CHAPTER 19

Mechanosensitive Ion Channels in Blood Pressure-Sensing Baroreceptor Neurons

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I. OVERVIEW

Baroreceptors (BRs) are mechanosensitive nerve endings in carotid sinuses and aortic arch that function as arterial blood pressure (BP) sensors. Changes in BR activity evoke reflex circulatory adjustments that reduce BP

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variability and its adverse consequences. BR activation during increases in BP involves three processes: (1) vascular distension and deformation of BR nerve endings, (2) depolarization of the nerve terminals consequent to opening of mechanosensitive ion channels (mechanoelectrical transduction), and (3) translation of mechanically induced depolarization into action potential discharge mediated by voltage-dependent Na^+ and K^+ channels. The mechanism of mechanoelectrical transduction (process 2) has been elusive. Recent studies have applied a variety of physiological, pharmacological, and molecular approaches to this problem, including studies in animals and isolated BR neurons in culture. In this chapter, we provide an overview of the molecular basis of BR mechanoelectrical transduction. Emerging evidence points to members of three evolutionarily conserved ion channel families in mediating BR activation: *e*pithelial *Na c*hannels (ENaCs), *a*cid *s*ensing *i*on *c*hannels (ASICs), and *t*ransient *r*eceptor *p*otential (TRP) channels. The precise composition of the BR mechanosensitive ion channel complex and the mechanism of channel gating remain to be determined. Translation of discoveries in lower "model" organisms to studies of mammalian BR function and use of multidisciplinary approaches including state-of-the-art spatial and temporal gene targeting are encouraged in order to move the field forward.

II. INTRODUCTION

Arterial BP provides the driving force for delivery of blood flow to tissues and is therefore essential for organ system function and life. Maintenance of a relatively normal BP is particularly important for the brain and heart due to the high metabolic rate of these organs and the need for a continuous supply of blood flow and oxygen to maintain their vital functions. Even transient decreases in BP can compromise cerebral and coronary blood flow with risk of losing consciousness (syncope), stroke, and myocardial infarction. Abnormally high levels of BP or increased BP variability cause "target organ" damage, for example, impairment of vascular endothelial function, vascular and cardiac hypertrophy, kidney disease, and stroke (Mancia and Parati, 2003). Many factors can alter BP, including acute stressors (e.g., hemorrhage, assumption of the upright posture, emotional stress) and chronic disease (e.g., hypertension, autonomic failure).

The arterial BR reflex is a key BP regulatory mechanism (Kirchheim, 1976; Chapleau *et al.*, 2001; Chapleau, 2003; Chapleau and Abboud, 2004). BRs are mechanosensitive nerve endings located primarily in adventitia of carotid sinuses and aortic arch (Fig. 1). Changes in arterial BP alter the degree of vascular distension, which is sensed by BR nerve endings via mechanical

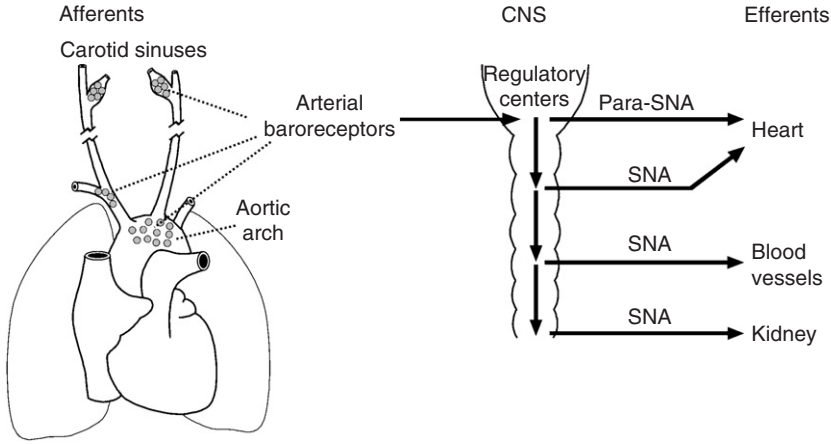

FIGURE 1 Baroreceptor reflex pathways. Shown are sites of afferent BR innervation (filled circles), and efferent parasympathetic (Para-SNA) and sympathetic (SNA) projections from the central nervous system (CNS) to the heart, blood vessels, and kidneys. Adapted and reprinted from Chapleau (2003, Fig. 1, p. 104) and Chapleau and Abboud (2004, Fig. 1, p. 2) with permission.

deformation. Afferent BR activity is transmitted along the carotid sinus and aortic depressor nerves (ADNs) to the nucleus tractus solitarii in the medulla oblongata where the signals are integrated and relayed through a network of central neurons controlling efferent parasympathetic and sympathetic nerve activity to the heart, vasculature, kidney, and other organs (Fig. 1).

Changes in the frequency of BR afferent discharge in response to changes in BP trigger reflex adjustments that buffer or oppose the change in BP (Kirchheim, 1976; Chapleau and Abboud, 2004). For example, a rise in BP increases BR activity leading to reflex inhibition of sympathetic activity, parasympathetic activation, and subsequent decreases in vascular resistance and heart rate. Conversely, a decrease in BP reduces BR activity thereby triggering a reflex increase in sympathetic activity, parasympathetic inhibition, and increases in vascular resistance and heart rate. Changes in BR activity also influence release of vasopressin and renin that contribute to the circulatory adjustment. Thus, the BR reflex provides a powerful moment-to-moment negative feedback regulation of BP.

In addition to regulating BP, the BR reflex exerts a significant influence on the electrical properties of the heart through modulation of parasympathetic and sympathetic nerve activity (Podrid *et al.*, 1990). Ventricular arrhythmias are a common cause of death during myocardial ischemia and after myocardial infarction. Animal and clinical studies have demonstrated that decreased BR

reflex sensitivity predicts susceptibility to arrhythmias and sudden death after acute myocardial infarction and heart failure (Kaye and Esler, 1995; LaRovere *et al.*, 1998). These findings suggest that the reflex may protect the heart from arrhythmias by providing appropriate and rapid modulation of cardiac autonomic tone.

The BR reflex is composed of three general components: afferent sensory transduction, central mediation of the reflex, and efferent neurocardiac and neurovascular transmission (Fig. 1). The focus of this chapter is on mechanisms mediating *mechanoelectrical transduction* at the BR sensory nerve terminals.

III. BR SENSORY TRANSDUCTION

BR activation during increase in BP involves three processes: (1) vascular distension and deformation of BR nerve endings, (2) subsequent depolarization of the nerve endings (mechanoelectrical transduction), and (3) translation of mechanically induced depolarization into action potential discharge mediated by voltage-dependent Na^+ and K^+ channels (Fig. 2).

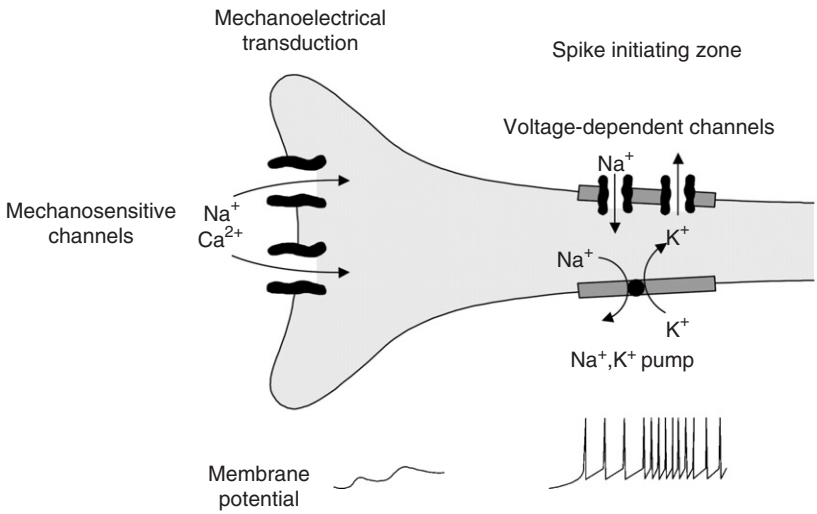

FIGURE 2 Mechanisms of BR activation in response to increase in arterial BP. Opening of mechanosensitive channels depolarizes the sensory nerve terminals (mechanoelectrical transduction). Depolarization of the SIZ sufficient to open voltage-dependent Na^+ and K^+ channels triggers action potential discharge. Adapted from Chapleau *et al.* (2001, Fig. 1, p. 3) with permission.

A. Vascular Compliance and Viscoelastic Coupling

The compliance of large arteries defines the extent of blood vessel distension for a given increase in BP and therefore is a major determinant of the magnitude of deformation and BR activity (Kirchheim, 1976). Decreased arterial compliance contributes to decreased BR sensitivity in diseases, such as atherosclerosis and chronic hypertension, and with aging (Kirchheim, 1976; Andresen and Yang, 1989). Viscoelastic coupling between elements in the arterial wall and the nerve endings also importantly influences BR activity (Coleridge *et al.*, 1984).

B. Mechanoelectrical Transduction

Elucidation of the mechanism of transducing mechanical deformation into membrane depolarization is of great interest in many areas of biology. The depolarization of mechanoreceptors, referred to as the "receptor" or "generator" potential, is graded in relation to the magnitude of mechanical stimulation and decays with time and distance from the point of stimulation (Katz, 1950; Grigg, 1986). The mechanoelectrical transduction is generally considered to involve opening of mechanosensitive ion channels gated by changes in membrane tension (Hamill and Martinac, 2001; Fig. 2).

1. Assessment of BR Sensitivity

The traditional approach to assess BR sensory function is to directly record the frequency or integrated voltage of action potential discharge from BR afferent nerve fibers *in vivo*, *in situ*, or *in vitro* (Kirchheim, 1976; Andresen and Yang, 1989; Chapleau *et al.*, 2001). The first approach used to test whether mechanosensitive channels mediate BR activation was to measure BR activity during ramp increases in pressure in the vascularly isolated carotid sinus of rabbits before and after intraluminal injection of gadolinium (Hajduczok *et al.*, 1994), an established blocker of mechanosensitive channels (Yang and Sachs, 1989). Gadolinium markedly attenuated the pressure-induced increases in BR activity without changing the compliance of the carotid sinus (Hajduczok *et al.*, 1994). Gadolinium did *not* block action potential discharge evoked by chemical stimulation of carotid sinus afferents with the Na^+ channel opener veratrine, indicating that the inhibition of pressure-induced BR activity by gadolinium was *not* caused by nonspecific suppression of neuronal excitability. In a separate study, gadolinium did not decrease the activity of rat aortic arch BR fibers (Andresen and Yang, 1992). The reason for the discrepant results is not known.

Action potential discharge in afferent fibers is measured away from the site of mechanoelectrical transduction in the sensory nerve terminals. The small size and complex architecture of the BR terminals embedded in the vascular wall have generally prevented the direct measurement of membrane potential in the terminals and limited investigation into mechanisms of BR activation *in vivo*. To our knowledge, only one study has reported direct measurements of membrane potential in BRs near the site of mechanoelectrical transduction (Matsuura, 1973). Furthermore, the presence of endothelium and vascular muscle along with other cell types in the vascular wall make it difficult to attribute changes in BR activity solely to direct actions on the nerve terminals. Factors released from nearby cells and changes in vascular smooth muscle tone can alter BR activity (Chapleau *et al.*, 2001; Chapleau and Abboud, 2004). These limitations motivated us and others to develop an *in vitro* preparation of isolated BR neurons in culture (Section III.B.2).

2. Study of Isolated BR Neurons in Culture

Aortic BR neurons can be labeled *in vivo* by application of a fluorescent dye (e.g., 1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanineperchlorate, diI) to the aortic arch adventitia or ADN of rats and mice (Mendelowitz and Kunze, 1992; Cunningham *et al.*, 1995, 1997; Li *et al.*, 1997; Sullivan *et al.*, 1997;

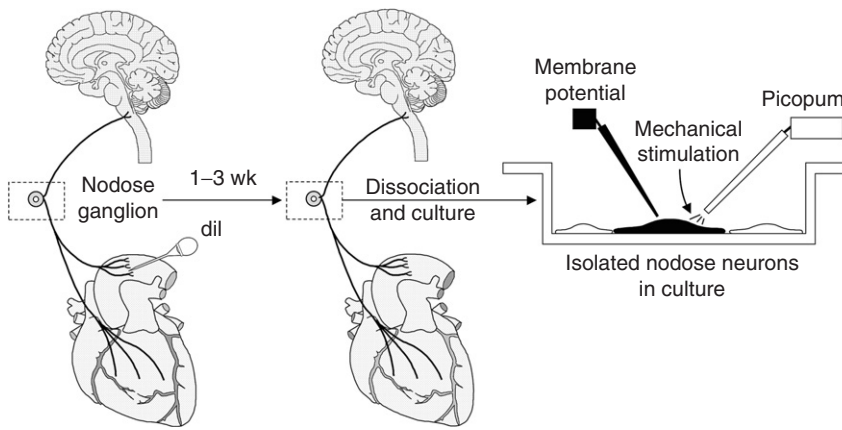

FIGURE 3 Study of isolated BR neurons in culture. BR neurons in nodose ganglia are labeled with a fluorescent dye (e.g., diI) applied to the aortic arch adventitia 1–3 weeks beforehand *in vivo*. Neurons are dissociated from nodose ganglia and fluorescently labeled BR neurons are studied *in vitro*. Adapted and reprinted from Chapleau *et al.* (2001, Fig.7, p.10) and Chapleau and Abboud (2004, Fig. 3, p. 8) with permission.

Drummond *et al.*, 1998; Kraske *et al.*, 1998; Li *et al.*, 1998; Fig. 3). One to 3 weeks later, nodose neurons are dissociated from nodose ganglia and maintained in culture. Functional studies are performed on individual diI-labeled aortic BR neurons (Fig. 3).

An important consideration is whether molecules expressed at the sensory terminals *in vivo* are also expressed in the cell membrane of isolated BR neurons in culture. Specific ligand receptors and ion channels present on the sensory nerve endings have been shown to be present on the soma of cultured nodose neurons (Fowler *et al.*, 1985; Stansfeld *et al.*, 1986; Christian *et al.*, 1989; Drummond *et al.*, 1998; Kraske *et al.*, 1998). Furthermore, spike frequency adaptation of dorsal root ganglion (DRG) neurons during sustained mechanical stimulation of cutaneous mechanoreceptor endings correlates with the adaptation during sustained depolarization of the same neuron by current injection into the soma (Harper, 1991).

Importantly, we have demonstrated that cultured BR neurons are mechanosensitive. Mechanical stimulation of isolated BR neurons evokes an inward cationic current (voltage clamp), depolarizes the membrane (current clamp), and increases cytosolic calcium concentration (Cunningham *et al.*, 1995, 1997; Sullivan *et al.*, 1997; Drummond *et al.*, 1998; Snitsarev *et al.*, 2002). Mechanosensitive channels have been identified in isolated BR neurons at the single-channel level (Kraske *et al.*, 1998). Similar to our findings *in vivo*, gadolinium blocks responses to mechanical stimulation of BR neurons *in vitro* (Cunningham *et al.*, 1995, 1997; Sullivan *et al.*, 1997). Gadolinium also inhibits mechanically induced responses in diI-labeled cardiac sensory neurons isolated from nodose ganglia (Linz and Veelken, 2002; Ditting *et al.*, 2003), and a subpopulation of neurons isolated from DRG (Gotoh and Takahashi, 1999; McCarter *et al.*, 1999; Raybould *et al.*, 1999; Cho *et al.*, 2002; Drew *et al.*, 2002). Differences in mechanosensitivity among different types of neurons isolated from nodose and DRG correspond to differences in mechanosensitivity of their respective nerve terminals (Cunningham *et al.*, 1995, 1997; Sharma *et al.*, 1995; Sullivan *et al.*, 1997; Drew *et al.*, 2002, 2004). Efferent autonomic neurons isolated from sympathetic ganglia do *not* generate inward currents or depolarize in response to mechanical stimulation (McCarter *et al.*, 1999; our unpublished observations).

Therefore, despite probable differences in expression and regulation of sensory molecules in sensory terminals vs isolated neuron somata, the isolated BR neuron appears to be a valid model for investigation of mechanisms of sensory transduction. The *in vivo* and *in vitro* findings reviewed above support the hypothesis that mechanosensitive channels mediate BR mechanoelectrical transduction (Fig. 2).

C. Encoding of Depolarization into Frequency of Action Potential Discharge

Depolarization of mechanosensitive nerve endings is localized to the sensory terminals and rapidly decays with distance from the site of stimulation (Katz, 1950; Grigg, 1986). The mechanically induced depolarization is translated into action potential discharge at the "spike initiating zone" (SIZ) near the nerve terminals (Katz, 1950; Grigg, 1986; Fig. 2). Action potentials are generated when the depolarization reaches a specific threshold for opening of voltage-dependent Na^+ and K^+ channels. The frequency of action potential discharge increases with further depolarization and is critically dependent on the expression and properties of voltage-dependent channels and membrane pumps near the SIZ. The role of voltage-dependent channels in modulating BR activity and their modulation by autocrine/paracrine factors have been reviewed elsewhere (Schild and Kunze, 1997; Chapleau *et al.*, 2001; Chapleau and Abboud, 2004; Schild *et al.*, 2005).

IV. MECHANOSENSITIVE CHANNELS IN BR NEURONS

Recent advances in gene discovery in diverse organisms and the development of methods to measure and selectively manipulate gene expression have enabled identification of several candidate genes that may encode BR mechanosensitive channels. Emerging evidence points to members of three evolutionarily conserved ion channel families.

A. Epithelial Na^+ Channels

The degenerin (DEG) genes MEC-4 and MEC-10 strongly influence touch sensitivity in the roundworm *Caenorhabditis elegans* (*C. elegans*) and null mutations in MEC-4 or accessory ion channel subunit genes (MEC-2 and MEC-6) eliminate mechanoreceptor currents in *C. elegans* sensory neurons (Tavernarakis and Driscoll, 2001; Goodman and Schwarz, 2003; O'Hagan *et al.*, 2005). Homology between *C. elegans* DEG genes and mammalian ENaC suggested that proteins of the DEG/ENaC superfamily may function as mechanosensors in mammals (Canessa *et al.*, 1993). Mammalian ENaCs are heteromultimers composed of α, β, and γ subunits that play an essential role in epithelial Na^+ transport (Kellenberger and Schild, 2002). Evidence that ENaCs may function as mechanosensors in a variety of cell types is accumulating (Awayda and Subramanyam, 1998; Kellenberger and Schild, 2002; Carattino *et al.*, 2004; Drummond *et al.*, 2004).

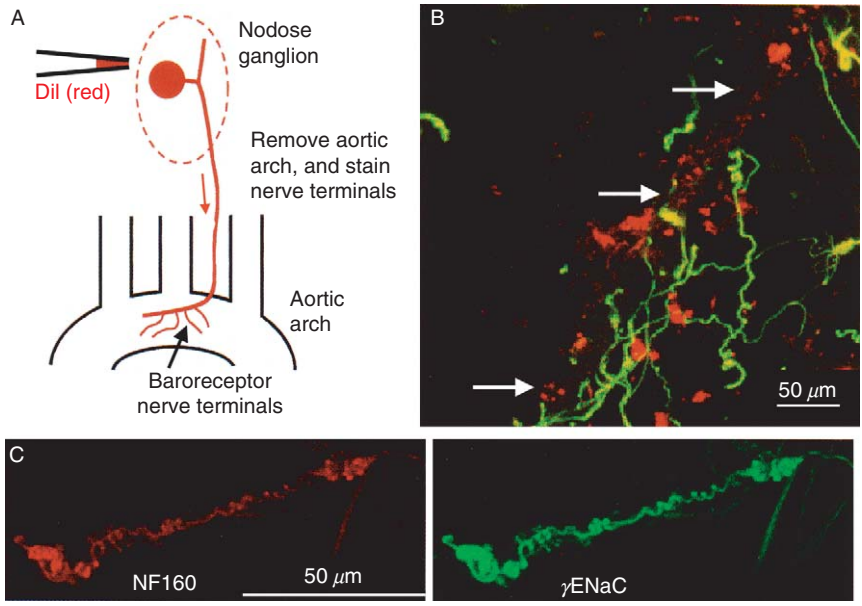

FIGURE 4 Gamma (γ) ENaC expression in aortic BR nerve terminals. (A) DiI was injected into rat nodose ganglion to label aortic BR fibers innervating aortic arch. (B) Two weeks later, colocalization of diI and γENaC is evident in BR nerve endings in aortic arch adventitia (150-μm optical section; diI—red, γENaC—green, diI + γENaC—yellow). (C) BR terminal in aortic arch immunolabeled with anti-NF160, a neuronal marker, and anti-γENaC. Reprinted from *Neuron*, Vol. 21, Drummond *et al.*, A molecular component of the arterial baroreceptor mechanotransducer (1998, Fig. 3A–C, p. 1438) with permission from Elsevier.

1. ENaCs Are Expressed in Nodose Neurons and BR Sensory Terminals

We hypothesized that the composition of the mechanosensitive ion channel complex in BR neurons may include ENaC subunits and that these subunits may therefore contribute to BR mechanoelectrical transduction. The first step was to determine if ENaC subunits are expressed in BR neurons. We demonstrated by RT-PCR the presence of mRNA for β and γ subunits of ENaC in rat nodose ganglia (Drummond *et al.*, 1998, 2001). Interestingly, the α subunit was not detected. Expression of γENaC protein was evident in diI-labeled BR neurons in nodose ganglia and, more importantly, was localized in BR sensory terminals in the adventitia of the aortic arch (Drummond *et al.*, 1998, 2001; Fig. 4). The apparent absence of the α subunit may reflect the expectation that mechanosensitive channels would be closed at rest and open only during mechanical stimulation; ENaCs containing α subunits are constitutively open (Kellenberger and Schild, 2002).

2. ENaC Blockers Attenuate BR Responses *In Vivo* and *In Vitro*

A common approach to determine whether ENaC or related channels contribute to functional responses is to obtain measurements before and after application of the DEG/ENaC blocker amiloride (Hamill *et al.*, 1992). Exposure of the isolated carotid sinus of rabbits to the amiloride analogue benzamil decreases BR activity in a dose-related manner and significantly attenuates the baroreflex-mediated fall in systemic BP evoked by increases in carotid sinus pressure (Drummond *et al.*, 1998, 2001; Fig. 5).

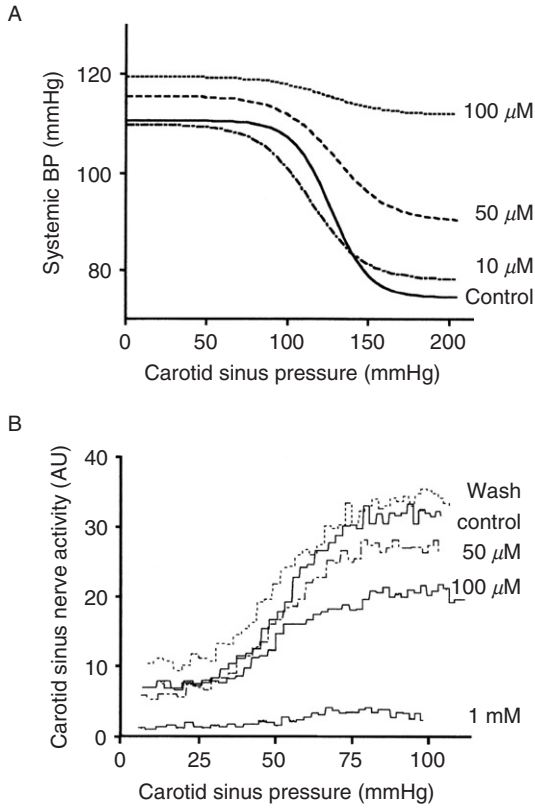

FIGURE 5 ENaC blocker benzamil attenuates BR activity and baroreflex control of BP. Exposure of isolated carotid sinus of rabbits to benzamil causes concentration-dependent inhibition of baroreflex-mediated decreases in systemic BP (A), and attenuation of pressure-induced increases in afferent carotid sinus BR activity (B). The BP curves represent average data from five experiments. Carotid sinus nerve activity curves are from one representative experiment. Reprinted from *Neuron*, Vol. 21, Drummond *et al.*, A molecular component of the arterial baroreceptor mechanotransducer (1998, Fig. 5, p. 1439) with permission from Elsevier.

The reflex inhibition of sympathetic nerve activity during blood volume expansion achieved by intravenous injection of saline in rats is also inhibited by benzamil injected into the pericardial sac surrounding the heart (Ditting *et al.*, 2003). The reflex response to blood volume expansion is mediated by activation of cardiac mechanoreceptors similar in structure and function to arterial BRs (Bishop *et al.*, 1983). Thus, amiloride-sensitive channels, presumably containing ENaC subunits, may contribute to activation of both arterial BRs and mechanosensitive afferents innervating the heart.

Consistent with our *in vivo* findings, amiloride essentially abolishes membrane depolarization and increases in cytosolic calcium evoked by puffing fluid onto isolated BR neurons (Drummond *et al.*, 1998; Snitsarev *et al.*, 2002; Fig. 6). Importantly, the concentration of amiloride used to inhibit mechanically induced responses (0.1–1.0 μM) does *not* attenuate action potential discharge evoked by depolarizing current injection (Snitsarev *et al.*, 2002; Fig. 6).

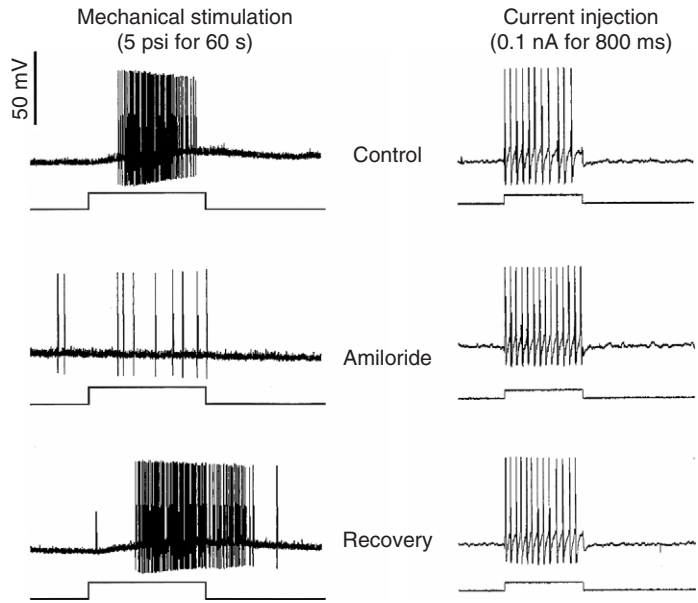

FIGURE 6 Amiloride (1 μM) selectively blocks mechanically induced depolarization of isolated rat nodose sensory neuron (left) without suppressing neuronal excitability (right). The neuron was mechanically stimulated by a stream of buffer ejected under pressure (5 psi) from a micropipette placed 50 μm from the cell surface. Membrane potential was recorded from a sharp microelectrode. Neuronal excitability was assessed by measuring the action potential response to depolarizing current injections (0.1 nA). Reprinted from Snitsarev *et al.* (2002, Fig. 1, p. 60) with permission.

Although amiloride can potentially inhibit neuronal excitability through effects on voltage-gated channels (Carr *et al.*, 2001; Ditting *et al.*, 2003), our results suggest that at lower concentrations (0.1–1.0 μM) amiloride is selective for mechanosensitive channels. Inhibitors of voltage-dependent channels do not attenuate inward ionic currents evoked by mechanical stimulation of isolated nodose neurons (Cunningham *et al.*, 1997).

Interestingly, amiloride and benzamil fail to block inward currents generated by exposure of isolated diI-labeled cardiac nodose sensory neurons to hypoosmotic stress (Ditting *et al.*, 2003). Different types of mechanosensitive channels may mediate responses to rapid mechanical stimulation with puffs of fluid vs slower cell swelling under hypoosmotic stress.

B. Acid Sensing Ion Channels

ASICs represent an additional mammalian subfamily of the DEG/ENaC superfamily (Price *et al.*, 1996; Waldmann *et al.*, 1997; Krishtal, 2003). As their name indicates, ASICs are H^+-gated ion channels. Several ASIC subunits have been identified including ASIC1 (1a and 1b), ASIC2 (2a and 2b), ASIC3, and ASIC4. ASIC1a and ASIC1b are splice variants of the same gene, as are ASIC2a and ASIC2b. Unlike ENaCs that are expressed in epithelium and a variety of other cell types, ASICs are expressed primarily in neurons. The ASIC subunits show varying degrees of acid sensitivity with ASIC3 and ASIC1a being the most sensitive and ASIC2 the least sensitive (Lingueglia *et al.*, 1997; Waldmann *et al.*, 1997; Hesselager *et al.*, 2004). The subunits can form homomultimers or heteromultimers; the specific combination of which influences the kinetics of channel inactivation and desensitization, H^+ sensitivity, cation selectivity, and susceptibility to amiloride and gadolinium blockade (Waldmann *et al.*, 1997; Babinski *et al.*, 2000; Alvarez de la Rosa *et al.*, 2002; Benson *et al.*, 2002; Hesselager *et al.*, 2004).

ASICs have been implicated in mechanotransduction in sensory nerves innervating a variety of tissues. ASIC2 (also named BNC1 and BNaC1) is expressed in DRG neurons innervating skin and has been shown to be transported from soma to mechanosensory nerve terminals (Price *et al.*, 2000; Garcia-Anoveros *et al.*, 2001). ASIC2$^{-/-}$ mice exhibit a selective decrease in sensitivity of low-threshold, rapidly adapting cutaneous mechanoreceptors (Price *et al.*, 2000; Welsh *et al.*, 2002), although this finding was not confirmed in a subsequent study (Roza *et al.*, 2004). In contrast, in ASIC3$^{-/-}$ mice, high-threshold mechanonociceptor sensitivity is impaired accompanied by *increased* sensitivity of the rapidly adapting cutaneous afferents (Price *et al.*, 2001). ASIC1, ASIC2, and ASIC3 have been shown to contribute differentially to mechanotransduction in different types of

visceral afferents innervating the gastrointestinal (GI) system (Jones *et al.*, 2005; Page *et al.*, 2005). Interestingly, ASIC3 contributes to mechanosensitivity in most types of GI afferents, ASIC1a exerts an *inhibitory* influence on mechanosensitivity in all types of GI afferents, and ASIC2 may either contribute to or restrain mechanosensitivity depending on the type of afferent (Jones *et al.*, 2005; Page *et al.*, 2005). Thus, ASIC subunits may contribute differentially to mechanotransduction and their contributions vary depending on the type of neuron and nature of the mechanical stimulus.

1. ASICs Are Expressed in Nodose Neurons and BR Terminals

ASIC subunits are expressed in nodose ganglia of mice at both mRNA and protein levels (Page *et al.*, 2005; Lu *et al.*, 2006). Preliminary results show similar relative mRNA levels of ASIC1a, ASIC1b, ASIC2a, and ASIC3, with higher expression of the ASIC2b subunit measured by real-time PCR (Lu *et al.*, 2006). ASIC2b alone does not form a pH-gated channel, suggesting that its functional significance may relate to modulation of other ASIC subunits (Lingueglia *et al.*, 1997; Hesselager *et al.*, 2004). ASIC1, ASIC2, and ASIC3 proteins are colocalized (immunohistochemistry) in some nodose neurons consistent with the presence of heteromultimeric channels (Lu *et al.*, 2006). ASIC2 staining is also evident in the ADN and nerve terminals in aortic arch. The presence of ASIC2 in BR and somatic afferent sensory terminals not sensitive to acid are consistent with it serving a mechanoreceptor function in these nerve endings (Price *et al.*, 2000; Garcia-Anoveros *et al.*, 2001; Welsh *et al.*, 2002; Lu *et al.*, 2006).

2. BR Function Is Impaired in ASIC2$^{-/-}$ Mice

Both ENaCs and ASICs are inhibited by amiloride, although ASICs are less sensitive with ASIC3 showing incomplete inhibition even at high concentrations of amiloride (Waldmann *et al.*, 1997; Kellenberger and Schild, 2002). Thus, inhibition of mechanically induced responses by amiloride cannot distinguish a specific contribution of ASICs. Therefore, we have chosen to investigate the role of ASICs in BR activation through study of ASIC-deficient mice.

This approach necessitated development of methods to assess BR function in mice. The sensitivity of BR afferents to changes in BP can be examined by directly recording afferent BR activity from the ADN and measuring changes in activity during pharmacologically induced changes in BP (Ma *et al.*, 2002; Fig. 7). Preliminary results obtained from ASIC2$^{-/-}$ mice support the hypothesis that ASIC2 contributes to BR activation (Ma *et al.*, 2001). The vasoconstrictor phenylephrine was injected intravenously in ASIC2$^{-/-}$ and wild-type mice in order to cause a sustained increase in BP. While the immediate increase in BR activity accompanying the rise in BP appears relatively

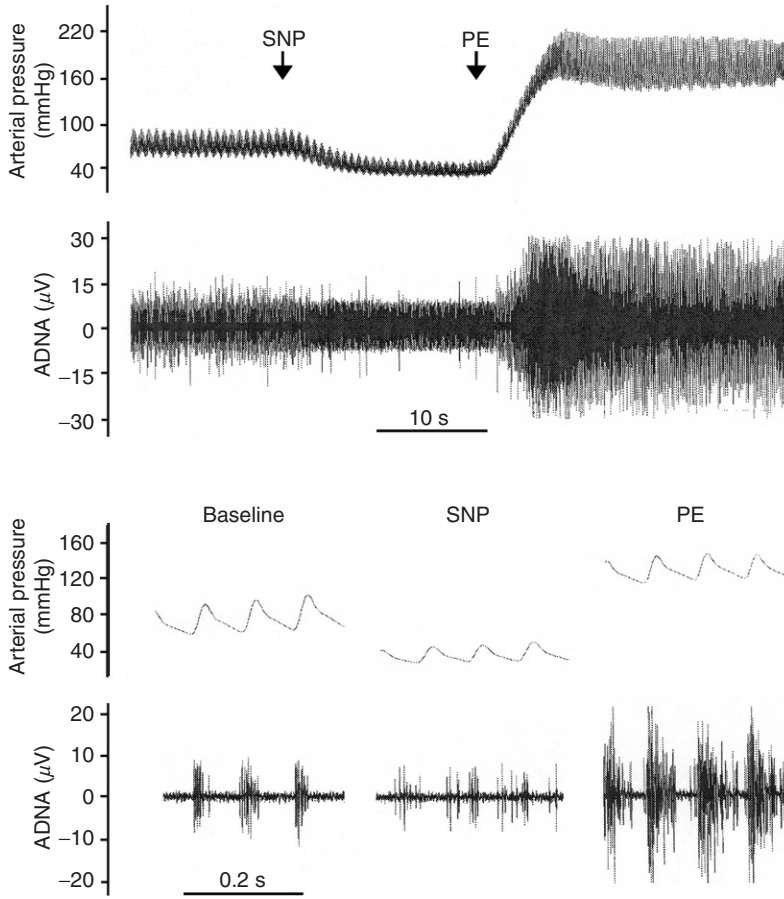

FIGURE 7 Assessment of BR afferent sensitivity to changes in BP in mice *in vivo*. Shown are recordings of BP and BR activity in ADN (ADNA) in an anesthetized mouse under baseline conditions, during sodium nitroprusside (SNP)-induced decreases in BP, and during phenylephrine (PE)-induced increases in BP. Reprinted from *Am. J. Physiol. Reg. Integr. Comp. Physiol.*, Vol. 283, Ma *et al.*, Analysis of afferent, central, and efferent components of the baroreceptor reflex in mice (2002, Fig. 4, p. R1037) with permission.

normal, the ability to sustain the increase in BR activity as BP is maintained at a high level is impaired in ASIC2$^{-/-}$ mice (Ma *et al.*, 2001). The results suggest that ASIC2 is essential for normal BR sensing of sustained increases in BP.

Impaired BR afferent sensitivity should translate to a defect in BR reflex control of heart rate and BP. Preliminary data suggest that baroreflex sensitivity for control of heart rate is decreased in conscious ASIC2$^{-/-}$ mice

(Sabharwal *et al.*, 2006). To evaluate reflex control of BP, we have measured the BP response to bilateral carotid artery occlusion (BCO) in anesthetized mice. BCO reduces carotid sinus pressure and BR activity thereby triggering a baroreflex-mediated increase in systemic BP. Activation of carotid body chemoreceptors consequent to ischemia during BCO can contribute to the reflex rise in BP (Alcayaga *et al.*, 1986). Therefore, measurements of the BP response to BCO were repeated while ventilating the mice with 100% oxygen to suppress chemoreceptor activity. This approach enables analysis of the relative contributions of the baroreflex and chemoreflex to the BCO-induced increase in BP (Sun *et al.*, 2000).

Our preliminary results indicate that the baroreflex component of the BCO reflex is significantly impaired in ASIC2$^{-/-}$ mice, while the chemoreflex component is enhanced (Sabharwal *et al.*, 2005a). The enhanced chemoreflex component of the BP rise suggests that efferent sympathetic-mediated vasoconstriction is preserved in ASIC2$^{-/-}$ mice. Thus, the defect in baroreflex control of BP likely resides in BR afferent nerves, or possibly within the central nervous system. The BR component of the BCO reflex was not altered in ASIC3$^{-/-}$ mice (Sabharwal *et al.*, 2005b).

The reciprocal relationship between baro- and chemoreflex sensitivity in ASIC2$^{-/-}$ mice is reminiscent of what has been observed in hypertension, heart failure, hypercholesterolemia, and aging (Trzebski *et al.*, 1982; Somers *et al.*, 1988; Franchini *et al.*, 1996; Ponikowski *et al.*, 1997; Sun *et al.*, 1999; Sun *et al.*, 2001a,b, 2002). We speculate that the functional reciprocity may reflect, in part, compensatory upregulation of expression or function of other ASIC subunits in ASIC2$^{-/-}$ mice and that dysregulation of ASICs may contribute to decreased baroreflex sensitivity and increased chemoreflex sensitivity in pathological states.

C. *TRP Channels*

TRP channels represent a superfamily of cation-selective channels (Clapham *et al.*, 2003; Desai and Clapham, 2005). These evolutionarily conserved channels are very weakly voltage dependent, are expressed in many types of cells, and have been implicated in sensing a variety of stimuli including light, temperature, pheromones, acidity, and osmolarity. TRP subfamilies include TRPC(1–7), TRPV(1–6), TRPM(1–8), TRPA, TRPN, TRPP, and TRPML. The subunits within subfamilies can form heteromultimers that influence the electrophysiological characteristics of the channels. TRPV1 is the well-known vanilloid receptor sensitive to capsaicin, heat, H^+, and endogenous cannabinoids.

Several TRP channels have been implicated in mechanotransduction (Lin and Corey, 2005; O'Neil and Heller, 2005). TRPV homologues mediate

osmosensory and mechanosensory responses in *C. elegans*, *Drosophila*, and mammals. Mammalian TRPV1 has been implicated in mechanosensory transduction in specific types of sensory afferents innervating the intestine and bladder (Birder *et al.*, 2002; Rong *et al.*, 2004; Jones *et al.*, 2005). TRPV4, cloned based on homology with *C. elegans* OSM-9, is activated by hypotonic cell swelling, shear stress, acidic pH, warm temperature, arachidonic acid, and 5′,6′-EET (5′,6′-epoxyeicosatrienoic acid), and is expressed in cutaneous mechanosensitive afferents (Liedtke *et al.*, 2003; Suzuki *et al.*, 2003a,b; Lin and Corey, 2005; O'Neil and Heller, 2005). TRPV2 is also activated by cell swelling and membrane stretch (Muraki *et al.*, 2003). The responsiveness of TRPV channels to both mechanical stimuli and chemical second messengers (e.g., 5′,6′-EET) suggests that their "mechanosensitivity" may involve *indirect* chemical activation of the channel.

TRPA1 and TRPC1 are components of mechanotransducer channels in vertebrate hair cells and *Xenopus* oocytes, respectively (Corey *et al.*, 2004; Maroto *et al.*, 2005). Activation of TRPA1 channels may involve transmission of force to the channel through accessory proteins (Corey *et al.*, 2004; Lin and Corey, 2005). In contrast, TRPC1 may be directly activated by lipid tension (Maroto *et al.*, 2005).

Thus, several TRP proteins should be considered as prime candidates for involvement in BR mechanoelectrical transduction.

1. TRP Proteins Are Expressed in Nodose Neurons and BR Terminals

TRPV1 and several TRPC proteins (TRPC1, TRPC3–7) are expressed in sensory neurons in rat nodose ganglia (Helliwell *et al.*, 1998; Glazebrook *et al.*, 2005). TRPV1 appears to be selectively expressed in C-fiber vagal afferents (Jin *et al.*, 2004). In contrast, both myelinated and unmyelinated fibers in the ADN contain TRPC1, TRPC3, TRPC4, and TRPC5 proteins with TRPC1, TRPC4, and TRPC5 being distinctly localized in sensory terminals in aortic arch adventitia, although the distribution of specific TRPC subunits may differ in sensory terminals of myelinated and unmyelinated BR afferents (Glazebrook *et al.*, 2005; Fig. 8). In preliminary experiments, we have confirmed expression of TRPC1 in nodose ganglia of adult rats and mice (our unpublished observation).

Although these results are consistent with TRP channels contributing to BR mechanoelectrical transduction, further studies are warranted. In addition to possibly functioning as a mechanosensor, TRP channels can be activated by ligand binding to G-protein–coupled receptors, tyrosine kinase activation, and second messengers (Clapham *et al.*, 2003; Desai and Clapham, 2005; Lin and Corey, 2005). Therefore, TRP channels may modulate BR sensitivity indirectly through their sensitivity to chemical factors and second messengers. Future studies of BR sensitivity in TRPV1- and TRPC-deficient mice and

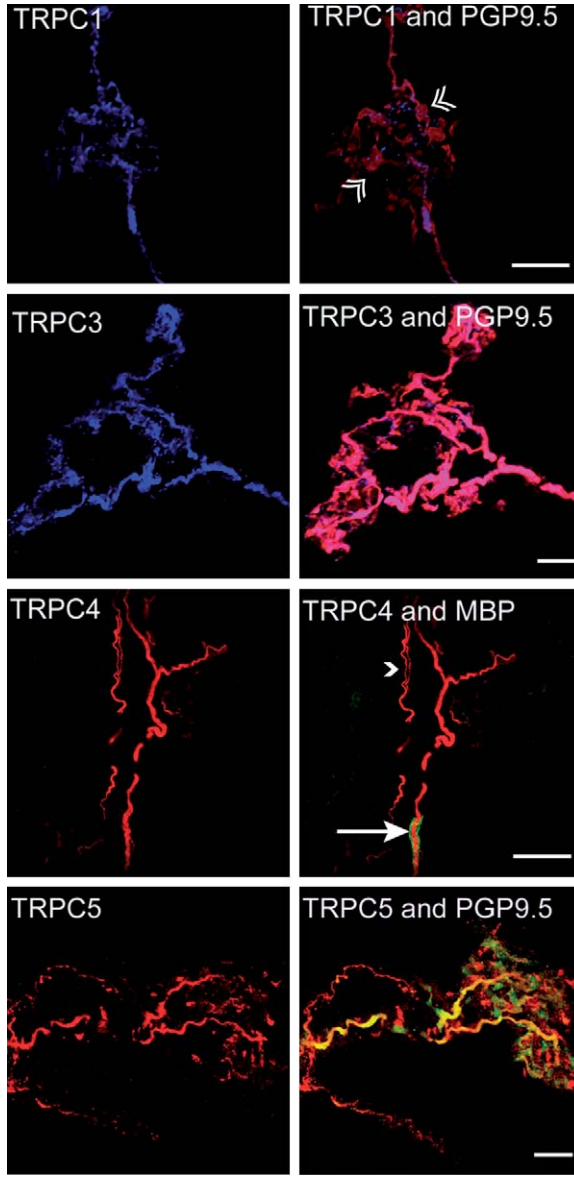

FIGURE 8 Expression of TRPC subunits in aortic BR nerve terminals. Shown are Z-series confocal images of BR terminals in aortic arch immunolabeled with antibodies to TRPC1, TRPC3, TRPC4, and TRPC5 (left panels). The same sections were stained with either the neuronal marker PGP9.5 to delineate the terminal or myelin basic protein (MBP) to show the edge of the myelin sheath (corresponding right panels). Scale bar = 20 μm. Reprinted from Glazebrook *et al.* (2005, Fig. 3, p. 128) with permission.

mechanoelectrical transduction in BR neurons lacking TRP subunits are needed to address the functional role of these proteins in BR afferent nerves.

V. METHODOLOGICAL LIMITATIONS AND CHALLENGES

The explosion of knowledge of genome sequences, technological advances, use of model systems in lower organisms amenable to genetic analysis (e.g., *Drosophila*, *C. elegans*), and identification of mammalian homologues as candidate mechanosensors have provided new and vast opportunities for discovery. Studies exploring the molecular basis of BR activation have begun to utilize these approaches. For example, ENaC, ASIC, and TRP subunits have been localized in BR sensory nerve terminals by immunohistochemistry. mRNA expression of these molecules has been confirmed in nodose and petrosal ganglia where BR somata reside by RT-PCR and quantitative real-time PCR. The impact of gene deletion on BR function is beginning to be explored using mutant mice. Recent and ongoing studies continue to rely heavily on the use of pharmacological blockers of mechanosensitive channels (e.g., gadolinium and amiloride). While these approaches have been productive, significant limitations of the methods are apparent and new challenges have arisen.

A. Need for Selective Pharmacological Antagonists

The absence or limited availability of selective pharmacological antagonists of ENaC, ASIC, and TRP subunits is a major limitation. The selectivity of drugs like gadolinium and amiloride within an experimental paradigm vary depending on a variety of factors, including the array of ion channels expressed in the neuron under study, the endpoint being measured (e.g., receptor potential vs cytosolic Ca^{2+} vs action potential firing), and the concentration of antagonist used. The utility of amiloride and its analogues to distinguish between ENaC and ASIC subunits is limited. ASIC3 is relatively resistant to blockade by amiloride (Waldmann *et al.*, 1997). Compound A-317567 has been reported to block ASICs with greater potency than amiloride (Dube *et al.*, 2005). Moreover, indirect evidence suggests that A-317567 does not block ENaCs (Dube *et al.*, 2005). Psalmotoxin 1 isolated from the tarantula spider and APETx2 from sea anemone selectively block ASIC1a and ASIC3 channels, respectively (Escoubas *et al.*, 2000; Diochot *et al.*, 2004). Future development of selective channel blockers should facilitate studies of BR mechanoelectrical transduction.

B. Complexity of Mechanosensitive Ion Channel Complex(es)

The complexity of the mechanosensitive ion channel complex and the potential involvement of multiple gene families create obstacles to both pharmacological and genetic approaches. ENaC, ASIC, and TRP subunits can form heteromultimeric channels of varying subunit composition. The diverse channels differ in many respects, including their sensitivity to different types of sensory input (e.g., mechanical, chemical, acidity, temperature) and their susceptibility to blockade by channel blockers (e.g., gadolinium and amiloride). The chemosensitivity of some of these proteins, particularly the TRP channels, creates the possibility that their activation by mechanical stimulation may be *indirect*, that is, via mechanically induced production of metabolites that subsequently open the channels (Lin and Corey, 2005; O'Neil and Heller, 2005). Heteromultimerization may occur not only between subunits within subfamilies with relatively homologous structures (Schaefer, 2005) but also between subunits from different families. For example, a preliminary report suggests heteromultimerization of ENaCs and ASICs (Meltzer *et al.*, 2006).

In addition to the mechanosensitive channel, mechanoelectrical transduction may depend on accessory tethering molecules that link the channel to the cytoskeleton and extracellular matrix (Tavernarakis and Driscoll, 2001; Chelur *et al.*, 2002; Goodman *et al.*, 2002; Goodman and Schwarz, 2003; Lin and Corey, 2005). For example, MEC-2 and MEC-6 are required for generation of mechanoreceptor currents in *C. elegans* sensory neurons (O'Hagan *et al.*, 2005). Homologous proteins such as stomatin and PICK1 may interact with and modulate mammalian ENaCs and ASICs (Mannsfeldt *et al.*, 1999; Fricke *et al.*, 2000; Duggan *et al.*, 2002; Deval *et al.*, 2004; Price *et al.*, 2004).

The complexity of the ion channel complexes poses significant challenges to experimental design and interpretation of data. The absence of obligatory accessory molecules in expression systems may prevent reconstitution of functional mechanosensitive channels. Tethering proteins or specific subunits may be selectively targeted to BR sensory terminals, thereby limiting the utility of studying channels in the soma. In fact, assessment of mechanosensitivity in neurons isolated from DRG of ASIC2- and ASIC3-deficient mice failed to detect an impaired response (Drew *et al.*, 2004). In knockout mice, other subunits may replace a deleted subunit to preserve channel function. Alternatively, increased expression of other heteromeric channels may compensate for loss of channel function. The consequences of manipulating one subunit of a heteromultimeric complex can be difficult to predict. The above factors may be particularly problematic in mice with life-long gene deletions.

C. Heterogeneity of Sensory Neurons

An additional factor that must be considered is the tremendous heterogeneity of mechanosensitive nerves. Mechanosensitive afferents innervating different organs (e.g., blood vessels, GI system, heart, bladder, skin, and muscle) differ to varying extent in their electrophysiological properties and ability to detect different modes of sensory stimuli. Differences in the molecular composition of the mechanosensitive channels likely contribute to the heterogeneity. For example, ASIC1a, ASIC2, and ASIC3 may each promote, oppose, or not influence mechanosensitivity depending on the tissue innervated (e.g., skin vs intestine) and functional type of sensory nerve within a given tissue (Price *et al.*, 2000, 2001; Jones *et al.*, 2005; Page *et al.*, 2005).

Subtypes of BR afferents can be distinguished based on conduction velocity, neuropeptide content, adaptation properties, and action potential discharge characteristics (Kirchheim, 1976; Chapleau *et al.*, 2001). The molecular composition of mechanosensitive channels may differ in subtypes of BR neurons.

VI. SUMMARY AND FUTURE DIRECTIONS

The process of BR activation by increases in BP involves vascular distension and BR deformation, depolarization of the nerve terminals, and encoding of the mechanically induced depolarization into action potential discharge. Emerging evidence suggests that members of the ENaC, ASIC, and TRP ion channel families mediate the BR depolarization (mechanoelectrical transduction), but the precise composition of the mechanosensitive ion channel complex (or complexes) in BR sensory terminals and the mechanism of channel gating remain to be determined.

Future studies should move toward more rapid translation of discoveries in lower ''model'' organisms to investigation of candidate genes mediating BR activation in mammals. Multidisciplinary state-of-the-art approaches including site-specific and temporal gene targeting are encouraged (Bockamp *et al.*, 2002). For example, use of inducible knockout technology can demonstrate reversibility of functional changes and avoid lethal phenotypes and long-term compensatory adaptations that may occur with life-long gene deletion. Generation of double and triple knockout mice may be necessary to entirely disrupt BR mechanosensitive channel function. Investigators are encouraged to make use of more selective ion channel blockers (e.g., for ENaC, ASIC, and TRP channel subunits) as they become available.

The presence of numerous types of neurons and other cells in nodose and petrosal ganglia and the recognition of the existence of BR subtypes underscore

the importance of studying individual identified BR neurons, preferably combining measurements of function (e.g., ionic currents, membrane depolarization, single-fiber discharge) with measurements of gene expression (e.g., single-cell PCR, immunocytochemistry). In addition to identifying the key molecules involved in BR mechanoelectrical transduction, it will be necessary to distinguish mechanisms of channel activation, albeit direct or indirect via chemical second messengers.

The recent discovery of genes regulating mechanosensitivity in lower organisms and the availability of powerful new technologies and experimental approaches make this a truly exciting time to pursue the goal of understanding the molecular basis of BR activation. The challenges are great, but so are the opportunities.

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