conducted to upstream sites (1200 μm distance) without attenuation in wild type. In marked contrast, the remote dilation was significantly reduced in Cx40ko (25±3%) and Cx40KI45 (24±2%). Likewise, dilations initiated by bradykinin application were conducted without attenuation up to 1200 μm in wild type but not in Cx40ko and Cx40KI45. Adenosine-induced dilations and K⁺-induced constrictions were conducted similarly with decaying amplitude in all genotypes. Arterial pressure was strongly elevated in Cx40ko (161±1 versus 116±2 mm Hg) but only moderately in Cx40KI45 (133±8 mm Hg). This demonstrates that Cx40 function is critical for the conduction of acetylcholine and bradykinin dilations and cannot be substituted by Cx45. Therefore, unique properties of Cx40 are required for endothelial signal conduction, whereas nonspecific restoration of function is critical for the conduction of acetylcholine and bradykinin dilations and cannot be substituted by Cx45.

Key Words: conducted responses ■ gap junctional communication ■ connexin40 ■ connexin45 ■ microcirculation

The adaptation of blood supply to changing tissue demands is a central function of the microcirculation. It requires the regulation of local blood flow in a wide dynamic range, which is provided by coordinated behavior of the cells along the arteriolar wall. Mechanisms that contribute to such coordination are locally generated signals at upstream sites that reflect downstream requirements (flow-induced dilation) and signal transfer through communication channels in the vascular wall itself. This latter communication pathway is provided by homocellular gap junctions composed of connexins (Cxs), which connect adjacent cells through low-resistance channels to a functional syncytium, enabling coordinated behavior. The coordination is reflected by conducted vascular responses that can be initiated by locally confined application of vasoactive compounds that not only cause dilation or constriction (depending on the stimulus) at the application site but also at up- and downstream sites several millimeters along the arteriole. Based on the high-transmission velocity and membrane potential measurements, it was concluded that the coordinating signal is the electrotonic transmission of membrane potential changes.

The pore-forming Cxs comprise different members of a large family that are classified according to their molecular weight. In vascular tissue, 4 different Cx isoforms (Cx37, Cx40, Cx43, and Cx45) have been identified. Of these, Cx40 is of special importance in arterioles because its deficiency impairs the conduction of locally initiated dilations in response to endothelium-dependent dilators. Cx40 is mainly expressed in endothelial cells, suggesting that dilations conduct along the endothelial cell layer. In addition, selective destruction of the endothelium prevented the conduction of dilations in response to acetylcholine (ACh) in the cremaster of mice, further highlighting the importance of the endothelial cells as a conduction pathway. Although Cx37 is also expressed in endothelial cells, it cannot compensate for the lack of Cx40, suggesting specific functions for different Cxs.

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Redundant, but also unique, functions have been revealed by targeted exchange of the coding regions of Cxs by genetic knock-in and generation of animals in which expression of a Cx is replaced by a different family member.7–9 For example, replacing Cx43 with Cx40 or Cx32 prevented perinatal lethality, but resulted in different phenotypes clearly demonstrating specific Cx requirements to serve special needs.7 It is unknown whether the special importance of Cx40 in endothelial cells is attributable to unique properties of this Cx. Whereas Cx40 channels exhibit a large conductance of 200 pS,10 Cx45 channels have a substantially lower conductivity (≈35 pS).11 Moreover, junctional gating and regulation varies between these Cxs.11–15 In the heart, Cx45 can partially replace the function of Cx40.9

In the present study, we assessed the functional equivalence of Cx40 and Cx45 in the microcirculation by studying conducted vascular responses in mice carrying a biallelic replacement of Cx40 by Cx45. Because the lack of Cx40 is associated with an elevated arterial pressure,16 arterial pressure was also assessed using telemetry in these mice and for comparison in Cx40-deficient (Cx40ko) animals.

**Materials and Methods**

**Experimental Setup**

Experiments were performed in accordance with the German animal protection law in mice carrying a biallelic replacement of Cx40 by Cx45 (Cx40K145)9 and Cx40ko mice.17 The animals were backcrossed at least 7 times into a C57BL6 genetic background, and wild-type C57BL6 mice (wt) served as controls. Animals at the age of 3 to 6 months were anesthetized by intraperitoneal injection of medetomidine (0.5 mg/kg), fentanyl (0.05 mg/kg), and midazolam (5 mg/kg), followed by continuous infusion via a catheter placed in the jugular vein. The animals were intubated by tracheotomy to ensure airway patency, and their esophageal temperature was maintained at 37°C by conductive heat. For intravital microscopy the cremaster muscle was prepared as described18 and superfused with bicarbonate-buffered (pH 7.4, 34°C) saline solution (in mmol/L: Na+ 143, K+ 1.2, Cl− 128, HCO3− 25, SO42− 1.2, and H2PO4− 1.2) gassed with 5% CO2 and 95% N2 (pCO2 40 mm Hg, pO2 100, where DTr is the diameter after treatment, DCo is the diameter before treatment, and Dmax is the maximal possible diameter that is the maximally dilated diameter for dilations or the minimum luminal diameter (0) for constrictions. The temporal characteristic of the responses was considered by calculation of the interval between stimulus application and attainment of peak diameter (time to peak) and of the response duration (interval from stimulation to recovery). Comparisons within groups were performed using paired t tests, and, for multiple comparisons, probability values were corrected according to Bonferroni. Data between groups were compared with ANOVA, followed by post hoc analysis of the means. Differences were considered significant at a corrected error probability of P<0.05. Data are presented as means±SEM.

**Experimental Protocols**

Conducted vasomotor responses were initiated in arterioles by locally confined stimulation with the vasoactive compounds Ach (1 mmol/L), adenosine (10 mmol/L), bradykinin (10 mmol/L), or KCl (3 mol/L), which were delivered through glass micropipettes (tip opening 1 to 2 μm) positioned in close proximity of the arterioles by a short pressure pulse (140 kPa, 100 to 500 ms). If a response at the application site (local) was obtained, the same stimulus was again applied and remote, upstream sites were recorded at distances of 300 to 1200 μm upstream. Each substance was studied in duplicate at a single vessel and examined under the microscope. Vascular tone is expressed as the quotient of resting and maximal diameter. Diameter changes were normalized to the maximal possible response: percentage of maximal response=(Dmax−Dtr)/Dmax×100, where Dtr is the diameter after treatment, Dmax is the diameter before treatment, and Dmax is the maximal possible diameter that is the maximally dilated diameter for dilations or the minimal luminal diameter (0) for constrictions. The temporal characteristic of the responses was considered by calculation of the interval between stimulus application and attainment of peak diameter (time to peak) and of the response duration (interval from stimulation to recovery). Comparisons within groups were performed using paired t tests, and, for multiple comparisons, probability values were corrected according to Bonferroni. Data between groups were compared with ANOVA, followed by post hoc analysis of the means. Differences were considered significant at a corrected error probability of P<0.05. Data are presented as means±SEM.

**Results**

**Basal Data**

A total of 102 arterioles with a maximal diameter of 35±1 μm were studied in 51 mice. Resting and maximal diameters of the arterioles studied (16±1 and 35±1 μm, respectively) were not significantly different between genotypes (Table 1). The arterioles exhibited various degrees of tone (from 0.20 to 0.86); however, resting tone was similar...
Table 1. Resting and Maximal Diameters of the Arterioles Studied in Different Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Animals, n</th>
<th>Vessels, n</th>
<th>Resting</th>
<th>Maximal</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>28</td>
<td>42</td>
<td>16±1</td>
<td>33±1</td>
</tr>
<tr>
<td>Cx40ko</td>
<td>11</td>
<td>30</td>
<td>16±1</td>
<td>35±1</td>
</tr>
<tr>
<td>Cx40KI45</td>
<td>12</td>
<td>30</td>
<td>17±1</td>
<td>36±1</td>
</tr>
</tbody>
</table>

Resting diameters were obtained before application of the stimulus and maximal diameter at the end of the experiment during superfusion of a combination of sodium nitroprusside, adenosine, and ACh (30 μM/L each); n indicates No. of investigated arterioles or animals. Values between genotypes were not different.

between genotypes (wt, 0.47±0.02; Cx40ko, 0.46±0.02; Cx40KI45, 0.46±0.03).

Conducted Dilations

Brief local stimulation of arterioles with ACh evoked a transient dilation in wt mice at the stimulation site that attained a maximum of 48±5% within 6.8±0.8 seconds (time to peak) and lasted for 19.3±2.7 seconds (response duration). The local response remained unchanged after treatment with L-NNA and indomethacin (Table 2). In genetically modified animals, ACh induced virtually identical responses at the stimulation site with a maximal dilation of 53±3% (Cx40ko) and 49±4% (Cx40KI45) and an equal time to peak (5.9±0.5 seconds). Likewise, the response duration was not different from wt (Cx40ko, 14±1.2 seconds; Cx40KI45, 15.9±1.6 seconds). In all 3 genotypes, the local dilation was conducted with a high velocity to distant upstream sites (Figure 1) independent of the presence of NO and prostaglandins (Table 2). Thus, the arterioles started to dilate at a distance of 1200 μm without measurable time delay (<1 seconds) in all mice. In wt, the amplitude of the dilation was not reduced even at the furthest distance studied (1200 μm, 44±4%), although there was a slight attenuation of the response duration (15.0±3.1 seconds). Not surprisingly, the amplitude of the dilation decreased in Cx40ko mice with increasing distance from the stimulation site (1200 μm, 25±3%; P<0.05 versus wt; Figure 2) and the response duration was attenuated (1200 μm, 8.8±0.6 seconds; P<0.05 versus wt). Interestingly, the responses at remote sites in Cx40KI45 mice exhibited very similar characteristics. The amplitude of the dilation decreased significantly with distance in these mice and the dilation at 1200 μm upstream was significantly attenuated compared with wt (Figure 2). The response duration was 10.1±0.7 seconds, which was comparable to Cx40ko.

A different endothelium-dependent dilator, bradykinin, also initiated a dilation after local application at the stimulation site, however, with slightly different characteristics. The initial phase resembled the ACh response, but thereafter the arterioles started to dilate at a distance of 600 μm (wt, 73±3%) that was attained later (28.6±1.3 seconds). Consequently, the dilation lasted longer (62±3 seconds; Figure 3). Importantly, and in contrast to ACh, the local dilation was attenuated after inhibition of NO synthase (Table 2). However, local dilations in genetically modified animals resembled the response in wt, and amplitudes and durations were not different (Cx40ko: 69±4%, 65±3 seconds; Cx40KI45: 76±3%, 69±1 seconds; Figure 3). Also, dilations in response to bradykinin conducted along the vessel wall and maintained their typical shape, with a secondary slowly developing dilation as well as their sensitivity toward inhibition of NO synthase (Table 2). However, the amplitude of the dilation was not attenuated at remote sites in untreated wt (1200 μm: 75±3%) but significantly reduced in untreated Cx40ko mice at upstream sites (600 μm: 60±5%, P=0.05; 1200 μm: 40±5%, P<0.001). In Cx40KI45 mice, a comparable attenuation of the amplitude at further distances from the application site was observed (600 μm: 69±1%, P=0.09; 1200 μm: 47±5%, P<0.001; Figure 3). In contrast to wt (59±3 seconds; P=NS versus local), the response duration was shortened in genetically modified mice at 1200 μm (Cx40ko: 42±4 seconds; Cx40KI45: 55±4 seconds; both P<0.001 versus local site).

After stimulation with adenosine, the arterioles dilated at the local site >50% in all genotypes (Figure 4). The response

Table 2. Effect of NO Synthase and Cyclooxygenase Inhibition on Local and Conducted Responses

<table>
<thead>
<tr>
<th>Agonist and Treatment</th>
<th>n</th>
<th>Maximum, μm</th>
<th>Rest, μm</th>
<th>Peak, μm</th>
<th>%</th>
<th>Rest, μm</th>
<th>Peak, μm</th>
<th>%</th>
<th>Rest, μm</th>
<th>Peak, μm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>33±1</td>
<td>15±2</td>
<td>23±2</td>
<td>50±7</td>
<td>14±2</td>
<td>22±2</td>
<td>44±6</td>
<td>18±3</td>
<td>23±3</td>
<td>45±6</td>
</tr>
<tr>
<td>LN+Indo</td>
<td>11</td>
<td>2*</td>
<td>20±1</td>
<td>44±3</td>
<td></td>
<td>11±2</td>
<td>20±1</td>
<td>43±4</td>
<td>12±2</td>
<td>21±2</td>
<td>46±4</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>7</td>
<td>30±1</td>
<td>15±2</td>
<td>27±1</td>
<td>86±2</td>
<td>12±1</td>
<td>24±1</td>
<td>70±6</td>
<td>14±1</td>
<td>25±1</td>
<td>72±6</td>
</tr>
<tr>
<td>LN+Indo</td>
<td>11</td>
<td>1*</td>
<td>20±1</td>
<td>47±6*</td>
<td></td>
<td>10±1</td>
<td>18±1</td>
<td>38±6*</td>
<td>12±1</td>
<td>19±1</td>
<td>43±5*</td>
</tr>
</tbody>
</table>

Arteriolar diameter before application of the stimulus (rest) and the peak during the transient dilation are given for local and upstream sites. These values and the maximal diameter of the vessel (maximum, determined at the end of the experiment) were used to calculate the normalized dilation (percentage of maximal response). n indicates No. of investigated arterioles and animals; LN, L-NNA; Indo, indomethacin. *P<0.05 vs respective control. Resting values were tested only for differences between treatments at the local site, and peak values were not tested.
duration of this dilation was in between that of ACh and bradykinin (50 seconds) and remained unchanged after inhibition of NO synthase and cyclooxygenase (Table 2). In contrast to ACh and bradykinin, the amplitude decreased in wt with increasing distance. A comparable attenuation of the amplitude was also observed in Cx40ko and Cx40KI45 (Figure 4). Thus, the amplitude was not significantly different between wt and genetically modified animals and likewise the response duration was identical (1200 μm: wt, 35±5 seconds; Cx40ko, 29±4 seconds; Cx40KI45, 30±5 seconds), suggesting that adenosine-induced dilations are conducted with similar properties in all genotypes.

Conducted Constrictions
Locally confined stimulation of the arterioles with a short pulse of depolarizing K+ solution (3 mol/L) induced a rapid, brief constriction at the local site with a response duration of ≈8 seconds. Neither the amplitude (Figure 5) nor the duration of the constriction was different between genotypes. This constriction was conducted to upstream sites without measurable delay, ie, at the same rate as the dilatory responses. However, the amplitude of the constriction decreased monotonically with increasing distance in wt as well as in Cx40ko and Cx40KI45 mice (Figure 5). The decline in amplitude was virtually identical in the different genotypes because the amplitude of the constrictions at all upstream sites in Cx40ko and Cx40KI45 was similar to wt.

Arterial Pressure
Arterial pressure was recorded by means of radiotelemetry in awake mice. Pressure was stable in all genotypes over the measurement period of 4 days after implantation. The mean pressure in wt mice (n = 14) ranged from 106 to 121 mm Hg with a mean of 116±2 mm Hg. As expected, mean arterial pressure was significantly elevated in Cx40ko animals (n = 5; ranging from 156 to 163 mm Hg; mean, 161±1 mm Hg; P<0.001). In Cx40KI45 mice, mean arterial pressure was also elevated to 133±8 mm Hg (P<0.001 versus wt; range, 100 to 156 mm Hg); however, mean pressure was significantly reduced compared with Cx40ko. Despite these large differences in pressure, heart rate was similar in all genotypes (wt, 579±23 bpm; Cx40ko, 553±34 bpm; Cx40KI45, 582±19 bpm).

Immunostaining
The expression of Cx40 in vascular cells was studied in cremaster vessels and the thoracic aorta. In both vessel types, Cx40 is mainly located at the borders of endothelial cells, which can be identified by their longitudinal shape and orientation to the vessel axis in the aorta (Figure 6A) and in cremaster arterioles (Figure 6B and 6C). As expected, Cx40 was not detected in Cx40ko or Cx40KI45 mice (Figure 6E and 6F). In wt mice, Cx45 staining was not detectable in endothelial cells; however, smooth muscle cells of arterioles were stained with Cx45.
exhibited a weak staining along cell borders (Figure 6H). In the aorta, specific staining for Cx45 was not detected in the endothelium (Figure 6G). In contrast to wt, Cx45 is abundantly expressed in endothelial cells of the aorta in Cx40KI45 mice and the staining was observed along the cell borders (Figure 6I). Similarly, Cx45 was located at endothelial cell borders in arterioles of Cx40KI45 (Figure 6J and 6K) but not in wt (Figure 6H). Thus, the expression pattern is similar to that of Cx40 in wt. Cx43 was located at smooth muscle but not endothelial cell borders in aortas and at cardiomyocyte borders in atria of wt and Cx40KI45 mice, but it was not detected in small arterioles in the cremaster muscle (Figure I in the online data supplement at http://circres.ahajournals.org).

**Discussion**

Cx40 is crucial for an efficient conduction of dilations initiated by endothelium-dependent stimuli (ACh, bradykinin), as previously demonstrated and confirmed in this study. Herein, we demonstrate that Cxs are not functionally exchangeable in arterioles and that the function of Cx40 cannot be replaced by Cx45, although Cx45 was expressed and present at endothelial cell borders in animals carrying a biallelic exchange of Cx40 by Cx45. This suggests that Cx40 serves specific functions in the endothelium that cannot be substituted by Cx45. However, Cx40 is not required for efficient conduction of every vasodilator because adenosine conduction was not hampered in arterioles deficient in Cx40 or after replacement of Cx40 by Cx45. Similarly, the conduction of vasoconstrictions also remained unaffected, suggesting that these responses are conducted in a Cx40-independent manner, possibly along the smooth muscle cell layer. In contrast to its role in arterioles, other functions of Cx40 may be rescued by Cx45 because the Cx40-associated increase in arterial pressure was significantly reduced in Cx40KI45 mice.
The rapid conduction of locally initiated dilations along the arterioles suggest that the electrotonic spread of local changes of the membrane potential underlies the diameter changes that are observed at distant sites. In fact, hyperpolarizations have been measured in endothelial and smooth muscle cells at distant locations, verifying the spread of membrane potential changes and the ability of vascular cells to conduct hyperpolarizations along the vessel wall. Changes in diameter show that Cx40 is important in the conduction of ACh-induced hyperpolarizations, as previously demonstrated and confirmed in the present study. The remaining dilation at remote sites may involve other Cxs in the endothelium or may be attributable to transmission along the smooth muscle cell layer. Similarly, Cx40 was required to transmit an electrically activated vasodilation along the vessel wall. Interestingly, the conduction of dilations initiated by ACh and bradykinin was impaired to a similar degree in Cx40ko and Cx40KI45 animals, and thus Cx45 did not even partially compensate the loss of Cx40. This lack of functional replacement was observed despite an integration of Cx45 into the membrane of endothelial cells because Cx45 localization in Cx40KI45 in the aorta as well as in the arterioles under study is similar to that of Cx40 in wt mice. Thus, these results are suggestive of a specific role of Cx40 in arterioles possibly related to different channel properties. Gap junctions formed by Cx40 have a large unitary conductance of ≈200 pS, whereas Cx45 channels exhibit a substantially lower conductance (≈35 pS). Furthermore, Cx40 channel gating is not very sensitive to the transjunctional voltage, as opposed to Cx45 channels, and the regulation of these channels by phosphorylation is distinct. Some of these specific properties may be required to support a sufficient charge transfer along the vessel wall, promote electrotonic conduction, and initiate dilations at remote sites in response to ACh. Other factors may contribute as well, eg, heteromeric gap junction channels. Furthermore, the distribution of Cx43 has been reported to depend on Cx40 expression. However, we did not detect expression of Cx43 in small cremaster arterioles in wt mice, in accordance with a previous study that excludes Cx40 as acting to assemble Cx43 in gap junctions in these vessels.

Alternatively, Cx40 may be required to form heterocellular gap junctions between endothelial and smooth muscle cells to support charge transfer between these cells and mediate
endothelium-derived hyperpolarizing factor–type dilations as reported in isolated mesenteric and cerebral arteries of the rat and in an in vitro model of myoendothelial junctions. However, in vivo the role of myoendothelial coupling is less well established. In fact, membrane potential measurements and studies on conduction with selective destruction of endothelial or smooth muscle cell layers suggest that myoendothelial coupling is not prominent in the microcirculation in vivo. Moreover, the present results clearly demonstrate the ability of ACh to initiate a dilation at the stimulation site that was of a similar amplitude in wt and genetically modified mice. Because the ACh-dilation (and its conduction; Table 2) is largely independent of NO and prostaglandins in these vessels, the local dilation is very likely attributable to an endothelium-derived hyperpolarizing factor–type dilation and obviously intact in these mice, indicating impaired longitudinal coupling rather than altered heterocellular coupling.

Recently, additional signaling mechanisms such as a spreading wave of NO release along the endothelium have been suggested to contribute to the conduction process. Such an “NO wave” was specifically important for conducted responses initiated by bradykinin. Also in the present study, local and conducted responses after bradykinin application were significantly impaired after inhibition of NO synthase, demonstrating a contribution by NO. Nevertheless, remote dilations were impaired in Cx40ko mice, as described previously, and attenuated to a similar extent in Cx40KI45. Assuming that the NO wave is related to an endothelial Ca2+ increase and a spreading Ca2+ wave, the present results suggest that Cx45 is also unable to support the spread of such a Ca2+ wave. In contrast to ACh and bradykinin, conducted dilations in response to adenosine remained unaffected by the loss of Cx40 or replacement of Cx40 by Cx45. Therefore, the conduction process involves a Cx40-independent signaling pathway, possibly the smooth muscle layer, which can act as a separate conduction pathway. This view is supported by the fact that the amplitude of the dilation diminishes more rapidly with distance than ACh or bradykinin responses. Thus, the adenosine response resembled the behavior of conducted constrictions initiated by local K+ depolarization. These were also independent of Cx40, intact in Cx40KI45 mice, and displayed a decreased amplitude with distance. Therefore, we suggest that conducted responses initiated by adenosine or high K+ solution are transmitted along the smooth muscle layer independent of Cx40, whereas ACh- and bradykinin-induced responses conduct along the endothelial cell layer in a Cx40-dependent manner. This latter function is not supported by Cx45, and the conduction is therefore similarly impaired in mice carrying a targeted replacement of Cx40 by Cx45. The persisting ACh- and bradykinin-responses at remote sites in mice devoid of Cx40 may reflect conduction along the smooth muscle cell layer and/or remaining endothelial conductivity supported by other Cxs.

Interestingly, Cx40KI45 mice exhibited only a moderate elevation of arterial pressure in contrast to the severe hypertension observed in Cx40ko animals, which was confirmed in this study. The hypertension in Cx40ko mice not only involves an attenuation of conducted responses but also an impairment of the pressure control of renin synthesis and secretion causing hyperreninemia. The lower arterial pressure in Cx40KI45 in comparison to Cx40ko suggests that Cx45 is able to nonspecifically substitute Cx40 functions. Similarly, Cx45 is able to replace Cx40 in the atrioventricular node and right atrium of the heart, whereas Cx40 function cannot be restored in the left atrium. It is tempting to speculate that Cx function with respect to renin suppression is related to mechanisms other than charge transfer or spreading of a Ca2+ wave. However, signaling distances required for this function are shorter and may therefore be rescued by low conductance Cx45 channels.

In summary, Cx45 cannot replace the function of Cx40 in arterioles; thus Cx40 serves unique functions in vascular tissue such as the conduction of signals along the endothelial cell layer. Although endothelial Cx45 expression in Cx40KI45 mice is similar to that of Cx40 in wt mice, the spread of dilations initiated by endothelium-dependent stimuli were impaired to a similar extent in the transgenic animals. The unhindered conduction of adenosine dilations and vasoconstrictions after depolarization suggest that in addition to endothelial cells, a further conduction pathway is provided by smooth muscle cells that is independent of Cx40. The remaining remote dilations on ACh and bradykinin stimulation observed in Cx40ko and Cx40KI45 mice possibly relies on transmission along this pathway. Thus, targeted replacement of Cxs provides a useful tool to dissect unique from shared functions of Cxs and clarifies the complex Cx diversity.

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Disclosures
None.

References


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Online Figure: Expression of connexin43 in tissues of wildtype and transgenic mice

Cx43 (red) is located at smooth muscle cell borders (visualized at the cutting edge of the preparation) in the aorta of wt (A, viewed en face) and Cx40KI45 mice (B) but not in endothelial cells or at endothelial cell borders. The Cx43 antibody also stained cell borders of cardiomyocytes in the atrium in both genotypes (C, D). However, Cx43 was not detected in small arterioles (diameter < 50 µm) of the cremaster muscle in wt (E) or Cx40KI45 mice (F). Dashed lines indicate arterioles, nuclei were stained in the cremaster preparation using bisbenzimide (blue). Thus, Cx43 expression is similar in wt and Cx40KI45 mice in these tissues.

Bars represent 20 µm. Images are representative for n = 3 experiments. The specificity of the antibody has been shown previously.\textsuperscript{1,2}

References

