

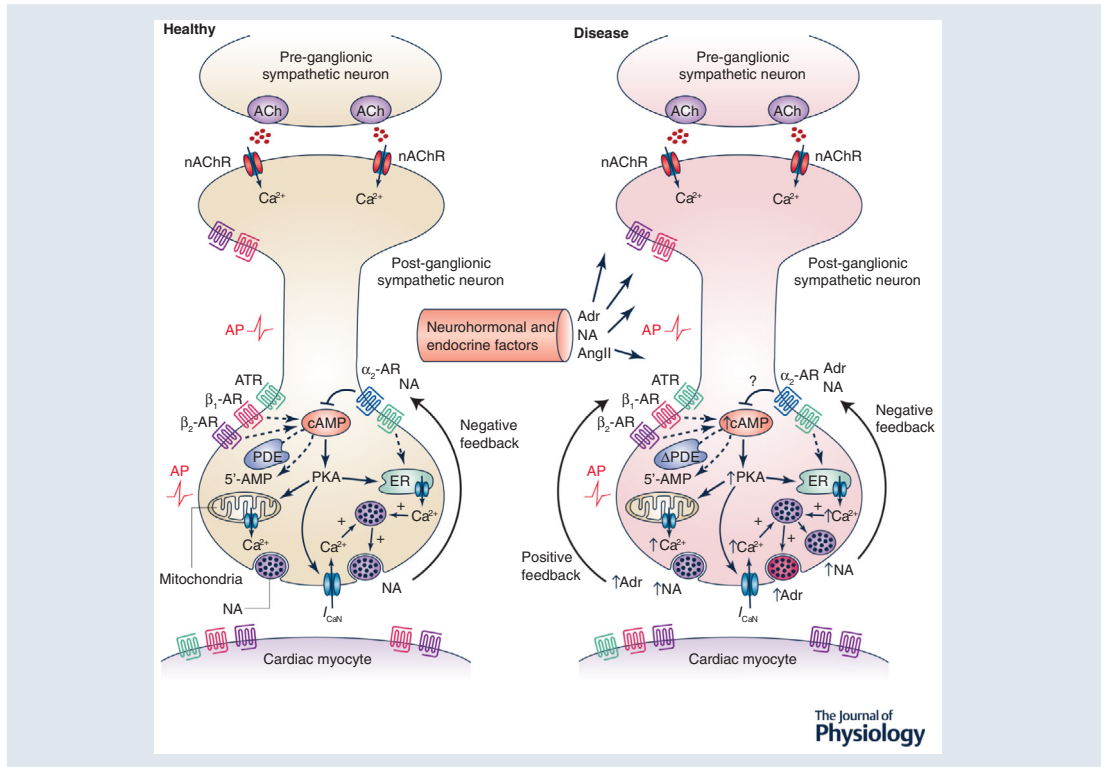
Neurocardiac regulation: from cardiac mechanisms to novel therapeutic approaches

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Abstract Cardiac sympathetic overactivity is a well-established contributor to the progression of neurogenic hypertension and heart failure, yet the underlying pathophysiology remains unclear. Recent studies have highlighted the importance of acutely regulated cyclic nucleotides and their effectors in the control of intracellular calcium and exocytosis. Emerging evidence now suggests that a significant component of sympathetic overactivity and enhanced transmission may arise from impaired cyclic nucleotide signalling, resulting from compromised phosphodiesterase activity, as well as alterations in receptor-coupled G-protein activation. In this review, we address some of the key cellular and molecular pathways that contribute to sympathetic overactivity in hypertension and discuss their potential for therapeutic targeting.

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Abstract figure legend In healthy stellate neurons, Ca^{2+} -dependent exocytosis facilitates the release of noradrenaline (NA) onto cardiac myocytes, where post-synaptic β_1 -ARs and β_2 -ARs are activated. Increases in extracellular NA acts on presynaptic α_2 -ARs, and presynaptic β_1 -ARs and β_2 -ARs to a small degree. Acute regulation of cAMP and cGMP signalling is maintained by phosphodiesterases (PDEs). cAMP generation and PKA activity increases $[\text{Ca}^{2+}]_i$ via phosphorylation of the N-type Ca^{2+} Channel (ICa_N ; $\text{Ca}_v2.2$); endoplasmic reticulum (ER) store and mitochondrial Ca^{2+} release. In diseased sympathetic neurons, Ca^{2+} -dependent exocytosis facilitates the release of NA and adrenaline (Adr) onto cardiac myocytes, where post-synaptic β_2 -AR signalling is upregulated and preferentially activated. Chronic elevations in NA and Adr release also acts on presynaptic β -ARs. Adr preferentially stimulates the β_2 -AR isoform, thus augmenting cAMP generation and PKA activity in prehypertension, in a potentiating feed-forward manner. Increased PKA activity raises $[\text{Ca}^{2+}]_i$ via phosphorylation of the N-type Ca^{2+} Channel (ICa_N ; $\text{Ca}_v2.2$), exacerbating the Ca^{2+} phenotype that may contribute to the initiation of hypertension.

Introduction

The autonomic nervous system, comprising the parasympathetic and sympathetic branches, provides a regulatory link between the central nervous system (CNS) and myocardium (Herring & Paterson, 2018). The notion of a mind–body connection has been proposed by many scientists throughout history, but it was perhaps first recorded in AD 30 by the Roman physician Celsus who wrote, ‘fear and anger and any other state of mind may often be apt to excite the pulse’ (Celsus & Spencer, 1935). Yet, the physiological mechanisms responsible for the relationship between the heart and the brain remained elusive until the 19th century, whereupon, it was discovered that heart rate could be accelerated or decelerated by stimulation of two antagonistic systems: sympathetic or parasympathetic nerve fibres (Gaskell, 1886; Langley, 1898; Woollard, 1926; Sheehan, 1936; Hoff, 1940). The ‘autonomic nervous system’, as coined by Langley in 1898 (Langley, 1898), is now known to play an integral role in cardiovascular homeostasis and cardiac responses to physical or emotional disturbances (Rozanski *et al.* 1999; Steptoe & Kivimaki, 2012; Tahsili-Fahadan & Geocadin, 2017; Herring & Paterson, 2018).

The cervicothoracic sympathetic stellate ganglion located adjacently to T1–T4 preferentially innervates the heart (Gaskell, 1886; Korzina *et al.* 2011) and, as such, exerts the greatest control over heart rate acceleration, contractility and conduction velocity at the

atrio-ventricular node (Shivkumar *et al.* 2016). Chronic alteration in sympathetic/parasympathetic balance (dysautonomia) is a well-established contributor to many cardiovascular diseases (CVDs) and is strongly linked to clinical outcome and prognosis (Brook & Julius, 2000; Palatini & Julius, 2004; Malpas, 2010; Parati & Esler, 2012; Mancina & Grassi, 2014). Increasing evidence suggests that essential hypertension is underpinned and maintained by sustained elevations in sympathetic nerve activity (SNA) and chronic end-organ transmission (Iriuchijima, 1973; Judy *et al.* 1979; Esler *et al.* 1986; 1988; Grassi & Esler, 1999; Johansson *et al.* 1999; Guyenet, 2006; Wang *et al.* 2006; Malpas, 2010; Parati & Esler, 2012; Shanks *et al.* 2013b; Esler, 2014; Oliveira-Sales *et al.* 2014; Grassi *et al.* 2015; Oliveira-Sales *et al.* 2016). Elevations in SNA are also frequently seen in normotensive progeny of hypertensive patients (Ferrara *et al.* 1988; Hausberg *et al.* 1998; Lopes *et al.* 2000; Piccirillo *et al.* 2000; Maver *et al.* 2004; Hamer, 2006; Pal *et al.* 2011; Johncy *et al.* 2015), suggesting a causative role and potential genetic basis (Judy *et al.* 1979; Horikoshi *et al.* 1985; Adams *et al.* 1989) for sympathetic overactivity in the aetiology of hypertension.

However, it is also well established that SNA is not uniformly altered within each ganglionic site (Grassi *et al.* 2015) and preclinical models have highlighted the critical role of elevated cardiac sympathetic nerve activity, specifically in the initiation and maintenance of hypertension (Souza *et al.* 2001; Petersson *et al.* 2002;

Tan *et al.* 2010; Shanks *et al.* 2013b; Larsen *et al.* 2016a; Tromp *et al.* 2018), cardiac arrhythmia (Meredith *et al.* 1991) and heart failure (Kaye *et al.* 1995; Rundqvist *et al.* 1997; Watson *et al.* 2007; Ramchandra *et al.* 2009; Tu *et al.* 2014). Multiple levels of the neural axis comprising several integrated feedback loops are involved in the regulation of autonomic transmission, and may be disturbed in hypertension. These include cardio-cardiac reflexes and intrinsic cardiac nerve activity that alter end-organ transmission within the myocardium directly, intrathoracic reflexes and feedback mechanisms that modify sympathetic ganglionic efferent transmission, and spinal and lower brainstem regulation that modulate autonomic outflow (Shivkumar *et al.* 2016; Hanna *et al.* 2017). Sustained alterations in one or several of these feedback processes may directly contribute to an elevation in SNA, yet it is difficult to dissociate the primary causative events from secondary consequential factors. Nevertheless, the dominance of cardiac sympathetic neurons over myocyte function is observed. This is illustrated in Fig. 1, where co-cultures of diseased stellate neurons and myocytes from rats predisposed to hypertension display enhanced myocyte cyclic adenosine monophosphate (cAMP) generation during neuronal stimulation compared to normal co-cultures (Larsen *et al.* 2016b). Moreover, cross-culturing diseased stellate neurons provokes healthy myocytes into a pre-hypertensive state partially recapitulating the elevation in cAMP observed in diseased myocytes. Critically, however, healthy neurons cultured with diseased myocytes rescues the aberrant myocardial cAMP response restoring cAMP to levels seen in normal myocytes (Larsen *et al.* 2016b). What are the mechanisms that underpin the sympathetic phenotype and lead to elevated cardiac sympathetic transmission?

In models of neurogenic hypertension, several key sympathetic adaptations are reported, including increased neuronal firing rate and burst frequency (Iriuchijima, 1973; Briant *et al.* 2015), elevated and aberrant regulation of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) that facilitates exocytosis (Li *et al.* 2013; Larsen *et al.* 2016a; Shanks *et al.* 2017; Tomek *et al.* 2017), decreased transmitter reuptake (Esler *et al.* 1981; Kimura *et al.* 1983; Esler *et al.* 1991; Rumantir *et al.* 2000b; Shanks *et al.* 2013a), and alterations in pre-synaptic feedback systems coupled to impaired intracellular signalling cascades (Wang *et al.* 2006; Shanks *et al.* 2013b; Bardsley *et al.* 2018b). In this brief review, we present the current evidence for the molecular and biochemical alterations that occur in stellate ganglia from rat and human patients that have a sympathetic phenotype and discuss their potential for therapeutic targeting.

Intrinsic excitability: control by cyclic nucleotides

The N-type Ca^{2+} channel is the primary neuronal voltage-gated Ca^{2+} channel (Catterall, 2003, 2011) and as

such plays a critical role in determining the cytosolic Ca^{2+} concentration during an action potential in sympathetic neurons (Pruneau & B  lichard, 1992; Ino *et al.* 2001; Mori *et al.* 2002; Uhrenholt & Nedergaard, 2003; Tu *et al.* 2014; Larsen *et al.* 2016a). Emerging evidence suggests that N-type Ca^{2+} channel activity is elevated in cardiac sympathetic ganglia in the prehypertensive SHR (Fig. 2; Larsen *et al.* 2016a) and in heart failure (Tu *et al.* 2014), indicating a synaptopathy that augments intracellular Ca^{2+} and raises the intrinsic excitability of these nerves (Briant *et al.* 2015). Voltage-gated Ca^{2+} channel conductance is differentially regulated by kinase phosphorylation (Gray *et al.* 1998; Schroder, 2003; Mahapatra *et al.* 2012; Larsen *et al.* 2016a) where processes that decrease cyclic guanosine monophosphate (cGMP)–protein kinase G (PKG) signalling, or elevate cAMP–protein kinase A (PKA) signalling result in a net increase in Ca^{2+} channel conductance (Brown & Birnbaumer, 1988; Leiser & Fleischer, 1996; Gray *et al.* 1998; D’Ascenzo *et al.* 2002; Schroder, 2003; Mahapatra *et al.* 2012; Zamponi *et al.* 2015; Sandoval *et al.* 2017). Thus, processes that selectively modulate the strength of cAMP or cGMP signals effectively regulate neuronal transmission (Pruneau & B  lichard, 1992; Leiser & Fleischer, 1996; Gray *et al.* 1998; Molderings *et al.* 2000; Ino *et al.* 2001; Mori *et al.* 2002; Tanaka *et al.* 2013; Yamada *et al.* 2014).

An increased cAMP–PKA/cGMP–PKG ratio exacerbates cardiac sympathetic activity

Nitric oxide (NO) is a significant neuronal modulator of sympatho-vagal activity (Sears *et al.* 1998; Wang *et al.* 2007). In the SHR, impaired NO generation via neuronal nitric oxide synthase (nNOS; Wang *et al.* 2007; Danson *et al.* 2009; Lee *et al.* 2009; Li *et al.* 2013, 2015; Lu *et al.* 2015) and down-regulation of soluble guanylyl cyclase (sGC; Li *et al.* 2013; Bardsley *et al.* 2018a) lead to significant reductions in cGMP production and PKG activity (Li *et al.* 2013, 2015; Larsen *et al.* 2016a). In the prehypertensive rat, deficits in cGMP–PKG signalling are directly linked to elevations in N-type Ca^{2+} channel Ca^{2+} conductance (Larsen *et al.* 2016a; Fig. 3) and may contribute to the increased firing rate and spike amplitude observed in models of disease (Briant *et al.* 2014; Tu *et al.* 2014). To understand the genetic basis for these observations, we carried out a comprehensive RNA sequencing study using ganglia from hypertensive and normotensive rats (Bardsley *et al.* 2018a) and found that transcripts within the cGMP–PKG pathway were significantly under-represented in the stellate ganglia of SHR with established hypertension. Notable transcripts included down-regulation of protein kinase G II (*Prkg2*) and the $\alpha 1$ -sGC subunit (*Gucyl1a3*). Genome wide association studies (GWAS) have also revealed a critical link between

mutations in loci containing the gene *Gucy1a3* and clinical hypertension (Ehret *et al.* 2011; Zheng *et al.* 2015; Wallace *et al.* 2016; Rippe *et al.* 2017; Seidel & Scholl, 2017), myocardial infarction (Erdmann *et al.* 2013; Wobst *et al.* 2015), atherosclerosis (Segura-Puimedon *et al.* 2016; Wobst *et al.* 2016) and coronary artery disease (CARDIo-

GRAMplusC4D Consortium *et al.* 2013; Nikpay *et al.* 2015; Kessler *et al.* 2017).

Reductions in cGMP–PKG or increases in cAMP–PKA augment Ca^{2+} conductance (Fig. 3) via site-specific phosphorylation of the N-type Ca^{2+} channel, where a shift towards cAMP–PKA signalling in hypertension

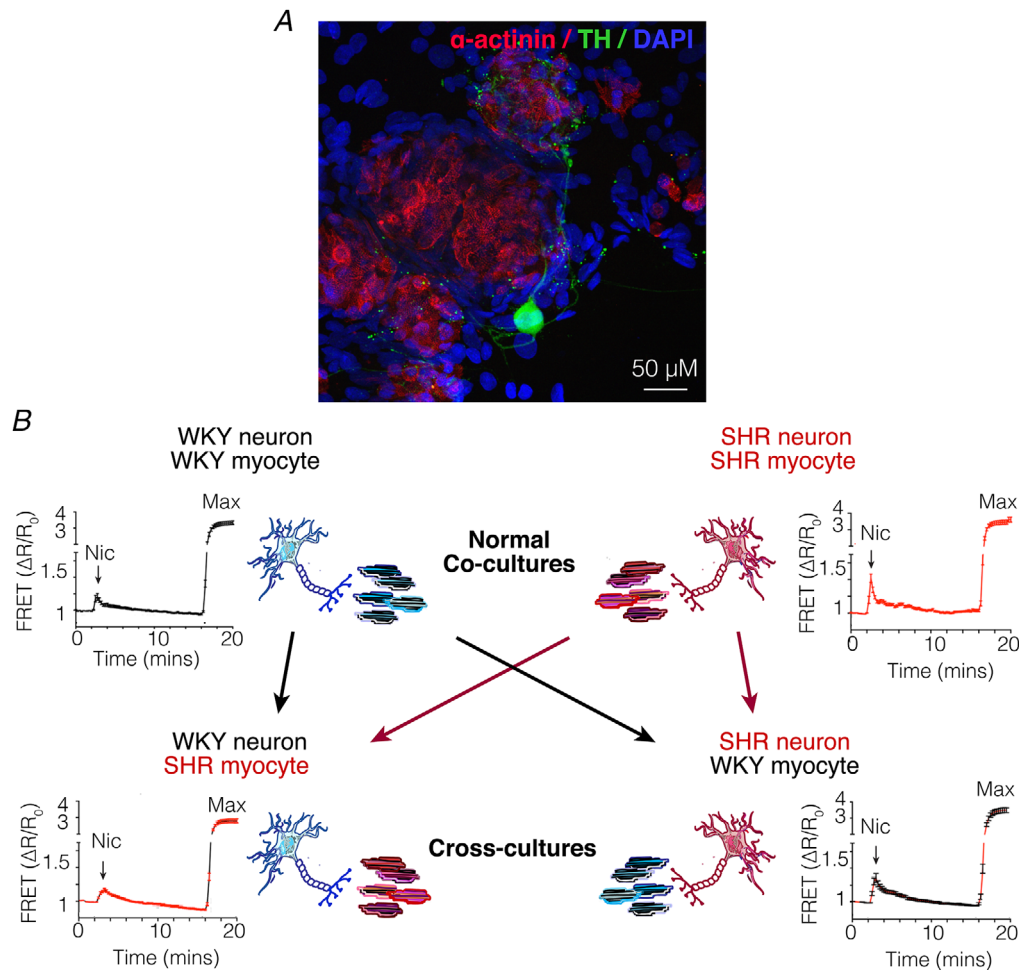


Figure 1. Sympathetic neurons are a powerful driver of myocyte function in cardiovascular disease

A, immunofluorescence depicting a co-culture of sympathetic neurons and ventricular myocytes (reproduced from Larsen *et al.* 2016b). Sympathetic neurons labelled with tyrosine hydroxylase (TH, green) densely innervate cultured cardiomyocytes labelled with sarcomeric α -actinin (red). B, Wistar-Kyoto (WKY) or SHR sympathetic neurons were stimulated with nicotine (Nic) and the resulting myocyte cAMP was measured as a surrogate for sympathetic transmission, in myocytes transduced with a cAMP Förster resonance energy transfer (FRET) sensor. FRET sensors were maximally stimulated (max) with an adenylyl cyclase (AC) activator forskolin (25 M) and a non-specific phosphodiesterase (PDE) inhibitor 3-isobutyl-1-methylxanthine (IBMX, 100 M). In healthy co-cultures (WKYn/WKYm), neuron-evoked myocyte cAMP (17.05 ± 3.715 , $n = 29$ cells) was significantly lower than cAMP measured in the diseased co-culture myocytes (SHRn/SHRm; 44.02 ± 5.310 , $n = 36$ cells; $P < 0.0001$). Cross-cultures were established by plating diseased SHR neurons on top of healthy WKY myocytes (SHRn/WKYm) or healthy WKY neurons on top of diseased SHR myocytes (WKYn/SHRm). In the first cross-culture (SHRn/WKYm), neuronal stimulation elevated myocyte cAMP (31.37 ± 5.194 , $n = 42$ cells) to levels that were not significantly different from measured in the diseased (SHRn/SHRm) co-cultures ($P = 0.094$), demonstrating that enhanced neuronal transmission elevates healthy-myocyte cAMP to levels observed in disease. Moreover, in the second cross-culture (WKYn/SHRm), stimulation of WKY neurons elevated SHR myocyte cAMP (15.67 ± 1.936 , $n = 24$ cells) to levels that were not significantly different from that measured in healthy (WKYn/WKYm) co-cultures ($P = 0.76$), demonstrating that healthy neurons attenuate the elevated myocyte cAMP response observed in SHR myocytes (modified from Larsen *et al.* 2016b).

facilitates exocytosis (Leiser & Fleischer, 1996; Gray *et al.* 1998; D'Ascenzo *et al.* 2002; Tanaka *et al.* 2013; Larsen *et al.* 2016a). In support of the evidence for elevated cAMP–PKA activity in hypertension, we identified a significant down-regulation in the gene encoding the type I α regulatory subunit of PKA (*Prkar1a*) in our RNA sequencing dataset. This subunit plays a dominant role as an endogenous inhibitor of kinase activity (Bardsley *et al.* 2018a) where loss-of-function mutations in *Prkar1a* are associated with a twofold greater responsiveness to cAMP and an excess of PKA type II activity (Stratakis *et al.* 2001). Knock-out mouse models of *Prkar1a* display impaired axonal sorting, myelination and proliferation (Guo *et al.* 2013). In humans, *Prkar1a* mutations are characterised by endocrine overactivity, neural dysfunction and cardiac complications, which result in dysregulation of arterial blood pressure homeostasis, arrhythmia and cardiomyopathies (Stratakis, 2002; Horvath *et al.* 2010), highlighting the importance of cAMP–PKA signalling in neuronal and cardiovascular regulation. Consequently,

it appears that in cardiac sympathetic nerves from pre-hypertensive rats, several processes that favour excitatory cAMP–PKA signalling are up-regulated, whereas pathways coupled to NO–cGMP are critically impaired early in disease, thus exacerbating or underpinning the observed Ca²⁺ phenotype (Li *et al.* 2013, 2015; Larsen *et al.* 2016a; Fig. 3D).

Phosphodiesterase enzymes: the centre of balance for cyclic nucleotides

Phosphodiesterase enzymes (PDEs) regulate ion channel activity through selective termination of cAMP and/or cGMP signalling (Tanaka *et al.* 2013; Zhao *et al.* 2016); therefore, the acute spatial and temporal regulation of cyclic nucleotide (cN) levels by PDEs is critical for maintaining a fine balance between PKA- and/or PKG-mediated effects (Zaccolo & Movsesian, 2007; Stangherlin & Zaccolo, 2012). The cN signal is acutely maintained by the PDE superfamily, comprising

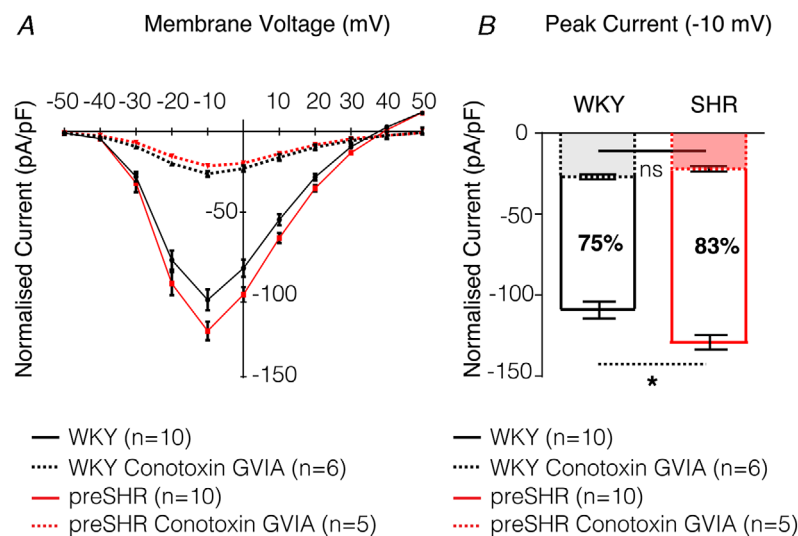


Figure 2. N-type Ca²⁺ channel conductance is elevated in preSHR cardiac sympathetic neurons

Whole cell voltage clamp was performed on cardiac sympathetic stellate neurons to investigate whole cell Ca²⁺ currents. *A*, the current–voltage relationship. Access to the cell was obtained in normal Tyrode's solution containing the following (in mM): 135 NaCl, 4.5 KCl, 11 glucose, 20 HEPES, 1MgCl₂, 2 CaCl₂, pH 7.4. To identify the Ca²⁺ current, normal Tyrode solution was replaced with a Ca²⁺-isolating solution using Ba²⁺ as the charge carrier, containing the following (in mM): 135 TEACl, 10 HEPES, 4.5 KCl, 1 MgCl₂, 4 glucose, 1 NaHCO₃, 2 BaCl₂, pH 7.40, either in the presence or absence of ω -conotoxin GVIA (1 μ M), which selectively blocks N-Type Ca²⁺ channels (IC₅₀ = 0.15 nM) (Sato *et al.* 1993). Ba²⁺ was used as the charge carrier to avoid Ca²⁺-dependent current inactivation (Imredy & Yue, 1994). The internal solution contained the following (in mM): 140 CsCl, 10 HEPES, 0.1 CaCl₂, 1 MgCl₂, 4 MgATP, 1 EGTA, pH 7.30. All solutions had osmolarities of 300 mOsm L⁻¹. *B*, the whole cell Ca²⁺ current is larger in preSHR sympathetic nerves (127.5 \pm 5.94 pA pF⁻¹, n = 10) compared to WKY cells (–108.0 \pm 6.80 pA pF⁻¹, n = 10, P = 0.045) where peak current was recorded at –10 mV. ω -Conotoxin GVIA (1 μ M), significantly reduced the N-type Ca²⁺ channel current to similar levels in both strains. A 75% reduction was observed in cells cultured from WKY stellate ganglia (–26.88 \pm 1.7 pA pF⁻¹, n = 6) and an 83% reduction was measured in neurons cultured from preSHR ganglia (–22.04 \pm 1.60 pA pF⁻¹, n = 5, ns) where peak current remained at –10 mV. Solid lines represent the mean of the WKY (black) and preSHR (red) control data. Dashed lines represent the mean of WKY (black) and preSHR (red) in the presence of ω -Conotoxin GVIA. Data are represented as mean \pm SEM. (*A* and *B* modified from Larsen *et al.* 2016a).

11 isoforms (Stangherlin & Zaccolo, 2012), which confine individual and unique cAMP/cGMP signals to distinct subcellular compartments, enabling the regulation of multiple effector responses at any given time (Lefkimiatis & Zaccolo, 2014). Indeed, cAMP is localised in close proximity to its effectors and regulators, where PKA, PDEs and phosphatases are tethered to A-kinase anchoring proteins forming signalosomes that restrict the duration and magnitude of the cAMP–PKA signal within specific subcellular domains (Musheshe

et al. 2018). Moreover, PDE isoforms are also subject to feedback inhibition and/or potentiation where specific isoforms are sensitive to cNs themselves (Zaccolo & Movsesian, 2007; Zhao *et al.* 2016), kinase activity (Zaccolo & Movsesian, 2007; Francis *et al.* 2011) and/or intracellular Ca^{2+} /calmodulin-dependent protein kinase signalling (Maurice, 2003; Bender, 2006; Francis *et al.* 2011). Sustained elevations in cAMP generation or alterations in PDE activity underpin several cardiovascular pathologies including cardiac hypertrophy (Zaccolo &

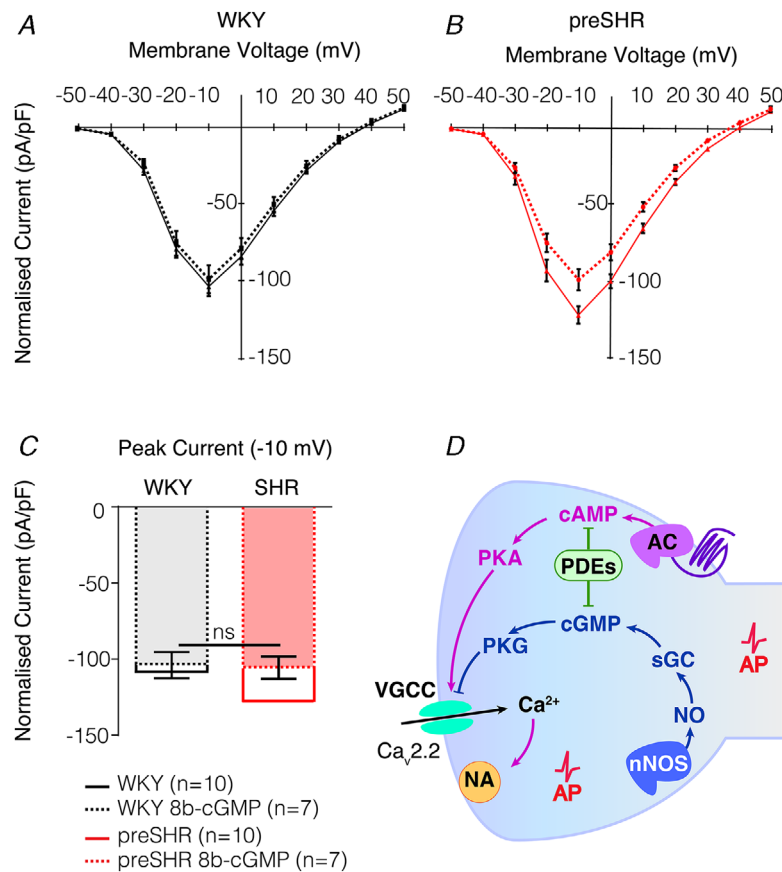


Figure 3. Elevated Ca^{2+} conductance in preSHR stellate neurons is rescued with cGMP administration

A–C, to ascertain whether cGMP signalling inhibits Ca^{2+} currents, whole cell voltage clamp was performed on sympathetic neurons from young normotensive WKY (A) and young prehypertensive SHR (B) in the presence of a cGMP analogue, 8-bromo-cGMP (8b-cGMP) (Larsen *et al.* 2016a). Access to the cell was obtained in normal Tyrode solution containing the following (in mM): 135 NaCl, 4.5 KCl, 11 glucose, 20 HEPES, 1 MgCl_2 , 2 CaCl_2 , pH 7.4. To identify the Ca^{2+} current, the solution was replaced with a Ca^{2+} -isolating solution using Ba^{2+} as the charge carrier, containing the following (in mM): 135 TEACl, 10 Hepes, 4.5 KCl, 1 MgCl_2 , 4 glucose, 1 NaHCO_3 , 2 BaCl_2 , pH 7.40, in either the presence or the absence of 8b-cGMP (100 μM). Ba^{2+} was used as the charge carrier to avoid Ca^{2+} -dependent current inactivation (Imredy & Yue, 1994). The internal solution contained the following (in mM): 140 CsCl, 10 Hepes, 0.1 CaCl_2 , 1 MgCl_2 , 4 MgATP, 1 EGTA, pH 7.30. All solutions had osmolarities of 300 mOsm L^{-1} . 8b-cGMP significantly reduced the elevated preSHR Ca^{2+} currents ($-127.5 \pm 5.94 \text{ pA pF}^{-1}$, $n = 10$ to $-105.2 \pm 7.79 \text{ pA pF}^{-1}$, $n = 7$) to levels that were no longer greater than WKY Ca^{2+} currents ($-108.0 \pm 6.80 \text{ pA pF}^{-1}$, $n = 10$). Moreover, 8b-cGMP had no significant effect on the WKY Ca^{2+} current, where peak currents were measured at -10 mV . Continuous lines represent the mean of the WKY (black) and preSHR (red) control data. Dashed lines represent the mean of WKY (black) and preSHR (red) in the presence of 8b-cGMP. Data are represented as mean \pm SEM. (A–C are reproduced from Larsen *et al.* 2016a). D, model diagram representing N-type Ca^{2+} channel control by PKA and PKG, where PKA augments and PKG inhibits channel conductance. Pathways that are decreased (blue) or increased (pink) in disease are represented. AP, action potential; NA, noradrenaline; VGCC, voltage-gated calcium channel; AC, adenylyl cyclase.

Movsesian, 2007; Sprenger *et al.* 2015; Zoccarato *et al.* 2015) and sympathetic overactivity in hypertension (Larsen *et al.* 2016a; Liu *et al.* 2018) where cAMP signals saturate the available PDEs and diffuse into neighbouring compartments leading to aberrant effector activity (Larsen *et al.* 2016a; Zhao *et al.* 2017).

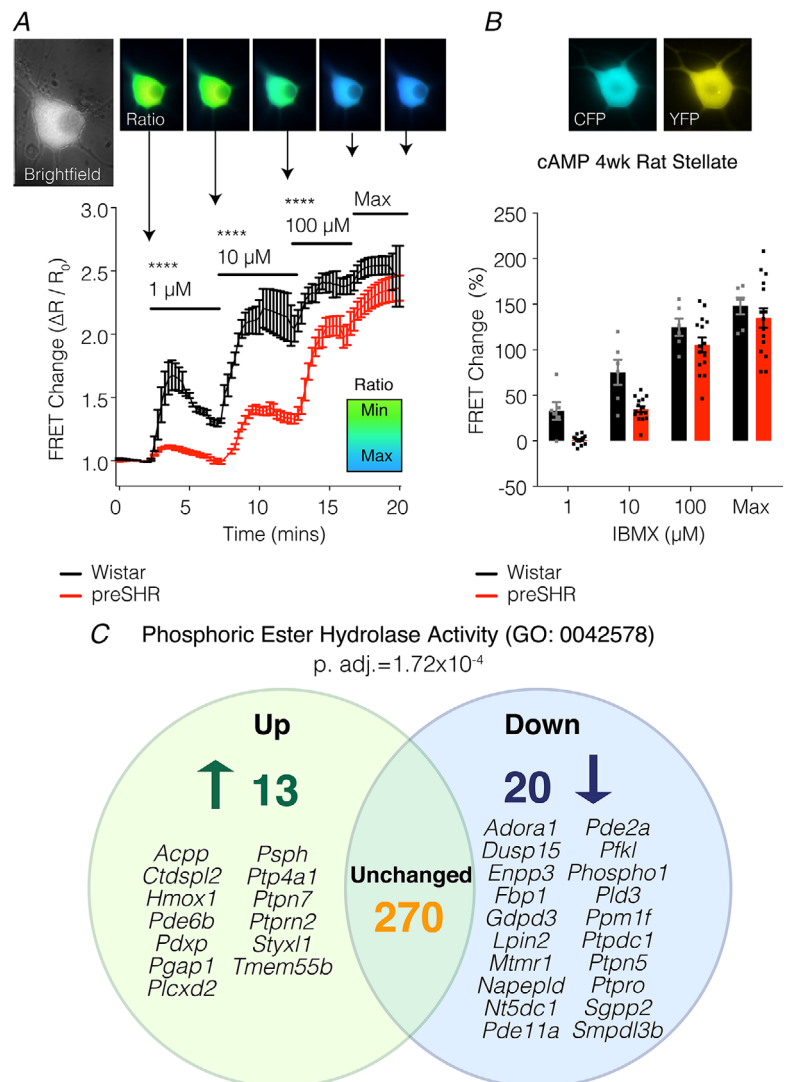
Phosphodiesterases in the cardiac sympathetic ganglia

We have previously reported that the activity of specific PDEs involved in the cross-talk between cAMP and cGMP pathways (PDE2a, PDE3) are impaired in cardiac sympathetic nerves in prehypertension (Li *et al.* 2015; Bardsley *et al.* 2016; Larsen *et al.* 2016a), and that cGMP pathways are preferentially diminished (Larsen *et al.* 2016a). However, a distinct contrast has also been identified in the hydrolysing activity of the wider PDE family within the sympathetic ganglia between

normotensive and prehypertensive strains (Fig. 4A). To understand the genetic basis for these observations, we carried out a gene ontology analysis from our RNA sequencing dataset and found that the genetic family representing 'phosphoric ester hydrolase activity' was significantly over-represented in established hypertension (Davis *et al.* 2018; Bardsley *et al.* 2018a), supporting pre-clinical reports and several clinical studies (Katz *et al.* 2000; Bender, 2006; Nagendran *et al.* 2007; Zaccolo & Movsesian, 2007; Lee *et al.* 2015; Maass *et al.* 2015; Zoccarato *et al.* 2015; Boda *et al.* 2016; Vettel *et al.* 2017; Assenza *et al.* 2018; Baliga *et al.* 2018; Bardsley *et al.* 2018a). It was observed that over 30 genes linked to the PDE superfamily are differentially expressed in the SHR stellate ganglia and that many of these mapped to regulators of PDE activity (Bardsley *et al.* 2018a; Fig. 4B), adding a further layer of complexity to the systems involved in cN control. Moreover, changes in transcripts do not necessarily lead to changes in protein

Figure 4. Phosphodiesterase (PDE) activity is impaired in preSHR neurons and has a genetic component

A and B, to investigate whether cytosolic PDE signalling is impaired in preSHR sympathetic neurons, a non-specific PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX; inhibits PDEs 1–7, 10–11), was administered to sympathetic stellate neurons (1–100 μ M). The resulting intracellular cAMP was measured using real-time Förster resonance energy transfer (FRET) in cells transduced with the adenovirus encoding the Epac- S^{H187} biosensor (Klarenbeek *et al.* 2015). A, there was significantly greater IBMX-stimulated cAMP in Wistar vs. preSHR neurons at all concentrations measured (two-way repeated measures ANOVA; $P < 0.05$) supporting the evidence that there is a differential PDE profile in preSHR vs. control stellate neurons. At 100 μ M IBMX, FRET responses were close to sensor saturation. B, peak FRET changes are depicted. Data are expressed as mean \pm SEM. C, we investigated whether transcriptomic changes could be identified in SHR stellate ganglia with established hypertension. Using RNA sequencing, it was observed that the molecular function gene ontology (GO) group encoding 'phosphoric ester hydrolase activity' (GO:0042578) was significantly over-represented in the SHR ganglia at 16 weeks. Thirty-three genes were found to be differentially expressed and many of these mapped to regulators of PDE and kinase activity (figure reproduced from Bardsley *et al.* 2018a).



levels. For example, RNA sequencing data revealed a decrease in *Pde2a* expression (Bardsley *et al.* 2018a), whereas PDE2A activity and protein levels are reportedly raised in SHR and human stellates (Li *et al.* 2015; Liu *et al.* 2018). Furthermore, over-expression of PDE2A in neuronal stellates recapitulates the Ca^{2+} phenotype and enhanced sympathetic response seen in disease (Li *et al.* 2015), illustrating complex interactions that may be related to microdomain signalling of various isoforms of PDE2A (Zhao *et al.* 2016).

Phosphodiesterases in the myocardium

Within the cardiac sympathetic axis, intrinsic electrical pacemaker activity arising from the sinoatrial node (SAN) dictates resting heart rate, which is increased by sympathetic noradrenaline and activation of myocardial G_α_s -coupled β -adrenergic receptors (β ARs). Elevation in myocardial cAMP–PKA activity regulates a large number of phospho-sensitive processes (Yaniv *et al.* 2015; Behar *et al.* 2016) and in particular plays a key role in elevating intracellular Ca^{2+} via phosphorylation of the L-type Ca^{2+} channel ($\text{Ca}_v1.2$, $\text{Ca}_v1.3$) (Zhao *et al.* 2016, 2017; Hua *et al.* 2012) as well as phospholamban, which increases Ca^{2+} reuptake by the sarcoplasmic reticulum (SR), facilitating rapid repolarisation (Simmerman & Jones, 1998; Mattiazzi & Kranias, 2014; Akaike *et al.* 2017). Conversely, mediators that elevate cGMP–PKG, such as NO coupled to sGCs or activation of membrane-bound particulate guanylyl cyclase (pGC) receptors (e.g. ANP, BNP), oppose the actions of cAMP–PKA, thus limiting intracellular Ca^{2+} . Sustained elevations in cAMP–PKA activity (Sprenger *et al.* 2015) and/or reductions in cardiac NO–cGMP signalling (Heaton *et al.* 2006; Dawson *et al.* 2008; Baliga *et al.* 2018) that elevate $[\text{Ca}^{2+}]_i$ (Leiser & Fleischer, 1996; Mattiazzi & Kranias, 2014; Zhao *et al.* 2016, 2017) are involved in cardiac remodelling and hypertrophy (Sprenger *et al.* 2015; Zoccarato *et al.* 2015), arrhythmia (Kalla *et al.* 2016) and heart failure (Kaye *et al.* 1995; Mehel *et al.* 2013; Florea & Cohn, 2014). In the SHR model, atrial myocytes display a greater cAMP response to β AR stimulation (Heaton *et al.* 2006), and lower basal levels of NO–cGMP (Heaton *et al.* 2006). Gene transfer approaches targeted to the SAN to up-regulate neuronal nitric oxide synthase (nNOS) or its anchoring protein CAPON (Lu *et al.* 2015) successfully reduce the surface density and activity of L-type Ca^{2+} currents (Danson *et al.* 2005) and decrease intracellular concentrations of cAMP via the proposed activation of PDE2a (Danson *et al.* 2005), highlighting a novel therapeutic potential for targeting cNs and their effectors within the myocardium directly. The intricacy of cN regulation, the inability to target specific PDE isoforms that reside in precise intracellular compartments, and the high-level of functional redundancy observed in the PDE superfamily perhaps help

to explain the lack of clinical efficacy achieved by selective PDE inhibitors. Computational protein design, protein engineering and the application of targeted vector systems may provide innovative solutions to these problems.

Neurohormonal and endocrine signalling: effects on presynaptic sympathetic nerves

Impaired neurohormonal regulation plays a critical role in the pathogenesis and progression of cardiovascular diseases (Malpas, 2010). Plasma and tissue levels of noradrenaline (NA), adrenaline (Adr), angiotensin II (AngII), aldosterone and other mediators are significantly altered in hypertension and heart failure and correlate with the severity of disease (Catt *et al.* 1971; Dang *et al.* 1999; Grassi & Esler, 1999; Romero & Reckelhoff, 1999; Rupp & Jäger, 2001; Schiffer *et al.* 2009; Riet *et al.* 2015; Shinohara *et al.* 2015; Najafi *et al.* 2016). Therapeutics aimed at opposing elevated adrenergic and/or antagonising renin–angiotensin–aldosterone signalling are gold-standard treatment strategies for blood pressure maintenance (van den Meiracker *et al.* 1995; Hansson *et al.* 1999; White *et al.* 2003; Flack *et al.* 2007; Ram, 2010; Nussberger & Bohlender, 2013; Williams *et al.* 2015; Frishman, 2016; Ghazi & Drawz, 2017; Rubattu *et al.* 2018; Wiysonge *et al.* 2018). Nevertheless, their precise mechanisms of action still remain unclear (Nussberger *et al.* 1986; van den Meiracker *et al.* 1995; Nussberger & Bohlender, 2013; Riet *et al.* 2015; Watanabe *et al.* 2017).

NA transmission plays a dominant role in vascular constriction and cardiac output (Herring & Paterson, 2018), whereas sustained elevations are involved in hypertension (Shanks *et al.* 2013b), arrhythmia (Meredith *et al.* 1991) and heart failure (Kaye *et al.* 1995; Florea & Cohn, 2014). In the 1980s, it was demonstrated that the activation of presynaptic β -ARs facilitates transmission within several peripheral ganglia (Lokhandwala & Eikenburg, 1983; Majewski, 1983; Misu & Kubo, 1986; Nedergaard & Abrahamsen, 1990; Apparsundaram & Eikenburg, 1995), yet little is known about the physiological or pathophysiological relevance of these receptors in hypertension. Recently, we demonstrated that activation of sympathetic stellate presynaptic β -AR receptors leads to cAMP–PKA activation that is significantly elevated in the prehypertensive SHR and is predominantly β_2 -AR mediated (Bardsley *et al.* 2018b) (Fig. 5). This increase in cAMP–PKA signalling augments high K^{+} -evoked Ca^{2+} liberation in neurons from prehypertensive rats, reflecting ion channel involvement (Bardsley *et al.* 2018b). These findings suggest a feed-forward potentiating mechanism exists for catecholaminergic regulation of cardiac sympathetic transmission, which exacerbates the cAMP/cGMP imbalance in disease (Fig. 6). To give these observations contextual relevance, we also confirmed the presence of β -ARs in human stellate ganglia, highlighting

an alternative site of action for the efficacy achieved with sustained clinical β -blocker therapy (Ram, 2010; Frishman, 2016; Wiysonge *et al.* 2018).

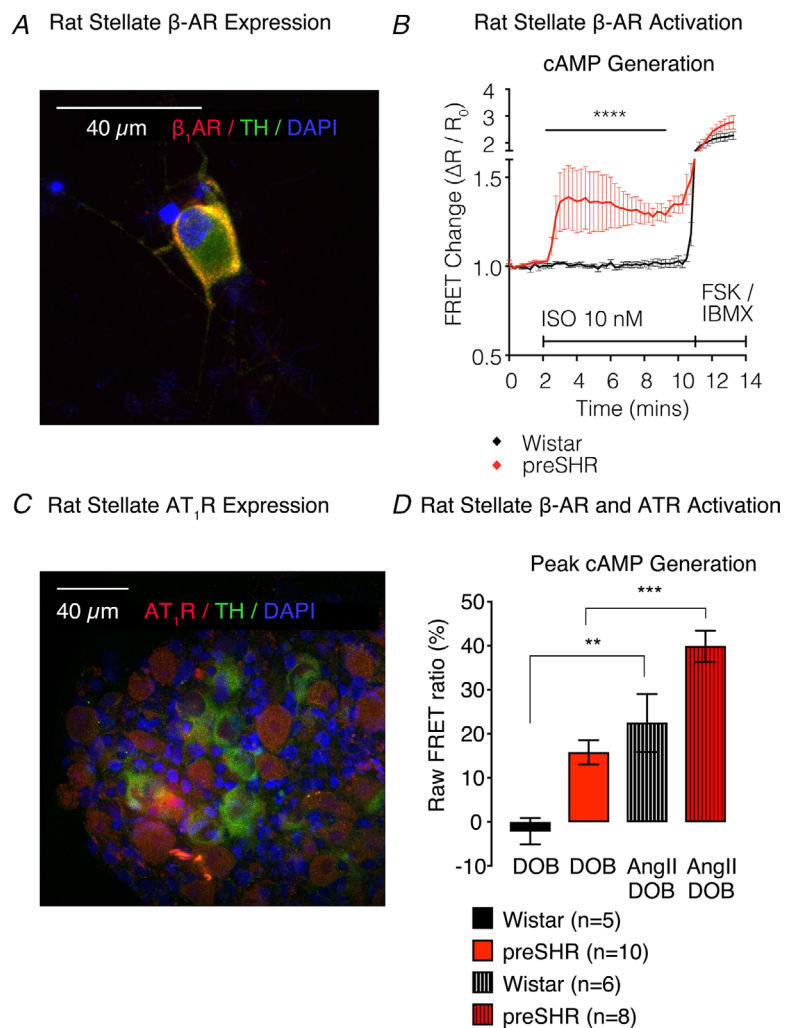
The renin–angiotensin system (RAS) is critically involved in blood pressure regulation and fluid volume homeostasis (Hall, 1986; Herring & Paterson, 2018) and alterations in RAS signalling are strongly associated with the aetiology of cardiovascular disease (Dang *et al.* 1999; Weir & Dzau, 1999; Rupp & Jäger, 2001; Crowley *et al.* 2006; Riet *et al.* 2015). AngII is a bioactive product of RAS that is synthesised through sequential cleavage of angiotensinogen and angiotensin I by the enzymes renin and angiotensin converting enzyme (ACE) (Weir & Dzau, 1999). Classically, AngII synthesis was thought to predominantly result from the activity of renal-derived renin, but emerging evidence has highlighted a critical role for ‘intracrine’ or intracellular RAS synthesis (Re & Bryan, 1984; Re, 2003) within several organ and tissue sites including the brain, heart and vasculature (Phillips *et al.* 1993). AngII and RAS peptide reactivity within the brain is primarily observed in areas involved in

sympathetic outflow and blood pressure control, including the paraventricular nucleus of the hypothalamus (Li *et al.* 2012; Biancardi *et al.* 2014) nucleus tractus solitarius (Li *et al.* 2012; Shan *et al.* 2013; Biancardi *et al.* 2014), rostro-ventral lateral medulla (Li *et al.* 2012; Biancardi *et al.* 2014) and subfornical organ (Hendel & Collister, 2005; Cao *et al.* 2012; Li *et al.* 2012), where the effects of AngII are primarily transduced via activation its cognate G_q -coupled receptor AT_1R (Sakai *et al.* 2004; Tan *et al.* 2004; Zhu *et al.* 2004; Sakai & Sigmund, 2005; Wang *et al.* 2012; Shan *et al.* 2013; Biancardi *et al.* 2014; Young & Davisson, 2015).

Evidence suggests that AngII signalling is enhanced in the CNS in hypertension (Chai *et al.* 1993; Gironacci *et al.* 2004; Schiffer *et al.* 2009; Young & Davisson, 2015; Santos *et al.* 2018), heart failure (Wang *et al.* 2012) and post-myocardial infarction (Tan *et al.* 2004). AngII also has a direct stimulatory effect on peripheral sympathetic neurons themselves (Cox *et al.* 2000; DiBona, 2000; Ma *et al.* 2001; Fernandez *et al.* 2003; Talaia *et al.* 2006; Wang *et al.* 2012; Berg, 2013). Critically, mice lacking the AngII receptor AT_1R within catecholaminergic

Figure 5. β -AR signalling is elevated in preSHR neurons

A, we identified the presence of β -adrenergic receptors (β -ARs) on tyrosine hydroxylase (TH) positive cardiac sympathetic neurons. B, activation of presynaptic β -ARs with isoprenaline (10 nM) led to a significantly larger cAMP generation in preSHR (56%; $n = 12$) vs. Wistar neurons (7%; $n = 12$; 2-way ANOVA; $P < 0.001$), which was measured using real-time cAMP in cells expressing the Epac- S^{H187} biosensor (A and B are reproduced from Bardsley *et al.* 2018b). C, we also identified the presence of AT_1R s on TH positive neurons. D, we investigated whether AT_1R could elevate β_1 -AR-evoked cAMP. Dobutamine (DOB) alone elevated cAMP in preSHR neurons (reproduced from Bardsley *et al.* 2018b). Moreover, AngII augments DOB-evoked cAMP generation in Wistar neurons ($n = 5$, 6; $P = 0.0073$) and SHR neurons ($n = 10$, 8; $P = 0.0005$). We also measured a strain-dependent effect following administration of DOB only ($P = 0.0015$) and in the presence of DOB with AngII ($P = 0.0283$). Data are represented as mean \pm SEM.



neurons develop fewer pathological effects following chronic AngII infusions. This includes attenuated sympathetic activation, reduced hypertensive responses and amelioration of ventricular hypertrophy (Jancovski *et al.* 2013). Collectively, this demonstrates the potential importance of neuronal AngII–AT₁R activation in the aetiology of sympathetic overactivity and neurogenic hypertension.

The close relationship between elevated AngII and sympathetic overactivity in cardiovascular disease is intriguing (Hilgers *et al.* 1993; Cox *et al.* 2000; Goldsmith, 2004; Berg, 2013) and has raised questions surrounding membrane level receptor–receptor interactions and cross-talk between AngII and adrenergic signalling cascades (Grant & McGrath, 1988; Barki-Harrington *et al.* 2003; Tilley, 2011; Saulière *et al.* 2012; Bellot *et al.* 2015; Liu *et al.* 2017; Tóth *et al.* 2018). Specifically, AT₁R- α_{2c} adrenergic receptor (AT₁R- α_{2c} -AR) heterodimers have been observed, where activation by NA promotes atypical enhanced cAMP–PKA signalling by converting an α_{2c} -AR autoinhibitory signal to excitatory positive feedback signalling (Bellot *et al.* 2015). Moreover, activation of the AT₁R- α_{2c} -AR heterodimer facilitates NA hypersecretion and sympathetic overactivity in sympathetic neurons *in vivo* (Bellot *et al.* 2015). Heterodimer formation has also been found to occur between AT₁R- β_2 -AR (Barki-Harrington *et al.* 2003; Tóth *et al.* 2017), which enhances the membrane stability of β_2 -AR and prolongs cAMP signalling. These results support our observations that AngII augments presynaptic β -AR-evoked cAMP (Fig. 5) and suggests a potential synergistic role for NA–AngII-mediated effects in provoking sympathetic overactivity in hypertension and cardiovascular pathophysiology (Barki-Harrington *et al.* 2003; Lourdes González-Hernández *et al.* 2010; Christensen *et al.* 2011; Berg, 2013; Bellot *et al.* 2015; Liu *et al.* 2017; Tóth *et al.* 2018).

Alterations in cardiac sympathetic transmitter release

Two simultaneous observations led to the concept of Adr as a pathological entity in the progression of hypertension. First, it was observed that Adr infusions underpin sustained increases in blood pressure post-infusion (Majewski *et al.* 1981; Brown & Macquin, 1982; Brown & Dollery, 1984); and secondly, that plasma Adr is elevated in hypertensive patients (Franco-Morselli *et al.* 1977; Brown & Macquin, 1981). Brown & Macquin (1981) proposed the ‘adrenaline hypothesis’ of essential hypertension (Brown & Dollery, 1984), which highlights a dominant role for Adr in facilitating NA release through actions at presynaptic β -ARs (Abboud *et al.* 1964; Floras *et al.* 1988, 1990). The source of Adr, however, was not fully resolved with reports suggesting chronic neuronal uptake and enhanced release of circulating Adr derived from the adrenals as

the primary site (Brown & Macquin, 1981; Majewski, 1983; Horikoshi *et al.* 1985; Blankestijn *et al.* 1988; Misu *et al.* 1988; Floras, 1992; Gudmundsdottir *et al.* 2008). Evidence has pointed to the possible synthesis of Adr in sympathetic nerves in patients with hypertension and stress disorder (Esler *et al.* 2008), but the *in situ* synthesis of Adr and a role for cardiac sympathetic Adr in the aetiology of hypertension are far from well-established.

Our RNA sequencing dataset provided a comprehensive profile of neurotransmitters and their respective synthesising enzymes in rat stellate ganglia (Bardsley *et al.* 2018a). Alongside the presence of classical transmitters and sympathetic markers, we also observed the transcript encoding phenylethanolamine *N*-methyltransferase (PNMT), the enzyme involved in the conversion of NA to Adr (Bardsley *et al.* 2018a). Protein concentrations of PNMT were detectable in rat and human stellate ganglia. To ascertain whether the presence of PNMT results in physiological concentrations and release of Adr, we electrically stimulated stellate ganglia from normotensive and hypertensive rats. Levels of both NA and Adr were elevated in the perfusate collected from prehypertensive SHR ganglia, whereas only NA could be detected in perfusate from healthy rat ganglia, and Adr was not observed (Fig. 6; Bardsley *et al.* 2018b). In support of this observation, a 20-year follow-up of the Oslo study on normotensive, prehypertensive and male patients with established hypertension has identified arterial Adr as an independent predictor of blood pressure elevation (Gudmundsdottir *et al.* 2008), re-raising the question of the importance of Adr in the pathophysiology of hypertension (Rumantir *et al.* 2000a). It is now evident that Adr synthesis occurs directly within cardiac sympathetic nerves in diseases associated with sympathetic overactivity (Esler *et al.* 2008), and that this neurotransmitter switching takes place before elevations in arterial blood pressure are observed (Bardsley *et al.* 2018b). In addition to the observed elevation in β -AR-mediated cAMP–PKA–Ca²⁺ signalling in prehypertensive rat stellate ganglia, these data support the notion of a causal role for Adr in the pathophysiology of neurogenic hypertension.

Targeting sympathetic overactivity: where are we now?

Hypertension is central in determining cardiovascular risk and is a strong predictive indicator of morbidity and mortality; however, there still remains an unmet clinical need for disease-modifying and prophylactic interventions. Cardiac sympathetic hyperactivity is a key feature of human hypertension that is also seen in animal models of cardiovascular disease (Esler, 2010; Larsen *et al.* 2016a), yet interventions that target this sympathetic phenotype are problematic to develop, due

to the anatomical location of the cardiac sympathetic ganglia (Kwon *et al.* 2018) and the challenge in unravelling the underlying pathophysiological mechanisms. Surgical techniques such as sympathectomy *per se*, provide symptomatic relief and lead to fewer cardiovascular co-morbidities in hypertension (Morrissey *et al.* 1953) and reduce the incidence of ventricular arrhythmia (Ajijola *et al.* 2014; Irie *et al.* 2017), yet these techniques are not without risk (Ajijola *et al.* 2014). Current pharmacological approaches including β -blockers and AngII inhibitors are mainstay therapeutic strategies for early hypertension and many other cardiovascular diseases associated with dysautonomia (Wysong *et al.* 2017). However, their efficacy may also be explained via reductions in peripheral sympatho-transmission. Approaches that aim to modulate sympathetic overactivity may have both a therapeutic and a physiological advantage over surgical techniques. Optimal neuromodulation of sympathetic tone will counteract hypertension-induced cardiovascular damage whilst retaining a level of sympathetic reserve that will still enable cardiac performance during physical

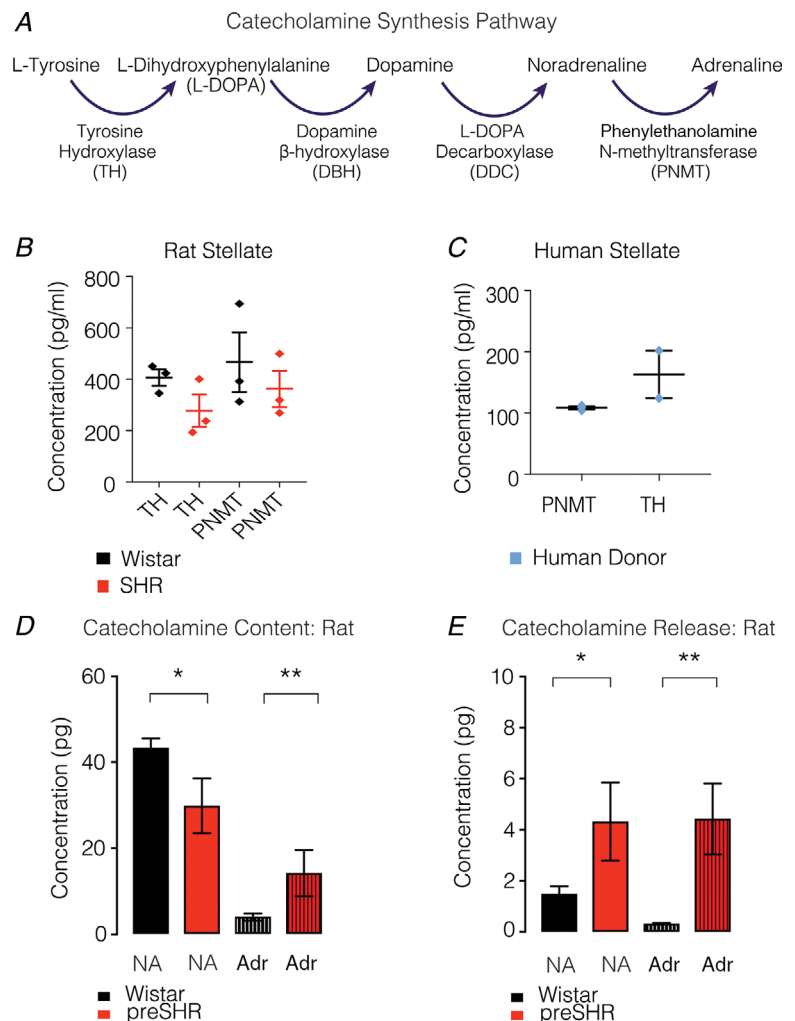
exertion. Gene transfer therapies that modulate cyclic nucleotide activity have had some success in improving neuronal activity, and a new era of genetic and protein modification techniques might be predicted to underpin the primary areas of advancement in this field. Moreover, the application of bioinformatics and the integration of machine-learning techniques with primary research may provide novel approaches for assisting diagnoses and prediction (LaFreniere *et al.* 2016; Kublanov *et al.* 2017; Savage, 2017; Poplin *et al.* 2018) as well as providing clarity regarding the complex interactions between pathways and their associated cellular and molecular processes (Cunningham, 2017; Wang *et al.* 2017; Xie *et al.* 2017; Cholley *et al.* 2018; Costello & Martin, 2018; Pavillon *et al.* 2018), as a way to facilitate precise therapeutic targeting.

Conclusion

Sympathetic overactivity is a well-established contributor to hypertension and CVD. Increased intracellular Ca^{2+} augments neurotransmission early in disease before

Figure 6. Adrenaline is released from preSHR neurons

A, the catecholamine synthesis pathway, highlights the role of Phenylethanolamine-*N*-methyltransferase (PNMT) in the conversion of noradrenaline (NA) to Adrenaline (Adr). B and C, tyrosine hydroxylase (TH) and PNMT were measured in adult rat (B) and human (C) stellate ganglia (reproduced from Bardsley *et al.* 2018b). D, using high pressure liquid chromatography with electrochemical detection (HPLC-EC), we measured significantly higher total NA in Wistar (43.3 \pm 2.173 pg; n = 8) compared with preSHR neurons (29.82 \pm 6.366 pg; n = 4; P = 0.0294). In the same samples, we also measured a significantly greater total content of Adr in preSHR (14.14 \pm 5.399 pg) compared with that measured in Wistar ganglia (3.937 \pm 0.820 pg, P = 0.0019). E, electrical field stimulation of whole rat stellate ganglia led to the release of NA that was significantly higher in samples obtained from preSHR (4.32 \pm 1.523 pg) vs. Wistar ganglia (1.477 \pm 0.316 pg; P = 0.0396). The concentrations of neurally mediated Adr release were also significantly higher in preSHR (4.424 \pm 1.391 pg, n = 4) compared with Wistar stellates (0.3201 \pm 0.0325 pg; n = 8; P = 0.0028) (figure reproduced from Bardsley *et al.* 2018b).



increases in blood pressure develop. This Ca^{2+} phenotype is underpinned by an impaired cAMP/cGMP balance that is weighted in favour of cAMP–PKA-dependent activity. Evidence suggests that this alteration in cN signalling results from changes in presynaptic receptor expression and signalling pathways, as well as critical changes in PDE activity. Pharmacological, surgical and genetic techniques aimed at reducing sympathetic tone or raising vagal transmission have had reasonable levels of success reducing hypertension and improving cardiac function (Morrissey *et al.* 1953; Heaton *et al.* 2007; Sabbah *et al.* 2011; Rath *et al.* 2013; Ajjola *et al.* 2014; Sverrisdottir *et al.* 2014; Shivkumar *et al.* 2016; Irie *et al.* 2017); nevertheless, no prophylactic strategies have yet successfully entered the clinical arena, emphasising a critical need for translational advancements in this field.

References

- Abboud FM, Eckstein JW & Zimmerman BG (1964). Effect of dichloroisoproterenol on vascular responses to catecholamines. *J Clin Invest* **43**, 316–322.
- Adams MA, Bobik A & Korner PI (1989). Differential development of vascular and cardiac hypertrophy in genetic hypertension. Relation to sympathetic function. *Hypertension* **14**, 191–202.
- Ajjola OA, Vaseghi M, Mahajan A & Shivkumar K (2014). Bilateral cardiac sympathetic denervation: why, who and when? *Expert Rev Cardiovasc Ther* **10**, 947–949.
- Akaike T, Du N, Lu G, Minamisawa S, Wang Y & Ruan H (2017). A sarcoplasmic reticulum localized protein phosphatase regulates phospholamban phosphorylation and promotes ischemia reperfusion injury in the heart. *JACC Basic Transl Sci* **2**, 160–180.
- Apparsundaram S & Eikenburg DC (1995). Role of prejunctional beta adrenoceptors in rat cardiac sympathetic neurotransmission. *J Pharmacol Exp Ther* **272**, 519–526.
- Assenza MR, Barbagallo F, Barrios F, Cornacchione M, Campolo F, Vivarelli E, Gianfrilli D, Auletta L, Soricelli A, Isidori AM, Lenzi A, Pellegrini M & Naro F (2018). Critical role of phosphodiesterase 2A in mouse congenital heart defects. *Cardiovasc Res* **114**, 830–845.
- Baliga RS, Preedy MEJ, Dukinfield MS, Chu SM, Aubdool AA, Bubb KJ, Moyes AJ, Tones MA & Hobbs AJ (2018). Phosphodiesterase 2 inhibition preferentially promotes NO/guanylyl cyclase/cGMP signaling to reverse the development of heart failure. *Proc Natl Acad Sci U S A* **115**, E7428–E7437.
- Bardsley EN, Davis H, Ajjola OA, Buckler KJ, Ardell JL, Shivkumar K & Paterson DJ (2018a). RNA sequencing reveals novel transcripts from sympathetic stellate ganglia during cardiac sympathetic hyperactivity. *Sci Rep* **8**, 8633.
- Bardsley EN, Davis H, Buckler KJ & Paterson DJ (2018b). Neurotransmitter switching coupled to β -adrenergic signaling in sympathetic neurons in prehypertensive states. *Hypertension* **71**, 1226–1238.
- Bardsley EN, Larsen HE & Paterson DJ (2016). Impaired cAMP–cGMP cross-talk during cardiac sympathetic dysautonomia. *Channels (Austin)* **11**, 178–180.
- Barki-Harrington L, Luttrell LM & Rockman HA (2003). Dual inhibition of beta-adrenergic and angiotensin II receptors by a single antagonist: a functional role for receptor–receptor interaction in vivo. *Circulation* **108**, 1611–1618.
- Behar J, Ganesan A, Zhang J & Yaniv Y (2016). The autonomic nervous system regulates the heart rate through cAMP–PKA dependent and independent coupled-clock pacemaker cell mechanisms. *Front Physiol* **7**, 419.
- Bellet M, Galandrin S, Boularan C, Matthies HJ, Despas F, Denis C, Javitch J, Mazères S, Sanni SJ, Pons V, Seguelas MH, Hansen JL, Pathak A, Galli A, S  nard JM & Gal  s C (2015). Dual agonist occupancy of AT1–R– α 2C–AR heterodimers results in atypical Gs–PKA signaling. *Nat Chem Biol* **11**, 271–279.
- Bender AT (2006). Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev* **58**, 488–520.
- Berg T (2013). Angiotensin AT1– α 2C-adrenoceptor interaction disturbs α 2A-auto-inhibition of catecholamine release in hypertensive rats. *Front Neurol* **4**, 70.
- Biancardi VC, Son SJ, Ahmadi S, Filosa JA & Stern JE (2014). Circulating angiotensin II gains access to the hypothalamus and brain stem during hypertension via breakdown of the blood–brain barrier. *Hypertension* **63**, 572–579.
- Blankestijn PJ, Man in't Veld AJ, Tulen J, van den Meiracker AH, Boomsma F, Moleman P, Ritsema van Eck HJ, Derckx FH, Mulder P & Lamberts SJ (1988). Support for adrenaline–hypertension hypothesis: 18 hour pressor effect after 6 hours adrenaline infusion. *Lancet* **2**, 1386–1389.
- Boda H, Uchida H, Takaiso N, Ouchi Y, Fujita N, Kuno A, Hata T, Nagatani A, Funamoto Y, Miyata M, Yoshikawa T, Kurahashi H & Inagaki H (2016). A PDE3A mutation in familial hypertension and brachydactyly syndrome. *J Hum Genet* **61**, 701–703.
- Briant LJB, Paton JFR, Pickering AE & Champneys AR (2015). Modelling the vascular response to sympathetic postganglionic nerve activity. *J Theor Biol* **371**, 102–116.
- Briant LJB, Stalbovskiy AO, Nolan MF, Champneys AR & Pickering AE (2014). Increased intrinsic excitability of muscle vasoconstrictor preganglionic neurons may contribute to the elevated sympathetic activity in hypertensive rats. *J Neurophysiol* **112**, 2756–2778.
- Brook RD & Julius S (2000). Autonomic imbalance, hypertension, and cardiovascular risk. *Am J Hypertens* **13**, 112S–122S.
- Brown AM & Birnbaumer L (1988). Direct G protein gating of ion channels. *Am J Physiol* **254**, H401–H410.
- Brown MJ & Dollery CT (1984). Adrenaline and hypertension. *Clin Exp Hypertens* **A6**, 539–549.
- Brown MJ & Macquin I (1981). Is adrenaline the cause of essential hypertension? *Lancet* **2**, 1079–1082.
- Brown MJ & Macquin I (1982). Catecholamine neurotransmitters and the heart. *Acta Medica Scandinavica* **211**, 34–39.

- Cao X, Peterson JR, Wang G, Anrather J, Young CN, Guraju MR, Burmeister MA, Iadecola C & Davisson RL (2012). Angiotensin II-dependent hypertension requires cyclooxygenase 1-derived prostaglandin E2 and EP1 receptor signaling in the subfornical organ of the brain. *Hypertension* **59**, 869–876.
- CARDIoGRAMplusC4D Consortium *et al.* (2013). Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Methods* **45**, 25–33.
- Catt KJ, Zimmet PZ, Cain MD, Cran E, Best JB & Coghlan JP (1971). Angiotensin II blood-levels in human hypertension. *Lancet* **297**, 459–464.
- Catterall WA (2003). International union of pharmacology. XL. Compendium of voltage-gated ion channels: calcium channels. *Pharmacol Rev* **55**, 579–581.
- Catterall WA (2011). Voltage-gated calcium channels. *Cold Spring Harb Perspect Biol* **3**, a003947.
- Celsus AC & Spencer WG (1935). *De Medicina (On Medicine)*. Loeb Classical Library. Harvard University Press, Cambridge, MA, USA/W. Heinemann, Ltd, London.
- Chai CY, Hellmann W, Tseng CJ & Luft FC (1993). Angiotensinogen mRNA and pressor reactions to angiotensin in brain stem areas of spontaneously hypertensive rats. *Clin Exp Hypertens* **15**, 709–725.
- Cholley P-E, Moehlin J, Rohmer A, Zilliox V, Nicaise S, Gronemeyer H & Mendoza-Parra MA (2018). Modeling gene-regulatory networks to describe cell fate transitions and predict master regulators. *NPJ Syst Biol Appl* **4**, 29.
- Christensen GL, Knudsen S, Schneider M, Aplin M, Gammeltoft S, Sheikh SP & Hansen JL (2011). AT1 receptor $G_{\alpha q}$ protein-independent signalling transcriptionally activates only a few genes directly, but robustly potentiates gene regulation from the β_2 -adrenergic receptor. *Mol Cell Endocrinol* **331**, 49–56.
- Costello Z & Martin HG (2018). A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data. *NPJ Syst Biol Appl* **4**, 19.
- Cox SL, Schelb V, Trendelenburg AU & Starke K (2000). Enhancement of noradrenaline release by angiotensin II and bradykinin in mouse atria: Evidence for cross-talk between $G_{q/11}$ protein- and $G_{i/o}$ protein-coupled receptors. *Br J Pharmacol* **129**, 1095–1102.
- Crowley SD, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, Kim H-S, Smithies O, Le TH & Coffman TM (2006). Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. *Proc Natl Acad Sci U S A* **103**, 17985–17990.
- Cunningham RJ (2017). The application of deep convolutional neural networks to ultrasound for modelling of dynamic states within human skeletal muscle. *bioRxiv*, doi: <http://doi.org/10.1101/157479>.
- Dang AM, Zheng DY, Wang B, Zhang YQ, Zhang PH, Yu MF, Liu GZ & Liu LS (1999). The role of the renin-angiotensin and cardiac sympathetic nervous systems in the development of hypertension and left ventricular hypertrophy in spontaneously hypertensive rats. *Hypertens Res* **22**, 217–221.
- D'Ascenzo M, Martinotti G, Azzena GB & Grassi C (2002). cGMP/protein kinase G-dependent inhibition of N-type Ca^{2+} channels induced by nitric oxide in human neuroblastoma IMR32 cells. *J Neurosci* **22**, 7485–7492.
- Danson E, Choate J & Paterson DJ (2005). Cardiac nitric oxide: Emerging role for nNOS in regulating physiological function. *Pharmacol Ther* **106**, 57–74.
- Danson EJ, Li D, Wang L, Dawson TA & Paterson DJ (2009). Targeting cardiac sympatho-vagal imbalance using gene transfer of nitric oxide synthase. *J Mol Cell Cardiol* **46**, 482–489.
- Davis H, Bardsley EN & Paterson DJ (2018). Data descriptor: Transcriptional profiling of stellate ganglia from normotensive and spontaneously hypertensive rat strains. *Sci Data*, <https://doi.org/10.1038/sdata.2018.123>.
- Dawson TA, Li D, Woodward T, Barber Z, Wang L & Paterson DJ (2008). Cardiac cholinergic NO-cGMP signaling following acute myocardial infarction and nNOS gene transfer. *Am J Physiol Heart Circ Physiol* **295**, H990–H998.
- DiBona GF (2000). Nervous kidney: Interaction between renal sympathetic nerves and the renin-angiotensin system in the control of renal function. *Hypertension* **36**, 1083–1088.
- Ehret GB *et al.* (2011). Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* **478**, 103–109.
- Erdmann J, Stark K, Esslinger UB, Rumpf PM, Koesling D, de Wit C, Kaiser FJ, Braunholz D, Medack A, Fischer M, Zimmermann ME, Tennstedt S, Graf E, Eck S, Aherrahrou Z, Nahrstaedt J, Willenborg C, Bruse P, Brænne I, Nöthen MM, Hofmann P, Braund PS, Mergia E, Reinhard W, Burgdorf C, Schreiber S, Balmforth AJ, Hall AS, Bertram L, Steinhagen-Thiessen E, Li SC, März W, Reilly M, Kathiresan S, McPherson R, Walter U; CARDIoGRAM, Ott J, Samani NJ, Strom TM, Meitinger T, Hengstenberg C & Schunkert H (2013). Dysfunctional nitric oxide signalling increases risk of myocardial infarction. *Nature* **504**, 432–436.
- Esler M (2010). Sympathetic nervous activation in essential hypertension: commonly neglected as a therapeutic target, usually ignored as a drug side effect. *Hypertension* **55**, 1090–1091.
- Esler M (2014). Sympathetic nervous system moves toward center stage in cardiovascular medicine from Thomas Willis to resistant hypertension. *Hypertension* **63**, e25–e32.
- Esler M, Eikelis N, Schlaich M, Lambert G, Alvarenga M, Kaye D, El-Osta A, Guo L, Barton D, Pier C, Brenchley C, Dawood T, Jennings G & Lambert E (2008). Human sympathetic nerve biology: parallel influences of stress and epigenetics in essential hypertension and panic disorder. *Ann N Y Acad Sci* **1148**, 338–348.
- Esler M, Jackman G, Bobik A, Leonard P, Kelleher D, Skews H, Jennings G & Korner P (1981). Norepinephrine kinetics in essential hypertension. Defective neuronal uptake of norepinephrine in some patients. *Hypertension* **3**, 149–156.
- Esler M, Jennings G, Biviano B, Lambert G & Hasking G (1986). Mechanism of elevated plasma noradrenaline in the course of essential hypertension. *J Cardiovasc Pharmacol* **8**, S39–S43.
- Esler M, Jennings G, Korner P, Willett I, Dudley F, Hasking G, Anderson W & Lambert G (1988). Assessment of human sympathetic nervous-system activity from measurements of norepinephrine turnover. *Hypertension* **11**, 3–20.

- Esler MD, Wallin G, Dorward PK, Eisenhofer G, Westerman R, Meredith I, Lambert G, Cox HS & Jennings G (1991). Effects of desipramine on sympathetic nerve firing and norepinephrine spillover to plasma in humans. *Am J Physiol* **260**, R817–R823.
- Fernandez SF, Huang M-H, Davidson BA, Knight PR & Izzo JL (2003). Modulation of angiotensin II responses in sympathetic neurons by cytosolic calcium. *Hypertension* **41**, 56–63.
- Ferrara LA, Moscato TS, Pisanti N, Marotta T, Krogh V, Capone D & Mancini M (1988). Is the sympathetic nervous system altered in children with familial history of arterial hypertension? *Cardiology* **75**, 200–205.
- Flack JM, Atlas SA, Pool JL & White WB (2007). Renin-angiotensin aldosterone system and hypertension: current approaches and future directions. *J Manag Care Spec Pharm* **13**, 1–39.
- Floras JS (1992). Epinephrine and the genesis of hypertension. *Hypertension* **19**, 1–18.
- Floras JS, Aylward PE, Mark AL & Abboud FM (1990). Adrenaline facilitates neurogenic vasoconstriction in borderline hypertensive subjects. *J Hypertens* **8**, 443–448.
- Floras JS, Aylward PE, Victor RG, Mark AL & Abboud FM (1988). Epinephrine facilitates neurogenic vasoconstriction in humans. *J Clin Invest* **81**, 1265–1274.
- Florea VG & Cohn JN (2014). The autonomic nervous system and heart failure. *Circ Res* **114**, 1815–1826.
- Francis SH, Blount MA & Corbin JD (2011). Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. *Physiol Rev* **91**, 651–690.
- Franco-Morselli R, Elghozi JL, Joly E, Di Giulio S & Meyer P (1977). Increased plasma adrenaline concentrations in benign essential hypertension. *Br Med J* **2**, 1251–1254.
- Frishman WH (2016). Beta-adrenergic receptor blockers in hypertension: alive and well. *Prog Cardiovasc Dis* **59**, 247–252.
- Gaskell WH (1886). On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. *J Physiol* **7**, 1–80.
- Ghazi L & Drawz P (2017). Advances in understanding the renin-angiotensin-aldosterone system (RAAS) in blood pressure control and recent pivotal trials of RAAS blockade in heart failure and diabetic nephropathy. *F1000Res* **6**, F1000 Faculty Rev-297.
- Gironacci MM, Valera MS, Yujnovsky I & Peña C (2004). Angiotensin-(1-7) inhibitory mechanism of norepinephrine release in hypertensive rats. *Hypertension* **44**, 783–787.
- Goldsmith SR (2004). Interactions between the sympathetic nervous system and the RAAS in heart failure. *Curr Heart Fail Rep* **1**, 45–50.
- Grant TL & McGrath JC (1988). Interactions between angiotensin-II and alpha-adrenoceptor agonists mediating pressor-responses in the pithed rat. *Br J Pharmacol* **95**, 1229–1240.
- Grassi G & Esler M (1999). How to assess sympathetic activity in humans. *J Hypertens* **17**, 719–734.
- Grassi G, Mark A & Esler M (2015). The sympathetic nervous system alterations in human hypertension. *Circ Res* **116**, 976–990.
- Gray PC, Scott JD & Catterall WA (1998). Regulation of ion channels by cAMP-dependent protein kinase and A-kinase anchoring proteins. *Curr Opin Neurobiol* **8**, 330–334.
- Gudmundsdottir H, Strand AH, Høiegggen A, Reims HM, Westheim AS, Eide IK, Kjeldsen SE & Os I (2008). Do screening blood pressure and plasma catecholamines predict development of hypertension? Twenty-year follow-up of middle-aged men. *Blood Press* **17**, 94–103.
- Guo L, Lee AA, Rizvi TA, Ratner N & Kirschner LS (2013). The protein kinase A regulatory subunit R1A (Prkar1a) plays critical roles in peripheral nerve development. *J Neurosci* **33**, 17967–17975.
- Guyenet PG (2006). The sympathetic control of blood pressure. *Nat Rev Neurosci* **7**, 335–346.
- Hall JE (1986). Control of sodium-excretion by angiotensin-II – intrarenal mechanisms and blood-pressure regulation. *Am J Physiol* **250**, R960–R972.
- Hamer M (2006). The effects of exercise on haemodynamic function in relation to the familial hypertension risk model. *J Hum Hypertens* **20**, 313–319.
- Hanna P, Rajendran PS, Ajiola OA, Vaseghi M, Armour JA, Ardell JL & Shivkumar K (2017). Cardiac neuroanatomy – Imaging nerves to define functional control. *Auton Neurosci* **207**, 48–58.
- Hansson L, Lindholm LH, Niskanen L, Lanke J, Hedner T, Niklason A, Luomanmäki K, Dahlöf B, de Faire U, Mörlin C, Karlberg BE, Wester PO, Björck J-E; the Captopril Prevention Project CAPPP study group (1999). Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: The Captopril Prevention Project (CAPPP) randomised trial. *Lancet* **353**, 611–616.
- Hausberg M, Sinkey CA, Mark AL, Hoffman RP & Anderson EA (1998). Sympathetic nerve activity and insulin sensitivity in normotensive offspring of hypertensive parents. *Am J Hypertens* **11**, 1312–1320.
- Heaton DA, Lei M, Li D, Golding S, Dawson TA, Mohan RM & Paterson DJ (2006). Remodeling of the cardiac pacemaker L-type calcium current and its beta-adrenergic responsiveness in hypertension after neuronal NO synthase gene transfer. *Hypertension* **48**, 443–452.
- Heaton DA, Li D, Almond SC, Dawson TA, Wang L, Channon KM & Paterson DJ (2007). Gene transfer of neuronal nitric oxide synthase into intracardiac ganglia reverses vagal impairment in hypertensive rats. *Hypertension* **49**, 380–388.
- Hendel MD & Collister JP (2005). Contribution of the subfornical organ to angiotensin II-induced hypertension. *Am J Physiol Heart Circ Physiol* **288**, H680–H685.
- Herring N & Paterson DJ (2018). *Levick's Introduction to Cardiovascular Physiology*, 6th edn. CRC Press, Boca Raton.
- Hilgers KF, Veelken R, Rupprecht G, Reeh PW, Luft FC & Mann JF (1993). Angiotensin II facilitates sympathetic transmission in rat hind limb circulation. *Hypertension* **21**, 322–328.
- Hoff HE (1940). The history of vagal inhibition. *Bull Hist Med* **8**, 461–496.
- Horikoshi Y, Tajima I, Igarashi H, Inui M, Kasahara K & Noguchi T (1985). The adreno-sympathetic system, the genetic predisposition to hypertension, and stress. *Am J Med Sci* **289**, 186–191.

- Horvath A, Bertherat J, Groussin L, Guillaud-Bataille M, Tsang K, Cazabat L, Libé R, Remmers E, René-Corail F, Faucez FR, Clauser E, Calender A, Bertagna X, Carney JA & Stratakis CA (2010). Mutations and polymorphisms in the gene encoding regulatory subunit type 1- α of protein kinase A (PRKAR1A): an update. *Hum Mutat* **31**, 369–379.
- Hua R, Adamczyk A, Robbins C, Ray G & Rose RA (2012). Distinct patterns of constitutive phosphodiesterase activity in mouse sinoatrial node and atrial myocardium. *PLoS One* **7**, e47652–12.
- Imredy JP & Yue DT (1994). Mechanism of Ca^{2+} -sensitive inactivation of L-type Ca^{2+} channels. *Neuron* **12**, 1301–1318.
- Ino M, Yoshinaga T, Wakamori M, Miyamoto N, Takahashi E, Sonoda J, Kagaya T, Oki T, Nagasu T, Nishizawa Y, Tanaka I, Imoto K, Aizawa S, Koch S, Schwartz A, Niidome T, Sawada K & Mori Y (2001). Functional disorders of the sympathetic nervous system in mice lacking the α_{1B} subunit ($\text{Ca}_v2.2$) of N-type calcium channels. *Proc Natl Acad Sci U S A* **98**, 5323–5328.
- Irie T, Yamakawa K, Hamon D, Nakamura K, Shivkumar K & Vaseghi M (2017). Cardiac sympathetic innervation via middle cervical and stellate ganglia and antiarrhythmic mechanism of bilateral slectectomy. *Am J Physiol Heart Circ Physiol* **312**, H392–H405.
- Iriuchijima J (1973). Sympathetic discharge rate in spontaneously hypertensive rats. *Jpn Heart J* **14**, 350–356.
- Jancovski N, Bassi JK, Carter DA, Choong Y-T, Connelly A, Nguyen T-P, Chen D, Lukoshkova EV, Menuet C, Head GA & Allen AM (2013). Stimulation of angiotensin type 1A receptors on catecholaminergic cells contributes to angiotensin-dependent hypertension. *Hypertension* **62**, 866–871.
- Johansson M, Elam M, Rundqvist B, Eisenhofer G, Herlitz H, Lambert G & Friberg P (1999). Increased sympathetic nerve activity in renovascular hypertension. *Circulation* **99**, 2537–2542.
- Johnny SS, Karthik CS, Bondade SY & Jayalakshmi MK (2015). Altered cardiovascular autonomic function in young normotensive offspring of hypertensive parents—Is obesity an additional risk factor? *J Basic Clin Physiol Pharmacol* **26**, 531–537.
- Judy WV, Watanabe AM, Murphy WR, Aprison BS & Yu PL (1979). Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rat. *Hypertension* **1**, 598–604.
- Kalla M, Chotalia M, Coughlan C, Hao G, Crabtree MJ, Tomek J, Bub G, Paterson DJ & Herring N (2016). Protection against ventricular fibrillation via cholinergic receptor stimulation and the generation of nitric oxide. *J Physiol* **594**, 3981–3992.
- Katz SD, Balidemaj K, Homma S, Wu H, Wang J & Maybaum S (2000). Acute type 5 phosphodiesterase inhibition with sildenafil enhances flow-mediated vasodilation in patients with chronic heart failure. *J Am Coll Cardiol* **36**, 845–851.
- Kaye DM, Lefkowitz J, Jennings GL, Bergin P, Broughton A & Esler MD (1995). Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol* **26**, 1257–1263.
- Kessler T, Wobst J, Wolf B, Eckhold J, Vilne B, Hollstein R, Ameln von S, Dang TA, Sager HB, Moritz Rumpf P, Aherrahrou R, Kastrati A, Björkegren JLM, Erdmann J, Lüscher AJ, Civelek M, Kaiser FJ & Schunkert H (2017). Functional characterization of the *GUCY1A3* coronary artery disease risk locus. *Circulation* **136**, 476–489.
- Kimura S, Miura Y, Adachi M, Nezu M, Toriyabe S, Sugawara T, Ishizuka Y, Noshiro T & Takahashi M (1983). The effect of sodium depletion on plasma norepinephrine kinetics in patients with essential hypertension. *Jpn Circ J* **47**, 1232–1241.
- Klarenbeek J, Goedhart J, van Batenburg A, Groenewald D & Jalink K (2015). Fourth-generation Epac-based FRET sensors for cAMP feature exceptional brightness, photostability and dynamic range: characterization of dedicated sensors for FLIM, for ratiometry and with high affinity. *PLoS One* **10**, e0122513.
- Korzina MB, Korobkin AA, Vasilieva OA & Maslyukov PM (2011). Morphological characteristics of the stellate ganglion in white rats. *Neurosci Behav Physiol* **41**, 436–439.
- Kublanov VS, Dolganov AY, Belo D & Gamboa H (2017). Comparison of machine learning methods for the arterial hypertension diagnostics. *Appl Bionics Biomech* **2017**, 5985479.
- Kwon OJ, Pendekanti S, Fox JN, Yanagawa J, Fishbein MC, Shivkumar K, Lambert HW & Ajijola OA (2018). Morphological spectra of adult human stellate ganglia: implications for thoracic sympathetic denervation. *Anat Rec (Hoboken)* **301**, 1244–1250.
- LaFreniere D, Zulkernine F, Barber D & Martin K (2016). Using machine learning to predict hypertension from a clinical dataset. *2016 IEEE Symposium Series on Computational Intelligence (SSCI)*. IEEE. <https://ieeexplore.ieee.org/document/7849886/>.
- Langley JN (1898). On the union of cranial autonomic (visceral) fibres with the nerve cells of the superior cervical ganglion. *J Physiol* **23**, 240–270.
- Larsen HE, Bardsley EN, Lefkimiatis K & Paterson DJ (2016a). Dysregulation of neuronal Ca^{2+} channel linked to heightened sympathetic phenotype in prohypertensive states. *J Neurosci* **36**, 8562–8573.
- Larsen HE, Lefkimiatis K & Paterson DJ (2016b). Sympathetic neurons are a powerful driver of myocyte function in cardiovascular disease. *Sci Rep* **6**, 38898.
- Lee C-W, Li D, Channon KM & Paterson DJ (2009). L-arginine supplementation reduces cardiac noradrenergic neurotransmission in spontaneously hypertensive rats. *J Mol Cell Cardiol* **47**, 149–155.
- Lee DI, Zhu G, Sasaki T, Cho GS, Hamdani N, Holewinski R, Jo SH, Danner T, Zhang M, Rainer PP, Bedja D, Kirk JA, Ranek MJ, Dostmann WR, Kwon C, Margulies KB, Van Eyk JE, Paulus WJ, Takimoto E & Kass DA (2015). Phosphodiesterase 9A controls nitric-oxide- independent cGMP and hypertrophic heart disease. *Nature* **519**, 472–476.
- Lefkimiatis K & Zaccolo M (2014). cAMP signaling in subcellular compartments. *Pharmacol Ther* **143**, 295–304.
- Leiser M & Fleischer N (1996). cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Diabetes* **45**, 1412–1418.

- Li D, Lu C-J, Hao G, Wright H, Woodward L, Liu K, Vergari E, Surdo NC, Herring N, Zaccolo M & Paterson DJ (2015). Efficacy of B-type natriuretic peptide is coupled to phosphodiesterase 2a in cardiac sympathetic neurons. *Hypertension* **66**, 190–198.
- Li D, Nikiforova N, Lu C-J, Wannop K, McMenamin M, Lee C-W, Buckler KJ & Paterson DJ (2013). Targeted neuronal nitric oxide synthase transgene delivery into stellate neurons reverses impaired intracellular calcium transients in prehypertensive rats. *Hypertension* **61**, 202–207.
- Li W, Peng H, Cao T, Sato R, McDaniels SJ, Kobori H, Navar LG & Feng Y (2012). Brain-targeted (pro)renin receptor knockdown attenuates angiotensin II-dependent hypertension. *Hypertension* **59**, 1188–1194.
- Liu C-H, Gong Z, Liang ZL, Liu ZX, Yang F, Sun YJ, Ma ML, Wang YJ, Ji CR, Wang YH, Wang MJ, Cui FA, Lin A, Zheng WS, He DF, Qu CX, Xiao P, Liu CY, Thomsen AR, Joseph Cahill T 3rd, Kahsai AW, Yi F, Xiao KH, Xue T, Zhou Z, Yu X & Sun JP (2017). Arrestin-biased AT1R agonism induces acute catecholamine secretion through TRPC3 coupling. *Nat Commun* **8**, 14335.
- Liu K, Li D, Hao G, McCaffary D, Neely O, Woodward L, Ioannides D, Lu C-J, Brescia M, Zaccolo M, Tandri H, Ajijola OA, Ardell JL, Shivkumar K & Paterson DJ (2018). Phosphodiesterase 2A as a therapeutic target to restore cardiac neurotransmission during sympathetic hyperactivity. *JCI Insight* **3**, 98694.
- Lokhandwala MF & Eikenburg DC (1983). Presynaptic receptors and alterations in norepinephrine release in spontaneously hypertensive rats. *Life Sci* **33**, 1527–1542.
- Lopes HF, Silva HB, Consolim-Colombo FM, Barreto Filho JA, Riccio GM, Giorgi DM & Krieger EM (2000). Autonomic abnormalities demonstrable in young normotensive subjects who are children of hypertensive parents. *Braz J Med Biol Res* **33**, 51–54.
- Lourdes González-Hernández Mde, Godínez-Hernández D, Bobadilla-Lugo RA & López-Sánchez P (2010). Angiotensin-II type 1 receptor (AT1R) and alpha-1D adrenoceptor form a heterodimer during pregnancy-induced hypertension. *Auton Autacoid Pharmacol* **30**, 167–172.
- Lu C-J, Hao G, Nikiforova N, Larsen HE, Liu K, Crabtree MJ, Li D, Herring N & Paterson DJ (2015). CAPON modulates neuronal calcium handling and cardiac sympathetic neurotransmission during dysautonomia in hypertension. *Hypertension* **65**, 1288–1297.
- Ma XY, Chapleau MW, Whiteis CA, Abboud FM & Bielefeldt K (2001). Angiotensin selectively activates a subpopulation of postganglionic sympathetic neurons in mice. *Circ Res* **88**, 787–793.
- Maass PG, Aydin A, Luft FC, Schächterle C, Weise A, Stricker S, Lindschau C, Vaegler M, Qadri F, Toka HR, Schulz H, Krawitz PM, Parkhomchuk D, Hecht J, Hollfinger I, Wefeld-Neuenfeld Y, Bartels-Klein E, Mühl A, Kann M, Schuster H, Chitayat D, Bialer MG, Wienker TF, Ott J, Rittscher K, Liehr T, Jordan J, Plessis G, Tank J, Mai K, Naraghi R, Hodge R, Hopp M, Hattenbach LO, Busjahn A, Rauch A, Vandeput F, Gong M, Rüschendorf F, Hübner N, Haller H, Mundlos S, Bilginturan N, Movsesian MA, Klusmann E, Toka O & Bähring S (2015). PDE3A mutations cause autosomal dominant hypertension with brachydactyly. *Nat Methods* **47**, 647–653.
- Mahapatra S, Marcantoni A, Zuccotti A, Carabelli V & Carbone E (2012). Equal sensitivity of Cav1.2 and Cav1.3 channels to the opposing modulations of PKA and PKG in mouse chromaffin cells. *J Physiol* **590**, 5053–5073.
- Majewski H (1983). Modulation of noradrenaline release through activation of presynaptic β -adrenoreceptors. *J Auton Pharmacol* **3**, 47–60.
- Majewski H, Tung LH & Rand MJ (1981). Adrenaline-induced hypertension in rats. *J Cardiovasc Pharmacol* **3**, 179–185.
- Malpas SC (2010). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol Rev* **90**, 513–557.
- Mancia G & Grassi G (2014). The autonomic nervous system and hypertension. *Circ Res* **114**, 1804–1814.
- Mattiazzi A & Kranias EG (2014). The role of CaMKII regulation of phospholamban activity in heart disease. *Front Pharmacol* **5**, 5.
- Maurice DH (2003). Cyclic nucleotide phosphodiesterase activity, expression, and targeting in cells of the cardiovascular system. *Mol Pharmacol* **64**, 533–546.
- Maver J, Štruel M & Accetto R (2004). Autonomic nervous system activity in normotensive subjects with a family history of hypertension. *Clin Auton Res* **14**, 369–375.
- Mehel H, Emons J, Vettel C, Wittköpper K, Seppelt D, Dewenter M, Lutz S, Sossalla S, Maier LS, Lechêne P, Leroy J, Lefebvre F, Varin A, Eschenhagen T, Nattel S, Dobrev D, Zimmermann WH, Nikolaev VO, Vandecasteele G, Fischmeister R & El-Armouche A (2013). Phosphodiesterase-2 is up-regulated in human failing hearts and blunts β -adrenergic responses in cardiomyocytes. *J Am Coll Cardiol* **62**, 1596–1606.
- Meredith IT, Broughton A, Jennings GL & Esler MD (1991). Evidence of a selective increase in cardiac sympathetic activity in patients with sustained ventricular arrhythmias. *N Engl J Med* **325**, 618–624.
- Misu Y & Kubo T (1986). Presynaptic β -adrenoceptors. *Med Res Rev* **6**, 197–225.
- Misu Y, Kuwahara M, Amano H & Kubo T (1988). Adrenaline as an endogenous agonist for presynaptic beta-adrenoceptors and their relevance to the development of hypertension in spontaneously hypertensive rats. *J Hypertens Suppl* **6**, S572–S574.
- Molderings GJ, Likungu J & Göthert M (2000). N-Type calcium channels control sympathetic neurotransmission in human heart atrium. *Circulation* **101**, 403–407.
- Mori Y, Nishida M, Shimizu S, Ishii M, Ino M, Sawada K & Niidome T (2002). Ca^{2+} channel α_{1B} subunit ($\text{Ca}_v2.2$) knockout mouse reveals a predominant role of N-type channels in the sympathetic regulation of the circulatory system. *Trends Cardiovasc Med* **12**, 270–275.
- Morrissey DM, Brookes VS & Cooke WT (1953). Sympathectomy in the treatment of hypertension – review of 122 cases. *Lancet* **264**, 403–412.
- Musheshe N, Schmidt M & Zaccolo M (2018). cAMP: from long-range second messenger to nanodomain signalling. *Trends Pharmacol Sci* **39**, 209–222.

- Nagendran J, Archer SL, Soliman D, Gurtu V, Moudgil R, Haromy A, St Aubin C, Webster L, Rebeyka IM, Ross DB, Light PE, Dyck JRB & Michelakis ED (2007). Phosphodiesterase type 5 is highly expressed in the hypertrophied human right ventricle, and acute inhibition of phosphodiesterase type 5 improves contractility. *Circulation* **116**, 238–248.
- Najafi A, Sequeira V, Kuster DWD & van der Velden J (2016). β -Adrenergic receptor signalling and its functional consequences in the diseased heart. *Eur J Clin Invest* **46**, 362–374.
- Nedergaard OA & Abrahamsen J (1990). Modulation of noradrenaline release by activation of presynaptic beta-adrenoceptors in the cardiovascular system. *Ann N Y Acad Sci* **604**, 528–544.
- Nikpay M *et al.* (2015). A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Methods* **47**, 1121–1130.
- Nussberger J & Bohlender J (2013). Pharmacotherapy: optimal blockade of the renin–angiotensin–aldosterone system. *Nat Methods* **10**, 183–184.
- Nussberger J, Brunner DB, Waeber B & Brunner HR (1986). Specific measurement of angiotensin metabolites and in vitro generated angiotensin II in plasma. *Hypertension* **8**, 476–482.
- Oliveira-Sales EB, Colombari E, Abdala AP, Campos RR & Paton JFR (2016). Sympathetic overactivity occurs before hypertension in the two-kidney, one-clip model. *Exp Physiol* **101**, 67–80.
- Oliveira-Sales EB, Toward MA, Campos RR & Paton JFR (2014). Revealing the role of the autonomic nervous system in the development and maintenance of Goldblatt hypertension in rats. *Auton Neurosci* **183**, 23–29.
- Pal GK, Pal P, Nanda N, Lalitha V, Dutta TK & Adithan C (2011). Sympathovagal imbalance in prehypertensive offspring of two parents versus one parent hypertensive. *Int J Hypertens* **2011**, 263170.
- Palatini P & Julius S (2004). Elevated heart rate: a major risk factor for cardiovascular disease. *Clin Exp Hypertens* **26**, 637–644.
- Parati G & Esler M (2012). The human sympathetic nervous system: Its relevance in hypertension and heart failure. *Eur Heart J* **33**, 1058–1066.
- Pavillon N, Hobro AJ, Akira S & Smith NI (2018). Noninvasive detection of macrophage activation with single-cell resolution through machine learning. *Proc Natl Acad Sci U S A* **115**, E2676–E2685.
- Petersson MJ, Rundqvist B, Johansson M, Eisenhofer G, Lambert G, Herlitz H, Jensen G & Friberg P (2002). Increased cardiac sympathetic drive in renovascular hypertension. *J Hypertens* **20**, 1181–1187.
- Phillips MI, Speakman EA & Kimura B (1993). Levels of angiotensin and molecular biology of the tissue renin angiotensin systems. *Regul Pept* **43**, 1–20.
- Piccirillo G, Viola E, Nocco M, Durante M, Tarantini S & Marigliano V (2000). Autonomic modulation of heart rate and blood pressure in normotensive offspring of hypertensive subjects. *J Lab Clin Med* **135**, 145–152.
- Poplin R, Varadarajan AV, Blumer K, Liu Y, McConnell MV, Corrado GS, Peng L & Webster DR (2018). Prediction of cardiovascular risk factors from retinal fundus photographs via deep learning. *Nat Biomed Eng* **2**, 158–164.
- Pruneau D & Bélitchard P (1992). Haemodynamic and humoral effects of ω -conotoxin GVIA in normotensive and spontaneously hypertensive rats. *Eur J Pharmacol* **211**, 329–335.
- Ram CVS (2010). Beta-blockers in hypertension. *Asian-Australas J Anim Sci* **106**, 1819–1825.
- Ramchandra R, Hood SG, Denton DA, Woods RL, McKinley MJ, McAllen RM & May CN (2009). Basis for the preferential activation of cardiac sympathetic nerve activity in heart failure. *Proc Natl Acad Sci U S A* **106**, 924–928.
- Rathi P, Agarwal V & Kumar A (2013). Sympathetic hyperactivity in children of hypertensive parents. *Ann Neurosci* **20**, 4–6.
- Re R & Bryan SE (1984). Functional intracellular renin–angiotensin systems may exist in multiple tissues. *Clin Exp Hypertens* **6**, 1739–1742.
- Re RN (2003). The intracrine hypothesis and intracellular peptide hormone action. *Bioessays* **25**, 401–409.
- Riet Te L, Van Esch JHM, Roks AJM, Van Den Meiracker AH & Danser AHJ (2015). Hypertension: renin–angiotensin–aldosterone system alterations. *Circ Res* **116**, 960–975.
- Rippe C, Zhu B, Krawczyk KK, Van Bavel E, Albinsson S, Iund JSX, Bakker ENTP & Rd KSX (2017). Hypertension reduces soluble guanylyl cyclase expression in the mouse aorta via the Notch signaling pathway. *Sci Rep*, 1–13.
- Romero JC & Reckelhoff JF (1999). Role of angiotensin and oxidative stress in essential hypertension. *Hypertension* **34**, 943–949.
- Rozanski A, Blumenthal JA & Kaplan J (1999). Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. *Circulation* **99**, 2192–2217.
- Rubattu S, Cotugno M, Forte M, Stanzione R, Bianchi F, Madonna M, Marchitti S & Volpe M (2018). Effects of dual angiotensin type 1 receptor/neprilysin inhibition vs. angiotensin type 1 receptor inhibition on target organ injury in the stroke-prone spontaneously hypertensive rat. *J Hypertens* **36**, 1902–1914.
- Rumantir MS, Jennings GL, Lambert GW, Kaye DM, Seals DR & Esler MD (2000a). The “adrenaline hypothesis” of hypertension revisited: evidence for adrenaline release from the heart of patients with essential hypertension. *J Hypertens* **18**, 717–723.
- Rumantir MS, Kaye DM, Jennings GL, Vaz M, Hastings JA & Esler MD (2000b). Phenotypic evidence of faulty neuronal norepinephrine reuptake in essential hypertension. *Hypertension* **36**, 824–829.
- Rundqvist B, Elam M, Bergmann-Sverrisdottir YB, Eisenhofer G & Friberg P (1997). Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. *Circulation* **95**, 169–175.
- Rupp H & Jäger B (2001). The renin–angiotensin system and the sympathetic nervous system in hypertension and congestive heart failure: Implications for therapeutic interventions. *J Clin Basic Cardiol* **4**, 47–51.

- Sabbah HN, Ilsar I, Zaretsky A, Rastogi S, Wang M & Gupta RC (2011). Vagus nerve stimulation in experimental heart failure. *Heart Fail Rev* **16**, 171–178.
- Sakai K, Chapleau MW, Morimoto S, Cassell MD & Sigmund CD (2004). Differential modulation of baroreflex control of heart rate by neuron- vs. glia-derived angiotensin II. *Physiol Genomics* **20**, 66–72.
- Sakai K & Sigmund CD (2005). Molecular evidence of tissue renin-angiotensin systems: A focus on the brain. *Curr Hypertens Rep* **7**, 135–140.
- Sandoval A, Duran P, Gandini MA, Andrade A, Almanza A, Kaja S & Felix R (2017). Regulation of L-type Cav1.3 channel activity and insulin secretion by the cGMP-PKG signaling pathway. *Cell Calcium* **66**, 1–9.
- Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M & Campagnole-Santos MJ (2018). The ACE2/angiotensin-(1–7)/mas axis of the renin-angiotensin system: focus on angiotensin-(1–7). *Physiol Rev* **98**, 505–553.
- Sato K, Park NG, Kohno T, Maeda T, Kim JI, Kato R & Takahashi M (1993). Role of basic residues for the binding of omega-conotoxin GVIA to N-type calcium channels. *Biochem Biophys Res Commun* **194**, 1292–1296.
- Saulière A, Bellot M, Paris H, Denis C, Finana F, Hansen JT, Altie M-F, Seguelas MH, Pathak A, Hansen JL, Sénard JM & Galés C (2012). Deciphering biased-agonism complexity reveals a new active AT1 receptor entity. *Nat Chem Biol* **8**, 622–630.
- Savage N (2017). Machine learning: Calculating disease. *Nature* **550**, S115–S117.
- Schiffer S, Pummer S, Witte K & Lemmer B (2009). Cardiovascular regulation in TGR(mREN2)27 rats: 24h variation in plasma catecholamines, angiotensin peptides, and telemetric heart rate variability. *Chronobiol Int* **18**, 461–474.
- Schroder F (2003). Single L-type Ca^{2+} channel regulation by cGMP-dependent protein kinase type I in adult cardiomyocytes from PKG I transgenic mice. *Cardiovasc Res* **60**, 268–277.
- Sears CE, Choate JK & Paterson DJ (1998). Effect of nitric oxide synthase inhibition on the sympatho-vagal control of heart rate. *J Auton Nerv Syst* **73**, 63–73.
- Segura-Puimedon M, Mergia E, Al-Hasani J, Aherrahrou R, Stoelting S, Kremer F, Freyer J, Koesling D, Erdmann J, Schunkert H, de Wit C & Aherrahrou Z (2016). Proatherosclerotic effect of the $\alpha 1$ -subunit of soluble guanylyl cyclase by promoting smooth muscle phenotypic switching. *Am J Pathol* **186**, 2220–2231.
- Seidel E & Scholl UI (2017). Genetic mechanisms of human hypertension and their implications for blood pressure physiology. *Physiol Genomics* **49**, 630–652.
- Shan Z, Zubcevic J, Shi P, Jun JY, Dong Y, Murça TM, Lamont GJ, Cuadra A, Yuan W, Qi Y, Li Q, Paton JFR, Katovich MJ, Sumners C & Raizada MK (2013). Chronic knockdown of the nucleus of the solitary tract AT1 receptors increases blood inflammatory-endothelial progenitor cell ratio and exacerbates hypertension in the spontaneously hypertensive rat. *Hypertension* **61**, 1328–1333.
- Shanks J, Herring N, Johnson E, Liu K, Li D & Paterson DJ (2017). Overexpression of sarcoendoplasmic reticulum calcium ATPase 2a promotes cardiac sympathetic neurotransmission via abnormal endoplasmic reticulum and mitochondria Ca^{2+} regulation. *Hypertension* **69**, 625–632.
- Shanks J, Mane S, Ryan R & Paterson DJ (2013a). Ganglion-specific impairment of the norepinephrine transporter in the hypertensive rat. *Hypertension* **61**, 197–193.
- Shanks J, Manou-Stathopoulou S, Lu C-J, Li D, Paterson DJ & Herring N (2013b). Cardiac sympathetic dysfunction in the prehypertensive spontaneously hypertensive rat. *Am J Physiol Heart Circ Physiol* **305**, H980–H986.
- Sheehan D (1936). Discovery of the autonomic nervous system. *Arch Neurol Psychiatry* **35**, 1081–1115.
- Shinohara K, Kishi T, Hirooka Y & Sunagawa K (2015). Circulating angiotensin II deteriorates left ventricular function with sympathoexcitation via brain angiotensin II receptor. *Physiol Rep* **3**, e12514–12.
- Shivkumar K, Ajjola OA, Anand I, Armour JA, Chen PS, Esler M, De Ferrari GM, Fishbein MC, Goldberger JJ, Harper RM, Joyner MJ, Khalsa SS, Kumar R, Lane R, Mahajan A, Po S, Schwartz PJ, Somers VK, Valderrabano M, Vaseghi M & Zipes DP (2016). Clinical neurocardiology defining the value of neuroscience-based cardiovascular therapeutics. *J Physiol* **594**, 3911–3954.
- Simmerman HK & Jones LR (1998). Phospholamban: protein structure, mechanism of action, and role in cardiac function. *Physiol Rev* **78**, 921–947.
- Souza HC, Ballejo G, Salgado MC, Da Silva VJ & Salgado HC (2001). Cardiac sympathetic overactivity and decreased baroreflex sensitivity in L-NAME hypertensive rats. *Am J Physiol Heart Circ Physiol* **280**, H844–H850.
- Sprenger JU, Perera RK, Steinbrecher JH, Lehnart SE, Maier LS, Hasenfuss G & Nikolaev VO (2015). In vivo model with targeted cAMP biosensor reveals changes in receptor-microdomain communication in cardiac disease. *Nat Commun* **6**, 6965.
- Stangherlin A & Zaccolo M (2012). Phosphodiesterases and subcellular compartmentalized cAMP signaling in the cardiovascular system. *Am J Physiol Heart Circ Physiol* **302**, H379–H390.
- Steptoe A & Kivimaki M (2012). Stress and cardiovascular disease. *Nat Rev Cardiol* **9**, 360–370.
- Stratakis CA (2002). Mutations of the gene encoding the protein kinase A type I- α regulatory subunit (PRKAR1A) in patients with the “complex of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas” (Carney complex). *Ann N Y Acad Sci* **968**, 3–21.
- Stratakis CA, Kirschner LS & Carney JA (2001). Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J Clin Endocrinol Metab* **86**, 4041–4046.
- Sverrisdottir YB, Green AL, Aziz TZ, Bahuri NFA, Hyam J, Basnayake SD & Paterson DJ (2014). Differentiated baroreflex modulation of sympathetic nerve activity during deep brain stimulation in humans. *Hypertension* **63**, 1000–1010.

- Tahsili-Fahadan P & Geocadin RG (2017). Heart-brain axis: effects of neurologic injury on cardiovascular function. *Circ Res* **120**, 559–572.
- Talaia C, Queiroz G, Pinheiro H, Moura D & Gonçalves J (2006). Involvement of G-protein $\beta\gamma$ subunits on the influence of inhibitory α_2 -autoreceptors on the angiotensin AT1-receptor modulation of noradrenaline release in the rat vas deferens. *Neurochem Int* **49**, 698–707.
- Tan J, Wang H & Leenen FHH (2004). Increases in brain and cardiac AT₁ receptor and ACE densities after myocardial infarct in rats. *Am J Physiol Heart Circ Physiol* **286**, H1665–H1671.
- Tan Z-Y, Lu Y, Whiteis CA, Simms AE, Paton JFR, Chapleau MW & Abboud FM (2010). Chemoreceptor hypersensitivity, sympathetic excitation, and overexpression of ASIC and TASK channels before the onset of hypertension in SHR. *Circ Res* **106**, 536–545.
- Tanaka S, Tanaka R, Harada S, Kohda Y, Matsumura H, Shimamoto C, Sawabe Y, Marunaka Y, Kuwabara H, Takahashi Y, Ito S & Nakahari T (2013). A PKG inhibitor increases Ca^{2+} -regulated exocytosis in guinea pig antral mucous cells: cAMP accumulation via PDE2A inhibition. *Am J Physiol Gastrointest Liver Physiol* **304**, G773–G780.
- Tilley DG (2011). Functional relevance of biased signaling at the angiotensin II type 1 receptor. *Endocr Metab Immune Disord Drug Targets* **11**, 99–111.
- Tomek J, Rodriguez B, Bub G & Heijman J (2017). β -Adrenergic receptor stimulation inhibits proarrhythmic alternans in postinfarction border zone cardiomyocytes: A computational analysis. *Am J Physiol Heart Circ Physiol* **313**, H338–H353.
- Tóth AD, Gyombolai P, Szalai B, Várnai P, Turu G & Hunyady L (2017). Angiotensin type 1A receptor regulates β -arrestin binding of the β_2 -adrenergic receptor via heterodimerization. *Mol Cell Endocrinol* **442**, 113–124.
- Tóth AD, Turu G, Hunyady L & Balla A (2018). Novel mechanisms of G-protein-coupled receptors functions: AT₁ angiotensin receptor acts as a signaling hub and focal point of receptor cross-talk. *Best Pract Res Clin Endocrinol Metab* **32**, 69–82.
- Tromp TR, Mahesh D, Joles JA & Ramchandra R (2018). Direct recording of cardiac and renal sympathetic nerve activity shows differential control in renovascular hypertension. *Hypertension* **71**, 1108–1116.
- Tu H, Liu J, Zhang D, Zheng H, Patel KP, Cornish KG, Wang W-Z, Muellemann RL & Li Y-L (2014). Heart failure-induced changes of voltage-gated Ca^{2+} channels and cell excitability in rat cardiac postganglionic neurons. *Am J Physiol Cell Physiol* **306**, C132–C142.
- Uhrenholt TR & Nedergaard OA (2003). Calcium channels involved in noradrenaline release from sympathetic neurones in rabbit carotid artery. *Pharmacol Toxicol* **92**, 226–233.
- van den Meiracker AH, Admiraal PJJ, Janssen JA, Kroodsmas JM, de Ronde WAM, Boomsma F, Sissmann J, Blankestijn PJ, Mulder PGM, Man in t Veld AJ & Schalekamp MADH (1995). Hemodynamic and biochemical effects of the AT1 receptor antagonist irbesartan in hypertension. *Hypertension* **25**, 22–29.
- Vettel C, Lindner M, Dewenter M, Lorenz K, Schanbacher C, Riedel M, Lämmle S, Meinecke S, Mason FE, Sossalla S, Geerts A, Hoffmann M, Wunder F, Brunner FJ, Wieland T, Mehel H, Karam S, Lechêne P, Leroy J, Vandecasteele G, Wagner M, Fischmeister R & El-Armouche A (2017). Phosphodiesterase 2 protects against catecholamine-induced arrhythmia and preserves contractile function after myocardial infarction. *Circ Res* **120**, 120–132.
- Wallace S, Guo DC, Regalado E, Mellor-Crummey L, Bamshad M, Nickerson DA, Dauser R, Hanchard N, Marom R, Martin E, Berka V, Sharina I, Ganesan V, Saunders D, Morris SA & Milewicz DM (2016). Disrupted nitric oxide signaling due to GUCY1A3 mutations increases risk for moyamoya disease, achalasia and hypertension. *Clin Genet* **90**, 351–360.
- Wang L, Henrich M, Buckler KJ, McMenamin M, Mee CJ, Sattelle DB & Paterson DJ (2007). Neuronal nitric oxide synthase gene transfer decreases $[\text{Ca}^{2+}]_i$ in cardiac sympathetic neurons. *J Mol Cell Cardiol* **43**, 717–725.
- Wang L, Li D, Plested CP, Dawson T, Teschemacher AG & Paterson DJ (2006). Noradrenergic neuron-specific overexpression of nNOS in cardiac sympathetic nerves decreases neurotransmission. *J Mol Cell Cardiol* **41**, 364–370.
- Wang W, Yang Y, Yin J & Gong X (2017). Different protein-protein interface patterns predicted by different machine learning methods. *Sci Rep* **7**, 16023.
- Wang Y, Seto S-W & Golledge J (2012). Angiotensin II, sympathetic nerve activity and chronic heart failure. *Heart Fail Rev* **19**, 187–198.
- Watanabe R, Suzuki J-I, Wakayama K, Maejima Y, Shimamura M, Koriyama H, Nakagami H, Kumagai H, Ikeda Y, Akazawa H, Morishita R, Komuro I & Isobe M (2017). A peptide vaccine targeting angiotensin II attenuates the cardiac dysfunction induced by myocardial infarction. *Sci Rep* **7**, 43920.
- Watson AMD, Hood SG, Ramchandra R, McAllen RM & May CN (2007). Increased cardiac sympathetic nerve activity in heart failure is not due to desensitization of the arterial baroreflex. *Am J Physiol Heart Circ Physiol* **293**, H798–H804.
- Weir MR & Dzau VJ (1999). The renin-angiotensin-aldosterone system: A specific target for hypertension management. *Am J Hypertens* **12**, 205S–213S.
- White WB, Carr AA, Krause S, Jordan R, Roniker B & Oigman W (2003). Assessment of the novel selective aldosterone blocker eplerenone using ambulatory and clinical blood pressure in patients with systemic hypertension. *Am J Cardiol* **92**, 38–42.
- Williams B, MacDonald TM, Morant S, Webb DJ, Sever P, McInnes G, Ford I, Cruickshank JK, Caulfield MJ, Salisbury J, Mackenzie I, Padmanabhan S, Brown MJ; The British Hypertension Society's PATHWAY Studies Group (2015). Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. *Lancet* **386**, 2059–2068.
- Wiysonge CS, Bradley HA, Volmink J & Mayosi BM (2018). Cochrane corner: beta-blockers for hypertension. *Heart* **104**, 282–283.

- Wysong CS, Bradley HA, Volmink J, Mayosi BM & Opie LH (2017). Beta-blockers for hypertension. *Cochrane Database Syst Rev* **285**, 2719–2796.
- Wobst J, Ameln S, Wolf B, Wierer M, Dang TA, Sager HB, Tennstedt S, Hengstenberg C, Koesling D, Friebe A, Braun SL, Erdmann J, Schunkert H & Kessler T (2016). Stimulators of the soluble guanylyl cyclase: promising functional insights from rare coding atherosclerosis-related *GUCY1A3* variants. *Basic Res Cardiol* **111**, 51.
- Wobst J, Rumpf PM, Dang TA, Segura-Puimedon M, Erdmann J & Schunkert H (2015). Molecular variants of soluble guanylyl cyclase affecting cardiovascular risk. *Circ J* **79**, 463–469.
- Woollard HH (1926). The innervation of the heart. *J Anat* **60**, 345–373.
- Xie Z-R, Chen J & Wu Y (2017). Predicting protein-protein association rates using coarse-grained simulation and machine learning. *Sci Rep* **7**, 46622.
- Yamada Y, Kinoshita H, Kuwahara K, Nakagawa Y, Kuwabara Y, Minami T, Yamada C, Shibata J, Nakao K, Cho K, Arai Y, Yasuno S, Nishikimi T, Ueshima K, Kamakura S, Nishida M, Kiyonaka S, Mori Y, Kimura T, Kangawa K & Nakao K (2014). Inhibition of N-type Ca^{2+} channels ameliorates an imbalance in cardiac autonomic nerve activity and prevents lethal arrhythmias in mice with heart failure. *Cardiovasc Res* **104**, 183–193.
- Yaniv Y, Ganesan A, Yang D, Ziman BD, Lyashkov AE, Levchenko A, Zhang J & Lakatta EG (2015). Real-time relationship between PKA biochemical signal network dynamics and increased action potential firing rate in heart pacemaker cells. Kinetics of PKA activation in heart pacemaker cells. *J Mol Cell Cardiol* **86**, 168–178.
- Young CN & Davisson RL (2015). Angiotensin-II, the brain, and hypertension: An update. *Hypertension* **66**, 920–926.
- Zaccolo M & Movsesian MA (2007). cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology. *Circ Res* **100**, 1569–1578.
- Zamponi GW, Striessnig J, Koschak A & Dolphin AC (2015). The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol Rev* **67**, 821–870.
- Zhao CY, Greenstein JL & Winslow RL (2016). Roles of phosphodiesterases in the regulation of the cardiac cyclic nucleotide cross-talk signaling network. *J Mol Cell Cardiol* **91**, 215–227.
- Zhao CY, Greenstein JL & Winslow RL (2017). Mechanisms of the cyclic nucleotide cross-talk signaling network in cardiac L-type calcium channel regulation. *J Mol Cell Cardiol* **106**, 29–44.
- Zheng J, Rao DC & Shi G (2015). An update on genome-wide association studies of hypertension. *Appl Inform* **2**, 10.
- Zhu G-Q, Gao L, Li Y, Patel KP, Zucker IH & Wang W (2004). AT_1 receptor mRNA antisense normalizes enhanced cardiac sympathetic afferent reflex in rats with chronic heart failure. *Am J Physiol Heart Circ Physiol* **287**, H1828–H1835.
- Zoccarato A, Surdo NC, Aronsen JM, Fields LA, Mancuso L, Dodoni G, Stangherlin A, Livie C, Jiang H, Sin YY, Gesellchen F, Terrin A, Baillie GS, Nicklin SA, Graham D, Szabo-Fresnais N, Krall J, Vandeput F, Movsesian M, Furlan L, Corsetti V, Hamilton G, Lefkimmatis K, Sjaastad I & Zaccolo M (2015). Cardiac hypertrophy is inhibited by a local pool of cAMP regulated by phosphodiesterase 2. *Circ Res* **117**, 707–719.

Additional information

Competing interests

None of the authors have any conflicts of interests.

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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