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MINI REVIEW

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Endogenous cardiac catecholaminergic systems in cardiac development, physiology, and pathophysiology

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Abstract

Catecholamines, canonically associated with the sympathetic nerves and the adrenal medulla, are also produced by neuroparacrine cells within the heart. Despite their putative importance, the precise functions of these neuroparacrine cells in the heart are not well understood and their clinical implications remain to be defined. In this review, we first explore the historical context and recent advances in research on intrinsic cardiac adrenergic (ICA) cells, focusing on their unique characteristics, distributions, and progenitor-like potential during cardiac development. We then examine their contributions to both physiological and pathological cardiac states. We further discuss a recently identified population of catecholaminergic cardiomyocytes; we discuss their involvement in cardiac development, maturation, and their potential interaction with sympathetic innervation during development. By reviewing these findings, we provide new insights into the endogenous production of catecholamines within the heart and its relevance to cardiac development, physiology and disease, and its potential clinical implications.

Keywords: Catecholamines, Intrinsic cardiac adrenergic cells, Catecholaminergic cardiomyocytes, *Dbh*, Pnmt

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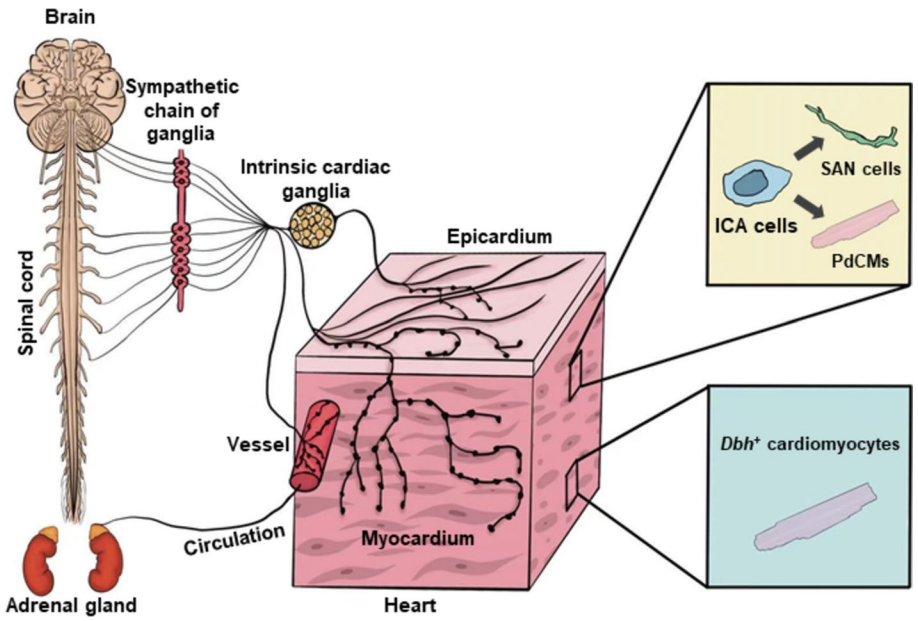
Graphical Abstract



Introduction

Catecholamines are a family of related small molecules, with certain members acting as signaling molecules. For example, dopamine, noradrenaline, and adrenaline function as hormones across autocrine, paracrine, and endocrine pathways both within and outside the nervous system [1–3]. The differing chemical structures of the catecholamines lead to differing receptor binding affinities, across a range of evolved catecholaminergic receptors. For example, noradrenaline and adrenaline have the highest potencies for the β_1-adrenoreceptors (β_1-AR) and β_2-adrenoreceptors (β_2-AR) expressed in cardiomyocytes in the heart [4, 5]. These G_s-coupled receptors act to stimulate cardiac pumping through positive chronotropic, inotropic, lusiotropic, and dromotropic effects that result in increased cardiac output. Canonically, these catecholamines are released from cardiac sympathetic nerve endings or chromaffin cells of the adrenal medulla [6–8]. However, non-neuronal catecholamines endogenous to the cardiac parenchyma are increasingly being discovered. This review aims to provide detailed insights into endogenous catecholamine signaling in the heart, including intrinsic cardiac adrenergic (ICA) cells, phenylethanolamine-*N*-methyl transferase$^+$ (Pnmt$^+$)-cell-derived cardiomyocytes, and dopamine β-hydroxylase$^+$ (*Dbh*$^+$) cardiomyocytes. A comprehensive understanding of cardiac catecholamine signaling may help find directions for further research.

Early studies of catecholamines in the heart

Catecholamines in the heart conventionally arise from sympathetic innervation and circulating endocrine catecholamines. The sympathetic innervation of the heart is part of the classical sympathoadrenal axis involved in cardiovascular regulation [9].

However, haematogenously-circulating catecholamines are released from the adrenal medulla, with a limited amount derived from the overflow of noradrenaline and dopamine secreted in synapses at target organs innervated by postganglionic sympathetic nerves [4]. However, pioneering studies investigating the cardiac response following interventions such as cardiac denervation, adrenalectomy, and transplantation demonstrate persistent cardiac adrenergic influence, supporting that an intrinsic cardiac catecholaminergic system may be present, in addition to the well-established sympathoadrenal system.

Specifically, Donald and Shepherd [10] presented intriguing findings regarding cardiac output in response to exercise in dogs subjected to chronic cardiac denervation and adrenalectomy. They reported that the cardiac response remained adequate and dynamic despite less optimal heart rate responses in these conditions with the primary increased being mediated through stroke volume increases - which was mimicked more by adrenaline infusion than noradrenaline. Subsequently, Elayan et al. found that sympathetically-denervated rat cardiac tissue retained adrenergic enzymatic activity and was capable of adrenaline production [11]. Extending this line of inquiry to human cardiac dynamics, investigations by Shaver et al., involving patients after cardiac transplantation, revealed normal cardiac function even in the absence of restored cardiac sympathetic innervation [12]. Collectively, although catecholamines are crucial for the normal function of the heart, intrinsic cardiac mechanisms ensure dynamic function can be maintained in the absence of sympathoadrenal axis dominance and these mechanisms resemble those elicited by adrenaline. The persistent, presumably non-neuronal presence, of adrenergic biosynthesis, supports the putative existence of an intrinsic cardiac adrenergic system operating in tandem with the sympathoadrenal system, contributing to the production of endogenous catecholamines within the heart [13].

Early investigations into catecholamines in the developing heart provided evidence suggesting that the heart may be a source of endogenous catecholamines during its early developmental stage. Precisely, pioneering work by Ignarro and Shideman [14] demonstrated the presence of dopamine, noradrenaline, and adrenaline in the embryonic developing chick heart during the initial days of incubation. Intriguingly, the endogenous concentrations of these catecholamines exhibited fluctuations throughout cardiac development and persisted for a short period post-hatching [13, 14]. In parallel, Thomas et al. [15] identified the expression of adrenaline in the embryonic developing mouse heart as early as embryonic day 10.5 (E10.5). Enzymes crucial for the biosynthesis and metabolism of catecholamines were also detected in the developing heart [13, 15]. For instance, López-Sánchez et al. observed tyrosine hydroxylase (TH), the first rate-limiting enzyme that catalyses the conversion of the amino acid L-tyrosine to L-DOPA in the catecholamine biosynthetic pathway—and is generally used as a neuronal marker—in the embryonic developing chick heart [13, 16]. Subsequently, Ignarro and Shideman confirmed the presence of dopamine β-hydroxylase (DBH), an enzyme responsible for catalyzing the conversion of dopamine to noradrenaline, in the embryonic developing chick heart [14]. Furthermore, Ebert et al. demonstrated the presence of phenylethanolamine-*N*-methyl transferase (Pnmt), a key enzyme responsible for catalyzing the conversion of noradrenaline to adrenaline in the final step of the catecholamine biosynthetic pathway, as early as E9.5 in the developing rat heart [17]. In addition, Ignarro and Shideman identified

the presence of catecholamine metabolic enzymes, including monoamine oxidase and catechol-*O*-methyl transferase, in the embryonic developing chick heart [13, 18].

Notably, during the early developmental stage, cardiac sympathetic innervation is absent. Cardiac sympathetic nerves, appearing after Hamburger–Hamilton (HH) stage 20 in chicks [19], E11.5 in mice [20], E16.0 to E17.0 in rats [21], and week 5/6 in humans [19], are established after the initiation of catecholamine production in the embryonic heart. Simultaneously, in addition to cardiac sympathetic nerves, the adrenal medulla begins its formation around E13.5 in mice, with the earliest expression of Pnmt detectable between E15.5 and E16.5 in rat adrenal medulla [22]. Consequently, these findings suggest the existence of intrinsic cardiac non-neuronal adrenergic cells in early developmental stages within the embryonic heart. These cells possess the potential capability to produce catecholamines, developmentally preceding their production in the adrenal medulla or cardiac sympathetic nerve endings. This intriguing interplay highlights the intricate regulatory mechanisms governing catecholamine dynamics during cardiac development. Across different embryonic species, one of the most striking findings is the detection of cardiac adrenergic signals well before cardiac sympathetic innervation and even before the first heartbeat. In fact, Fujinaga and Scott used reverse transcription polymerase chain reaction (RT-PCR) to detect *Th* and *Dbh* mRNA (prior to development of the classical sympathoadrenal lineage) in rat embryos as early as E7.0, more than 2 days before heartbeat initiation at E9.5, indicating adrenergic preparedness prior to pacemaker cell development and functioning [23].

Endogenous cardiac catecholamine secreting cells

Intrinsic cardiac adrenergic cells

In early 1990s, Huang et al. [24] pioneered the idea of an intrinsic cardiac adrenergic system within the mammalian heart, supplementing the well-established sympathoadrenal system. This groundbreaking work involved comprehensive in situ and in vitro immunohistochemistry experiments of neonatal and adult rat hearts, as well as fetal human hearts. In their analysis, the researchers identified a distinct class of cardiac cells, unrelated to cardiac sympathetic axons or neurites, which exhibited positive staining with an anti-TH antibody in both rat and human hearts. These cells contained the major catecholamine biosynthetic enzymes TH, DBH, and PNMT [24]. These cells were subsequently named intrinsic cardiac adrenergic (ICA) cells.

To unravel the intricacies of ICA cells within the cardiac microenvironment, previous researchers have developed two distinct mouse models, the Pnmt-GFP knock-in mouse model and the Pnmt-reporter mouse model [13, 25, 26]. The first genetic modified mouse model designed for the investigation of ICA cells was the Pnmt-GFP knock-in mouse model. Mice harboring this Pnmt-GFP mutation exhibit green fluorescence in cells expressing the Pnmt gene, thereby facilitating the identification, isolation, and characterization of all Pnmt-expressing cells, including ICA cells. The second genetically modified mouse model, the Pnmt-Cre/R26R mouse model, was generated by crossing the Pnmt-Cre mouse model with the Rosa26 reporter (R26R) mouse model. The R26R mouse model involves a specific targeted insertion of the LacZ reporter gene into the Rosa26 locus, which is ubiquitously expressed but initially silenced by a loxP-flanked STOP cassette [27]. When Pnmt-Cre mice are crossed with

R26R mice, the offspring have both genetic modifications. In cells expressing Pnmt, Cre recombinase is produced, which excises the STOP cassette at the Rosa26 locus. This leads to the activation of the LacZ reporter gene specifically in Pnmt-expressing cells. The expression of LacZ results in the production of β-galactosidase, which can be detected using histochemical staining with XGAL, producing a blue color in Pnmt-expressing cells. Due to the irreversible genetic change induced by the Cre recombinase enzyme, continuous expression is unnecessary for activating LacZ expression. Cells can be permanently marked as β-galactosidase positive, even with transient Cre recombinase expression. As the Cre recombinase enzyme is introduced into the Pnmt locus, cells transiently expressing Pnmt during mouse development exhibit β-galactosidase positivity. Consequently, the Pnmt-Cre/R26R mouse model serves as a valuable tool for conducting genetic fate-mapping experiments for ICA cells [13].

Characteristics of ICA cells

To elucidate the morphology and ultrastructure of ICA cells, a series of electron microscopic investigations have been undertaken, primarily focusing on primary isolates of ICA cells. Huang et al. contributed significantly to this understanding, demonstrating that isolated ICA cells, when stained positively with anti-Th and anti-neuron-specific enolase (Nse) antibodies in the adult rat heart, exhibited a distinct roundish-shaped gross morphology (Fig. 1A). Expanding the scope beyond adult rat

(See figure on next page.)

Fig. 1 Morphology, distribution, co-staining, and noradrenaline (NA) uptake of ICA cells. (**A**) Isolated ICA cells in the adult rat heart stained positively with anti-Th antibodies (left) and anti-Nse antibodies (right). Scale bar: 10 µm. Pictures were reprinted from ref. [24] with permission obtained from the copyright holder. (**B**) The co-localizing immunoreactivity of noradrenaline transporter (Nat; left) and Th (middle) in fetal rat ICA cell–myocyte co-cultures. The nuclei represent ICA cells and myocytes (right). Scale bar: 20 µm. (**C**) [^{3}H] noradrenaline uptake in control conditions and in the presence of exogenous noradrenaline (1 µM) and nisoxetine (1 µM). * $P < 0.05$ and ** $P < 0.01$ (ANOVA). (**B** and **C**) were reprinted from ref. [30] with permission obtained from the copyright holder. (**D**) Co-expression of XGAL and Hcn4 in the sinoatrial node (SAN) in the embryonic Pnmt-Cre/R26R mouse heart at E15.5. XGAL staining (left), Hcn4 staining (middle), and overlay graph (right) of the SAN. Scale bar: 0.1 mm. Pictures were reprinted from ref. [26] with permission. (**E**) Distribution pattern of the XGAL staining in the adult Pnmt-Cre/R26R mouse heart. Left is a 2D section at 20 µm. Right is 3D image generated from the 2D section. Pictures were reprinted from ref. [35] with permission obtained from the copyright holder. (**F**) Co-expression of Hcn4 and Th in TGAC8 and wild-type mouse models. Scale bar: 20 µm. Pictures were reprinted from ref. [34] with permission obtained from the copyright holder. (**G**) Exclusive expression of *CGRP* mRNA in ICA cells and co-expression of CGRP and DOR in both human and rat LV myocardium. Human section (i–vi): (i) *TH* and *CGRP* mRNA in left ventricular myocardium. H, human LV myocardium; Neu, human neuroblastoma cell line. (ii) ICA cells (arrow) stained positively with anti-CGRP antibody (brown). (iii) The expression of *CGRP* mRNA (green) in an ICA cell cluster. (iv) An ICA cell co-stained positively with anti-CGRP (green) and anti-TH (red) antibodies. (v) Another ICA cell co-stained positively with anti-CGRP (green) and anti-TH (red) antibodies. (vi) An ICA cell co-stained positively with anti-DOR (red) and anti-TH (green) antibodies. (vii) A rat ICA cell co-stained positively with anti-Cgrp (green) and anti-Th (red) antibodies. Scale bar: 10 µm. Pictures were reprinted from ref. [44] with permission obtained from the copyright holder. (**H**) (i and ii) Immunofluorescent dual labeling of TH and C-KIT (a progenitor cell marker) highlights their colocalization (insert) in dividing (binucleated) ICA cells from an autopsy-derived LV myocardial sample. (iii) Immunoperoxidase labeling of TH (brown DAB staining) in two ICA cell clusters in another autopsy-derived LV sample of a human heart allograft, with the upper cluster located in close proximity to an arteriole (A) and a venule (v). (iv) High-magnification (100×) image of the ICA cell cluster shown in (iii). Scale bar in **C**: 50 mm, all others 10 mm. (Pictures are unpublished observations of Minghe Huang)

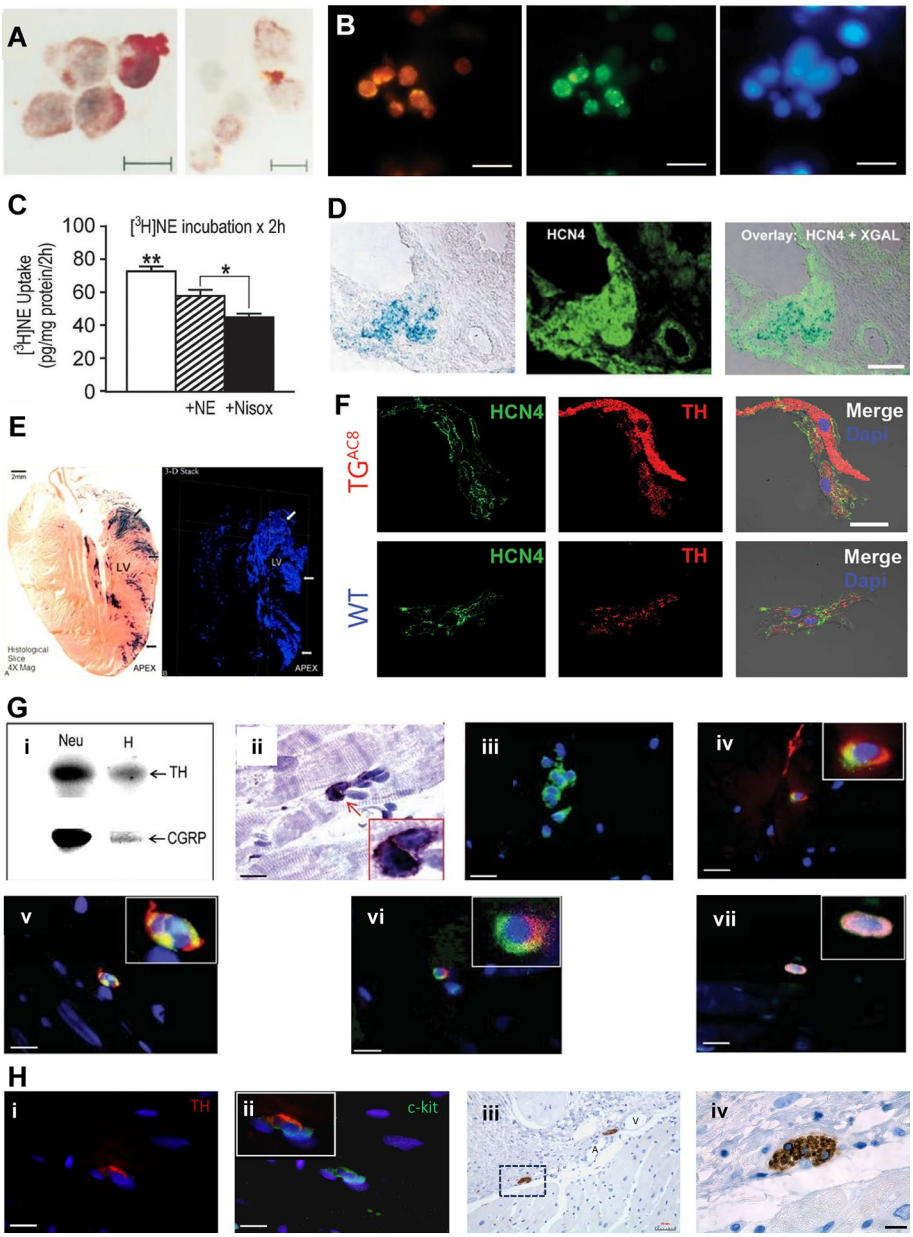


Fig. 1 (See legend on previous page.)

hearts, the same researchers identified ICA cells with a similar roundish-shaped gross morphology, stained positively with an anti-TH antibody, in two fetal human hearts [24]. Building upon these observations, Natarajan et al. not only validated the roundish shape of ICA cells but also introduced a new dimension to their morphology. In their study on neonatal rat hearts, ICA cells were not only co-stained positively with anti-Pnmt, anti-Th, and anti-Dbh antibodies but also exhibited a triangular-shaped gross morphology. This discovery has expanded the understanding of ICA cell diversity, revealing distinct shapes in different developmental stages [28]. These electron microscopic studies collectively provide a comprehensive insight into the morphological variations of ICA cells, laying the foundation for a nuanced understanding of

their structural characteristics and potential functional implications in various cardiac contexts.

Distribution of ICA cells

To elucidate the spatial organization of ICA cells within the intact heart, researchers have conducted immunofluorescent histochemical staining experiments utilizing antibodies specific to catecholamine biosynthetic enzymes, including TH, DBH, and PNMT, alongside the cardiomyocyte-specific marker, sarcomeric α-actinin. A consistent distribution pattern emerged across studies, revealing a predominant presence of ICA cells in perivascular and intramyocardial regions. Specifically, Huang et al. observed clusters of ICA cells in close proximity to coronary microvasculature, including venules, arterioles, and capillaries, as well as clusters adjacent to atrial and ventricular myocytes in the adult rat heart. This observation aligns with the findings of Natarajan et al., who demonstrated a similar spatial arrangement of neonatal rat ICA cells, frequently situated alongside cardiomyocytes within ventricular myocardium. Using immunofluorescent staining techniques, Ebert et al. studied the distribution pattern of ICA cells in the embryonic rat heart across various embryonic stages. At E10.5, ICA cells were found in truncus arteriosus as well as atrial and ventricular chambers, and dorsal venous valve and atrioventricular canal regions at E11.5. Subsequently, at E12.5–E13.5, they turned out to be in the atrioventricular node (AVN) and then in the crest of the interventricular septum, particularly the dorsal limb (early bundle of His), at E16.5 [29]. The perivascular distribution of ICA cells implies a potential role as cardiac chemoreceptors, where neurotransmitters or circulating molecules may modulate the function of ICA cells, regulating the release of endogenous catecholamines from these cells. Owing to the unique perivascular and intramural distribution of ICA cells, it is conceivable that they may participate in regulating coronary blood flow, especially in scenarios involving compromised blood supply, such as in ischemic heart diseases [24, 28].

Huang et al. revealed the diffuse distribution of ICA cells throughout the entire rat heart, with a notable concentration in the left atrium. A similar distribution pattern was observed in both neonatal and adult stages, where ICA cells exhibited a diffuse and sporadic presence across all four chambers of the rat heart [30], as documented by Huang et al. and Natarajan et al. However, these experiments do not comprehensively capture the dynamic distribution pattern of ICA cells within intact heart tissue throughout a more integrated life cycle, spanning from the embryonic stage to the neonatal stage and progressing into the adult stage.

Catecholamine biosynthesis, release, and uptake by ICA cells

ICA cells, recognized as constitutively active entities, possess neuroendocrine properties, including the biosynthesis, release, and uptake of catecholamines. Specifically, Huang et al. conducted seminal studies revealing that the levels of endogenous noradrenaline and adrenaline in rat ICA cells closely resembled those found in sympathetic neurons of the rat stellate ganglion. However, the dopamine content in ICA cells was notably lower compared with sympathetic neurons, with noradrenaline being the predominant catecholamine. Analysis of endogenous catecholamine levels in ICA cells and whole cardiac tissue indicated that ICA cells contributed to approximately 16%,

13%, and 18% of the total cardiac contents of dopamine, noradrenaline, and adrenaline, respectively [13]. The substantial catecholamine content in ICA cells implies their potential for catecholamine synthesis, a notion reinforced by the presence of catecholamine biosynthetic enzymes in these cells. The same researchers conducted comprehensive northern and western blot analyses, revealing the existence of both mRNA and proteins for key catecholamine biosynthetic enzymes, including Th, Dbh, and Pnmt, in adult rat ICA cells [24]. Similarly, in fetal rat ICA cells, Huang et al. observed the presence of *Th* and *Pnmt* mRNA at E16.0, even in the absence of Th-positive sympathetic nerve endings, indicating the absence of cardiac sympathetic innervation at this developmental stage [13]. Collectively, these findings underscore the unique catecholamine biosynthetic system of ICA cells within the heart, operating independently of cardiac sympathetic innervation. This distinctive feature suggests a potential role for ICA cells in regulating cardiac adrenergic function during the early developmental stages [30].

In addition to their capacity for catecholamine biosynthesis, ICA cells exhibit the ability to release catecholamines in vitro, as demonstrated by Huang et al. Notably, the release ratio of noradrenaline to adrenaline in these cells differed from their content ratio, hinting at potential differences in storage and secretion mechanisms for each catecholamine. The intricacies of these mechanisms warrant further investigation in future experiments. The catecholamines released by ICA cells have the potential to stimulate G protein-coupled receptors in both autocrine and paracrine manners [31]. Significantly, Huang et al. highlighted the co-localized immunoreactivity of TH and noradrenaline transporter (NAT) in ICA cell–myocyte co-cultures, indicating the presence of noradrenaline uptake mechanisms in these cells. [^{3}H] noradrenaline uptake assays demonstrated that exogenous noradrenaline and the NAT inhibitor nisoxetine partially inhibited [^{3}H] noradrenaline uptake in ICA cells by 20% and 36%, respectively (Fig. 1B, C). This suggests that ICA cells are capable of noradrenaline uptake via NAT, but the partial inhibition by nisoxetine suggests potential structural or functional distinctions in NAT compared with sympathetic nerve endings. Future experiments are required to explore the specific structure and function of ICA-cell-specific NAT and to investigate the presence of possible NAT subtypes [30]. Consistent with previous studies, Huang et al. reaffirmed that ICA cells spontaneously released [^{3}H] noradrenaline following uptake, further supporting the conclusion that ICA cells possess neuroendocrine properties, encompassing the biosynthesis, release, and uptake of catecholamines [13].

Cardiac development and potential progenitor roles of ICA cells

Several studies have contributed evidence highlighting that certain subsets of cardiomyocytes have developmentally adrenergic progenitors. It is interesting to consider whether a subset of ICA cells may undergo transdifferentiation to become pacemaker and conduction system cells during cardiac development. In a seminal study, Ebert et al. employed genetic fate-mapping experiments utilizing the Pnmt-Cre/R26R reporter mouse model, revealing that approximately 50% of pacemaker cells in the SAN, identified by positive staining with the anti-hyperpolarization-activated cyclic nucleotide-gated channel isoform 4 (Hcn4) antibody, co-stained positively with blue XGAL in the embryonic mouse heart at E15.5 (Fig. 1D) [26]. The XGAL staining served to detect LacZ expression, marking cells with a history of Pnmt expression, including ICA cells

[32]. Hcn4, a pivotal pacemaker channel protein, exhibits its highest expression in the SAN [33]. Although Hcn4 staining is not exclusive to SAN pacemaker cells, the predominant Hcn4 expression in the SAN region, supports the conclusion that a subset of pacemaker cells in the SAN originates from adrenergic cells, possibly ICA cells given their high concentration in the embryonic SAN [13]. In a recent study, Moen et al. introduced an innovative genetically modified mouse model, termed the TG^{AC8} mouse model, designed to exclusively overexpress adenylyl cyclase type 8 (Ac8) in the heart. Ac8 is a key regulator of intrinsic cyclic AMP–protein kinase A (PKA)–Ca^{2+}-mediated pacemaker function. Using this mouse model, the researchers demonstrated a noteworthy increase in the concentrations of Th in the TG^{AC8} mouse's Hcn4-stained SAN cells when compared with the wild-type HCN4-stained SAN cells (Fig. 1F). This intriguing finding further supports the prevalence of cardiomyocyte catecholamine production and its dynamic response to physiological and pathophysiological stressors. It is tempting to theorise whethera subset of catecholaminergic pacemaker cells within the adult SAN may even trace their origin back to ICA cells [34].

Some have also proposed that a fraction of conduction system cells may also derive from ICA cells. Ebert et al. found that in Pnmt-Cre/Rosa26R mouse embryos, LacZ-positive cells appeared in specific areas of the heart from E8.5 onward. By E10.5, LacZ staining was present in all parts of the heart, especially at the atrial and ventricular junction [26]. Osuala et al. [35] found that in hearts separated at 8–10 weeks of age after hybridization of Pnmt-Cre-Rosa26 Lacz mice, XGAL staining was found primarily in the left atrium and left ventricle. The left atrial myocardium had extensive XGAL staining, while the staining in the left ventricle was more concentrated, the apical XGAL staining was stronger, the dorsal section was stronger, and the staining inside the heart was more pronounced than around it. In the most dorsal part, there was more extensive staining throughout the bottom of the left ventricle (Fig. 1E) [35].

In 2017, by crossing Pnmt-Cre mice with channelrhodopsin-2 (ChR2)-tdTomato mice, our group generated a mouse model in which $Pnmt^+$ cells not only expressed tdTomato fluorescence but also ChR2 channels. On the basis of morphology, surprisingly, we found another kind of elongated, rod-shaped $Pnmt^+$ cells, other than the small triangular-shaped ICA cells, that expressed α-actinin. These were termed $Pnmt^+$ cell-derived cardiomyocytes (PdCMs). Cardiomyocyte-like cells that expressed tdTomato fluorescence were mainly found in the left side of the heart and conduction system. To be specific, 50% of the total myocytes in left atrium (LA), 21% in left ventricle (LV), 7% in right atrium (RA), and 2% in right ventricle (RV). In the AVN region, these cells showed partial co-expression with HCN4, but in the SAN they existed in peripheral regions and showed little co-expression with HCN4 [36]. Ren et al. performed real-time 3D cardiac imaging reconstruction by combining a modified cardiac transparency technology with high-resolution light-sheet fluorescence microscopy (LSFM) using the Zeiss Zen program. Using heart samples from transgenic mouse models (HCN4-DreER/tdTomato and Pnmt-Cre/ChR2-tdTomato), they successfully reconstructed the 3D spatial distribution of $HCN4^+$ pacemaker cells and PdCMs. They found that the distribution patterns of $HCN4^+$ cells in HCN4-DreER/tdTomato heart and $Pnmt^+$ cells in Pnmt-Cre/ChR2-tdTomato heart had an identical expression pattern [37]. In general, PdCMs are preferentially distributed in the left side of the adult heart and partially co-expressed with

HCN4 [36, 38]. This expression pattern closely aligns with the anticipated distribution of ventricular conduction system cells, providing further, albeit limited, support for the hypothesis that a subset of these conduction system cells might originate from ICA cells [26, 39]. These findings collectively highlight the close relationship between the developing cardiac conduction system and endogenous cardiac catecholamines - of which ICA cells may likely be an important source of catecholamines, and potentially even act as progenitors during the cardiac development.

Physiological and pathological roles of ICA cells

ICA cells regulate cardiac contractile and pacemaker function Given the proximity of ICA cells to clusters of atrial and ventricular myocytes within the intramyocardial distribution, they are believed to play a pivotal role in regulating the cardiac contractile and pacemaker functions of cardiomyocytes. In a seminal study, Huang et al. demonstrated that the application of the β-adrenoreceptor (β-AR) antagonist timolol (1 μM) to neonatal rat ventricular myocyte primary isolates, which included ICA cells, significantly reduced the spontaneous beating rate of these myocytes by approximately 58% in vitro. While the specific signalling mechanism underlying this reduction remains unclear, this groundbreaking study marked the first instance of showcasing the role of ICA cells in regulating the beating rate of neonatal cardiomyocytes [24].

During the neonatal stage, the sympathetic nervous system has a minimal impact on the beating rate of rat cardiomyocytes, as the maturation of cardiac sympathetic innervation is completed after the second week of rat birth [13, 40]. Nonetheless, the beating rate of neonatal cardiomyocytes can be stimulated by both α-adrenergic receptor (α-AR) agonists and β-AR agonists, indicating the presence of functional α-ARs and β-ARs in the neonatal rat heart [13, 41]. Subsequently, as cardiac sympathetic innervation matures, the beating rate of cardiomyocytes becomes predominantly under β-AR control, as observed in the adult stage [42]. Building on these insights, Natarajan et al. expanded upon Huang's research and demonstrated that the beating rate of neonatal rat cardiomyocyte cultures containing ICA cells was significantly decreased in the presence of the catecholamine-depleting agent reserpine, the α1-AR antagonist prazosin, and the β-AR antagonist timolol in a concentration-dependent manner. Intriguingly, the reduction induced by reserpine could be reversed by the addition of exogenous noradrenaline [13, 28]. These results collectively suggest that ICA cells, as catecholamine-synthesizing cells, can regulate the beating rate of neonatal cardiomyocytes by releasing endogenous catecholamines to stimulate α-AR or β-AR in the developing heart before the establishment of cardiac sympathetic innervation [13]. The presence of ICA cells underscores their importance in early fetal heart development. Fetal blood carries less oxygen than postnatal blood because it is oxygenated by the placenta rather than the lungs. The fetal heart is small and relatively weak, limiting the amount of blood it can pump with each beat. To maintain adequate cardiac output (CO = heart rate × stroke volume), the fetal heart compensates by beating at a much faster rate, reaching a peak of 170–180 bpm by 9–10 weeks of pregnancy, to ensure sufficient delivery of oxygen and nutrients. In the absence of a fully developed sympathetic nervous system, ICA cells likely play a pivotal role in supporting this elevated cardiac activity. Consistent with cardiac adrenergic gene expression [23], Ebert et al. demonstrated that, in mice, ICA cells emerge as early as

E8.5, 2 days prior to the development of SAN pacemaker cells and continue to accumulate in substantial numbers through birth [43].

In the adult heart, the coexistence of intrinsic and extrinsic cardiac catecholaminergic systems likely holds distinct physiological significance. Under resting parasympathetic-dominance, cardiac sympathetic nerves release minimal noradrenaline. Instead, constitutive catecholamine release from ICA cells may suffice to support basal heart function. During stress, ICA cells and sympathetic nerves may act synergistically to achieve optimal cardiac augmentation. It is intriguing that despite the crucial role of catecholamines, widely used clinical therapies involving β-blockers do not significantly impair cardiac performance in patients with cardiovascular diseases. This is because β-blockers are predominantly selective for β_1-AR, with a lower affinity for β_2-AR. As a result, β_2-mediated chronotropic and inotropic effects of ICA cell-derived adrenaline remain largely intact during β-blocker therapy. In addition, ICA cells secrete calcitonin gene-related peptide (CGRP) [44], a neuropeptide also known to enhance contractility in ventricular myocytes [45]. Adrenaline can further stimulate CGRP production from ICA cells via β_2-AR-mediated gene upregulation [46]. These mechanisms highlight the human heart's remarkable and multi-layered neurohormonal backup systems that preserve cardiac performance under stress.

Nonetheless, the specific signalling mechanism responsible for triggering the release of endogenous catecholamines from ICA cells remains unknown. Given that the release of neurotransmitters from neuroendocrine cells typically requires Ca^{2+} influx [47], it is reasonable to assume that ICA cells similarly necessitate Ca^{2+} influx for the release of endogenous catecholamines. In support of this hypothesis, Huang et al. demonstrated that ICA cells generated spontaneous Ca^{2+} influx-mediated $[Ca^{2+}]_i$ transients characterized by a lower basal frequency (compared with canonical cardiomyocytes) and a significantly irregular rhythm in ICA cell–myocyte co-cultures. Their experiments further revealed that the addition of the voltage-gated sodium channel blocker tetrodotoxin abolished the $[Ca^{2+}]_i$ transients of ICA cells, and the L-type calcium channel blocker nifedipine significantly decreased the amplitude of ICA cell $[Ca^{2+}]_i$ transients [13, 30]. This implies that the generation of $[Ca^{2+}]_i$ transients by ICA cells involves the activation of both membrane voltage-gated sodium channels and L-type calcium channels. This, in turn, stimulates the release of endogenous catecholamines, activating α-AR or β-AR in the developing heart before cardiac sympathetic innervation [13].

ICA cells possess an oxygen-sensing function ICA cells possess the remarkable ability to sense various oxygen tensions, including hypoxia and reoxygenation. Hypoxia, characterized by reduced oxygen levels, typically leads to increases in heart rate. However, in certain situations, there can be decreases in heart rate despite hypoxia. For instance, elite divers experience rapid reductions in heart rate during deep breath-hold dives at sea, as observed by Kiviniemi et al. [48]. However, the specific molecular mechanisms underlying bradycardia despite hypoxia remain a subject of debate and likely involve multiple interacting organ systems. Shedding light on this mechanism, Huang et al. demonstrated that acute hypoxia promptly inhibited the generation of $[Ca^{2+}]_i$ transients by ICA cells in co-cultures with myocytes [30]. This inhibition suggests a potential mechanism for hypoxic bradycardia, possibly mediated by the reduced release of endogenous catechola-

mines from ICA cells. Interestingly, this hypoxia-induced inhibitory response of ICA cells contrasts with the behavior of adrenal chromaffin cells, which increase catecholamine release during hypoxia [49].

Following hypoxia, reoxygenation often triggers a surge in myocardial interstitial catecholamine concentrations. Killingsworth et al. demonstrated a significant increase in noradrenaline and adrenaline concentrations after reperfusion in anesthetized pigs. This surge, which far exceeds the increase achieved through cardiac sympathetic stimulation alone, suggests the involvement of an alternative mechanism [50]. Further elucidating this mechanism, Huang et al. showed that reoxygenation after hypoxia substantially increased the frequency of $[Ca^{2+}]_i$ transients in ICA cells in co-cultures with myocytes, with a sustained response [30]. This heightened activity, unique to ICA cells, caused temporal summation of $[Ca^{2+}]_i$ transients upon reoxygenation, potentially contributing to the surge in catecholamine concentrations observed after reperfusion in pigs. Collectively, these findings highlight the oxygen-sensing capabilities of ICA cells and their pivotal role in regulating cardiac function under both resting and stress conditions. By modulating their $[Ca^{2+}]_i$ transient activities, ICA cells can dynamically adjust the release of endogenous catecholamines, thereby influencing cardiac contractility and pacemaker functions.

In response to hypoxia–reoxygenation, cardiac tissues must deploy various mechanisms to enhance oxygen delivery, one of which involves the activation of myocardial angiogenesis [51]. Given the oxygen-sensing capabilities of ICA cells, it is reasonable to speculate that these cells may play a role in angiogenesis underlying myocardial infarction, in addition to their established roles in regulating in cardiac contractility and pacemaker functions. ICA cells might potentially trigger signaling pathways involved in the promotion of angiogenesis, leading to the formation of new blood vessels in ischemic cardiac tissues. This hypothesis is supported by emerging evidence suggesting that catecholamines, which are synthesized and released by ICA cells, can modulate angiogenesis in various physiological and pathological contexts [52]. Furthermore, studies have demonstrated that catecholamines can influence endothelial cell function, including proliferation, migration, and tube formation, all of which are crucial steps in angiogenesis [53]. In addition, catecholamines have been implicated in the regulation of vascular endothelial growth factor (VEGF) expression, a key player in angiogenesis [54]. Therefore, it is conceivable that ICA cells, through their catecholamine production and release, may exert paracrine effects on endothelial cells, thereby promoting angiogenesis in the ischemic heart. Alternatively, ICA cells may serve as progenitor cells that directly transdifferentiate into vascular-specific cells, including smooth muscle cells and endothelial cells. However, further research is warranted to elucidate the precise mechanisms by which ICA cells contribute to angiogenesis in the ischemic heart. Understanding the role of ICA cells in this process may pave the way for novel therapeutic strategies aimed at harnessing the angiogenic potential of these cells to promote cardiac repair and regeneration following myocardial infarction.

ICA cells mediate cardiac protection Indeed, ICA cells, endowed with their oxygen-sensing function, are believed to play a crucial role in mediating cardioprotective mechanisms against ischemia–reperfusion injury in the heart. Numerous studies have impli-

cated the stimulation of the δ-opioid receptor (DOR) in mediating the cardioprotective effects of ischemic preconditioning across various experimental models [13, 55]. Similarly, activation of the β_2-AR during ischemia has been shown to confer cardioprotection by reducing infarct size and improving LV function in murine hearts [56, 57]. Interestingly, the cardioprotective effects mediated by DOR are partly attributed to β_2-AR activation within the heart [58].

While the expression of β_2-AR in the heart is well-documented, there is ongoing debate regarding the specific cardiac cell types expressing DOR. However, Huang et al. provided compelling evidence indicating that over 90% of ICA cells, identified by positive staining with anti-TH antibody, also expressed DOR, as demonstrated through co-staining with an anti-DOR antibody in both rat and human hearts. Building on this co-expression pattern, this research group demonstrated that the addition of the selective DOR agonist [D-Pen^2, D-Pen^5] enkephalin (DPDPE) resulted in a concentration-dependent increase in both $[Ca^{2+}]_i$ transient spikes of ICA cells and the release of endogenous adrenaline from these cells. Furthermore, pretreatment with DPDPE before coronary artery occlusion significantly reduced LV infarct size and ischemia-induced myocyte death by approximately 54% and 26%, respectively, in ICA cell–myocyte co-cultures [13]. However, this protective effect was not observed in ventricular myocytes depleted of ICA cells, underscoring the significant role of ICA cells in mediating cardiac protection against ischemia–reperfusion injury in the heart [59].

In summary, these findings suggest that ICA cells contribute significantly to the cardioprotective response against ischemia–reperfusion injury by stimulating their DOR, leading to increased adrenaline release and subsequent activation of the β_2-AR-mediated protective mechanisms within the heart.

In addition to the cardiac DOR, CGRP, a neuropeptide, has emerged as a mediator of the cardioprotective mechanism against ischemia–reperfusion injury in the heart [60]. However, similar to the ambiguity surrounding the cellular origins of cardiac DOR expression, it remains unclear which specific cardiac cell types produce CGRP. Notably, Huang et al. provided compelling evidence of the exclusive expression of *CGRP* mRNA in ICA cells, alongside the co-expression of CGRP and DOR in both human and rat LV myocardium (Fig. 1G) [44]. Furthermore, their study demonstrated that both rat and human ICA cells constitutively release CGRP, with an increase observed following the addition of DPDPE, suggesting the existence of a δ-opioid-regulated paracrine system within ICA cells crucial for the release of both catecholamines and CGRP. Of particular significance, this study reported that concurrent administration of β_2-AR and CGRP receptor (CGRP-R) antagonists resulted in a more pronounced increase in infarct size (by 62%) compared with individual treatment with either the β_2-AR antagonist (by 46%) or CGRP-R antagonist (by 40%). Interestingly, in the presence of either the β_2-AR antagonist or CGRP-R antagonist, the addition of DPDPE showed no significant effect on infarct size. These findings strongly suggest that the δ-opioid-mediated cardioprotective mechanism requires co-signalling through both β_2-AR and CGRP-R [60]. These findings shed light on the intricate interplay between multiple signaling pathways orchestrated by ICA cells to confer cardioprotection against ischemia–reperfusion injury, emphasizing their pivotal role in maintaining cardiac homeostasis under stress conditions.

ICA cells restore cardiac adrenergic function by proliferation and transdifferentiation ICA cells, as part of the intrinsic cardiac adrenergic signaling system, possess the remarkable capacity to biosynthesize, release, and uptake endogenous catecholamines, thus playing a pivotal role in cardiac adrenergic function, particularly in situations where sympathetic innervation is compromised, such as in transplanted hearts [61]. Following cardiac transplantation, sympathetic denervation is a common occurrence due to surgical procedures that involve the blockade of parasympathetic vagal neurons and postganglionic sympathetic nerve fibers [62]. Despite this denervation, studies have shown partial restoration of cardiac adrenergic function in transplanted hearts, characterized by the recovery of catecholamine uptake and storage [63]. However, the possibility of cardiac sympathetic reinnervation remains a topic of debate. Tamura et al. offered significant insights into the adaptive capabilities of ICA cells following cardiac transplantation. They demonstrated that during the second week post-transplantation, ICA cells underwent robust proliferation, indicating an active response to the altered cardiac microenvironment. Furthermore, a subset of these proliferating ICA cells underwent transdifferentiation into adrenergic neurosecretory-like cells, suggesting a remarkable plasticity within the cardiac adrenergic system. This was evidenced by elevated mRNA levels of key enzymes involved in catecholamine synthesis (TH, DBH, and PNMT), enhanced catecholamine uptake and storage capacity, and heightened heart rate responses to tyramine stimulation. This process occurred concurrently with the re-establishment of cardiac adrenergic function in transplanted mouse hearts, highlighting the dynamic nature of ICA cells in adapting to changes in sympathetic innervation status [64]. These findings underscore the role of ICA cells in partially restoring cardiac adrenergic function in transplanted hearts in the absence of or during the process of sympathetic innervation recovery.

Clinical implications **Role of ICA cells in cardiac allograft hypertrophy and vasculopathy**

Human cardiac allograft hypertrophy has remained a perplexing clinical challenge since Christiaan Barnard performed the first human-to-human heart transplantation nearly six decades ago. Severe allograft hypertrophy is a strong, independent predictor of increased mortality in heart transplant recipients [65]. Despite its clinical significance, the underlying mechanisms driving this condition remain unknown, leaving it without treatment. Endogenous catecholamines are well-established neurohormones that promote left ventricular hypertrophy (LVH) [61]. Chronic α-AR overstimulation is implicated in LVH development [66, 67]. As discussed above [64], donor hearts exhibit sustained ICA cell hyperproliferation post-transplantation. This hyperproliferation is accompanied by a robust upregulation of *Th* and *Pnmt* mRNA, along with a sustained rise in myocardial noradrenaline and adrenaline levels. If this phenomenon is conserved in human cardiac allografts, as indicated by our preliminary clinical data (Fig. 1H, unpublished), chronic ICA cell hyperproliferation and adrenergic overcompensation could play a key role in the development of allograft LVH.

Further supporting this hypothesis, Huang et al. [68] identified a positive correlation between myocardial CGRP levels in cardiac allografts and the severity of allograft LVH. In patients with the most severe LVH, coronary sinus CGRP levels were approximately ten times higher than in those with minimal LVH. CGRP, a neuropeptide known to

promote myocyte hypertrophy [69], is exclusively synthesized and released by ICA cells in the human heart [44]. Our data suggest that enhanced adrenaline release from ICA cells or exogenous adrenaline administration enhances ICA cell *Cgrp* mRNA expression by 4- and 16-fold, respectively, via an autocrine β_2-adrenoreceptor-mediated loop [46]. This interplay between adrenaline and CGRP may synergistically drive myocardial growth, contributing to allograft LVH. Further investigations into ICA-cell-mediated pathways in allograft pathology are warranted. If confirmed, a combination therapy targeting α-AR and CGRP receptor signaling may offer a promising strategy to prevent or mitigate allograft LVH.

Another important major long-term complication of heart transplantation is allograft vasculopathy, a leading cause of morbidity and death in heart transplant recipients, particularly beyond the first-year post-transplant [70]. Allograft vasculopathy is an accelerated atherosclerotic process, affecting both epicardial and intramyocardial coronary arteries. Intimal hyperplasia driven by excessive proliferation of vascular smooth muscle cells (VSMCs) and migration is a key pathological feature and contributing factor of allograft vasculopathy. Among several proposed mechanisms responsible for intimal hyperplasia, chronic α-AR overstimulation is a well-established neurohormonal signaling mechanism [71, 72]. Given that chronic α-AR overstimulation may contribute to the pathogenesis of both LVH and intimal hyperplasia, it is important to investigate whether chronic catecholamine overproduction by ICA cells occurs in human heart allografts. Uncovering this ICA-cell-driven mechanism could support the development of a unified α-AR blockade strategy to simultaneously target both cardiac allograft LVH and vasculopathy.

Dbh$^+$ catecholaminergic cardiomyocytes

Since Blaschko first discovered this enzyme in mammalian adrenal chromaffin granules in the 1950s, the exploration of DBH has never ceased [73]. For decades, DBH was believed to be distributed in postganglionic sympathetic nerve fibers, noradrenergic cells of the locus coeruleus, and chromaffin cells in the adrenal medulla [74]. Recently we performed single-cell RNA sequencing (scRNA-seq) on embryos at 8.5 days (E8.5) and 10.5 days (E10.5), and whole hearts at 12.5 days (E12.5), 14.5 days (E14.5), 16.5 days (E16.5), and 3 days after birth (P3), and for the first time we discovered a population of previously unrecognized cardiomyocytes expressing the catecholaminergic gene *Dbh* that encodes dopamine β-hydroxylase in the mouse heart. These cells were termed *Dbh*$^+$ catecholaminergic cardiomyocytes (*Dbh*$^+$-CMs) through cardiac genetic fate analysis and lineage tracing. The main types of *Dbh*$^+$ cells were identified as: atrial (2.13%) and ventricular (3.90%) cardiomyocytes, early (2.26%) and mature (4.18%) trabecular ventricular cardiomyocytes, sinoatrial (8.39%) and atrioventricular nodal (12.14%) cells, and nonspecific atrial conduction system (3.82%) and Purkinje fiber cells (12.76%). These data were the initial evidence of the correlation between *Dbh*$^+$- CMs and the cardiac conduction system (CCS) [75, 76].

Genetic fate mapping of their development and distribution

To trace and verify *Dbh*$^+$-CMs, a reporter line *Dbh*^{Cre}/Rosa26-tdTomato mouse model was established for genetic fate mapping, complemented by spatial distribution

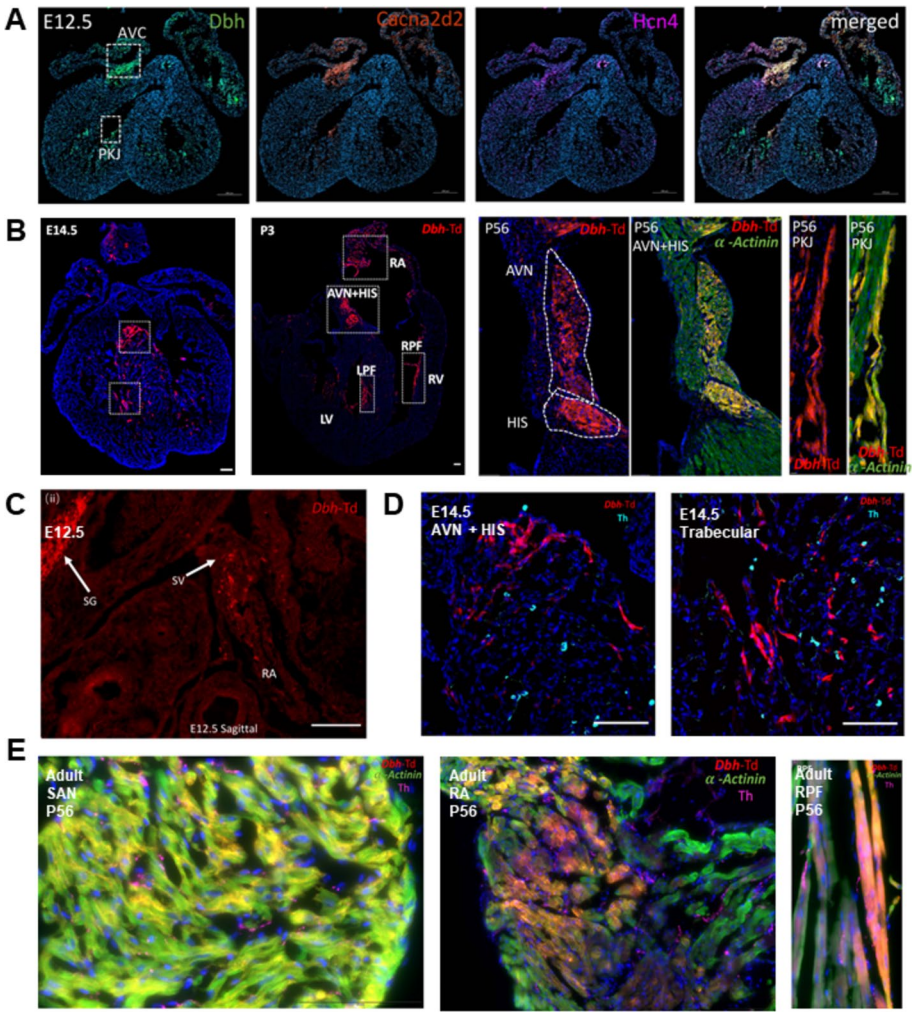


Fig. 2 *Dbh*$^{+}$-CMs were co-localized with CCS markers and co-developed with sympathetic innervation. (**A**) Representative RNAscope images of *Dbh* (green), CCS marker Cacna2d2 (red), and HCN4 (magenta) distribution in E12.5 heart. Scale bar: 200 μm. (**B**) Immunostaining results showing tdTomato (red) and α-actinin (green) co-expression of *Dbh*$^{+}$-derived CMs in CCS at E14.5, P3, and P56. Scale bar: 10 μm for PKJ and 100 μm for the rest. (**C**) Representative images showing tdTomato signals in RA and SV regions of an E12.5 embryo. Scale bar: 200 μm. (**D**) Immunofluorescence of *Dbh*$^{+}$-CMs stained with TH (cyan) in the AVN + HIS bundle and trabecular regions at E14.5. Scale bar: 100 μm. (**E**) Immunofluorescence of *Dbh*$^{+}$-derived CMs stained for α-actinin (green) and TH (magenta) in SAN, RA, and RPF regions at the adult stage. Scale bar: 100 μm. Panels **A**–**E** were reprinted from ref. [75] with permission under the Creative Commons license (https://creativecommons.org/licenses/by/4.0/)

analysis using spatio-temporal enhanced resolution-omics sequencing (Stereo-seq). Using this technique, we found that from E8.5 to P3, the *Dbh* gene had a spatial co-localization with CCS marker genes, especially ventricular CCS, suggesting that the *Dbh* gene was related to the developing CCS. As tdTomato-coding sequences were not readily detectable, we studied the spatial distribution of *Dbh* in the heart using *Wpre*, a transcript derived from tdTomato. We also performed Stereo-seq on heart slices of adult mice (P56), the results further confirmed that in adulthood, *Dbh* was also expressed in both working and non-working myocardial cell types and was colocalized with CCS marker genes in a pattern similar to that of developing hearts.

Further validation was performed through RNAscope, immunohistochemical staining, and confocal microscopy (Fig. 2A, B), using the *Dbh*^{Cre}/Rosa26-tdTomato mouse model from E8.5 to P56, it was found that *Dbh*$^{+}$ cells first appeared in the dorsal neural tube at E10.5 and migrated through the pharyngeal arches to the sinus venous (SV) and right atrium at E12.5. RNAscope co-staining showed that *Dbh* was abundant in CCS components such as SV, HIS bundles, and purkinje (PKJ) fibers, overlapping with CCS markers from E12.5 to P3, which suggested *Dbh*$^{+}$ cells were potentially involved in CCS development and function. Immunofluorescence staining results showed that cells expressing tdTomato were enriched in the ventricular trabecular area and CCS (AVN and HIS bundles) at E14.5, P3, and P56 stages, and these cells expressed α-actinin, confirming that the majority of tdTomato-expressing cells were cardiomyocytes, thus further confirming the identity of the cells as *Dbh*$^{+}$-derived CMs.

To further investigate the expression and localization of *Dbh*$^{+}$-CMs in developing and adult hearts, we established a *Dbh*-knock-in-CFP (*Dbh*^{CFP}) mouse model and used anti-Flag or anti-CFP antibodies to enhance CFP signaling. The results showed that *Dbh*$^{+}$-CMs were mainly distributed in the AVN and ventricular CCS at E14.5 and P56. We then established a *Dbh*^{CreERT}/Rosa26-tdTomato inducible reporter mouse line to map the *Dbh*$^{+}$-CMs in a temporally controlled manner and found that tdTomato fluorescence was mainly concentrated in the AVN and HIS regions of the CCS, supporting the results of the *Dbh*^{Cre}/Rosa26-tdTomato and *Dbh*^{CFP} lines.

To confirm whether *Dbh*$^{+}$-CMs were involved in the formation of PKJ networks, we established *Dbh*^{Cre}/Rosa26-tdTomato/Cx40-eGFP mice. In the left and right ventricular PKJ networks, we clearly found co-localization between tdTomato and eGFP signals, but the tdTomato signal was significantly wider, supporting more than Purkinje conductive cells, suggesting that *Dbh*$^{+}$-derived CMs also formed other cardiomyocyte populations. It was validated that the majority of *Dbh*$^{+}$-CMs and *Dbh*$^{+}$-derived CMs in various stages of hearts were co-localized with CCS markers, which suggested that they played a vital role in the physiology of CCS, especially in His bundle and PKJ fibers, where *Dbh*$^{+}$-derived CMs were found co-localized with Cx40^{+}-cells [75, 76].

Co-development with sympathetic innervation

Our study highlighted the close developmental relationship between *Dbh*$^{+}$-CMs and sympathetic innervation, which is critical for CCS formation and function. This relationship underscores the interplay between neurocardiac signaling and heart physiology.

Utilizing the *Dbh*^{Cre}/Rosa26-tdTomato mouse line, we first observed tdTomato signals in the dorsal neural tube at E10.5, which reflected neural expression in the developing CNS. At E12.5, the tdTomato signal was observed in the sympathetic ganglion near the sinus venosus and RA. From E14.5 to the postnatal period into adulthood, *Dbh*$^{+}$-derived CMs were found widely distributed in CCS regions that were also rich in sympathetic innervation, as detected by anti-TH antibody immunostaining. For the P68-induced *Dbh*^{CreERT}/Rosa26-tdTomato reporter mouse line, the tdTomato fluorescence was observed in the CCS, especially in the His bundle and RBB regions, which have rich sympathetic innervation, and cardiomyocyte identity was validated by α-actinin staining

[75]. As is shown in Fig. 2C–E, the results suggested that Dbh^{+}-CMs co-develop with sympathetic innervation in the heart.

Electrophysiological characteristics

Using optogenetics and conditional gene knockout models, we demonstrated that Dbh^{+}-CMs were integral to the ventricular conduction system. Specifically, to study the electrophysiological characterization of Dbh^{+}-CMs and Dbh^{+}-derived CMs, two series of mouse models were developed: Dbh^{Cre}/ChR2-tdTomato (Dbh-ChR2), $Cx40^{CreERT}$/ChR2-Tomato (Cx40-ChR2), and MHC^{Cre}/ChR2-tdTomato (MHC-ChR2) mouse models; and the mice with specific deletion of *Dbh* in cardiomyocytes (Dbh^{cko}) and their control littermates ($Dbh^{f/f}$).

ECGs were recorded and analyzed in Langendorff-perfused hearts of the three genotypes (MHC-ChR2, Dbh-ChR2, and Cx40-ChR2) and illuminated directly towards the epicardium in LA, RA, LV, and RV regions with 470 nm light pulses generated by a time-controlled light emitting diode (LED). The results showed that the QRS waveform characteristics induced by light stimulation of the RV of Dbh-ChR2 mice were similar to those of Cx40-ChR2 mouse hearts. However, the effective refractory periods (ERPs) of RV in Dbh-ChR2 and Cx40-ChR2 mouse hearts were significantly longer than those that in MHC-ChR2 hearts, indicating partial overlap between Dbh^{+}-CMs and $Cx40^{+}$-derived cardiomyocytes and Purkinje fibers in RV. In order to further investigate the special association between Dbh^{+}-CMs and Purkinje network in RV, Lugol's solution was applied to ablate Purkinje fibers in LV. After ablation, the electrocardiogram results showed that the QRS complex of Dbh-ChR2 and Cx40-ChR2 mouse hearts was prolonged, and the heart could no longer be paced in the RV. These results indicate that the electrophysiological characteristics induced by light stimulation in the hearts of Dbh-ChR2 and Cx40-ChR2 mice are basically similar and the association of Dbh^{+}-derived CMs with Purkinje fibers is similar to that of $Cx40^{+}$-derived cardiomyocytes [75, 76].

Furthermore, recording the ECGs of $Dbh^{f/f}$ and Dbh^{cko} isolated hearts, it found that compared with $Dbh^{f/f}$ hearts, Dbh^{cko} hearts had a longer P–R interval, AVN effective refractory period (AVNERP), and atrial ventricular (A–V) conduction Wenckebach block period. This result provides the first evidence that the specific deletion of the *Dbh* gene in cardiomyocytes affects AV conduction and AVN electrophysiological properties [75, 76].

Catecholamine characteristics

Transmission electron microscopy (TEM) imaging and energy dispersive X-ray spectroscopy (EDS) analysis found that the cardiomyocytes from the Dbh^{+}-CM-rich atrioventricular junction, especially the pacemaker type cell, contained vesicles akin to those in chromaffin cells, although smaller in number and weaker in signal. Therefore, for the first time, we found that Dbh^{+}-CMs exhibit catecholaminergic endocrine-like functions, suggesting a dual role in neurotransmitter release and cardiac signal transmission. This provides a potential mechanism for how these cells influence the CCS and overall cardiac electrophysiology [75, 76].

Implications of Dbh deficiency

In 1995, Thomas et al. replaced 3.4 kilobases (kb) of genomic sequence, which included 0.6 kb of the *Dbh* proximal promoter and the first exon, with a neomycin-resistance cassette, thus generating mice lacking dopamine β-hydroxylase. Utilizing this mouse line, it was found that all embryos died in utero in homozygous mothers, and most homozygous embryos died in the uterus in heterozygous mothers—only about 5% reached adulthood. These mutant embryos were similar to embryos deficient in TH in the histological phenotype, which suggested that cardiovascular failure might cause death. The experiment indicated the vital effect of *Dbh* for the development of the heart. Pregnant heterozygous females treated with dihydroxyphenyl serine (DOPS), a synthetic amino acid that can be converted into noradrenaline by L-aromatic-amino-acid decarboxylase (AADC) in drinking water, led to the survival of all homozygotes. However, when the inhibitor of AADC, carbidopa, was added along with DOPS, the rescue failed. The result suggested the effect of DOPS depended on its conversion to noradrenaline [15].

In 2004, using radiotelemetry to monitor the cardiovascular parameters of *Dbh* (−/−) and *Dbh* (+/−) mice, Swoap et al. reported that under normal circumstances at an ambient temperature of 29 °C, *Dbh* (−/−) mice had low heart rates, severe hypotension, and a weakened circadian rhythm of blood pressure. They then put these mice under a 50% caloric restriction. The blood pressures and the heart rates of *Dbh* (−/−) mice did not fall significantly, while *Dbh* (+/−) mice showed decreases in heart rate and average blood pressure. In response to an open-field test, there were weak changes in blood pressures in *Dbh* (−/−) mice, while no change in heart rate was found, in contrast, the *Dbh* (+/−) mice had a substantial and rapid increase in blood pressure and heart rate. These results suggest the effect of *Dbh* in mediating the hypotension induced by dieting, and its effect in caloric-restriction-induced bradycardia. Moreover, in an open field, *Dbh* was required for tachycardia but not required for blood pressure increases [77].

In 2012, utilizing the same mouse line along with immunofluorescent histochemical staining, Baker et al. also reported that there was a decrease in the major gap junction protein (Cx43) expression in adrenergic-deficient myocardium compared with adrenergic-competent hearts after E10.5, while no significant decrease was detected in E9.5 mice between the two groups. Culturing E10.5 hearts of the two types and measuring extracellular field potentials of varied regions by microelectrode arrays (MEAs), it was found that in adrenergic-deficient E10.5 hearts, the atrioventricular conduction velocity was selectively slowed, while there was no significant changes in atrial conduction and average beating rates. Subsequently, treating the isolated hearts with isoproterenol and measuring rhythmicity using the arrhythmic index (AI), a significant increase of 225% ($P < 0.05$) in AI was found in the adrenergic-deficient mouse hearts at E10.5, while in either group at E9.5 no significant differences in AI were discovered. That is, by E10.5, after acute isoproterenol attack, the degree of cardiac arrhythmia in adrenergic-deficient mice was significantly higher than that in the adrenergic-competent controls. From all of the above, it was indicated that adrenergic hormones had the ability to stimulate Cx43 expression, promote atrioventricular conduction, and assist in maintaining heart rhythm during the critical early stages of embryonic heart development [78].

In 2015, Baker et al. further examined the concentration of adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP) in *Dbh* (+/+), *Dbh* (+/−), and *Dbh* (−/−) mouse embryos, and found in E11.5, the concentration of ATP decreased dramatically in *Dbh* (−/−) mice, whereas ADP concentrations rose, resulting in an ATP/ADP ratio in *Dbh* (−/−) mice was nearly 50-fold less than that found in *Dbh* (+/+) and *Dbh* (+/−) mice. Furthermore, in *Dbh* (−/−) hearts, a significant decrease in cardiac extracellular acidification and oxygen consumption rates were found, and there were larger and more branched mitochondria. However, treating the mother with adrenergic receptor agonists isoproterenol or L-phenylephrine could reduce the decreases in ATP in *Dbh* (−/−) embryos, suggesting that adrenergic hormones stimulate cardiac energy metabolism during a critical period of embryonic development [79].

In general, mouse experiments have demonstrated that noradrenaline, generated through DBH catalysis, plays a critical role in ensuring cardiac development and embryonic survival. Deficiency in DBH can lead to reduced energy metabolism in the embryonic heart, slowed atrioventricular conduction, and dysregulation of heart rate and blood pressure control.

Clinical relevance

Besides the above evidence in mice, there are also some human case reports, showing that *Dbh* deficiency may lead to disrupted electrical activity, increasing the risk of arrhythmias and sudden cardiac death [80].

In 1995, it was found in human that during supine exercise, blood pressure increased in control groups and returned to resting levels after 5 min post-exercise, however, in a *Dbh*-deficient group, blood pressure was unchanged throughout. Blood samples were taken to evaluate catecholamines in the control and *Dbh*-deficient groups, and it was found that plasma noradrenaline and adrenaline were undetectable at rest and at all stages of post-exercise, but plasma dopamine was elevated and rose further with exercise in *Dbh*-deficient group. As for heart rate, people with *Dbh* deficiency had a lower basal heart rate than the control group, and after exercise, it took a longer time for *Dbh*-deficient people to return to a normal heart rate. Subsequently, Smith et al. found that with exercise, cardiac output showed a larger increase in *Dbh*-deficient people than controls [81]. They also studied postural hypotension. In the control group, exercise increased supine blood pressure, and no postural fall was observed before or after exercise. In the *Dbh*-deficient group, there was little change in blood pressure with exercise as reported before, but blood pressure fell to a lower level during standing after exercise. These results suggested that there were more symptoms of postural hypotension on standing after exercise in people with *Dbh* deficiency. Because NE plays a crucial role in maintaining vascular tension and blood pressure stability, its deficiency means that blood vessels are unable to contract normally to maintain blood pressure, especially when the body position changes [82]. One of the reasons for disabling orthostatic hypotension may be mutations of CYB561 causing a congenital absence of noradrenaline [83]. Above all, it can be found that humans with *Dbh* deficiency have impaired heart rate and blood pressure regulation, and are more prone to orthostatic hypotension, especially after exercise.

Fortunately, the exploration for treatments of *Dbh* deficiency has not come to a standstill. In 2016, Cubells et al. generated a transgenic mice line that carried a bacterial artificial chromosome (BAC) containing the human DBH coding locus. By measuring levels of NE and DA in the brain and other peripheral organs in $Dbh^{+/-}$, $Dbh^{-/-}$, and transgenic $Dbh^{-/-}$ BT mice, it was found that with the BAC transgene, NE increased close to control levels while DA decreased to near normal. Furthermore, they also found that BAC transgenesis rescued the disorders associated with *Dbh* deficiency, such as embryonic lethality, growth delay, and ptosis, which demonstrated that BAC transgenesis of the human *Dbh* gene may be a useful therapeutic target to the decline of noradrenergic function caused by *Dbh* expression deficiency [84].

Future perspectives

Future research should focus on two critical areas: (1) elucidating the transcriptomic landscape and epigenetics of ICA cells through scRNA-seq of both healthy and diseased human hearts, taking advantage of several comprehensive datasets now available in public repositories, and (2) advancing translational and clinical research to explore ICA-cell-targeted therapies. Since endogenous catecholamines, particularly via chronic α-AR overstimulation, contribute to LVH and vascular intimal hyperplasia, it is crucial to assess whether ICA-cell-derived catecholamine overproduction occurs in human heart transplant recipients. If confirmed, a proof-of-concept trial should test whether α-AR blockade can effectively mitigate allograft hypertrophy and vasculopathy.

Conclusions

ICA cells and Dbh^{+}-CMs may represent different cell types in the heart and contribute to the regulated release of endogenous catecholamines. ICA cells are non-cardiomyocytes distributed around blood vessels and intramyocardial regions, with the functions of secreting catecholamines and chemoreceptors. ICA cells can regulate adrenaline function during early developmental stages, regulate calcium transients by sensing oxygen levels (thereby regulating endogenous catecholamine release), and modulating cardiac pacing and contraction. In addition, some ICA cells may also develop into SAN cells and PdCMs, forming the CCS together with Dbh^{+}-CMs. Dbh^{+}-CMs are mainly distributed in the AVN and ventricular CCS and partially co-localized with the sympathetic nervous system. Both mice with a cardiac-specific deletion of the *Dbh* gene and patients with *Dbh* deficiency present altered cardiac electrophysiology and function, suggesting an important role of Dbh^{+}-CMs in cardiac physiology. However, further research is needed to determine how they modulate cardiac function by endogenous catecholamines and whether such regulation has any clinical implications.

Abbreviation

ICA	Intrinsic cardiac adrenergic
VEGF	Vascular endothelial growth factor
β-AR	β-adrenoreceptor
DOR	δ-opioid receptor
Th	Tyrosine hydroxylase
DPDPE	DOR agonist [D-Pen^2, D-Pen^5] enkephalin
Dbh	Dopamine β-hydroxylase
CGRP	Calcitonin gene-related peptide
Pnmt	Phenylethanolamine-*N*-methyl transferase

LVH	Left ventricular hypertrophy
HH	Hamburger–Hamilton
VSMCs	Vascular smooth muscle cells
R26R	Rosa26 reporter
scRNA-Seq	Single-cell RNA sequencing
NSE	Neuron-specific enolase
Dbh$^+$-CM	*Dbh*$^+$ catecholaminergic cardiomyocyte
AVN	Atrioventricular node
Stereo-seq	Spatial enhanced resolution omics-sequencing
NAT	Noradrenaline transporter
CCS	Cardiac conduction system
SAN	Sinoatrial node
SV	Sinus venous
HCN4	Hyperpolarization activated cyclic nucleotide gated channel 4
PKJ	Purkinje
AC8	Adenylyl cyclase type 8
LED	Light emitting diode
PKA	Protein kinase A
ERP	Effective refractory period
ChR2	Channelrhodopsin 2
TEM	Transmission electron microscopy
PdCM	Pnmt$^+$ cell-derived cardiomyocyte
EDS	Energy dispersive X-ray spectroscopy
LA	Left atrium
DOPS	Dihydroxyphenyl serine
LV	Left ventricle
AADC	L-aromatic-amino-acid decarboxylase
RA	Right atrium
MEA	Microelectrode array
RV	Right ventricle
AI	Arrhythmic index
LSFM	Light-sheet fluorescence microscopy
BAC	Bacterial artificial chromosome
α-AR	α-adrenergic receptor
ATP	Adenosine 5′-triphosphate
CO	Cardiac output
ADP	Adenosine 5′-diphosphate

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M.L. designed the topic and structure of the paper. Y.Z., R.Y.R., X.W., F.Z., and M.L. drafted the paper. Y.Z., M.L., M.H., and A.G.R. revised the draft. T.S., X.O., and X.T. contributed to the revision and editing of the manuscript.

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References

1. Bucolo C, Leggio GM, Drago F, Salomone S. Dopamine outside the brain: the eye, cardiovascular system and endocrine pancreas. Pharmacol Ther. 2019;203: 107392.
2. Goldstein DS, Mezey E, Yamamoto T, Aneman A, Friberg P, Eisenhofer G. Is there a third peripheral catecholaminergic system? Endogenous dopamine as an autocrine/paracrine substance derived from plasma DOPA and inactivated by conjugation. Hypertens Res. 1995;18(Suppl 1):S93–9.
3. Neumann J, Hofmann B, Dhein S, Gergs U. Role of dopamine in the heart in health and disease. Int J Mol Sci. 2023;24(5): 5042.
4. Tank AW, Lee WD. Peripheral and central effects of circulating catecholamines. Compr Physiol. 2015;5(9):1–15.
5. Park DH, Kashimoto T, Ebstein RP, Goldstein M. Purification and immunochemical characterization of dopamine beta-hydroxylase from human pheochromocytoma. Mol Pharmacol. 1976;12(1):73–81.
6. Lizot G, Pasqualin C, Tissot A, Pagès S, Faivre JF, Chatelier A. Molecular and functional characterization of the mouse intrinsic cardiac nervous system. Heart Rhythm. 2022;19(10):1352–62.
7. Goldberger JJ, Arora R, Buckley U, Shivkumar K. Autonomic nervous system dysfunction: JACC Focus Seminar. J Am Coll Cardiol. 2019;73(11):1189–206.
8. de Lucia C, Eguchi A, Koch WJ. New insights in cardiac β-adrenergic signaling during heart failure and aging. Front Pharmacol. 2018;9:904.
9. Shen MJ, Choi EK, Tan AY, Lin SF, Fishbein MC, Chen LS, et al. Neural mechanisms of atrial arrhythmias. Nat Rev Cardiol. 2011;9(6):30–9.
10. Donald DE, Shepherd JT. Response to exercise in dogs with cardiac denervation. Am J Physiol. 1963;205(3):393–400.
11. Elayan HH, Kennedy BP, Ziegler MG. Cardiac atria and ventricles contain different inducible adrenaline synthesising enzymes. Cardiovasc Res. 1990;24(2):53–6.
12. Shaver JA, Leon DF, Gray S 3rd, Leonard JJ, Bahnson HT. Hemodynamic observations after cardiac transplantation. N Engl J Med. 1969;281(15):822–7.
13. Yue R, Ming L. The roles of intrinsic cardiac adrenergic (ICA) cells in cardiac repair. Oxford: University of Oxford; 2024.
14. Ignarro LJ, Shideman FE. Appearance and concentrations of catecholamines and their biosynthesis in the embryonic and developing chick. J Pharmacol Exp Ther. 1968;159(1):38–48.
15. Thomas SA, Matsumoto AM, Palmiter RD. Noradrenaline is essential for mouse fetal development. Nature. 1995;374(32):643–6.
16. López-Sánchez C, Bártulos O, Martínez-Campos E, Gañán C, Valenciano AI, García-Martínez V, et al. Tyrosine hydroxylase is expressed during early heart development and is required for cardiac chamber formation. Cardiovasc Res. 2010;88(1):111–20.
17. Ebert SN, Baden JM, Mathers LH, Siddall BJ, Wong DL. Expression of phenylethanolamine *N*-methyltransferase in the embryonic rat heart. J Mol Cell Cardiol. 1996;28(8):1653–8.
18. Ignarro LJ, Shideman FE. Catechol-O-methyl transferase and monoamine oxidase activities in the heart and liver of the embryonic and developing chick. J Pharmacol Exp Ther. 1968;159(1):29–37.
19. Végh AMD, Duim SN, Smits AM, Poelmann RE, Ten Harkel ADJ, DeRuiter MC, et al. Part and parcel of the cardiac autonomic nerve system: unravelling its cellular building blocks during development. J Cardiovasc Dev Dis. 2016;3(3):1–29.
20. de Boer BA, van den Berg G, de Boer PA, Moorman AF, Ruijter JM. Growth of the developing mouse heart: an interactive qualitative and quantitative 3D atlas. Dev Biol. 2012;368(2):203–13.
21. Shigenobu K, Tanaka H, Kasuya Y. Changes in sensitivity of rat heart to norepinephrine and isoproterenol during pre- and postnatal development and its relation to sympathetic innervation. Dev Pharmacol Ther. 1988;11(4):226–36.
22. Teitelman G, Baker H, Joh TH, Reis DJ. Appearance of catecholamine-synthesizing enzymes during development of rat sympathetic nervous system: possible role of tissue environment. Proc Natl Acad Sci U S A. 1979;76(1):509–13.
23. Fujinaga M, Scott JC. Gene expression of catecholamine synthesizing enzymes and beta adrenoceptor subtypes during rat embryogenesis. Neurosci Lett. 1997;231(2):108–12.
24. Huang MH, Friend DS, Sunday ME, Singh K, Haley K, Austen KF, et al. An intrinsic adrenergic system in mammalian heart. J Clin Invest. 1996;98(24):1298–303.
25. Pfeifer K, Boe SP, Rong Q, Ebert SN. Generating mouse models for studying the function and fate of intrinsic cardiac adrenergic cells. Ann N Y Acad Sci. 2004;1018(33):418–23.
26. Ebert SN, Rong Q, Boe S, Thompson RP, Grinberg A, Pfeifer K. Targeted insertion of the Cre-recombinase gene at the phenylethanolamine *N*-methyltransferase locus: a new model for studying the developmental distribution of adrenergic cells. Dev Dyn. 2004;231(29):849–58.
27. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet. 1999;21(1):70–1.
28. Natarajan AR, Rong Q, Katchman AN, Ebert SN. Intrinsic cardiac catecholamines help maintain beating activity in neonatal rat cardiomyocyte cultures. Pediatr Res. 2004;56(25):411–7.
29. Ebert SN, Thompson RP. Embryonic epinephrine synthesis in the rat heart before innervation: association with pacemaking and conduction tissue development. Circ Res. 2001;88(1):117–24.
30. Huang MH, Bahl JJ, Wu Y, Hu F, Larson DF, Roeske WR, et al. Neuroendocrine properties of intrinsic cardiac adrenergic cells in fetal rat heart. Am J Physiol Heart Circ Physiol. 2005;288(26):H497-503.
31. Peoples JN, Taylor DG, Katchman AN, Ebert SN. Intact calcium signaling in adrenergic-deficient embryonic mouse hearts. Biochem Biophys Res Commun. 2018;495(4):2547–52.
32. Burn SF. Detection of β-galactosidase activity: X-gal staining. Methods Mol Biol. 2012;886:241–50.
33. Garcia-Frigola C, Shi Y, Evans SM. Expression of the hyperpolarization-activated cyclic nucleotide-gated cation channel HCN4 during mouse heart development. Gene Expr Patterns. 2003;3(6):777–83.
34. Moen JM, Matt MG, Ramirez C, Tarasov KV, Chakir K, Tarasova YS, et al. Overexpression of a neuronal type adenylyl cyclase (type 8) in sinoatrial node markedly impacts heart rate and rhythm. Front Neurosci. 2019;13: 615.
35. Osuala K, Telusma K, Khan SM, Wu S, Shah M, Baker C, et al. Distinctive left-sided distribution of adrenergic-derived cells in the adult mouse heart. PLoS ONE. 2011;6(7): e22811.

36. Wang Y, Lin WK, Crawford W, Ni H, Bolton EL, Khan H, et al. Optogenetic control of heart rhythm by selective stimulation of cardiomyocytes derived from Pnmt(+) cells in murine heart. Sci Rep. 2017;7:40687.
37. Ren H, Pu Z, Sun T, Chen T, Liu L, Liu Z, et al. High-resolution 3D heart models of cardiomyocyte subpopulations in cleared murine heart. Front Physiol. 2022;13(46): 779514.
38. Ni H, Wang Y, Crawford W, Zhang S, Cheng L, Zhang H, et al. Three-dimensional image reconstruction of distribution of Pnmt(+) cell-derived cells in murine heart. Sci Data. 2017;4: 170134.
39. Rentschler S, Vaidya DM, Tamaddon H, Degenhardt K, Sassoon D, Morley GE, et al. Visualization and functional characterization of the developing murine cardiac conduction system. Development. 2001;128(10):1785–92.
40. Bareis DL, Morgan RE, Lau C, Slotkin TA. Maturation of sympathetic neurotransmission in the rat heart. IV. Effects guanethidine-induced sympathectomy on neonatal development of synaptic vesicles, synaptic terminal function and heart growth. Dev Neurosci. 1981;4(1):15–24.
41. Simpson P. Stimulation of hypertrophy of cultured neonatal rat heart cells through an alpha 1-adrenergic receptor and induction of beating through an alpha 1- and beta 1-adrenergic receptor interaction. Evidence for independent regulation of growth and beating. Circ Res. 1985;56(6):884–94.
42. Drugge ED, Rosen MR, Robinson RB. Neuronal regulation of the development of the alpha-adrenergic chronotropic response in the rat heart. Circ Res. 1985;57(3):415–23.
43. Ebert SN, Rong Q, Boe S, Pfeifer K. Catecholamine-synthesizing cells in the embryonic mouse heart. Ann N Y Acad Sci. 2008;1148:317–24.
44. Huang MH, Nguyen V, Wu Y, Rastogi S, Lui CY, Birnbaum Y, et al. Reducing ischaemia/reperfusion injury through delta-opioid-regulated intrinsic cardiac adrenergic cells: adrenopeptidergic co-signalling. Cardiovasc Res. 2009;84(3):452–60.
45. Huang MH, Knight PR 3rd, Izzo JL Jr. Ca^{2+}-induced Ca^{2+} release involved in positive inotropic effect mediated by CGRP in ventricular myocytes. Am J Physiol. 1999;276(1):R259–64.
46. Nguyen V, Zarraga IG, Rastogi S, Birnbaum Y, Uretsky B, Huang M-H. Abstract 820: Autoregulation of CGRP Expression via β_2-adrenoreceptor signaling in intrinsic cardiac adrenergic cells. Circulation. 2007;116(16):159.
47. Zucker RS, Delaney KR, Mulkey R, Tank DW. Presynaptic calcium in transmitter release and posttetanic potentiation. Ann N Y Acad Sci. 1991;635:191–207.
48. Kiviniemi AM, Breskovic T, Uglesic L, Kuch B, Maslov PZ, Sieber A, et al. Heart rate variability during static and dynamic breath-hold dives in elite divers. Auton Neurosci. 2012;169(2):95–101.
49. Mochizuki-Oda N, Takeuchi Y, Matsumura K, Oosawa Y, Watanabe Y. Hypoxia-induced catecholamine release and intracellular Ca2+ increase via suppression of K+ channels in cultured rat adrenal chromaffin cells. J Neurochem. 1997;69(1):377–87.
50. Killingsworth CR, Wei CC, Dell'Italia LJ, Ardell JL, Kingsley MA, Smith WM, et al. Short-acting beta-adrenergic antagonist esmolol given at reperfusion improves survival after prolonged ventricular fibrillation. Circulation. 2004;109(20):2469–74.
51. Sasaki H, Ray PS, Zhu L, Otani H, Asahara T, Maulik N. Hypoxia/reoxygenation promotes myocardial angiogenesis via an NF kappa B-dependent mechanism in a rat model of chronic myocardial infarction. J Mol Cell Cardiol. 2001;33(2):283–94.
52. Wong GT, Ling LJ, Irwin MG. Activation of central opioid receptors induces cardioprotection against ischemia-reperfusion injury. Anesth Analg. 2010;111(1):24–8.
53. Sorriento D, Santulli G, Del Giudice C, Anastasio A, Trimarco B, Iaccarino G. Endothelial cells are able to synthesize and release catecholamines both *in vitro* and *in vivo*. Hypertension. 2012;60(1):129–36.
54. Garg J, Feng YX, Jansen SR, Friedrich J, Lezoualc'h F, Schmidt M, et al. Catecholamines facilitate VEGF-dependent angiogenesis via β2-adrenoceptor-induced Epac1 and PKA activation. Oncotarget. 2017;8(27):44732–48.
55. Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by delta1- but not mu- or kappa-opioid receptors. Circulation. 1998;97(13):1282–9.
56. Mieno S, Horimoto H, Sawa Y, Watanabe F, Furuya E, Horimoto S, et al. Activation of beta2-adrenergic receptor plays a pivotal role in generating the protective effect of ischemic preconditioning in rat hearts. Scand Cardiovasc J. 2005;39(5):313–9.
57. Tong H, Bernstein D, Murphy E, Steenbergen C. The role of beta-adrenergic receptor signaling in cardioprotection. FASEB J. 2005;19(8):983–5.
58. Peart JN, Gross GJ. Cardioprotective effects of acute and chronic opioid treatment are mediated via different signaling pathways. Am J Physiol Heart Circ Physiol. 2006;291(4):H1746–53.
59. Huang MH, Wang HQ, Roeske WR, Birnbaum Y, Wu Y, Yang NP, et al. Mediating delta-opioid-initiated heart protection via the beta2-adrenergic receptor: role of the intrinsic cardiac adrenergic cell. Am J Physiol Heart Circ Physiol. 2007;293(34):H376–84.
60. Chai W, Mehrotra S, Danser AH, Schoemaker RG. The role of calcitonin gene-related peptide (CGRP) in ischemic preconditioning in isolated rat hearts. Eur J Pharmacol. 2006;531(1–3):246–53.
61. Rapacciuolo A, Esposito G, Caron K, Mao L, Thomas SA, Rockman HA. Important role of endogenous norepinephrine and epinephrine in the development of in vivo pressure-overload cardiac hypertrophy. J Am Coll Cardiol. 2001;38(3):876–82.
62. Cooper T, Willman VL, Jellinek M, Hanlon CR. Heart autotransplantation: effect on myocardial catecholamine and histamine. Science. 1962;138(3536):40–1.
63. Bengel FM, Ueberfuhr P, Schiepel N, Nekolla SG, Reichart B, Schwaiger M. Effect of sympathetic reinnervation on cardiac performance after heart transplantation. N Engl J Med. 2001;345(10):731–8.
64. Tamura Y, Sano M, Nakamura H, Ito K, Sato Y, Shinmura K, et al. Neural crest-derived resident cardiac cells contribute to the restoration of adrenergic function of transplanted heart in rodent. Cardiovasc Res. 2016;109(3):350–7.
65. Goodroe R, Bonnema DD, Lunsford S, Anderson P, Ryan-Baille B, Uber W, et al. Severe left ventricular hypertrophy 1 year after transplant predicts mortality in cardiac transplant recipients. J Heart Lung Transplant. 2007;26(2):145–51.

66. Milano CA, Dolber PC, Rockman HA, Bond RA, Venable ME, Allen LF, et al. Myocardial expression of a constitutively active alpha 1B-adrenergic receptor in transgenic mice induces cardiac hypertrophy. Proc Natl Acad Sci U S A. 1994;91(21):10109–13.
67. Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, et al. Alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. Circulation. 2005;111(9):1192–8.
68. Huang MH, Lui CY, Abusaid GH, Poh KK, Barbagelata AN, Uretsky BF, et al. Cardiac calcitonin gene-related peptide and left ventricular hypertrophy in the cardiac allograft. J Heart Lung Transplant. 2010;29(4):487–8.
69. Bell D, Schlüter KD, Zhou XJ, McDermott BJ, Piper HM. Hypertrophic effects of calcitonin gene-related peptide (CGRP) and amylin on adult mammalian ventricular cardiomyocytes. J Mol Cell Cardiol. 1995;27(11):2433–43.
70. Dhaliwal A, Thohan V. Cardiac allograft vasculopathy: the Achilles' heel of long-term survival after cardiac transplantation. Curr Atheroscler Rep. 2006;8(2):119–30.
71. O'Malley MK, McDermott EW, Mehigan D, O'Higgins NJ. Role for prazosin in reducing the development of rabbit intimal hyperplasia after endothelial denudation. Br J Surg. 1989;76(9):936–8.
72. deBlois D, Schwartz SM, van Kleef EM, Su JE, Griffin KA, Bidani AK, et al. Chronic alpha 1-adrenoreceptor stimulation increases DNA synthesis in rat arterial wall. Modulation of responsiveness after vascular injury. Arterioscler Thromb Vasc Biol. 1996;16(9):1122.
73. Blaschko H, Hagen JM, Hagen P. Mitochondrial enzymes and chromaffin granules. J Physiol. 1957;139(2):316–22.
74. Gonzalez-Lopez E, Vrana KE. Dopamine beta-hydroxylase and its genetic variants in human health and disease. J Neurochem. 2020;152(2):157–81.
75. Sun T, Grassam-Rowe A, Pu Z, Li Y, Ren H, An Y, et al. Dbh^{+} catecholaminergic cardiomyocytes contribute to the structure and function of the cardiac conduction system in murine heart. Nat Commun. 2023;14(1):7801.
76. Sun T, Ming L. Study of catecholaminergic cells in murine heart. Oxford: University of Oxford; 2021.
77. Swoap SJ, Weinshenker D, Palmiter RD, Garber G. Dbh(−/−) mice are hypotensive, have altered circadian rhythms, and have abnormal responses to dieting and stress. Am J Physiol Regul Integr Comp Physiol. 2004;286(3):R108–13.
78. Baker C, Taylor DG, Osuala K, Natarajan A, Molnar PJ, Hickman J, et al. Adrenergic deficiency leads to impaired electrical conduction and increased arrhythmic potential in the embryonic mouse heart. Biochem Biophys Res Commun. 2012;423(4):536–41.
79. Baker CN, Gidus SA, Price GF, Peoples JN, Ebert SN. Impaired cardiac energy metabolism in embryos lacking adrenergic stimulation. Am J Physiol Endocrinol Metab. 2015;308(5):E402–13.
80. Wassenberg T, Deinum J, van Ittersum FJ, Kamsteeg EJ, Pennings M, Verbeek MM, et al. Clinical presentation and long-term follow-up of dopamine beta hydroxylase deficiency. J Inherit Metab Dis. 2021;44(6):554–65.
81. Smith GD, Watson LP, Pavitt DV, Mathias CJ. Abnormal cardiovascular and catecholamine responses to supine exercise in human subjects with sympathetic dysfunction. J Physiol. 1995;484(7):255–65.
82. Smith GD, Mathias CJ. Postural hypotension enhanced by exercise in patients with chronic autonomic failure. QJM. 1995;88(8):251–6.
83. Shibao CA, Garland EM, Black BK, Mathias CJ, Grant MB, Root AW, et al. Congenital absence of norepinephrine due to CYB561 mutations. Neurology. 2020;94(9):e200–4.
84. Cubells JF, Schroeder JP, Barrie ES, Manvich DF, Sadee W, Berg T, et al. Human bacterial artificial chromosome (BAC) transgenesis fully rescues noradrenergic function in dopamine β-hydroxylase knockout mice. PLoS ONE. 2016;11(10): e0154864.

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