Four evolving concepts in molecular and clinical autonomic research

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In reflecting on decades of autonomic research, I recall the 2009 Walter B. Cannon Memorial Award Lecture, which I entitled *In Search of Autonomic Balance: The Good, The Bad and The Ugly* [1]. The Good was the inhibition of excessive sympathetic nerve activity by baroreceptors and by cardiac and respiratory vagal afferents. The Bad was the loss of these inhibitory signals. The Ugly was the fact that the loss of the inhibitory signals enhances the excitatory signals (e.g., from chemoreceptors and somatic afferents). The discovery of the molecular determinants of afferent mechanical and chemical signaling, their paracrine activation, and their genetic regulation have revealed new mechanisms of cardiovascular autonomic dysfunction with potential therapeutic interventions. Namely, sophisticated optogenetic techniques allow activation of specific afferent autonomic nerves [16]; and novel surgical devices and approaches are used in clinical trials involving carotid sinus, glossopharyngeal, or vagus nerve stimulation, renal denervation, carotid body resection, and cardiac neuroablation.

In this invited commentary commemorating the 30th anniversary of Clinical Autonomic Research, I will highlight progress in clinical autonomic research by briefly reviewing four evolving concepts in the field. I ask for your indulgence, as I focus on experimental themes specifically pursued at the University of Iowa Cardiovascular Research Center. Consequently, other relevant autonomic concepts may not have been discussed here.

First evolving concept: the neurosensory integration of human sympathetic activity and its clinical implications

Hypertension, heart failure and obstructive sleep apnea are disorders with excessive sympathetic nerve activity as a major contributor to cardiovascular mortality. The convergence of multiple afferent signals, integrated centrally, defines the pattern of peripheral sympathetic activity. Additional examples of clinically significant sensory interactions abound. Thermal sensing by cutaneous afferents prevents reflex sympathetic vasoconstriction during hypotension to sustain preferentially heat dissipation, even though this could contribute to heat exhaustion and fainting [13]. Similarly, the reflex vasoconstrictor response to hypoxia is abrogated as a result of hyperventilation but is conversely enhanced, alarmingly, with the suppression of respiratory afferent activity during apnea. Using direct microneurography, we documented a dramatic and potentially fatal accentuation of sympathetic nerve activity in humans during obstructive sleep apnea [20]. (Fig. 1A).

Cardiac afferents are major regulators of the autonomic system supporting the concept that the heart is a sensory organ. Low-pressure mechanosensory vagal endings in the heart suppress sympathetic and enhance parasympathetic activity, regulating both blood volume and arterial pressure [2]. We established their role in neurocardiogenic syncope and syncope in patients with aortic stenosis. The distribution of these sensory endings in the posterior–inferior wall of the left ventricle explains the preponderance of bradycardia and hypotension in patients with inferior wall myocardial infarction in contrast to anterior wall infarction [22]. In patients with heart failure, treatment with cardiac glycosides, such as digitalis, restores the depressed sensitivity of these endings and thereby beneficially reduces excessive sympathetic nerve activity. Impairment of these endings is also seen with aging, hypertension, and diabetes mellitus. Although cardiac

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Fig. 1 Evolving concepts in molecular and clinical autonomic research. **A** Obstructive Sleep Apnea (OSA). Increased sympathetic nerve activity (SNA) in a patient with OSA is seen in the awake state and is enhanced alarmingly by episodes of OSA during the rapid eye movement (REM) sleep state. Positive airway pressure (CPAP) suppresses the increase in SNA during REM. Adapted from [20]. **B** Degenerin (DEG)/Epithelial Sodium Channel (ENaC) Family Members. Evolutionary conservation of mechanosensitive (MEC) and acid-sensing ion channels (ASICs). Adapted from [1]. **C** Mechanically induced Depolarization of aortic baroreceptor neurons is ASIC2 dependent. Adapted from [15]. **D** Cytokine release from innate

immune cells in response to Toll-like receptor (TLR) ligands is regulated by nicotine and angiotensin II (Ang II) receptors. Adapted from [3]. **E** Response of splenocytes to Toll-like receptor (TLR) activation is suppressed by nicotine in normotensive rats (WKY) and enhanced by both nicotine and angiotensin II (Ang II) in spontaneously hypertensive rats (SHR). Adapted from [3]. **F** Pressor responses to angiotensin II (Ang II) are dependent on two adaptor proteins that mediate Toll-like receptor (TLR) activation of innate immune responses. MyD88 suppresses and TRIF mediates the pressor response and the pro-inflammatory gene expression. Adapted from [19]

mechanosensors and arterial baroreceptors suppress sympathetic activity, the cardiac sensory terminals which are activated by the expansion of central blood volume target preferentially renal sympathetic nerves to achieve diuresis and volume homeostasis. An important interaction that I refer to as “reciprocal dysautonomia” is the one between baroreceptors and chemoreceptors. The decreased baroreceptor activity in hypertension and heart failure increases sympathetic activity and enhances the response of chemoreceptors to hypoxia that synergistically increases further sympathetic nerve activity and its morbidity [14]. This central interaction clarifies a dual sympatho-inhibitory mechanism by which carotid sinus nerve stimulation may be beneficial in patients with resistant hypertension or heart failure.

White adipose tissue is connected to the CNS via afferent and efferent fibers. White adipose tissue afferents are unmyelinated fibers sensitive to lipolysis products in adipocytes. The neuronal bodies of these afferent fibers are located in dorsal root ganglia which are, in turn, connected to relevant

CNS nuclei to activate the sympathetic nervous system to white and brown adipose tissues in a positive feedback reflex termed adipose afferent reflex. This reflex provides a neural control of energy balance. When lipolysis is high in white adipose tissue, the thermogenic machinery in brown adipose tissue is engaged [9].

Second evolving concept: the molecular determinants of afferent sensory signals

In the past three decades, our work led to: i) the discovery that, in addition to the classical baro- and chemoreceptors, rheoreceptors, specialized receptors that respond to changes in blood flow, contribute to carotid sinus nerve activity [10]; and ii) that endocrine, paracrine-endothelial, and autocrine ionic and oxidative processes are powerful modulators of neurosensory activity. For example, decreased synthesis of endogenous prostanoids as well as

increased reactive oxygen species suppresses baroreceptor afferent signals in hypertension and atherosclerosis. We demonstrated that the resetting of the baroreceptors is predominantly neuronal rather than vascular, identified mechanosensitive ion channels in isolated patch-clamped baroreceptor neurons, and found that pulsatility of the arterial pressure signal prevents baroreceptor afferent adaptation and sustains reflex sympathoinhibition. We also reported that the mechanoelectrical transduction in baroreceptor neurons is mediated by a new class of gadolinium-sensitive stretch-activated channels. These mechanosensitive channels, initially identified in differentiated touch receptor neurons of *C. elegans* [5], are members of an invertebrate subfamily of the Degnerin (DEG)/epithelial sodium channel (ENaC) family (Fig. 1B). In mammals, a subfamily of the DEG/ENaC family includes the Acid Sensing Ion Channels (ASIC), which are expressed in human peripheral nerve and brain [25]. ASIC have a broad range of sensitivities not just to acid pH and protons, but also to mechanical deformation, temperature change, and nociception. The ASIC subunits 1, 2, and 3 in different heteromeric combinations form various channels in different nerve terminals and define the nature of the sensory signals. Using gene deletion and overexpression strategies, we defined a critically important role of these and other sensory molecules [8] in maintaining autonomic balance. For instance, in aortic baroreceptor neurons, ASIC 2 is an important determinant of mechanically induced depolarization [15] (Fig. 1C). And the ASIC 2 knock-out mouse develops neurogenic hypertension. We also reported that blockade of potassium channels enhances the generation of action potentials in nodose ganglion neurons [6], and that activation of the sodium–potassium ATPase causes baroreceptor resetting during acute hypertension [12]. These ionic mechanisms play a role in the impaired baroreceptor activity in hypertension.

Patch-clamping glomus cells of the carotid bodies uncovered ion channels that are hypersensitive to hypoxia and low pH in these peripheral chemoreceptors. Specifically, non-voltage gated acid-sensing ion channels (ASIC 1 and ASIC 3) and two-pore domain transient acid-sensing potassium channels (TASK) are overexpressed and linked to the exaggerated chemoreceptor sensitivity in the spontaneously hypertensive rat compared to normotensive rats [21]. In skeletal muscle afferents, ASIC heteromers contribute significantly to the exercise pressor reflex, and, in cardiac afferents of the dorsal root ganglia, ASICs contribute to nociception and the detrimental sympathetic activation during cardiac ischemia and heart failure. The translational importance of activation of ASIC 2 in baroreceptors is a beneficial suppression of the enhanced sympathetic excitatory reflexes of chemoreceptors and skeletal muscle afferents, as well as the ASIC1a-mediated excitation of

CNS neurons during emotional states of fear, threat, or panic.

Piezo1 and Piezo2 are mechanically activated ion channels that play crucial roles in several mechanotransduction processes. In collaboration with Drs. Liberles and Patapoutian, we recently reported that Piezo ion channels are expressed in sensory neurons that contribute to the baroreflex. Conditional double knock-out of the *PIEZO1* and *PIEZO2* genes in mice causes baroreflex failure severely disrupting control of blood pressure and heart rate [26]. Patients with *PIEZO2* mutations have severely decreased proprioception [7]. Combined loss of function of Piezo1 and Piezo2 presumably causes baroreflex failure in humans, although these patients are yet to be identified.

Third evolving concept: the autonomic neuro-immune synapse

Autonomic regulation of the immune system has a major influence on cardiovascular disease and clinical outcomes. Increases in the sympathetic and the renin–angiotensin system (RAS) activities and decreases in parasympathetic cholinergic activity are vital in acute circulatory adjustments to hemodynamic, hypotensive stresses. When sustained and prolonged, these autonomic changes have nefarious consequences [1]. The destructive tissue damage is not the result of the hemodynamic influence per se but rather a powerful autonomic pro-inflammatory modulation of a genetically vulnerable immune system [18] (Fig. 1D and 1E). Activation of the sympathetic and the renin–angiotensin systems is pro-inflammatory, whereas parasympathetic activation is anti-inflammatory and protective. The anti-inflammatory effect is mediated by the $\alpha 7$ nicotinic cholinergic receptor ($\alpha 7nAChR$) on innate immune cells [23]. Deletion of this receptor enhances end-organ damage in different models of hypertension, whereas its pharmacologic stimulation is protective.

This can be studied in the spontaneously hypertensive rat (SHR), a clinically relevant genetic animal model of hypertension. During a pre-hypertensive period of up to 6 weeks after birth, when the SHR newborn is still normotensive, significant autonomic, metabolic, and immunological abnormalities occur. For example, SHR splenocytes exposed to CpG, a ligand of Toll-like receptor 9 (TLR9) activation, release large quantities of pro-inflammatory IL-6 and IL-1 β when co-stimulated with nicotine. This pro-inflammatory reaction is not observed in the normotensive control Wistar-Kyoto rat, in which nicotine suppresses the pro-inflammatory response to CpG. Harwani and colleagues, at the University of Iowa, propose that nicotinic cholinergic receptors mediate either anti- or pro-inflammatory responses depending on the activation of selective nicotinic receptors

that may be differentially expressed in innate immune cells of SHR vs. normotensive control rats [11] (Fig. 1E). Abnormal innate immune responses may also be defined by the TLRs that are activated, their ligands, and their adaptor proteins. For example, we recently confirmed that angiotensin II (Ang II) responses in mice depend on two major adaptor proteins: MyD88 (myeloid protein 88) and TRIF (TIR-domain-containing adaptor-inducing interferon- β) [19]. A non-functional TRIF abrogates Ang II-induced hypertension, cardiac hypertrophy, and pro-inflammatory gene expression, whereas deletion of MyD88 enhances these responses (Fig. 1F). This indicates that MyD88 is a protective negative regulator of the inflammatory responses to Ang II.

Fourth evolving concept: the regulation of intestinal vagal afferents and their microbiome interactions

The satiety effects and metabolic actions of cholecystokinin (CCK) have been viewed as potential therapeutic targets for obesity. We identified a Ca^{2+} -activated chloride (Cl^-) current (CaCC) that is evoked by CCK in intestinal vagal afferents of nodose neurons [24]. The CaCC subunit *Anoctamin 2* (*Ano2*/TMEM16B) is the dominant contributor to this current. Reduced expression of TMEM16B in the heterozygote knock-out of the channel in sensory neurons results in obese rats with loss of CCK sensitivity in intestinal nodose neurons, loss of CCK-induced satiety, and decreased energy expenditure. Our findings reveal that *Ano2*/TMEM16B expressed in sensory neurons selectively promotes a reflex increase in sympathetic nerve activity to brown adipose tissue and corresponding thermogenesis [24].

Chemical signals from the gut microbiota activate vagal afferent terminals beneath the gut epithelium and affect food intake and metabolism. They may trigger inflammatory responses in vagal afferent neurons with cell bodies located in nodose ganglia, causing autonomic dysfunction [4]. In addition, nodose ganglia microglial activation by microbiota dysbiosis may suppress vagal afferent signals to the CNS. We found remarkable differences in neonatal gut microbiota between SHR and normotensive control rats that do not persist into adulthood and yet may have a lasting effect on the immune response and the development of hypertension [16]. More importantly, cross-fostering of SHR with normotensive control rats changes their neonatal microbiota to reflect that of the normotensive control rats and favorably reduces the inflammatory response and hypertension. Although cross-fostering also induces changes in the microbiota of normotensive control rats toward neonatal SHR microbiota, these changes are not sufficient to cause hypertension [17]. We suspect that the likely source of the

neonatal diversity could be the mother's milk, the genetic predisposition of the pups in supporting the growth of specific early gut bacteria, or both. Therefore, cross-fostering is a robust method of altering the neonatal gut microbiota with distinct and measurable long-term beneficial outcomes, with potential implications in sympathetic-mediated disorders such as hypertension.

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