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Neuronal soma-satellite glial cell interactions in sensory ganglia and the participation of purinergic receptors

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Abstract

It has been known for some time that the somata of neurons in sensory ganglia respond to electrical or chemical stimulation and release transmitters in a Ca^{2+} -dependent manner. The function of the somatic release has not been well delineated. A unique characteristic of the ganglia is that each neuronal soma is tightly enwrapped by satellite glial cells (SGCs). The somatic membrane of a sensory neuron rarely makes synaptic contact with another neuron. As a result, the influence of somatic release on the activity of adjacent neurons is likely to be indirect and/or slow. Recent studies of neuron-SGC interactions have demonstrated that ATP released from the somata of dorsal root ganglion neurons activates SGCs. They in turn exert complex excitatory and inhibitory modulation of neuronal activity. Thus, SGCs are actively involved in the processing of afferent information. In this review, we summarize our understanding of bidirectional communication between neuronal somata and SGCs in sensory ganglia and its possible role in afferent signaling under normal and injurious conditions. The participation of purinergic receptors is emphasized because of their dominant roles in the communication.

Keywords

somatic ATP release; P2X7 receptor; cytokine release; dorsal root ganglia; pathological nociception

Characteristics of neuronal somata in sensory ganglia

Dorsal root ganglion (DRG) and trigeminal (TG) sensory neurons are the first relay in the somatosensory pathway for the transmission of pain signals from the periphery, including the skin and internal organs, to the brain (Willis & Coggeshall, 2004). As a result of the pseudounipolar structure of the sensory neuron, afferent spikes flowing through the peripheral branch of the axonal process can bypass the cell body, i.e., soma, and directly reach the central branch of the process and its terminals (Amir & Devor, 2003a). Nevertheless, these neurons rely on afferent spike invasion into their somata for regulation (Amir & Devor, 2003a). Spike invasion provides the necessary cues for sensory neurons to synthesize, transport and maintain optimal levels of ion channels, receptors and proteins at

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STATEMENT OF INTEREST

None

the peripheral and central terminals. The roles of somata in afferent signaling, however, are far from passive. In addition to responding afferent inputs, neuronal somata have been found to actively affect the signal getting into the spinal cord. During tetanic stimulation of afferent fibers, somatic spikes become delayed or distorted as the stimulation frequency approaches the refractory period of the soma. This results in the generation of extra spikes in the peripheral or central processes, thus disrupting the fidelity of signal transmission (Amir & Devor, 2003b). Following injury, the somata in DRG neurons become spontaneously active and fire action potentials for a prolonged period of time (Liu *et al.*, 1999). The ectopic discharges in the somata are thought to trigger and maintain the sensitization in the spinal cord and underlie neuropathic pain generated after nerve injury (Liu *et al.*, 2000; Sukhotinsky *et al.*, 2004; Devor, 2009).

The somata of sensory neurons are densely packed in DRGs and TGs. However, they do not form morphologically defined synapses with one another. Each soma is tightly enwrapped by a layer of satellite glial cells (SGCs) (Pannese, 1981), which are often coupled with each other with gap junctions (Pannese *et al.*, 2003; Huang *et al.*, 2005). Individual neuronal soma with its surrounding SGCs is enclosed by a connective tissue sheath and form a functional unit (Hanani, 2005). A lack of synaptic contacts among neuronal somata together with the close apposition between neurons and surrounding SGCs suggests that bidirectional neuron-SGC communication may have an essential role in afferent signaling. It is therefore of great interest to understand how the soma in a neuron communicates with surrounding SGCs, how SGCs influence neuronal activity and how changes in the communication under injurious conditions contribute to ectopic discharge of sensory neurons and production of chronic pain.

ATP is the major fast-acting transmitter released from the somata of sensory neurons

Neurons communicate with each other and with glia through the release of transmitters. In response to KCl depolarization or capsaicin stimulation, neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP) have been found to be released from cultured DRG and TG neuronal preparations, which contain neuronal cell bodies as well as neurites (Mason *et al.*, 1984; Hingtgen & Vasko, 1994). To determine if neuronal somatic release indeed occurs, we used DRG preparations devoid of neurites and combined membrane capacitance and intracellular Ca^{2+} dye measurements to show that the somata of DRG neurons undergo robust Ca^{2+} -dependent exocytosis in response to membrane depolarization (Huang & Neher, 1996). Ca^{2+} -independent exocytosis was later found to also occur in the same preparation (Zhang & Zhou, 2002). Using the single-cell immunoblot technique, we further showed that SP is released from individual neuronal somata (Huang & Neher, 1996). Radioimmunoassay of SP or CGRP released from neuron-enriched cultured TG cells (Matsuka *et al.*, 2001) or TG slices (Ulrich-Lai *et al.*, 2001) confirmed the conclusion by showing that a substantial portion of the neuropeptide release is from the somata of sensory neurons. Since most peptides function as modulators of neuronal transmission, it is important to determine if fast acting neurotransmitters such as ATP or glutamate are released from neuronal somata and if they are directly involved in the neuron-SGC communication in sensory ganglia. ATP was found to be released from pre-myelinated axons of cultured DRG neurons (Stevens & Fields, 2000; Ishibashi *et al.*, 2006) and co-released with SP from acutely dissociated TG neurons (Matsuka *et al.*, 2001). We used the sniffer patch recording method to test whether vesicular release of ATP or glutamate in fact takes place in the somata of DRG neurons (Zhang *et al.*, 2007). The method consists of pulling an outside-out patch from a HEK cell that is overexpressed with purinergic P2X2R-EGFP or glutamatergic GluR6-EGFP receptors and pressing it onto the surface of the soma of a DRG neuron. The expressed receptors on the HEK cell patch then become the

biosensors that detect the release of ATP or glutamate (Fig. 1). Following electric stimulation of the somata of DRG neurons, miniature excitatory postsynaptic current (mEPSC)-like activity can be detected by the sniffer pipette, thus suggesting that ATP or glutamate is vesicularly released. Because of the abundance and robustness of ATP release, it has been studied in great detail (Zhang *et al.*, 2007). The identification of ATP as the released substance is further substantiated by the observations that mEPSC-like activity disappears when ATP and ADP are degraded by the enzyme, apyrase, or when the P2XR antagonist, PPADS, are applied (Zhang *et al.*, 2007). The ATP release depends on the presence of external Ca^{2+} and the activation of L-type Ca^{2+} channels in neurons. With increased stimulus frequency, the release activity increases and the latency between the stimulus and release is shortened. The amplitude distribution of ATP release events can be fitted with multiple Gaussians of equally spaced peaks, further suggesting the quantal nature of the ATP release (Zhang *et al.*, 2007).

Neuronal soma communicates with SGCs through somatic release of ATP

One of the likely functions of neuronal somatic release is to communicate with SGCs. Although there is a great deal of evidence that astrocytes and glial cells in the central nervous system (CNS) respond to synaptic inputs (Matsui & Jahr, 2004; Wang *et al.*, 2006), few have studied the direct activation of glial cells by neuronal somata. To establish direct neuron to SGC communication in sensory ganglia, it is essential to establish that SGCs surrounding the soma of a neuron respond to afferent nerve stimulation. A straightforward approach is to determine Ca^{2+} signaling between a neuron and surrounding SGCs in ganglia. When a Ca^{2+} dye, e.g. Fluo4, is loaded into DRGs, changes in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) in both the neuron and SGCs can be monitored simultaneously as afferent fibers of DRG neurons are electrically stimulated. Following nerve stimulation, the $[\text{Ca}^{2+}]_i$ in the neuron invariably increases first; the $[\text{Ca}^{2+}]_i$ in surrounding SGCs then increases with a delay (Fig. 2) (Zhang *et al.*, 2007). As stimulus frequency of afferent nerves increases, the magnitudes of $[\text{Ca}^{2+}]_i$ in both the neuron and SGCs increase and the delay between Ca^{2+} signals is shortened. These observations suggest that the somata of sensory neurons indeed communicate with SGCs. Since the L-type Ca^{2+} channel blocker, nimodipine, completely blocks the $[\text{Ca}^{2+}]_i$ increase in both the neuron and SGCs, activation of L-type Ca^{2+} channels in the neuron is essential for neuron-SGC communication (Zhang *et al.*, 2007). Because apyrase abolishes the $[\text{Ca}^{2+}]_i$ increase in SGCs, but had no effect on $[\text{Ca}^{2+}]_i$ in the neuron, ATP is the major transmitter mediating the neuron-SGC communication (Zhang *et al.*, 2007).

ATP signals sensory inputs in DRGs by activating purinergic ionotropic P2X receptors (P2XRs) and metabotropic P2YRs (Burnstock, 2000; North, 2002; Ruan & Burnstock, 2003; Fields & Burnstock, 2006). Both P2XRs and P2YRs are found in the somata, axons and terminals of DRG neurons and in surrounding SGCs and Schwann cells (Grubb & Evans, 1999; Hanani, 2005; Kobayashi *et al.*, 2005; Kobayashi *et al.*, 2006). P2X2–P2X6 mRNAs are found in DRG neurons (Kobayashi *et al.*, 2005). Homomeric P2X3Rs and heteromeric P2X2/3Rs receptors are the major P2XRs in the somata, peripheral and central terminals of small and medium sized sensory neurons (Burgard *et al.*, 1999; North, 2002). These receptors are responsible for transmitting sensory information from the periphery to the spinal cord (Bardoni *et al.*, 1997; Gu, 2003; Nakatsuka & Gu, 2006) and from the orofacial areas to the trigeminal subnucleus caudalis (Jennings *et al.*, 2006). P2X7 and P2X4 are the primary P2XR subtypes found in SGCs (Kobayashi *et al.*, 2005). A unique feature of sensory ganglia is that P2X7Rs are abundantly expressed in SGCs, but are not expressed in DRG neurons (Kobayashi *et al.*, 2005; Zhang *et al.*, 2005; Chen *et al.*, 2008). At the same time, P2X3Rs are the highest expressed P2XRs in DRG neurons but are not found in SGCs (Kobayashi *et al.*, 2005; Chen *et al.*, 2008). This separate expression of the P2X3R and

P2X7R provides us with a convenient way to differentiate the actions of ATP on neurons and on SGCs in DRGs. From Ca^{2+} imaging experiments, we found that the P2X7R antagonists, brilliant blue G (BBG) and oxATP, blocked afferent fiber-induced $[\text{Ca}^{2+}]_i$ increase only in SGCs, but not in the neuron (Fig. 3) (Zhang *et al.*, 2007). When Ca^{2+} channels in the neuron were activated by high KCl (80 mM), $[\text{Ca}^{2+}]_i$ in both neuron and SGCs were increased. BBG again blocked the KCl-induced $[\text{Ca}^{2+}]_i$ increase in SGCs without affecting $[\text{Ca}^{2+}]_i$ changes in the neuron (Zhang *et al.*, 2007). These observations suggest that in response to afferent stimulation or KCl-induced depolarization, activation of P2X7Rs in SGCs is the key event mediating the responses of neuronal soma to SGC communication.

Satellite glial cells exert feedback control of neuronal somatic activity through the activation of P2X7Rs

To understand the role the SGCs in sensory information processing, it is important to study SGCs responses to neuronal signaling and to determine if SGCs provide active feedback control of neuronal activity in sensory neurons. There is a great deal of information concerning the influence of neuronal activity by glia in the CNS (Volterra & Meldolesi, 2005; Haydon & Carmignoto, 2006; Perea & Araque, 2010). Glial cells respond to electrical and mechanical stimulation by releasing a variety of gliotransmitters, e.g., ATP, glutamate, GABA and prostaglandin E2 (PGE2) (Volterra & Meldolesi, 2005). ATP released from astrocytes has been found to initiate $[\text{Ca}^{2+}]_i$ oscillation and Ca^{2+} waves that propagate among astrocytes (Guthrie *et al.*, 1999; Cotrina *et al.*, 2000). When ATP released from astrocyte is converted to adenosine by extracellular adenosine triphosphatases (ATPases), A1 receptors in hippocampal neurons become activated and glutamatergic synaptic responses are depressed (Zhang *et al.*, 2003). Such heterosynaptic suppression is absent in transgenic mice in which ATP release from astrocytes is impaired (Pascual *et al.*, 2005). When caged Ca^{2+} or ATP-induced glutamate release from astrocytes coincides temporally with the postsynaptic depolarization of CA1 neurons in the hippocampus, long term potentiation can be evoked at CA3-CA1 synapses (Perea & Araque, 2007). In addition, glutamate released from astrocytes has been found to evoke slow inward currents through extrasynaptic NMDA receptors and to activate multiple hippocampal CA1 neurons synchronously (Fellin *et al.*, 2004).

In sensory ganglia, the influence of SGC activation on neuronal activity is not well understood. Electrical properties of SGCs in DRG and TG neurons have been studied. Unlike neurons, SGCs do not express voltage-dependent Na^+ channels, but express Ca^{2+} -dependent, inwardly rectifying and voltage-dependent K^+ channels (Cherkas *et al.*, 2004; Vit *et al.*, 2006; Zhang *et al.*, 2009). Thus, SGCs are not electrically excitable. On the other hand, SGCs express a variety of receptors. They respond to external stimuli by changes in cytosolic Ca^{2+} through the opening of Ca^{2+} -permeable receptors, e.g., ionotropic purinergic P2XRs (Zhang *et al.*, 2007; Ceruti *et al.*, 2008) or by mobilizing Ca^{2+} from intracellular Ca^{2+} stores through the activation of metabotropic receptors, e.g., P2YRs (Weick *et al.*, 2003; Ceruti *et al.*, 2008). Therefore, SGCs use intracellular Ca^{2+} for signaling, similarly to glial cells in the CNS. In trigeminal neuron-SGC mixed cultures, mechanical stimulation of SGCs has been shown to evoke Ca^{2+} waves that spread to the neighboring neuron and among the nearby SGCs. The Ca^{2+} signaling is mediated by P2Rs and gap junctions (Suadicani *et al.*, 2009).

Since P2X7Rs in SGC cells directly respond to somatic ATP release (Fig. 3), we examined whether activation of P2X7Rs affects neuronal activity (Zhang *et al.*, 2007; Chen *et al.*, 2008). When nerve fibers of DRGs are subject to tetanic trains of stimulation, a large increase in TNF α release can be observed (Zhang *et al.*, 2007). The release is greatly

diminished in the presence of the P2X7R antagonist, oxATP, suggesting that activation of P2X7Rs in SGCs mediates the TNF α release. Studying the action of TNF α on the activity of DRG neurons, we found that TNF α potentiates P2X3R-mediated currents and enhances the action potential firing of somata. Therefore, the SGC P2X7R-mediated release of TNF α exerts an excitatory action on DRG neurons (Zhang *et al.*, 2007).

P2X7R activation has been directly linked to the post-translational maturation and release of cytokines, e.g., TNF α and interleukin 1b (IL-1b), from glial cells after injury (Colomar *et al.*, 2003; Ferrari *et al.*, 2006; McGaraughty *et al.*, 2007; Zhang *et al.*, 2007; Skaper *et al.*, 2010). P2X7R knock-out mutant mice lack the ability to release IL-1b from peritoneal macrophages in response to ATP stimulation (Solle *et al.*, 2001) and fail to develop swollen paws or joint cartilage lesions as wild-type animals do when treated with collagen monoclonal antibodies to induce arthritis (Labasi *et al.*, 2002). Furthermore, in mice lacking P2X7Rs, thermal and mechanical hypersensitivity following inflammation, nerve ligation or lipopolysaccharide (LPS) treatment are absent (Chessell *et al.*, 2005; Clark *et al.*, 2010) and LPS- or BzATP-induced release of IL-1b is greatly diminished (Clark *et al.*, 2010). Most cytokines released from immune cells are through the endoplasmic reticulum-to-Golgi exocytic pathway (Moqbel & Coughlin, 2006). However, P2X7 receptor-mediated cytokine release appears to occur through alternate routes. They include the ATP binding cassette transporter which is linked to a P2X7R-associated chloride conductance (Marty *et al.*, 2005), microvesicle shedding (MacKenzie *et al.*, 2001; Bianco *et al.*, 2005) and membrane blebbing (Wilson *et al.*, 2002). Recently, a hemichannel protein, pannexin-1 was found to be associated with P2X7Rs and is required for P2X7R-mediated release of cytokine (Pelegrin & Surprenant, 2006; Iglesias *et al.*, 2008; Iglesias *et al.*, 2009; Surprenant & North, 2009).

In addition to the excitatory effect, activation of P2X7R in SGCs was also found to exert inhibitory actions on DRG neurons (Chen *et al.*, 2008). Studying the basal ATP release from DRGs using a luciferase assay, we showed that the ganglia endogenously released ATP and the release was greatly reduced by treating DRGs with oxATP. Thus, P2X7Rs mediate a significant portion of the ATP release from SGCs. When P2X7Rs and their mediated ATP release were blocked, an increase in P2X3R expression in DRG neurons was observed. Furthermore, reducing P2X7R expression with P2X7R-siRNA also increased the P2X3R expression. Thus, P2X7R activation tonically suppresses the expression of P2X3Rs in DRG neurons. In addition, we found that the suppression was largely eliminated by treating ganglia with the P2Y1R antagonist, MRS2179, suggesting that the activation of metabotropic P2Y1Rs in neurons is required for the inhibitory P2X7R-P2X3R expression control. Treatment of DRGs with the Krebs cycle inhibitor, fluorocitrate, disrupted SGC activity and hence blocked the activation of P2X7Rs. Under such a condition, P2X3R expression was enhanced (Chen *et al.*, 2008). Preincubation of DRGs with the P2Y1R agonist, 2MeSADP, in the presence of fluorocitrate restored the inhibition of P2X3R expression. Thus, activation of neuronal P2Y1Rs, which is downstream of P2X7Rs, is sufficient for the P2X7R-P2X3R inhibitory control. Taken together, the observations suggest that activation of P2Y1Rs is necessary and sufficient for the inhibitory control of P2X3R expression by P2X7Rs (Chen *et al.*, 2008).

Ca²⁺ imaging analyses of DRGs were also used to determine the functional effect of the SGC-neuronal soma control (Fig. 4) (Chen *et al.*, 2008). When P2X7Rs in SGCs were activated by the application of the P2X7R agonist BzATP, we observed a large [Ca²⁺]_i increase in SGCs (Fig. 4. Left), but only a moderate [Ca²⁺]_i increase in the neuron (Fig. 4, Right). Preincubation of the P2Y1R antagonist, reactive blue 2 (RB) for 1 hr did not change [Ca²⁺]_i in SGCs further, but greatly increased [Ca²⁺]_i in the neuron. The [Ca²⁺]_i increase was P2X3R mediated because the [Ca²⁺]_i change in the neuron was blocked by the P2X3R antagonists, A317491. Thus, P2Y1R activation in neurons is required for glial P2X7Rs to

exert their inhibitory control on P2X3Rs. This conclusion is further supported by behavioral observations (Chen *et al.*, 2008). Application of α,β meATP to the rat hindpaw elicited repetitive paw lifting (flinching), a nocifensive behavior known to be mediated by P2X3Rs. Down-regulation of P2X7R with P2X7-siRNA elicited a large increase in flinching responses. The increase was abolished when P2Y1Rs was activated by 2MeSADP. Thus, activation of P2Y1R effectively curtails the P2X3R-mediated nociceptive responses.

P2X7Rs, which are richly expressed in SGCs, have two unique channel properties (Sperlagh *et al.*, 2006; Jarvis & Khakh, 2009). First, the P2X7R is relatively insensitive to ATP. The EC_{50} (~100 μ M) of ATP for P2X7R is ~10 times higher than that for the P2X2R and P2X3R. Second, prolonged P2X7R activation renders the receptor progressively non-selective to cations (pore dilation), thus allowing passage of large molecules (Virginio *et al.*, 1999; Khakh & North, 2006). These properties make P2X7R activation dependent on cell activity. Strong stimulation or injury increases ATP release from activated neurons (Cook & McCleskey, 2002), glia and immune cells (Bianco *et al.*, 2005; Franke *et al.*, 2006). The level of extracellular ATP concentration would reach a sufficient level to activate P2X7Rs. Neurotransmitters, such as glutamate (Sperlagh *et al.*, 2002; Duan *et al.*, 2003), GABA (Sperlagh *et al.*, 2002; Papp *et al.*, 2004) and ATP (Zhang *et al.*, 2003) and neuromodulators, such as D-serine (Yang *et al.*, 2003) and endocannabinoid 2-AG (Walter *et al.*, 2004), are found to be released from glia following the activation of P2X7Rs. In addition to vesicular release, glial cells use additional mechanisms for transmitter release. They include outflow through anion channels, leakage from connexin and hemichannels, reversal of uptake of transporters and efflux through the P2X7R pore (Duan & Neary, 2006; Scemes *et al.*, 2007; Malarkey & Parpura, 2008). Unlike Ca^{2+} -dependent exocytosis observed at nerve terminals, P2X7R-mediated release from glial cells does not depend on the extracellular Ca^{2+} concentration (Duan *et al.*, 2003). Because of its unique requirement of high ATP concentration for activation, P2X7Rs are thought to specifically respond to high neuronal activity associated with neuronal degeneration (Le Feuvre *et al.*, 2002; Sperlagh *et al.*, 2006), spinal cord injury (Wang *et al.*, 2004) and abnormal pain (McGaraughty *et al.*, 2007; Skaper *et al.*, 2010).

Very few studies show that activation of P2X7R is neuroprotective (Suzuki *et al.*, 2004; Chen *et al.*, 2008). Due to the requirement of high ATP concentration for P2X7R activation, it is generally assumed that P2X7Rs play a minor role in influencing neuronal activity under normal conditions. Our study of SGC to neuron communication in DRGs suggests that this is not the case. We found that P2X7Rs are endogenously activated by basal ATP release and tonically down-regulate P2X3R expression in DRG neurons under normal conditions (Chen *et al.*, 2008). Since extracellular ATP concentration is kept at a low level in the presence ecto-ATPase and ecto-apyrase under these conditions, it remains to be determined how P2X7Rs are activated endogenously in DRGs. P2X7R activation depends on the location of the receptor relative to ATP release sites. SGCs are tightly wrapped around the somata of DRG neurons with a narrow gap (~20 nm) (Pannese, 1981; Hanani, 2005). If P2X7Rs are located in the vicinity of ATP release sites, the local ATP concentration at the P2X7R can be sufficiently high (5–500 μ M) (Pankratov *et al.*, 2006) for their activation without requiring a high level of neuronal activity. Thus, the extent of P2X7R activation may not be a direct function of “global” extracellular ATP concentration.

Satellite glial cell-neuronal soma interactions after injury

It is well documented that inflammation and nerve injury alter the properties of sensory neurons. As the results of a reduction of firing threshold and an increase in excitability after injury, these neurons become spontaneously active and/or fire action potentials at high frequencies in response to external stimuli (Zhang *et al.*, 1999; Cherkas *et al.*, 2004; Ma &

LaMotte, 2005; Dublin & Hanani, 2007). These abnormalities contribute to pathological nociceptive (pain) behaviors, e.g., hyperalgesia and allodynia. Since SGCs can have profound effects on neuronal activity (Zhang *et al.*, 2007; Chen *et al.*, 2008), it is important to understand if the communication between SGCs and the somata of sensory neurons changes after injury.

In the somatosensory system, inflammation and nerve injury initiate many changes in glial cells. They include several-fold increase in the coupling between SGCs through gap junctions in DRGs (Pannese *et al.*, 2003; Cherkas *et al.*, 2004; Hanani, 2005; Dublin & Hanani, 2007; Zhang *et al.*, 2009), a decrease in inwardly rectified K⁺ currents in SGCs (Takeda *et al.*, 2008b; Zhang *et al.*, 2009), activation of extracellular signal-regulated kinase (ERK) in astrocytes and microglia (Zhuang *et al.*, 2005), a large increase in the number of activated microglia in the spinal cord (Beggs & Salter, 2007), upregulation of P2X4Rs in microglia (Tsuda *et al.*, 2003; Trang *et al.*, 2006) and induction of BDNF release from microglia to reduce GABA inhibitory control in dorsal horn neurons (Coull *et al.*, 2005). In addition, glial cells facilitate immune responses after injury. Cytokines have been found to exaggerate nociceptive responses in the spinal cord (Watkins *et al.*, 2003; Raghavendra *et al.*, 2004; Marchand *et al.*, 2005; McMahon *et al.*, 2005; Takeda *et al.*, 2009). TNF α and IL-1b are important pain-mediators in sensory neurons (Marchand *et al.*, 2005; Takeda *et al.*, 2009). Only low levels of TNF α and IL-1b are detected in DRG neurons and SGCs of normal rats and mice (Ohtori *et al.*, 2004; Xu *et al.*, 2006). After inflammation or nerve injury, these cytokines are upregulated rapidly in both neurons and SGCs (Ohtori *et al.*, 2004; Miyagi *et al.*, 2006; Xu *et al.*, 2006; Takeda *et al.*, 2007). Similarly, TNF α type 1 receptors (TNFR1s) and IL1R1 in DRG or TG neurons are expressed only in modest amount in neurons and SGCs normally, but are increased several-fold after nerve injury or inflammation (Ohtori *et al.*, 2004; Inglis *et al.*, 2005; Takeda *et al.*, 2007). The increase in TNF α and TNFRs following inflammation and nerve injury was found to coincide with the production of hyperalgesia or allodynia (Wieseler-Frank *et al.*, 2005). Treatment of the rat paw with these cytokines reduces nociceptive thresholds and produces nociceptive hypersensitivity (Sachs *et al.*, 2002). Acute treatment of DRG with TNF α and IL-1b increases intracellular Ca²⁺ transients in neurons (Pollock *et al.*, 2002), depolarizes membrane potentials, reduces threshold currents for action potentials and elicits spontaneous firing in DRG neurons (Liu *et al.*, 2002; Schafer *et al.*, 2003; Takeda *et al.*, 2007). The mechanisms underlying these direct actions of TNF α and IL-1b on sensory neurons include a reduction of voltage-dependent K⁺ currents (Diem *et al.*, 2001; Takeda *et al.*, 2008a) and an increase in Ca²⁺-dependent inward currents (Pollock *et al.*, 2002). The effects of TNF α can be long-lasting. A single injection of TNF α into DRGs was found to produce allodynia that lasts for more than ten days (Schafer *et al.*, 2003). Long term exposure to TNF α results in an increase in PGE2 production (Sachs *et al.*, 2002; Fehrenbacher *et al.*, 2005) and potentiates TRPV1 responses in DRG neurons (Nicol *et al.*, 1997). Our observations that TNF α is released from SGCs following strong stimulation, a condition that invariably occurs following injury, and TNF α treatment increases neuronal excitability are consistent with the view that injury-induced release of cytokines from SGCs markedly affects the activity of somata in DRG neurons.

Since SGCs are found to exert inhibitory action on neuronal activity under normal conditions (Chen *et al.*, 2008), it is of interest to determine the P2X7R-P2X3 inhibitory control after injury. We studied the effect of P2X7R-P2X3R control on von-Frey filament induced allodynia and on P2X3R-mediated α,β meATP-induced paw flinching in inflamed rats (Chen *et al.*, 2008) and nerve injury rats (Preliminary studies). Following inflammation and nerve injury, allodynia and flinch behaviors were exaggerated. When P2X7Rs were activated by the P2X7R agonist, BzATP, the exaggerated responses became greatly diminished in both rat models. In rats treated with BzATP plus the P2X7R antagonist,

oxATP or BBG, the protective action of BzATP was much reduced (Preliminary studies). Thus, P2X7R-P2X3R inhibitory control persists after injury. Activation of P2X7Rs is effective in curbing the inflammation and nerve-injury-induced hyperalgesia.

We also determined the P2X7-P2X3R expression control in DRGs isolated from inflamed rats. P2X3R expression is increased after inflammation (Xu & Huang, 2002; Chen *et al.*, 2008). Activation of P2X7Rs by BzATP reduces P2X3R expression. The reduction is blocked following the oxATP+BzATP treatment. Thus, P2X7R exerts inhibitory control on P2X3R expression in inflamed rats, similar to that observed in normal rats (Chen *et al.*, 2008). We are investigating if P2X7R activation by BzATP affects the P2X3R expression in nerve injured rats

CONCLUSIONS

Studies of neuronal soma-SGC interactions in sensory ganglia in the last few years have led investigators to appreciate the interdependence between neuronal somata and SGCs in regulating peripheral signaling and the prominent role that P2X7Rs play in the neuron-SGC communication (Hanani, 2005; Zhang *et al.*, 2007; Chen *et al.*, 2008; Miller *et al.*, 2009; Suadicani *et al.*, 2009; Takeda *et al.*, 2009; Skaper *et al.*, 2010). In response to afferent nerve fiber stimulation, somatic release of ATP from DRG neurons activates P2X7Rs in SGCs (Zhang *et al.*, 2007). Because P2X7Rs are expressed only in SGCs (Kobayashi *et al.*, 2005; Chen *et al.*, 2008), they exert feedback control on neuronal activity exclusively through SGC-neuron interactions. Studies of SGC-neuron control suggest that P2X7R activation exerts both inhibitory and excitatory influences on the activity of the somata of sensory neurons (Fig. 5) (Zhang *et al.*, 2007; Chen *et al.*, 2008). Under normal conditions, the P2X7R-P2X3R inhibitory control keeps P2X3R activity in neurons at an optimal level (Chen *et al.*, 2008). After injury, P2X7Rs are involved in cytokine release to promote wound healing while maintaining P2X7R-P2X3R inhibitory control. Following severe injury, an increased P2X7R-mediated cytokine release can overwhelm the P2X7R inhibitory control and give rise to the overexcitation of sensory neurons. The challenge is to determine how to balance the inhibitory and excitatory SGC-neuron controls in order to prevent the production of abnormal neuronal activity and pathological pain conditions. Because of the complex modulation by neuronal activity by P2X7R activation, direct inhibition of P2X7R would not be an effective way to control chronic pain or neurodegeneration disorders induced by tissue or nerve injury. Blocking the P2X7R-associated protein, pannexin-1 hemichannel, which is found to be involved in the P2X7R-mediated cytokine release or pore dilation (Iglesias *et al.*, 2009; Pelegrin & Surprenant, 2009), is likely to be a better strategy to curb the deleterious effects of P2X7R overexcitation. P2X7Rs have also been found to be upregulated in SGCs of avulsion-injured DRGs isolated from chronic neuropathic pain patients (Chessell *et al.*, 2005). The P2X7R appears to have an equally central role in SGC-neuronal soma signaling in human DRGs. Judicious choice of a site for controlling P2X7R activity would be essential for making the P2X7R a useful therapeutic target. Glial cells are found to wrap around the soma of neurons in the CNS (Perea *et al.*, 2009). Similar neuronal soma-glial cell interactions and controls may well occur in the brain and in the spinal cord.

Acknowledgments

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Fig. 1. Stimulation of DRG neurons evokes ATP or glutamate release

(A) (Upper) Examples of action potentials evoked in a DRG neuron by a current stimulus train under current clamp conditions. (Lower) Activation of the DRG neuron evoked mEPSC-like activity in a sniffer patch expressing P2X2-EGFP receptors and held at -80 mV. The same stimulus did not evoke any activity in another outside-out patch excised from a nontransfected HEK cell. Adapted from Zhang et al. (2007) (Copyright © 2007 by National Academy of Sciences, U.S.A.) (B) Stimulation of another DRG neuron evoked mEPSC-like activity in a sniffer patch expressing GluR6-EGFP receptors. The activity was blocked by the application of the GluR antagonist, NBQX. The activity resumed when the antagonist was washed out.

Fig. 2. Neuronal somata communicate with SGCs

(A) (Upper) $[Ca^{2+}]_i$ responses in a neuron (N) and in one of the SGCs surrounding the soma (arrow) following afferent nerve fiber stimulation (200 Hz, 30s). The time at which the image was taken is indicated by the number at the upper left corner in each frame. Enlarged views of the SGC are shown directly below. Color-coded intensity calibration bar is shown on the right. A different color-coded calibration is used in the enlarged view to highlight the fluorescence change in the SGC. Neuron diameter = 19.3 μm . (Lower) Time courses of the $[Ca^{2+}]_i$ -induced fluorescence changes, i.e., $[(F-F_0)/F_0] = \Delta F/F_0$, in the neuron (red) ($\Delta F/F_{0(N)}$) and in the SGC (blue) ($\Delta F/F_{0(S)}$). F_0 is the basal fluorescence in either the neuron or the SGC before nerve stimulation. The horizontal line indicates the period of nerve stimulation. Responses in the dotted box are shown on an expanded scale to the right. Nerve stimulation evoked $[Ca^{2+}]_i$ increase in the neuron first and then in the SGC with a delay. The $[Ca^{2+}]_i$ signal in the SGC had a later onset and a slower recovery than the $[Ca^{2+}]_i$ increase in the neuron. (B) Neuronal soma-SGC interactions depend on the frequency of nerve stimulation. With increasing stimulus frequency, the peak $[Ca^{2+}]_i$ in both neurons and SGCs increased and the delay between the soma and SGCs was shortened ($n = 4-6$). Adapted from Zhang et al. (2007) (Copyright © 2007 by National Academy of Sciences, U.S.A.)

Fig. 3. Neuronal soma-SGC cell communication depends on the activation of P2X7Rs
 (A) The reversible P2X7R antagonist, BBG (1 μ M) inhibited the $[Ca^{2+}]_i$ -induced fluorescence increase in a SGC, i.e., ($\Delta F/F_{0(S)}$), but had no effect on the $[Ca^{2+}]_i$ increase in a neuron, i.e., ($\Delta F/F_{0(N)}$). The blocking effect of BBG was reversed after wash. (B) Average blocking actions of BBG and the irreversible P2X7R antagonist, oxATP. BBG had no effect on $\Delta F/F_{0(N)}$ in neuronal somata (n=7), but reduced $\Delta F/F_{0(S)}$ increase by 78.6% (n=15). Irreversible oxATP (100 μ M) had no effect on $\Delta F/F_{0(N)}$ increase in neuronal somata (n= 4), but decreased $\Delta F/F_{0(S)}$ increase in SGCs by 73.8% (n=9). Adapted from Chen et al. (2008) (Copyright © 2008 by National Academy of Sciences, U.S.A.)

Fig. 4. Blocking the P2YR enhances P2X3R activity in neurons

Activation of P2X7Rs by acute application of BzATP (100 μ M) elicited a $[Ca^{2+}]_i$ -induced fluorescence increase in SGCs (Left), but a rather small $[Ca^{2+}]_i$ increase in neurons (Right). Following 1 hr preincubation of DRGs with the P2YR antagonist, RB (1 μ M), BzATP did not change $[Ca^{2+}]_i$ in SGCs, but produced a large increase in $[Ca^{2+}]_i$ signals in neurons. This observed $[Ca^{2+}]_i$ increase in neurons is mediated by P2X3Rs because the increase was blocked when the P2X3 antagonist, A317491 (60 μ M), was applied with BzATP. The thick lines represent the average $[Ca^{2+}]_i$ responses of 10 to 15 neurons or those of 16 to 26 SGCs. The thin lines are the SEs of the average values. The arrows indicate the starting time of drug applications. Adapted from Chen et al. (2008) (Copyright © 2008 by National Academy of Sciences, U.S.A.)

Fig. 5. Neuronal soma-SGC communication

A schematic drawing illustrates the afferent-stimulated ATP release from the soma of a DRG neuron and the excitatory (red arrow) and inhibitory (black arrow) control of the neuronal activity by SGCs.