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Neuromodulation in the Spiral Ganglion: Shaping Signals from the Organ of Corti to the CNS

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Introduction

Hidden deep within the temporal bone, neurons of the spiral ganglion have long resisted attempts to characterize their endogenous electrophysiological properties. Single-unit recordings revealed heterogeneity in responses to sounds of similar frequency (Kiang et al., 1965; Liberman, 1978) suggesting that these first-order neurons may be intrinsically "tuned" to different parameters of acoustic stimuli, but it was not until the first voltage-clamp recordings were obtained (Santos-Sacchi, 1993) that this issue could be addressed directly. Indeed, given the propensity of peripheral sensory neurons to enhance detection of environmental features of interest, it would be most surprising if these unique bipolar neurons did not possess mechanisms to tailor their responsiveness to parameters such as sound frequency and intensity. In this review we describe recent progress made possible by recording techniques that elucidate the characteristics of intrinsic ion channels and receptors which govern the firing and synaptic responses of type I spiral ganglion neurons. These studies reveal extensive neuromodulatory effects of compounds ranging from ATP and protons to neurotransmitters and neurotrophins that undoubtedly serve to optimize the signaling capabilities of the spiral ganglion and allow them to carry out their critical role in the perception of sound.

Key words: ACh — ASIC — ATP — BDNF — Cochlea — Glutamate — Metabotropic — Neurotrophin — NT-3 — P2X — Purinergic — Substance P — TRPC

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Signaling via P2X Receptors: Effects on Spiral Ganglion Neuron Threshold Level and Firing Properties

ATP has been implicated in the function of various peripheral and central neurons. The localization of ionotropic P2X receptors to post-synaptic dendritic spines in the brain suggests it acts as a fast excitatory neurotransmitter (Rubio & Soto, 2001), and significant divalent cation permeability allows P2X receptors to provide Ca^{2+} entry at the resting potential of neurons (Burnashev, 1998). Thus, ATP may also act as a neuromodulator, altering the response of the postsynaptic neuron to co-transmitters such as glutamate.

P2X receptors have been localized to various sensory and non-sensory tissues in the mammalian cochlea (Housley et al., 1999). ATP is thus thought to have extensive effects on normal hearing function (Housley et al., 2002), and it may contribute to pathological states in the cochlea (Thorne et al., 2002). Most pertinent to the current review is the widespread and complex expression pattern of various P2X receptor subtypes in cochlear afferent tissue. In several species at different stages of development $P2X_1$ (Nikolic et al., 2001), $P2X_2$ (Housley et al., 1999; Jarlebark, et al., 2000), $P2X_3$ (Huang et al., 2005) and $P2X_7$ (Nikolic Housley & Thorne, 2003) receptors have all been localized to the spiral ganglion neurons, including their sensory terminals under hair cells. This leads us to expect that ATP may play diverse roles in synapse development, and in primary afferent coding of auditory stimuli.

What are the effects of P2X receptor activation on spiral ganglion neuron membrane function? Recent patch-clamp studies of spiral ganglion neurons in

cochlear slices have shown that the somata of these cells are exquisitely and consistently sensitive to micromolar applications of ATP, resulting in a short-latency desensitizing inward current at the resting membrane potential (Jagger, Robertson & Housley, 2000). Several purinergic agonists activate the current, including α,β-methyleneATP, 2-methylthioATP, and ADP (Salih, Jagger & Housley, 2002). In addition, low concentrations of Bz-ATP are effective (D. Jagger, *unpublished observations*). The current is strongly inwardly rectifying, blocked by the purinergic antagonists PPADS and TNP-ATP, and strongly potentiated by extracellular acidification (Salih et al., 2002). Repetitive application of ATP desensitizes the current, but delays between applications allow receptor resensitization. These characteristics present a conundrum, as they do not match those of characterized recombinant P2X receptors. The answer may lie in a specific heteromultimeric P2X receptor subunit assembly. In agreement with this proposal, RT-PCR analysis of single spiral ganglion neurons has detected the mRNA for a $P2X_{2/3}$ heteromultimer (D. Greenwood, G. Housley & D. Jagger, in preparation).

It remains to be determined whether P2X receptors contribute to normal hearing by shaping neuronal firing properties by the direct action of ATP at the hair cell afferent synapse. Robust desensitizing inward ATP-gated currents in inner hair cell and outer hair cell afferent neurons persist to just prior to the onset of hearing (Jagger & Housley, 2003). These single neurons respond to exogenous application of both purinergic and AMPA receptor agonists. The contribution of the heteromultimeric assembly to adult hearing function seems unlikely though, as both $P2X_3$ mRNA and protein expression are seen to decline beyond postnatal day 14 in rat cochlea (Huang et al., 2005). However, the $P2X_2$ subunit has been localized to the spiral ganglion and the afferent synapse under both inner and outer hair cells in adult guinea pigs (Housley et al., 1999), and rats (Wang et al., 2003). Also, the $P2X_7$ subunit has been localized to the spiral ganglion and the afferent synapse under both inner and outer hair cells in adult rats (Nikolic et al., 2003). A hypothetical neuromodulatory role for ATP is supported by the localization of ectonucleoside triphosphate diphosphorylases in adult spiral ganglion neurons (Vlajkovic et al., 2002). Hydrolysis of endogenous extracellular ATP would enable indefatigable purinergic signaling at the synapse by the prevention of $P2X_2$ receptor desensitization.

Insights into the effects of P2X receptor activation on spiral ganglion neuron neurotransmission come from in vivo studies of cochlear activity. Perilymphatic perfusion (including the afferent synapse) with purinergic agonists has complex effects on cochlear potentials and spiral ganglion neuron firing characteristics. The overall effect is a suppression of cochlear responses to sounds (Kujawa et al., 1994; Sueta et al., 2003). This may be explained in part by dramatic alterations of spontaneous firing rates of individual spiral ganglion neurons (Sueta et al., 2003), resulting in increased thresholds for neuron tuning curves. The agonist-dependence of these effects is consistent with an action on $P2X_2$ receptors.

In summary, endogenous ATP has complex effects on cochlear physiology, including direct actions on the membrane function of spiral ganglion neurons. In neonatal neurons ATP analogues activate depolarizing currents with characteristics compatible with heteromultimeric P2X receptor assemblies, probably comprising $P2X_2$ and $P2X_3$ subunits. There is a body of evidence that $P2X_2$ receptor activation continues to regulate spiral ganglion neuron firing in the mature cochlea. Future research should clarify the mechanism by which these effects are achieved at the hair cell afferent synapse. It seems likely that P2X receptors play a neuromodulatory role, conditioning the response of spiral ganglion neurons to glutamate.

Activation of Group I mGluRs (mGluRI) in Spiral Ganglion Neurons Constitutes a Component of the Postsynaptic Excitatory Response at Suprathreshold Levels

Glutamate-mediated fast neurotransmission between the hair cells and the dendrites of spiral ganglion neurons is carried out by the activity of postsynaptic ionotropic glutamate receptors (iGluRs; Nakagawa et al., 1991; Ruel et al., 1999). The localization of metabotropic glutamate receptors (mGluRs) in spiral ganglion neurons by immunolabeling (Niedzielski, Saffieddine & Wenthold, 1997; Kleinlogel et al., 1999) suggests that the glutamate released by hair cells may also activate mGluRs, which are generally coupled to second messenger systems via G-protein-coupled pathways to modulate neuronal excitability.

Eight mGluR subtypes have been cloned and they are classified into three distinct groups (Pin & Duvoisin, 1995; Conn & Pin, 1997). Groups II & III mGluRs are found primarily at presynaptic terminals. They modulate synaptic efficacy by regulating neurotransmitter release. The group I mGluR (mGluRI), which includes mGluR1 and mGluR5, is typically located postsynaptically. They couple to PTX-insensitive G_q and phospholipase C (PLC) pathway (Masu et al., 1991; Abe et al., 1992; Baude et al., 1993; Petralia et al., 1996; Lujan et al., 1997; De Blasi et al., 2001). The activation of mGluRI generates inositol-1,4,5-triphosphate (IP_3) and releases Ca^{++} from intracellular stores (Houamed et al., 1991; Masu et al., 1991), which can be measured directly by ratiometric Ca^{++} imaging.

Assayed by quantitative PCR and low-density DNA array hybridization methods, Peng et al. (2004b) found that the mRNA expression level of

mGluR1 in the cochlea is about 30 times more abundant than that of the mGluR5. Antibody against mGluR1 labeled spiral ganglion neurons. They also showed that the common G-protein partner (G_q) with the mGluRI is one of the predominant forms of G-proteins found in the cochlea. Direct evidence supporting mGluRs as part of the postsynaptic response element in cochlear neurotransmission was demonstrated in cultured spiral ganglion neurons using the ratiometric Ca^{++} imaging and patch-clamp recording techniques. Bath applications of agonists to mGluRIs to the isolated spiral ganglion neurons in cultures resulted in transient increases of intracellular Ca^{++} concentration and transient inward currents that give rise to firings of action potentials, and these responses showed mGluRI pharmacological specificity and quickly desensitized.

Intracochlear perfusion of pharmacological agents that block the non-N-methyl-D-aspartic acid glutamate receptors was sufficient to eliminate compound action potentials of the auditory nerve reversibly. In contrast, pharmacologically inhibiting mGluRIs in the cochlea did not significantly affect the hearing threshold, suggesting that mGluRIs did not initiate fast cochlear neurotransmission. Blocking mGluRIs lowered the amplitude of compound action potentials at louder sound levels and reduced the noise-induced temporary threshold shift (TTS). These in vivo data show that mGluRIs contribute to excitatory cochlear neurotransmission at suprathreshold sound levels. Thus, excitatory responses added by activation of mGluRIs at high-intensity sounds may contribute to excitotoxic neuronal degeneration observed at the synapse between the hair cells and spiral ganglion neurons (Puel et al., 1998). The demonstration that a mGluRI antagonist, AIDA, reduced noise-induced TTS (Peng et al., 2004b) is consistent with this notion.

Modulation of peripheral sensory inputs by activation of mGluRs is found in many sensory modalities including spinal nociception (Meller, Dykstra & Gebhart, 1993; Neugebauer, Lucke & Schaible, 1994), light responses in the retinal ganglion neurons (Awatramani & Slaughter, 2000), olfactory sensory transmission (Hayashi et al., 1993). Activation of mGluRs in vestibular hair cells augments the sound-evoked transmitter release at suprathreshold levels (Guth et al., 1998). mGluRs are present in every center along the auditory pathway (Kotak & Sanes, 1995; Sanes, McGee & Walsh, 1998; Schwarz, Tennigkeit & Puil, 2000; Zirpel, Lachica & Rubel, 1995). Because activation of mGluRIs triggers many intracellular processes via the PLC pathway, which may have long-lasting modulatory effects, it seems that simply viewing spiral ganglion neurons as faithful relay neurons in the cochlea is an oversimplified summary for the function of these neurons.

Multiple Neurotransmitters Activate a Non-Specific Cationic Channel Type that Increases Neuronal Excitability

In addition to the previously mentioned glutamatergic (mGluR; Peng et al., 2004b) and P2X receptors (Housley et al., 1999), adult and developing spiral ganglion neurons have been shown to express other types of neurotransmitter and neuropeptide-activated G-protein–coupled receptors (GPCR). Muscarinic receptors (mAChR; Safieddine et al., 1996; Khan et al., 2002; Ito & Dulon, 2002); purinergic receptors (P2YR, Ito & Dulon, 2002), GABAergic receptors ($GABA_B$; Lin, Chen & Chen, 2000) and tachykinin receptors to substance P (SP;Ito et al., 2002) have all been localized to the spiral ganglion neurons. The expression of such a large variety of metabotropic GPCR further emphasizes the complex regulation of the auditory signal at the afferent nerve fibers.

Alteration of the concentration of intracellular calcium via GPCR is a key regulator of many cellular processes. In auditory neurons, the physiological consequences of changes in intracellular calcium are not clearly established but we believe that they could regulate fundamental processes such as neurite outgrowth during development, and cell survival (Hegarty, Kay & Green, 1997; Hansen et al., 2003; Lallemend et al., 2003) and regulate neuronal firing by the modulation of calcium-sensitive potassium channels known to be expressed by these neurons (Adamson, Reid & Davis, 2002; Skinner et al., 2003). Activation of GPCR by ACh or ATP could also be a way of controlling neurotransmitter release from spiral ganglion fibers at the central cochlear nucleus synapses, as recently proposed in chromaffin cells (Chen et al., 2005).

The external application of micromolar concentrations of ACh, ATP or SP to isolated spiral ganglion neurons, freshly from the rat cochlea, produces transient increases of intracellular calcium (Rome, Luo & Dulon, 1999; Ito & Dulon, 2002; Ito et al., 2002). These calcium responses are observed in the soma of isolated neurons at different stages of development from postnatal age P1 to mature stage P25. These calcium responses show all the characteristics of activation of G-protein–coupled receptors: desensitization during repetitive or prolonged application of the agonist and non-inhibition in calcium-free saline. Furthermore, N-ethylmaleimide and U-73122, two potent inhibitors of G-proteins and phospholipase C (PLC) respectively, inhibit the calcium responses. These results indicate that multiple neurotransmitters can release intracellular calcium via a G-protein/PLC pathway. This PLC signaling mediates the hydrolysis of phosphatidylinositol 4,5-biphosphate into the diffusible messengers Ins(1,4,5)P3 and diaglycerol. These two messengers trigger calcium release from internal

stores and activation of protein kinase C, respectively. A similar calcium mechanism has been proposed during the activation of G-protein–coupled glutamate receptors in mice cochlear neurons (Peng et al., 2004b).

Under voltage-clamp conditions near resting membrane potential, application of ACh (or muscarine), ATP or SP also activates a large inward conductance (Ito & Dulon, 2002). The depolarizing current shows a long latency, low frequency oscillations and reverses near zero mV, suggesting a metabotropic activation of a non-selective cation channel. Replacement of extracellular Na^+ with large cations such as $NMDG^+$ or TEA does not inhibit the inward current, indicating the involvement of channels with a relatively large pore. Chelating intracellular calcium with the fast buffer BAPTA or using U-73122 do not affect the non-selective cation current, indicating that it is not activated directly by a rise of intracellular calcium or by a conventional PLC pathway. The presence of N-ethylmaleimide or GDP-βS, however, blocked the SP-evoked depolarizing current, suggesting the implication of a G-protein–related process in the activation of the non-selective conductance. Interestingly, central auditory neurons have also been shown to express a non-selective cation conductance that can be activated during application of SP (Wang & Robertson, 1998a). The precise physiological role of GPCR–activated non-selective cation channels remains now to be determined. A GPCR-induced depolarization near action potential threshold would have the consequence to increase neuronal excitability of spiral ganglion neurons as demonstrated in experiments using cochlear perfusions of neurotransmitters in vivo (Felix & Ehrenberger, 1992; Schickinger et al., 1996). Such an excitatory effect with SP has also been shown in the rat auditory brainstem (Wang & Robertson, 1998a,b).

The molecular nature of the non-selective cation conductance expressed in spiral ganglion neurons remains to be discovered. Transient receptor potential (TRP) channels (TRPC) have been shown to be good candidates for the non-selective cation channels in other cell types. Indeed, TRPC can be activated by muscarinic GPCR in smooth muscle cells (Lee et al., 2003), and in neuronal PC12 cells (Kim & Saffen, 2005). The coexpression of tachykinin GPCR and TRPC in HEK cells has demonstrated the activation of such TRP non-selective cationic conductance with the application of substance P (Oh et al., 2003). The expression and characterization of TRPC should be now investigated in cochlear neurons in order to determine their potential implication in GPCR-activated non-selective cation channels.

Independent of the identity of the non-selective cation channels, there appears to be cross-talk between GPCR receptors. The depolarizing and calcium responses evoked by ACh, ATP or SP in freshly isolated cochlear neurons show mutual desensitization and non-additivity (Ito et al., 2002). P2YR, mAChR and tachykinin receptors appear to share the activation of common effector channels, i.e., large non-selective cation channels. These results suggest a functional cross-talk between these different neurotransmitter-activated GPCR and that they are closely associated with their effector channels. As discussed below, spiral ganglion neurons are also known to be under a complex regulation by neurotrophins, in particular neurotrophin-3 (NT-3) via the high-affinity tyrosine kinase receptors, trkB and trkC (Zhou, Liu & Davis, 2005). Further investigations are needed to determine whether there is possible cross-talk between GPCR signaling and NT-3 regulation.

Most of the studies discussed in this chapter have been carried out in vitro on freshly dissociated neurons that have lost most of their peripheral and central axon (Ito & Dulon, 2002). We know from these studies that GPCR and non-selective channels are expressed at the perikaryon of the bipolar cochlear neurons, but we don't know, because of the lack of good specific antibodies, whether they are also expressed along the peripheral or central axon and at their synaptic endings.

A particularity of spiral ganglion neurons is that their myelinated ganglion cell bodies (perikaryon) lie directly in the middle of the pathway of incoming nerve impulses. At first glance, this perikaryon appears as a physical barrier that could disturb the propagation of action potentials. However, a great density of internodes (discontinuities in the myelin sheaths), that should help to reduce membrane impedance, is present in this area (Rosenbluth, 1962). At these internode-like structures, the expression of specific sodium and potassium channels could help to regenerate action potentials (Santos-Sacchi, 1993). Interestingly, the contactin-associated protein (Caspr), a protein potentially important for the expression and localization of ion channels, is strongly expressed in the ganglion cell soma (Hossain et al., 2005). It is possible that the expression of GPCRs and non-selective cation channels also participate in reducing membrane resistance and regenerate action potentials at the perikaryon. The origin of neurotransmitter release in this area is unknown. Speculatively, it could arise from an autocrine mechanism or from a paracrine mechanism between the ganglion cell body and its surrounding Schwann cells.

Further investigations are needed to determine whether GPCR and their associated non-selective cation channels are expressed at other locations of the bipolar neurons, such as at the post-synaptic afferent endings below the inner hair cells. Indeed, the unmyelinated efferent fibers of the lateral olivocochlear complex (LOC) contact these synaptic endings and they are certainly the best candidates for a complex neurotransmitter regulation via GPCRs.

Acid-sensing Ion Channels (ASICs) in Spiral Ganglion Neurons Provide a Molecular Sensor of Extracellular pH

ASICs belong to the Degenerin/epithelial sodium channel (DEG/ENaC) family. Many members in this superfamily play roles in mechanosensation in the mammalian skin (Drummond, Abboud & Welsh, 2000; Price et al., 2000, 2001). Until recently ASIC2 and 3 have been considered candidates constituting at least part of the mechanoreceptor for mammalian auditory mechano-transduction (Hille, 2001). A number of groups showed that the hearing threshold is normal in ASIC2&3 knockout mice (Drew et al., 2004; Hildebrand et al., 2004; Peng et al., 2004a; Roza et al., 2004), excluding ASICs' direct involvement in mechanotransduction in the cochlea. However, immunolabeling results clearly showed ASIC2 expression in both the dendritic and soma regions of spiral ganglion neurons in the adult cochlea. Comparisons of proton-evoked excitatory responses of cultured spiral ganglion neurons obtained from wild-type and ASIC2$^{-/-}$ mice identified ASIC2 as the major contributor to proton-activated excitatory responses. The functional role of ASIC2 was revealed by testing the noise sensitivity of ASIC null mice, which are considerably more resistant to noise-induced temporary threshold shifts. Synaptic vesicles contain a high concentration of protons (pH around 5.7; Yuste et al., 2000). With high-intensity sound stimuli to release a large number of synaptic vesicles, local extracellular pH at the synaptic cleft may be transiently lowered (Krishtal et al., 1987; DeVries, 2001; Traynelis & Chesler, 2001). The direct excitation by proton to the ASIC2s in spiral ganglion neuron at higher sound levels may constitute one additional component of the postsynaptic excitatory responses. The observation that absence of the ASIC2 gene reduced noise sensitivity in mice supports this notion (Peng et al., 2004a).

The ASIC2 in the membrane of spiral ganglion neurons could provide cellular sensors to directly convert extracellular acidosis to excitatory responses. This is a mechanism to excite spiral ganglion neurons by a pathway unrelated to mechano-sensory inputs from the hair cells. Many types of cochlear pathology (e.g., inflammation and ischemia) are likely to be accompanied by local acidosis of the extracellular space. Therefore, ASICs offer a cellular apparatus linking hearing losses caused by many enigmatic causes (e.g., ischemia or inflammation of the inner ear) to excitotoxicity.

Neurotrophins Regulate Voltage-gated Ion Channels that Modulate Spiral Ganglion Neuron Firing Patterns

In addition to ATP, neurotransmitters, neuropeptides, and protons, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) also act as modulators of spiral ganglion neuron firing patterns. Although many studies have shown that these members of the neurotrophin family are present within the cochlea during development (for review, *see* Rubel & Fritzsch, 2002), the scope of their actions are only now becoming clear. It is known that all spiral ganglion neurons possess both cognate high-affinity tyrosine kinase receptors trkB and trkC (Ylikoski et al., 1993; Mou et al., 1997; Cochran et al., 1999; Gestwa et al., 1999), and that the survival and outgrowth of these neurons depends upon the presence of BDNF and NT-3 (for review, *see* Fritzsch et al., 2004). However, the role of BDNF and NT-3 goes beyond these functions to determine the electrophysiological phenotype of neurons within the ganglion.

Neurotrophins have been shown to affect ion channel composition in the spiral ganglion and other neuronal cell types (Levine et al., 1995; Jimenez et al., 1997; Baldelli et al., 2000); yet, what is unique about the dual modulatory effects of BDNF and NT-3 on spiral ganglion neuron firing is that they act in concert while having opposite actions. BDNF application for 3–6 days at physiological concentrations (5 ng/ml) causes spiral ganglion neurons to display a consistently 'fast' phenotype characterized by rapid accommodation and abbreviated action potential durations and latencies at threshold. In contrast to this, neurons exposed to NT-3 (3–6 days; 5 ng/ml) displayed firing patterns that were typified by slow accommodation with longer action potential durations and latencies at threshold (Adamson, Reid & Davis, 2002a).

Commensurate with these electrophysiological findings, ion channel distributions, determined with immunocytochemistry, showed predictable patterns. Staining of antibodies raised against voltage-gated ion channels that could contribute to rapid accommodation, abbreviated action potential durations, and rapid afterhyperpolarizations (Kv1.1, Kv3.1 and the large-conductance calcium-activated K^+ channels, respectively) was enriched in neurons exposed to 5 ng/ml BDNF. When staining intensity for these same antibodies was assessed in neurons exposed to NT-3, the apparent levels of these K^+ channels were either reduced or unchanged. The converse result was obtained for an antibody raised against a voltage-gated ion channel that could contribute to prolonging the neuronal response latency (Kv4.2), such that staining intensity was increased in spiral ganglion neurons exposed to 5 ng/ml NT-3 (Adamson et al., 2002a).

Taken together, these findings suggested that the heterogeneous firing properties of spiral ganglion neurons (Mo & Davis, 1997a, b; Mo, Adamson & Davis, 2002) could represent a systematic variation in firing pattern along the length of the cochlea. By

isolating neurons from restricted regions of the cochlea, we confirmed this prediction: cells obtained from the basal and apical regions of the cochlea showed properties similar to cells exposed to BDNF and NT-3, respectively (Adamson et al., 2002b). Moreover, these data indicate that BDNF, and/or its cognate receptor, trkB, should be localized preferentially in the basal regions, and that NT-3, and/or its cognate receptor, trkC, is localized in the opposite orientation (Davis, 2003). These patterns have indeed been found to exist in both postnatal and adult mammalian inner ears for NT-3 and BDNF (Fritzsch, Farinas & Reichatdt, 1997b; Schimmang et al., 2003; Sugawara et al., 2005).

Based upon findings that spiral ganglion neurons are exposed to opposing gradients of BDNF and NT-3 in postnatal and adult animals, and that these neurotrophins have powerful effects upon neuronal firing properties, it is clear that neurotrophin modulation is a key organizational principle in the ganglion. Indeed, the correlation of fast and slow firing features with the preferred high and low sound frequencies in the basal and apical regions of the cochlea suggest strongly that spiral ganglion neuron firing patterns are specialized in a location-dependent manner. However, it is not yet clear why dual gradients are required and whether the electrophysiological phenotypes of neurons themselves are graded in a simple linear fashion or display a more complex organization. In order to approach these types of questions additional studies were undertaken to evaluate the concentration dependence of the effects of neurotrophins on spiral ganglion neuron electrophysiology.

Compared to other neurotrophins, NT-3 is unique because of its promiscuous binding to other high-affinity trk receptors (Segal, 2003). Therefore, exposure to increasing concentrations of NT-3 might produce mixed responses due to its binding to trkB as well as trkC receptors at high concentrations. In a recent study (Zhou, Liu & Davis, 2005) low NT-3 concentrations (5, 7, and 10 ng/ml) caused neurons to exhibit the expected 'slow' electrophysiological phenotype. Once the NT-3 concentration exceeded 10 ng/ml, however, firing patterns became progressively faster, thus generating more of a BDNF-like response. These results open up the possibility that firing pattern distribution along the tonotopic gradient may depend critically upon the concentration of NT-3. For example, if NT-3 is absent or at low concentration in the base of the cochlea, then increases and peaks at the equivalent of 10 ng/ml in the apex, one would expect that the spiral ganglion firing phenotype would be distributed in a smooth gradient, analogous to observations of non-mammalian vertebrate hair cells (Fettiplace & Fuchs, 1999). On the other hand, if NT-3 concentrations exceed 10 ng/ml in mid-cochlear regions and peak at a higher value at the apex of the cochlea, then non-monotonic electrophysiological distributions would be predicted, such that slow firing features would be most prominent in mid-cochlear regions. Studies of ganglion cultures in which multiple identified spiral ganglion neurons can be assessed according to their relative location within the ganglion are currently being carried out to directly assess this issue (Liu & Davis, 2005).

The effects of neurotrophins have revealed a complex interaction between the electrophysiological phenotype of spiral ganglion neurons and their cochlear location. Moreover, these studies have shed some light on fundamental principles of neurotrophin interactions. For example, the effects of BDNF and NT-3 on spiral ganglion neurons provides one of the best examples that cognate high-affinity neurotrophin receptors (trkB and trkC) can signal independently through distinct signaling cascades (Huang & Reichardt, 2003). Furthermore, these studies may address the possible functional significance of NT-3 promiscuous binding to other high-affinity receptors; as a possible mechanism to convert a simple neurotrophin gradient into a complex electrophysiological pattern.

In summary, electrical signaling from the periphery to the auditory brainstem is subject to modulation at many levels, ranging from the acute effects of ATP and protons, the intermediate effects of neurotransmitter- and neuropeptide-activated G-protein–coupled receptors, to the long-term shaping of firing patterns by neurotrophins. The substrates for this modulation are the spiral ganglion neurons, which possess a diverse array of voltage-gated ion channels and neurotransmitter receptors. These studies all support the conclusion that spiral ganglion neurons do not passively convey the synaptic input that they receive from the hair cell receptors, but that they actually refine the electrical signals they transmit into the brain.

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