A Novel APJ Signaling Cascade That Regulates Cardiovascular Development

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The apelin receptor, APJ, is a G-protein–coupled receptor that was discovered in 1993 based on its homology to the angiotensin II type 1 receptor. The endogenous APJ ligands were identified 5 years later and are a group of peptides referred to as apelins that are all derived from a single prepeptide. The apelins vary in length and in their ability to induce APJ signaling and trafficking but are often treated as a single entity, with apelin referring to the most potent 13-amino-acid fragment. Canonical apelin-stimulated APJ signaling involves activation of pertussis toxin-sensitive G-proteins (G\(_i\) or G\(_o\)) leading to reduced cAMP production, as well as the activation of phospholipase C, protein kinase C, and the extracellular signal–regulated kinases 1 and 2. Some studies also show that part of apelin–APJ signaling is Gi-dependent. Apelin induces an inotropic response in cardiomyocytes by increasing the amplitude of the [Ca\(_{\text{2+}}\)] transient in a protein kinase C–dependent manner. Correspondingly, cardiomyocytes from apelin-deficient mice are unable to compensate for increased pressure load and the development of cardiac failure is accelerated in these mice. In addition, stimulation of APJ with apelin leads to recruitment of β-arrrestins, a group of scaffold proteins that not only regulate receptor desensitization and internalization but also activate alternative G-protein–independent signaling pathways downstream of G-protein–coupled receptors. Recent studies have highlighted the sophistication of APJ signaling and the exposure of cardiomyocytes to stretch, for instance, biases APJ to β-arrestin recruitment rather than G\(_i\) activation, shifting the balance away from cardioprotection and toward hypertrophy and the transcription of genes associated with the fetal gene program (Figure). Like many other G-protein–coupled receptors, APJ can form heterodimers with the angiotensin II type 1 receptor. G\(_{\text{q/13}}\)-dependent activation of APJ causes HDAC phosphorylation and nuclear translocation (Figure). These in vitro observations were confirmed in vivo because Apj\(^{-/-}\) embryos show increased endothelial nuclear accumulation of HDAC4 and HDAC5. How exactly G\(_{\text{q/13}}\) activation by APJ causes HDAC phosphorylation is unclear. The activation of G\(_{\text{q/13}}\) by apelin–APJ signaling elicited the phosphorylation and nuclear export of class II histone deacetylases (HDACs) in cultured endothelial cells (Figure). These in vitro observations were confirmed in vivo because Apj\(^{-/-}\) embryos show increased endothelial nuclear accumulation of HDAC4 and HDAC5. How exactly G\(_{\text{q/13}}\) activation by APJ causes HDAC phosphorylation is unclear. Class II HDACs are generally phosphorylated by protein kinase D or the Ca\(_{\text{2+}}\)/calmodulin-dependent kinase II, but there are only few accounts of G\(_{\text{q/13}}\) activation and no previous reports of G\(_{\text{q/13}}\)-dependent regulation of HDAC5.

One currently unanswered question is the role of the agonist apelin in the newly described APJ signaling pathway. In endothelial cells that endogenously express APJ, the G\(_{\text{q/13}}\)/HDAC4 or HDAC5/MEF pathway could be enhanced with the addition of apelin or reduced by the small interfering RNA–mediated downregulation of APJ. However, Kang et al reported that the genetic deletion of APJ in mice previously was reported to result in normal embryonic development and normal adult appearance and cardiovascular parameters, low numbers of homozygous Apj\(^{-/-}\) offspring were born in some genetic backgrounds, and the absence of APJ in other model organisms has resulted in significant impairment of cardiovascular development. In this issue of Circulation Research, Kang et al had a closer look at embryonic development in Apj\(^{-/-}\) mice and report that it is far from normal. The authors have documented many cardiovascular malformations of varying severity, including incomplete looping of the primitive heart tube and delayed atrioventricular cushion formation, resulting in significant lethality at different embryonic and neonatal stages. Furthermore, many of the Apj\(^{-/-}\) animals that do survive to adulthood present with cardiac malformations, including ventricular septal defects.

In the developing heart, APJ is predominantly, but not exclusively, expressed in the endothelial and endocardial cell layers. Previous publications have described the apelin-dependent regulation of Krippel-like factor-2 (KLF2) expression in endothelial cells and, correspondingly, KLF2 expression is reduced in Apj\(^{-/-}\) embryos. KLF2 is upregulated in the endocardium at approximately embryonic day 9.5, when it plays an important role in atrioventricular cushion formation, and KLF2\(^{-/-}\) embryos display cardiac defects similar to those seen in Apj\(^{-/-}\) embryos, albeit with stronger penetrance. Given the known link between KLF2 and myocyte enhancer factor (MEF) 2 transcription factors, the authors hypothesized that apelin–APJ signaling induces MEF-2 activation. This turned out to be the case, and Kang et al found the 2 G-proteins most frequently linked to APJ signaling, that is, G\(_{\text{q/13}}\) and G\(_{\text{q/13}}\), were not involved in the apelin–APJ-mediated activation of MEF-2, whereas G\(_{\text{q/13}}\) was involved. Furthermore, the activation of G\(_{\text{q/13}}\) by apelin–APJ signaling elicited the phosphorylation and nuclear export of class II histone deacetylases (HDACs) in cultured endothelial cells (Figure). These in vitro observations were confirmed in vivo because Apj\(^{-/-}\) embryos show increased endothelial nuclear accumulation of HDAC4 and HDAC5. How exactly G\(_{\text{q/13}}\) activation by APJ causes HDAC phosphorylation is unclear. Class II HDACs are generally phosphorylated by protein kinase D or the Ca\(_{\text{2+}}\)/calmodulin-dependent kinase II, but there are only few accounts of G\(_{\text{q/13}}\) activation and no previous reports of G\(_{\text{q/13}}\)-dependent regulation of HDAC5.

The apelin–APJ axis plays an important role in cardiovascular physiology, regulating angiogenesis and vascular and embryonic maturation, blood pressure control, and cardiac performance.
overexpression of APJ alone was sufficient to activate this pathway in COS7 or human endothelial cells and the dual overexpression of APJ and apelin failed to enhance the response. Such findings may imply that either APJ shows intrinsic activity or sufficient ligand is produced by the cells and present in the culture medium to induce significant APJ signaling. At least in vivo, apelin is mainly expressed by endothelial cells, making the latter a plausible option. There is, however, at least circumstantial evidence for the existence of apelin-independent APJ signaling because the phenotype of apelin-deficient (apln−/−) mice is distinctly different from that of Apj−/− mice. In contrast to the Apj−/− mice, apln−/− mice on the same genetic background are fully viable and do not show any developmental defects. Furthermore, although Apj−/− mice are protected from pressure overload–induced heart failure, apln−/− mice show accelerated cardiac failure. This discrepancy has been attributed to stretch-induced β-arrestin recruitment to APJ leading to prohypertrophic gene expression that can be overcome by apelin. At present, it is not known whether endothelial APJ signaling also interferes with mechanotransduction, but the exposure of endocardial cells to fluid shear stress is vital in the early stages of cardiac development and drives the expression of KLF226,20 that also depends, at least in part, on APJ.14

Given the predominant endothelial and endocardiac expression of APJ, Kang et al13 focused their attention on endothelial cell signaling. APJ expression, however, is not restricted to these cells and an important remaining question is how other cell types in the developing heart are affected by APJ deficiency. Certainly, the signaling defects found in cultured APJ-deficient endothelial cells could be confirmed in the Apj−/− embryos and deletion of KLF2, which in embryonic development is selectively expressed in endocardial cells,21 causes a highly similar phenotype. On the contrary, the importance of apelin–APJ signaling in adult cardiomyocytes is well-documented and both in vivo and in vitro models have shown that APJ is also important in driving cardiomyocyte differentiation of progenitor cells, particularly for correct expression of proteins building the contractile apparatus,12,13,22,23 making it highly probable that APJ deficiency affects more than endothelial signaling even during embryonic development. The cell type–specific Apj−/− mice that currently are being generated should shed more light on these issues.14

In summary, Kang et al13 add another layer of complexity to APJ signaling by describing a novel APJ signaling route in endothelial cells through Gα13, leading to phosphorylation of class II HDACs and subsequent activation of MEF-2a/c–dependent transcription. The described developmental defects of variable severity provide more insight into the redundancy in signaling pathways contributing to congenital heart defects. However, they open our eyes to a completely novel signaling pathway downstream of APJ that should be taken into account when considering the therapeutic potential of apelin in the therapy of heart failure.24

**Sources of Funding**

Studies performed in the author’s own laboratory were supported by the Deutsche Forschungsgemeinschaft (SFB 834/A4 and Exzellenzcluster 147 Cardio-Pulmonary System) to LF.

**Disclosures**

None.

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Key Words: apelin receptor APJ mouse cardiovascular development Gq13 GTP-binding proteins MEF2A and MEF2C
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Circ Res. 2013;113:4-6
doi: 10.1161/CIRCRESAHA.113.301632

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