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Targeting Voltage Gated Sodium Channels $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ for Treatment of Pathological Cough

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Abstract

Recent advances in our understanding of voltage-gated sodium channels (Na_VS) lead to the rational hypothesis that drugs capable of selective blockade of Na_V subtypes may be a safe and effective strategy for the treatment of unwanted cough. Among the nine Na_V subtypes ($Na_V1.1$–$Na_V1.9$), the afferent nerves involved in initiating cough, in common with nociceptive neurons in the somatosensory system, express mainly $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$. Although knowledge about the effect of selectively blocking these channels on the cough reflex is limited, their biophysical properties indicate that each may contribute to the hypertussive and allotussive state that typifies subacute and chronic nonproductive cough.

Keywords

Vagus; C-fiber; Sodium channel

Introduction

Cough can be initiated from activation of peripheral afferent (sensory) nerves or initiated from processes occurring within the central nervous system (CNS) independently of afferent nerves. Cough initiated from the CNS can be subcategorized as voluntary cough or psychogenic cough. Cough initiated from the periphery can be subcategorized into the protective cough reflex vital for airway defense on one hand, and the irritating, itchy, urge-to-cough that serves essentially no useful purpose, on the other. From a therapeutic perspective, it is logical to focus attention on psychogenic cough and the afferent initiated nonproductive urge-to-cough sensations and avoid inhibiting protective cough or voluntary cough. With respect to inhibiting cough initiated by activation of peripheral sensory nerves, the voltage-gated sodium channels (Na_VS) are particularly attractive therapeutic targets.

Cough associated with respiratory viral infections, respiratory diseases, and esophageal reflux are initiated by peripheral afferent nerves. Often the cough associated with these disorders develops into an itchy, nonproductive cough. These sensations and the resulting

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Conflict of interest None.

nonproductive coughing can be mimicked by inhalation of low concentrations of agents known to stimulate vagal afferent C-fibers. Yet, there is little evidence for the existence of a truly effective peripherally acting antitussive drug.

Na_Vs are a *sine qua non* of action potential discharge [1]. They provide the current for the action potential spike, and they are determinant in voltage thresholds, spike frequency, and conduction. A breakthrough in our understanding of Na_Vs came with the unraveling of their molecular biology [2]. The Na_Vs comprise large α subunits with four homologous domains and two noncovalently linked β subunits. The α subunits are the pore-forming proteins and are encoded by nine distinct genes. The channels formed are referred to $Na_V1.1$–1.9. These channels can be blocked nonselectively with the class of drugs known as local anesthetics. They are "local" because systemic blockade of all Na_Vs is lethal, limiting their utility in the treatment of visceral diseases.

Proof of Concept

Local anesthetics have been tried in proof-of-concept trials for Na_V blockade in cough. Although local treatment with lidocaine inhibits cough evoked by mechanical stimulation of the larynx/trachea, and nebulized lidocaine causes a short-lasting partial inhibition of cough induced by inhaled capsaicin [3], it has thus far shown to be of little use in the treatment of pathological cough. Case reports show the antitussive efficacy of nebulized lidocaine in patients with chronic cough [4, 5], but in a large study examining cough in COPD, lidocaine was not effective [6]. An oral formulation of benzonatate, a derivative of the local anesthetic procaine, is used for the treatment of cough, but the evidence of its effectiveness is scanty [7]. The lack of impressive efficacy of nebulized lidocaine or oral benzonatate should not, however, be taken as a lack of "proof-of-concept" for Na_Vs in the treatment of cough. Local anesthetics have relatively low affinities for Na_Vs, and it is likely that at the doses that can be administered safely, they only weakly and briefly inhibit Na_Vs in the afferent C-fiber and A-fiber terminals involved in cough. More potent nonselective Na_Vs are available, but severe toxicity prevents their use in humans. For example, tetrodotoxin (TTX) blocks seven of the nine Na_Vs (all except $Na_V1.5$, 1.8 and 1.9) with ~1000 times greater potency than lidocaine. Systemic administration of this toxin however leads to rapid death that is likely secondary the paralysis of respiratory muscles.

All peripherally acting stimuli for cough must first interact with afferent nerve terminals to cause a membrane depolarization. This initial stimulus-dependent depolarization is referred to as a "generator potential" (Fig. 1). The nature of the activating stimuli for the Aδ-fiber "cough receptor" terminal in the large airways appears to be relatively limited [8]. In guinea pigs, the A-fiber cough receptor is stimulated by punctuate mechanical perturbation of the epithelium and by rapid decreases in pH; it is by in large not stimulated by mediators of inflammation. The vagal airway afferent C-fibers, by contrast, have a much more promiscuous activation profile. Depending on the C-fiber subtype, these nerves can be activated by numerous chemicals and inflammatory mediators. In many cases, these stimuli lead to generator potentials by stimulating ionotropic receptors. Stimuli that gate ionotropic receptors include ATP acting via P2X2/3 receptors, 5-HT acting via 5-HT3 receptors, nicotine via nicotinic receptors, and the panoply of irritating substances that can activate

TRPV1 or TRPA1 channels. Certain mediators that stimulate G-protein receptors also can lead to generator potentials in vagal afferent C-fibers in the airways. These include bradykinin via B2 receptors and adenosine via A1 and A2A receptors [9–14].

Generator potentials are of little consequence unless they are of a rate and magnitude sufficient to trigger an action potential. Short of this, generator potentials electronically decline to the resting potential over a time and distance that is based on the time and space constant of the nerve membrane. The triggering of the action potential occurs only when the voltage threshold of Na_Vs is reached, the channels are opened, and the rush of sodium ion traverses the membrane (Fig. 1). When Na_Vs in vagal afferent nerves are blocked, action potentials are not generated, and the communication between the innervated organ and CNS is silenced. In other words, blockade of Na_Vs is a form of “chemical denervation” every bit as effective as surgical sectioning of the nerve. If you silence the communication between the respiratory tract (and perhaps esophagus) and the brainstem, there will be no urge-to-cough initiated from peripheral sources (although voluntary cough and psychogenic cough would be unaffected). In a sense, the proof of concept for Na_V blockade in cough can be found in double-lung-transplant patients, where indeed it is not excessive coughing but a lack of coughing that poses the potential problem [15].

Selective Na_V Blockade for Cough

The Na_V1 subtypes ($Na_V1.1$–$Na_V1.9$) are differentially distributed among neurons, cardiac muscle, and skeletal muscle [2]. For example, the Na_V involved in action potential generation in skeletal muscle is largely $Na_V1.4$, the Na_V expressed in cardiac myocytes is principally 1.5, and the neurons in the brain express largely $Na_V1.1$, 1.2, 1.3, and 1.6. This differential distribution paves the way for developing selective Na_V blockers with therapeutic indexes much greater than can be obtained with nonselective local anesthetics. The question arises, what types of Na_Vs are expressed by afferent neurons involved in the cough reflex? This important issue has received relatively little experimental attention, but in guinea pigs, the vagal afferent A-fiber cough receptors and C-fiber neurons innervating the respiratory tract were found to express primarily $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ [16, 17] (Fig. 2). This is potentially very encouraging information, because these channels have a relatively limited expression elsewhere in the body [18]. They are not expressed by skeletal or cardiac muscle and scantly expressed in the CNS. They are strongly expressed in small diameter, presumed nociceptive, neurons in the dorsal root ganglia. This has led to the hypothesis that $Na_V1.7$, 1.8, and 1.9 may play important roles in transmitting pain signals to the brain [19–21]. Accordingly, the past decade has witnessed intensive efforts within the pharmaceutical industry towards discovering selective $Na_V1.7$, 1.8, and 1.9 blockers for the treatment of neuropathic and inflammatory pain. Several companies have developed safe Na_V blockers that are in various stages of development [21]. The time is ripe to investigate the potential of these same products for peripheral acting antitussive agents.

$Na_V1.7$

Among the three principal Na_Vs expressed in airway specific vagal afferent neurons (Na_V 1.7, 1.8, and 1.9), $Na_V1.7$ is the only one that is sensitive to blockade with TTX. $Na_V1.8$ and 1.9 are TTX-resistant channels. TTX is effective at blocking action potential conduction in

vagal afferent nerve fibers [17, 22]; therefore, it is likely that $Na_V1.7$ plays an important role in this regard as well. This hypothesis was addressed using $Na_V1.7$ shRNA delivered to the vagal sensory neurons via adeno-associated virus (AAV) in vivo [17]. This treatment nearly abolished expression of $Na_V1.7$ without influencing expression of other Na_Vs in the transfected vagal neurons. In the absence of $Na_V1.7$, the neurons were much less excitable (required a much larger depolarizing stimulus to evoke an action potential), and they were incapable of firing action potentials at high frequencies (Fig. 3). This latter point is relevant in that cough is preferentially triggered by high frequency action potential input to the brainstem [23, 24]. When the vagus nerve was isolated from animals in which the expression of $Na_V1.7$ was silenced, the number of afferent nerve fibers capable of conducting action potentials was substantially inhibited. Thus, inhibiting the expression of $Na_V1.7$ recapitulates many of the effects of TTX on vagal afferent nerves. It was not surprising therefore, that a lack of $Na_V1.7$ was associated with inhibition of C-fiber and Aδ-fiber mediated cough in guinea pigs [17, 25].

Another point making $Na_V1.7$ an attractive target for inflammatory associated cough is the observation that P38 mitogen-activated protein kinase and ERK kinases can lead to channel phosphorylation and a consequent increase in the sodium current density [26]. Inflammation also has been associated with an increase in expression of $Na_V1.7$ [27]. A $Na_V1.7$ blocker, at the right dose, therefore may normalize the hyper-excitable state of afferent terminals at the sites of inflammation. A rare loss-of-function mutation in $Na_V1.7$ leads to a congenital insensitivity to pain, yet with otherwise normal neuronal function and normal sensations to other non-painful stimuli [28], although a recent report indicates that those with this mutation also have a diminished sense of smell [29]. The cough sensitivity in these subjects has not been evaluated.

$Na_V1.8$

In the presence of TTX (that blocks $Na_V1.1$–$Na_V1.7$), depolarizing current leads to a relatively large, fast, inactivating sodium current, in airway-specific sensory neurons that is likely secondary to the gating of $Na_V1.8$. Elegant modeling has indicated that along with $Na_V1.7$, $Na_V1.8$ has a substantive effect on the neurons excitability and capacity for action potential discharge in vagal sensory neurons [30]. Studies in both spinal afferent neurons [31] and in vagal afferent neurons [32] have demonstrated that inflammatory mediators known to increase the excitability of afferent C-fibers and increase cough sensitivity [33] can, by multiple mechanisms, lead to $Na_V1.8$ phosphorylation and an increase in sodium current density. $Na_V1.8$ (as with $Na_V1.7$ and $Na_V1.9$) also appears to be upregulated transcriptionally by inflammatory processes [27]. As with $Na_V1.7$, inhibiting $Na_V1.8$ may therefore normalize C-fibers that are in a hyperexcitable state due to an inflammatory reaction. Consistent with this idea, blocking $Na_V1.8$ has little effect on the response to painful stimuli in healthy animals, but profoundly inhibits the *hyperalgesia* in response to inflammatory lesions [19, 34]. There is no information on the effect of $Na_V1.8$ blockade specifically on cough associated afferent nerves.

$Na_V1.9$

The other TTX-resistant current in vagal afferent neurons involved in cough is activated at membrane potentials closer to the resting potential, and unlike $Na_V1.7$ and $Na_V1.8$ results in a persistent very slowly inactivating current. The slowly inactivating Na_V current is largely gone in neurons from $Na_V1.9$ knockout mice [35]. $Na_V1.9$ is strongly expressed in C-fibers and A-fibers involved in the cough reflex in guinea pigs [16], but there is no information on how this channel modulates cough sensitivity. Once again, we are left with studies on pain sensations for potential analogies [36]. Inflammatory mediators, such as PGE2, shifts the steady-state activation of this channel in a hyperpolarizing direction, which might be expected for a mechanisms that could increase nerve excitability [37]. As with $Na_V1.8$, when $Na_V1.9$ is genetically deleted, there is little change in the response of the healthy mouse to various painful stimuli. However, the hyperalgesia that accompanies the injection of various inflammatory mediators, such as bradykinin and PGE2, is absent in $Na_V1.9$ −/− mice [35]. Certain models of inflammation-induced hyperalgesia are normalized in $Na_V1.9$ −/− mice [35]. Likewise, inflammation associated bladder hyperreactivity is diminished in $Na_V1.9$ −/− mice [38].

Advantages of Targeting Na_Vs for Cough

Blocking Na_Vs will decrease the efficacy by which generator potentials lead to action potentials in all afferent nerve terminals. It will also lead to a decrease in the action potential discharge frequency conducted in the vagal afferent nerves to the brain stem. This will be the case irrespective of the nature of the stimuli that induces the generator potential. A decided advantage of Na_V blockers in cough is that in theory they will be equally effective at quelling cough evoked by stimuli as disparate as mechanical perturbations, acid, osmotic changes, TRPV1 stimulants, TRPA1 stimulants, bradykinin, ATP, and various inhaled irritants.

Na_V blockers could conceivably be administered topically via aerosol, but due to the relatively limited expression of $Na_V1.7$, 1.8, and 1.9, these drugs are likely to be relatively safe given systemically. This could be an advantage when the afferent nerve driving the pathological cough is situated outside the airway wall, e.g., in the nasal mucosa, oral pharynx, or esophagus.

The third advantage of targeting Na_Vs is based on the studies showing that $Na_V1.7$, 1.8, and 1.9 are all “upregulated” in the presence of inflammation, and specific inflammatory mediators [27]. It is possible that targeting these channels could block the hypertussivity and allotussivity associated with pathological cough without inhibiting the protective cough reflex.

Disadvantages of Targeting Na_Vs for Cough

Although the Na_Vs expressed in cough causing afferent nerves have a limited distribution in the body, there is little evidence that subtypes of vagal afferent C-fibers and A-fibers differentially express Na_Vs. Therefore, if the Na_Vs were maximally blocked, one would anticipate that the protective cough reflex could be compromised. Based on the limited

information available, this is likely to be truer for $Na_V1.7$ than either $Na_V1.8$ or $Na_V1.9$. This concern would need to be addressed in dose-ranging studies where the goal is to normalize the hypertussive state, without severely compromising the protective cough reflex. Studies performed in guinea pigs suggest that $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ are not only expressed in vagal C-fibers and A-fiber cough receptors, but also expressed in rapidly and slowly adapting low threshold stretch sensitive nerves in the lungs (RARs and SARs) [16]. The effect of inhibiting Na_Vs in these afferent nerve subtypes may at least potentially lead to unwanted side effects. Little is known about the Na_V expression in autonomic nerves in the airways. Inhibition of neural control of vascular tone in the respiratory tract could potentially raise concerns.

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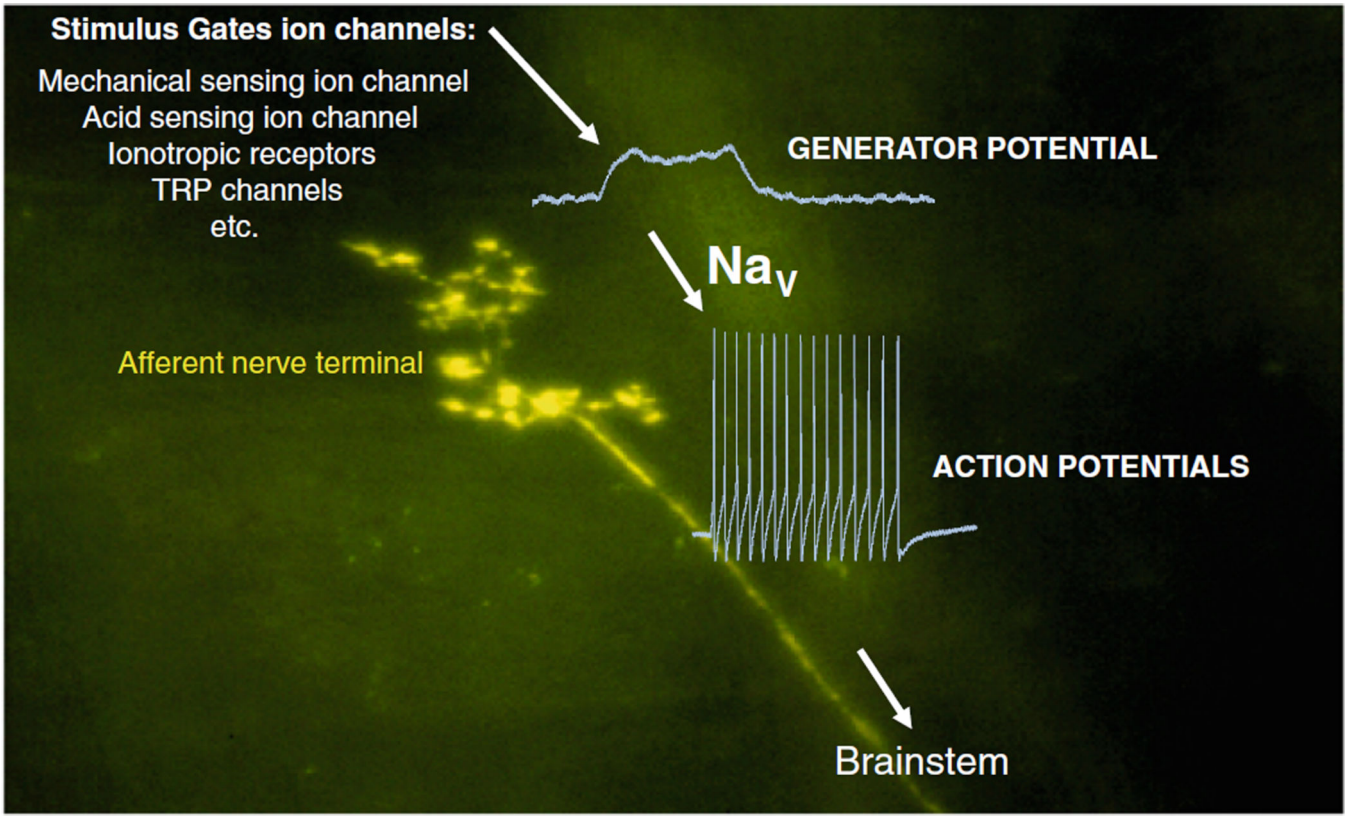

Fig. 1.

Illustration of the concept of afferent nerve terminal activation. Stimuli act on various receptors and ion channels to cause a membrane depolarization that is referred to as the generator potential. This in turn activates voltage-gated sodium channels (Na_V) that are responsible for action potential generation and conduction to the central terminal in the brainstem. The *yellow image* is an actual guinea pig vagal afferent nerve terminal. The example of a generator potential is actually a depolarizing potential recorded with patch clamp technology at the level of the cell soma (due to technical difficulties, generator potentials have not yet been recorded at vagal afferent nerve terminals)

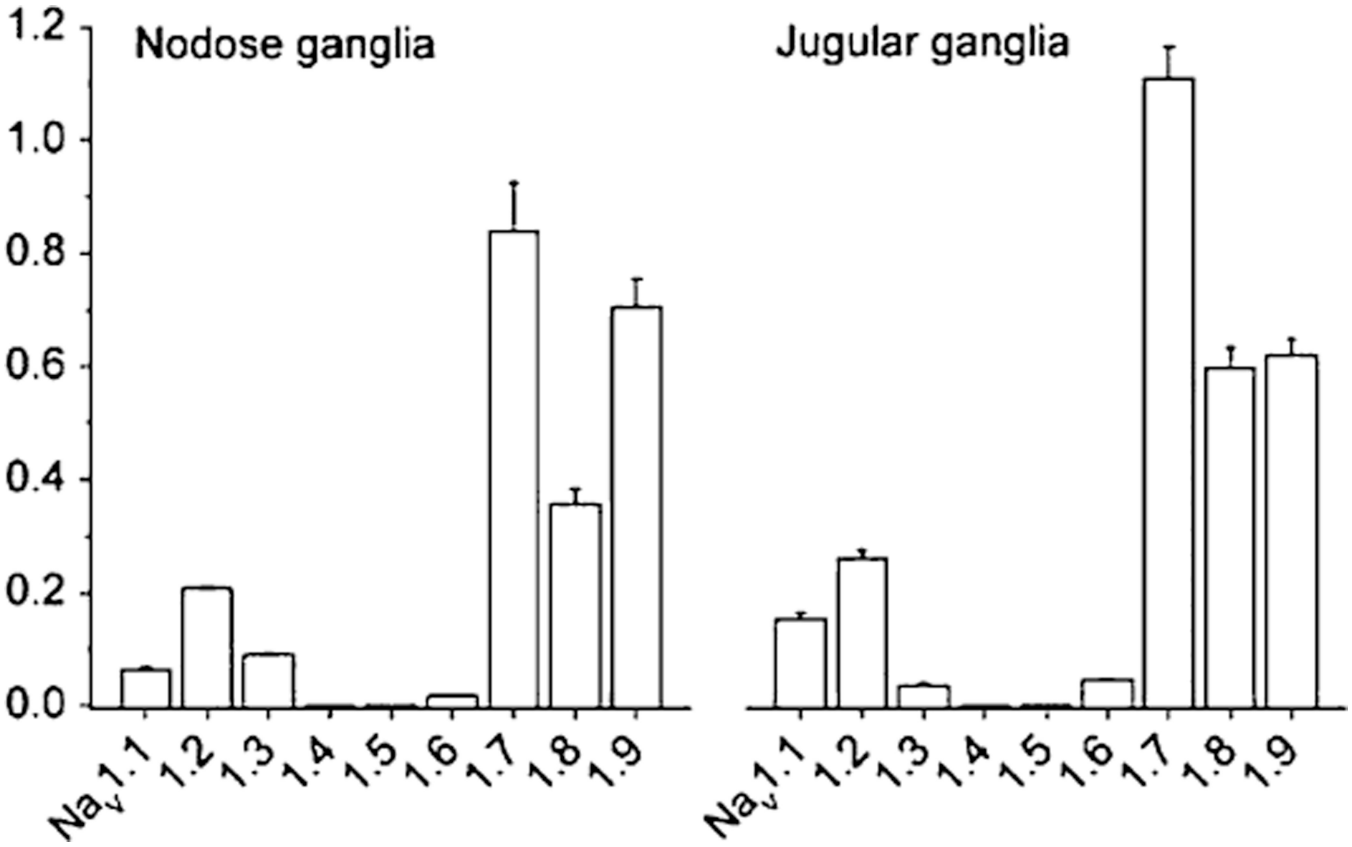

Fig. 2.

Relative expression of $Na_V1.1–1.9$ in neurons situated in the guinea pig *nodose and jugular ganglia* as determined using real time quantitative PCR. For more details of Na_V expression in airway-specific nodose and jugular ganglia see [16] and [17]

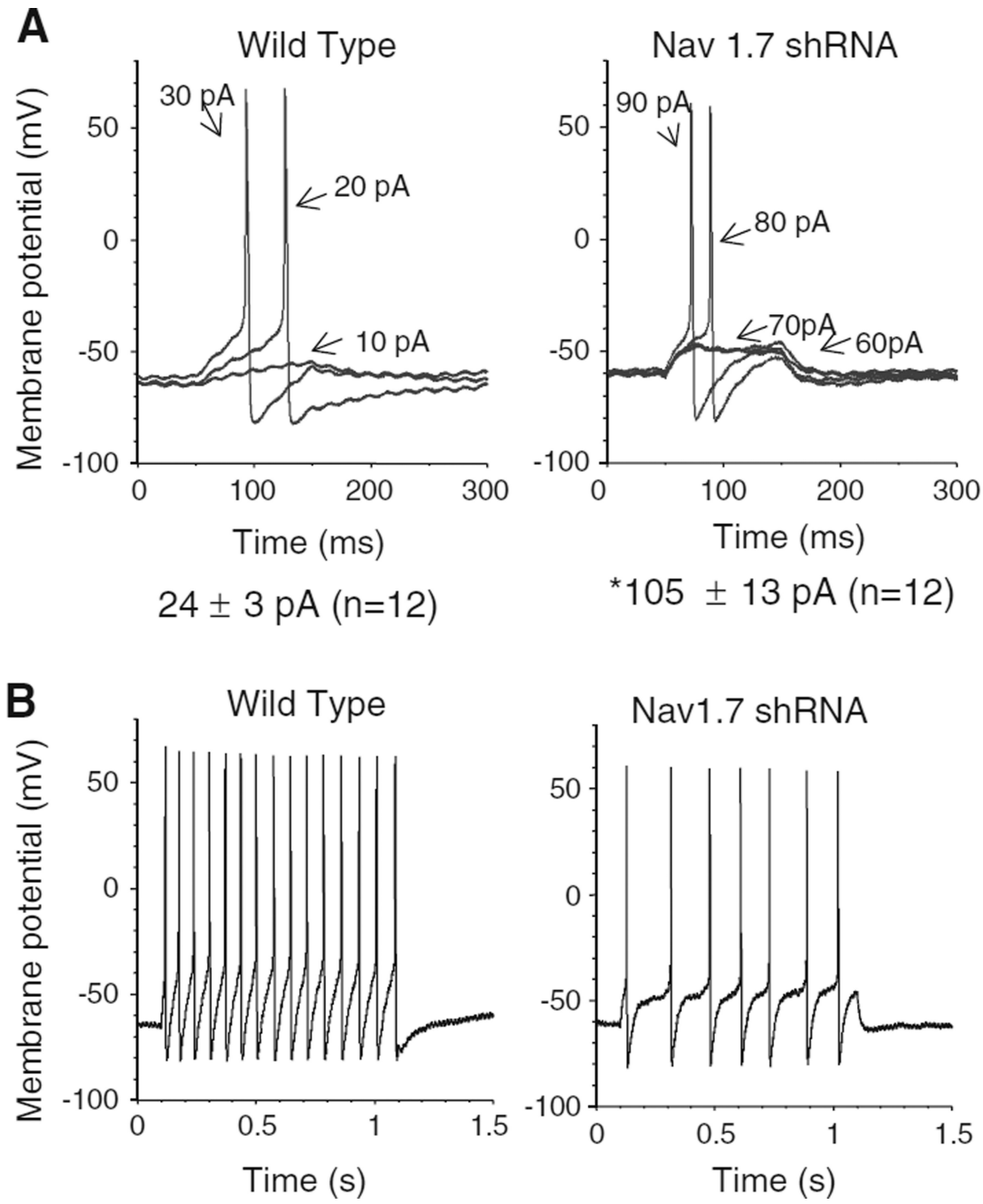

Fig. 3.

Patch clamp recordings of a vagal sensory nodose neurons isolate from a control guinea pig (*left*) or a guinea pig that was previously treated with $Na_V1.7$shRNA to block expression of $Na_V1.7$ channels (*right*). **a** Example of an experiment in which the amount of depolarizing current required to evoke an action potential was determined. In this control neuron 20 pA was required, whereas in the $Na_V1.7$ shRNA treated neuron 80 pA was required. The average ± SEM from 12 experiments is stated below the figures. **b** The frequency of action

potential discharge in response to a supramaximal 1 s depolarizing current was determined; note how the frequency of firing is reduced in the absence of $Na_V1.7$. For details see [17]