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tered gap junction signaling has been associated with endothelial dysfunction, reduced capacity for arterioles to vasodilate, impaired conduction, and hypertension. However, the way in which the proteins composing gap junctions, connexins (Cx), may be able to differentially regulate the vascular physiology, especially in regards to conduction and possibly different forms of blood pressure control, is not well understood. In this edition of Hypertension, Morton and colleagues aimed to investigate how Cx40 in the endothelium could regulate endothelial conduction and how it may affect blood pressure regulation.1 Although several other studies have focused on this (eg, Ref 2–4), the authors attempted a more nuanced approach to their work by inserting novel mutations in Cx40. What sets this work apart is that although the Cx40 is still present, the introduced mutations are reported to produce non-functional Cx40 gap junction channels in vitro and leads to an elevation in a specific form of blood pressure in mice after a loss of conducted vasodilation.

Vasodilation is required during exercise to coordinate the perfusion of active muscles (functional hyperemia) and can possibly contribute to overall blood pressure by reducing peripheral resistance and, by extension, likely increase the cardiac output. This concept of activity-associated increases in blood pressure, for example, activity-associated hypertension, has been hypothesized as a possible prognostic tool to identify hypertension-prone, normotensive patients.5 Morton et al examined these phenomena by developing an endothelial-specific Cx40 transgenic mouse model (Cx40T152ATg), which expresses both wild-type and mutant Cx40. In the Cx40T252ATg mice, radiotelemetry measurements revealed a significant elevation in systolic blood pressure associated with increases in cardiac output during exercise.6 As cardiac output plays a significant role in the responses, the authors also described increases in systolic blood pressure in the transgenic mice during rest without a significant alteration in heart rate, suggesting that disruption in the conducted response in the endothelium plays a role in increased blood pressure independent of cardiac output.

In this study, the authors attribute the change observed in blood pressure in the Cx40T152ATg mice with a change in conducted vasodilation. In the 1980s, studies by Segal and Duling demonstrated that conducted (ascending) vasodilation against the flow of blood occurred in the endothelium of resistance arteries.6 This was the first indication for a possible role for intracellular communication in the regulation of blood vessel dilation. In the resistance vasculature, endothelial cells, coupled by gap junctions, propagate signals along the length of the endothelium and directly with vascular smooth muscle cells through the myoendothelial junction. Hexameric association of the Cx proteins produces a plasma membrane hemichannel, which docks with adjacent hemichannels on surrounding cells to form a gap junction. Within the vasculature, there are 24 Cx: Cx37 and Cx40 predominantly expressed in endothelial cells and Cx43 and Cx45 in vascular smooth muscle cells. These channels allow for direct, intracellular passage of small molecules (around 1 kDa). In particular, the endothelial Cx, Cx40, has been identified as having a critical role in the conducted vasodilation responses.7

In the current study by Morton et al, the authors show that a functional Cx40 channel is essential in ascending vasodilation and blood pressure regulation during activity in mice. Investigating the role of Cx40 in the vasculature has been complicated through compensation mechanisms of the renin-secreting system. De Wit et al were the first to show that Cx40−/− mice presented with a significantly hypertensive phenotype.2 However, it was later demonstrated that the hypertension in these mice was related to an increase in renin secretion.3 Over the following years, several Cx40 models were developed to test the role of Cx40 in hypertension, for example, site-specific mutations Cx40A96S but similarly resulted in renin-dependent hypertension.7 This work has complicated studies investigating the importance of the endothelium-derived hyperpolarization response and vasodilation in the development of vascular disease.8 In a recent study by Wagner et al, 2 mouse models were generated to test the effects of Cx40 by specifically knocking the protein out in renin-producing cells (Ren-Cre Cx40fl/fl) or in endothelial cells (Tie2-Cre Cx40fl/fl). The endothelial-specific Cx40 knockout mice did not present with increased arterial pressure or with increases in renin secretion.3 Their results suggesting that vascular communication through Cx40 channels only play a limited role in vasodilation and hypertension. In the study by Morton et al, the authors aimed to take a closer look at the roles for Cx40 both in vitro and in vivo. The authors suggest that the Tie2Cx40Ko mice published by
Wagner et al were complicated not by a loss of renin, but by reductions in the Cx37 gap junction proteins, suggesting that other elements of the conduction system were affected in their mice. The endothelial-targeted Cx40T152ATg mice generated by Morton et al express a nonfunctional Cx40 gap junctions, which can target the membrane and stabilize the Cx37 gap junction channels, resulting in no alterations in Cx37 expression. As discussed above, the Cx40T152ATg mice display elevated systolic blood pressure at rest and during activity, indicating that Cx40 gap junction channels were important for regulating vasodilation. In part, the differences between the Morton and Wagner studies could be explained by a loss of endothelial Cx37 in the Tie2Cx40ko mice. However, the contribution of Cx37 gap junction channels to ascending dilation seems to be minimal as shown in both the Wagner and Morton studies. Thus, it is not clear how the alteration in targeting of this channel effects on vasodilation remains to be thoroughly investigated.

The study performed by Morton focuses on the molecular questions regarding whether Cx40 can alter the endothelial conducted response and vasodilation, yet the question remains as to how these findings relate to endothelial dysfunction and the development of hypertension in humans. In 2006, a study by Firouzi et al reported that a mutation in the promoter region of Cx40 lead to reduced Cx40 expression and suggested that this mutation could be a genetic susceptibility factor for essential hypertension in men. Later studies by Pfenniger et al failed to identify this correlation, but the authors suggested that alterations in channel function may be important in regulating cardiovascular disease. Thus, currently there are no clear genetic links between these channels and the development of hypertension. The Cx40T152A mutation used in the study by Morton et al have not been identified in human disease, although there are parallels with this mutation and the Cx40A96S mutations, which has been shown to produce atrial fibrillation in humans and renin-dependent hypertension in mice. In vitro studies by Morton et al demonstrated that the Cx40T152A gap junction channels are functionally dominant over wild-type Cx40 channels, chemical and electrically inactive, and importantly renin secretion is not altered in the Cx40T152ATg mice. Similar to the Cx40KO mice, the ascending vasodilation in Cx40T152ATg mice is significantly attenuated, demonstrating the essential role for Cx40 in endothelium-derived vasodilation. Thus, their model has some specific advantages over previously mentioned models in altering channel function, allowing for a more accurate assessment of the role of Cx40 in conducted vasodilation, which may prove a valuable tool for future studies in evaluating their role in the development of hypertension.

The work by Morton et al further demonstrates a role for Cx40 in conducted vasodilation. However, many questions still remain to be answered as to whether this relates to the development of hypertension. The study does highlight the dependence of properly targeted Cx40 in stabilizing Cx37 at the cell membrane in endothelial cells, but it is not clear what the role for this is. In the in vitro studies performed by Morton, C37-Cx40T152ATg heteromeric channels were functional, indicating that Cx37 may in some way rescues the phenotype of the nonfunctional Cx40 channels. Yet, in vivo this did not occur. It was suggested that, although possible in vitro, Cx37-Cx40 channels did not occur in vivo, potentially explaining why the conducted vasodilation is not resuved by Cx37 in vivo, yet this remains to be explored in much more detail. As shown in this study, Cx37 is highly expressed within the endothelium, forming a tight network of connectivity between endothelial cells; yet, these gap junctions do not confer a significant effect on ascending vasodilation, leading to the question of what these proteins are doing in the endothelium. It is possible that the Cx37 channels are post-translationally modified, for example, nitrosylation or phosphorylation, closing the Cx37 gap junctions in vivo; yet, this remains to be investigated. Thus, it remains unknown what the functional interplay between Cx40 and Cx37 in modulating endothelial cell conducted responses is exactly. The studies by Morton et al do show an important function of Cx40, independent of renin secretion, in regulating vascular dilation; however, the effects on blood pressure are still difficult to compare with that seen when Cx40 is deleted from renin-secreting cells or Cx40KO mice. Further studies in the Cx40T152ATg mice, looking at contributing factors to hypertension, such as gender, genetic factors, protein pathways, and more, may provide greater insight as to how Cx40 alters the vascular reactivity in hypertension and may provide a valuable tool in identifying markers in patients at risk of developing hypertension.

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References


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