

Endogenous Dynorphins: Possible Role in Peripheral Tinnitus

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Abstract: Tinnitus has been defined as the perceptual correlate of altered spontaneous neural activity occurring without an external auditory stimulus. Hyperacusis, defined as a collapse of tolerance to sound, is present in 40–86% of those who suffer from disabling forms of tinnitus. Both phenomena often are induced or exacerbated by physical or psychological stress. Biological systems known to regulate the body's overall response to stress use and release endogenous neuroactive opioid peptides. These stress-related neuromodulators consist of products derived from three genetically distinct precursor hormones. Two of these precursor hormones are proenkephalin and prodynorphin. Enkephalin and dynorphin-related peptides exist within the efferent olivocochlear systems (lateral and medial) of several mammalian species, including humans. Prodynorphin derivatives, however, may be restricted exclusively to lateral efferent neurons. Descending lateral efferent axons terminate solely on primary (type I) auditory dendrites innervating cochlear inner hair cells in most species. This action indicates that they play an important role in modulating auditory nerve sensitivity and spontaneous discharge. In a fashion similar to that exhibited by the observed excitatory mechanism of action of dynorphins in the spinal cord, sodium salicylate (aspirin) recently was shown to facilitate the excitatory effects of glutamate in the cochlea. This article provides support for a neurochemical model in which endogenous dynorphins may induce hyperacusis and can contribute to the induction, maintenance, or exacerbation of tinnitus in the auditory periphery by altering auditory type I neural excitability to glutamate.

Keywords: dynorphins; lateral efferent olivocochlear system; NMDA receptors; peripheral tinnitus model; stress

Tinnitus is a serious clinical symptom that may affect 30–40 million individuals nationally [1,2]. In approximately 10 million individuals, the symptom is severe enough to compromise the quality of life significantly [1]. Tinnitus has been described as

the perceptual correlate of altered spontaneous neural activity occurring in the absence of an external auditory stimulus [3–6]. Some 80% of tinnitus sufferers have problems that can be traced to or are associated with changes in the inner ear [1]. Hyperacusis has been defined as a collapse of tolerance to sound, often occurring with no significant loss in hearing sensitivity [7,8]. Hyperacusis is present in approximately 40–86% of those who suffer from disruptive forms of tinnitus; it also can compromise the quality of life in those individuals so afflicted [7–9]. In a large percentage of those suffering from both phenomena, the debility has been traced to the auditory periphery [10–12]. For instance, in one investigation, hyperacusis was shown to involve the peripheral auditory system in 59% of cases [6]. Furthermore, both auditory phenomena are associated with

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Portions of this manuscript were presented at the Second International Tinnitus Think Tank: The Neurochemistry of Tinnitus, Martha Entenmann Tinnitus Research Center and The State University of New York Health Sciences Center at Brooklyn, NY, November 5–6, 1998.

or are induced or exacerbated by physical fatigue or psychological stress [2,6,13–16]. An association has been drawn also between clinical depression (dysphoria) and severe forms of tinnitus, with an incidence reported as high as 60–80% [16–18]. As discussed later, opioid k-receptor ligands administered into the cochlea have been observed to alter auditory neural sensitivity to low-intensity sounds [19]. In a fashion similar to that of the reported effects of dynorphins in the spinal cord, sodium salicylate recently was shown to facilitate the excitatory effects of glutamate in the cochlea [20]. Furthermore, salicylate produces tinnitus concomitant with quantifiable spectral alterations in the “ensemble spontaneous discharge activity” of the auditory nerve [4,5,21]. Our position is that endogenous dynorphin peptides are liberated into the inner hair cell (IHC) synaptic region by the lateral efferent olivocochlear system. These neuroactive substances then interact with nearby excitatory neurotransmitter receptors, producing quantifiable alterations in stimulus-evoked and spontaneous auditory neural activity. During periods of physical or emotional stress, this interaction may be especially pertinent. Furthermore, this hypothesized association may not be restricted only to tinnitus generated at the level of the auditory periphery.

SIGNIFICANCE OF THE LATERAL EFFERENT OLIVOCOCHLEAR SYSTEM

Our understanding of the functional biochemistry of the medial efferent olivocochlear system has advanced at a steady rate [22–24], whereas comparatively little in the way of function is known of the lateral efferent olivocochlear system. Dynorphin-like and enkephalin-like opioid peptides are codistributed within lateral efferent brainstem nuclei, in their descending fiber bundles and, in lateral efferent terminal varicosities, in the cochlea of such species as the guinea pig and rat [25–27]. Though the existence of prodynorphin peptides has yet to be shown in the human auditory system, products of proenkephalin synthesis have been detected in the lateral efferent terminal varicosities of the human cochlea [28]. Preliminary work has indicated also that dynorphin-related derivatives are codistributed within lateral efferent brainstem nuclei of the chinchilla [29], though it remains to be determined whether the descending axons that project into the cochlea also contain dynorphins in this species.

What possible auditory function might be served by these endogenous neuroactive peptides? The axons of descending, lateral efferent brainstem neurons [30] are well-known to terminate on the auditory spiral ganglion type I dendrites that, in turn, innervate the cochlear in-

ner hair cells [31,32]. The axodendritic innervation pattern of these dynorphin-containing terminals suggests that the lateral efferent system participates in modulating auditory neural sensitivity [33–35]. Data from recent pharmacologic studies [36,37] and developmental studies [38,39] also suggest an important role for this system in modulating auditory sensitivity. Consistent with what is currently known regarding neuroactive opioid peptides is our view that auditory neural modulation occurs at axodendritic synapses beneath the IHCs and involves (at least in part) the actions of endogenous opioid substances [40].

BACKGROUND: THE ENDOGENOUS OPIOID PEPTIDES

Identification of three genetically distinct opioid peptide families was brought about by the 1979 application of recombinant DNA biochemistry for the characterization of adrenocorticotrophic and melanocyte-stimulating hormone (ACTH/MSH) [41] and by the 1982 characterization of opioid peptides from endocrine tissue [42–45]. These three identified peptide families are (1) proopiomelanocortin (2) proenkephalin (proenkephalin A), and (3) prodynorphin (proenkephalin B). Neuroactive opioid peptides modulate sensory input through separate and often distinct opioid receptors. These receptors can exist either presynaptically or postsynaptically [46, 47]. Opioid receptors consist of at least four general types: the alkaloid (morphinelike) mu (μ) receptors, the endorphin-sensitive epsilon (ϵ) receptors, the enkephalin-selective delta (δ) receptors, and the dynorphin-selective (ketocyclazocine) kappa (κ) receptors [48–50]. Recently, two new μ -selective peptide ligands were isolated. They are endomorphin-1 (Tyr-Pro-Trp-Phe-NH₂) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH₂) [51]; likely, the endomorphins are the endogenous ligands for the (morphine) μ -opioid receptor [52].

Opioid peptides and their unique receptors exhibit a widespread distribution in central and peripheral nervous system structures involved in vision, audition, olfaction, and somatic sensation. This fact has underscored their neurotransmitter-neuromodulatory role in the filtering of sensory information and in the enhancement and fine tuning of the regulatory functions exerted by other neurotransmitters or hormones [53–58]. Antinociception (analgesia) is the most familiar and most intensively investigated property of such opioid substances as morphine; by definition, all opioids are analgesics [49,59]. Also well-known is that opioid peptides function as neurotransmitters or neuromodulators and that they play an important role in modulating nociceptive sensory input [59–61]. In addition to modulating the sensation-perception of pain, opioid peptides

exert a wide spectrum of physiological-behavioral effects on autonomic function, affect, mood, attention, and physical or psychological stress [62,63]. Indeed, considerable evidence indicates that endogenous opioid peptides are found within, and are released from, neural systems that regulate the body's overall biological response to stress [53,64,65]. In addition to the wide range of observed behavioral responses, a wide range of neurophysiological responses also is produced by the binding of endogenous opioid ligands to their receptors.

Receptor Properties of Opioid Substances

The activation of opioid receptors by endogenous opioid substances produces alterations in intercellular ionic conductances, resulting in either neural inhibition or neural excitation [50,66]. As with most neuropeptides, the receptor-activated ion-channel properties of opioid peptides are mediated by a class of membrane-bound, pertussis toxin-sensitive "G"-proteins [50,61,66]. The G-proteins derive their name from the fact that they bind the guanine nucleotides, guanosine triphosphate, and guanosine diphosphate [67].

More specifically, G-proteins exist in numerous types (e.g., the heterotrimeric Gi-, Go-, and Gs-proteins) [67,68]. As is often the case in the central nervous system [69], the ion channel actions of opioid-receptor linked G-proteins are mediated by the regulation of adenylate cyclase that catalyzes the synthesis of the second messenger, cyclic adenosine 3',5'-monophosphate (cAMP) [47,61]. Therefore, the binding of opioid ligands to their respective receptors often activates a Gi-protein that inhibits adenylate cyclase and decreases cAMP [61,67]. In many other instances, the opioid receptor-linked stimulation of a G-protein may regulate a specific type of ion channel directly, such as a potassium (K⁺) or a calcium (Ca⁺⁺) channel, independent of adenylate cyclase-cAMP. These (other) G-proteins are designated as Go-proteins [48,61,67]. Therefore, in many neural systems, opioid receptors are coupled to either the Gi- or Go-protein, wherein the actions of opioid ligands inhibit adenylate cyclase and K⁺ or Ca⁺⁺ channels are regulated [61]. Opioid receptor activation of the Gi- or the Go-protein has been linked to neural inhibition through an activation of voltage- or Ca⁺⁺-dependent, inward-rectifying K⁺ channel conductances or inhibition of voltage-dependent inward Ca⁺⁺ channel conductances [61,66,70-72].

In contrast to their more widely studied inhibitory properties, opioid peptides also produce neural excitation [70,73,74]. For instance, opioid receptors can be linked to a Gs-protein that, when stimulated, activates adenylate cyclase and increases cAMP [61,67]. Opioid

receptor activation of a Gs-protein has been associated with neural excitation (prolonged action potentials) through a μ -, δ -, or κ -receptor-mediated reduction in an outward voltage-dependent K⁺ conductance or through a κ -receptor-mediated increase in a voltage-dependent inward Ca⁺⁺ conductance [66,75-78].

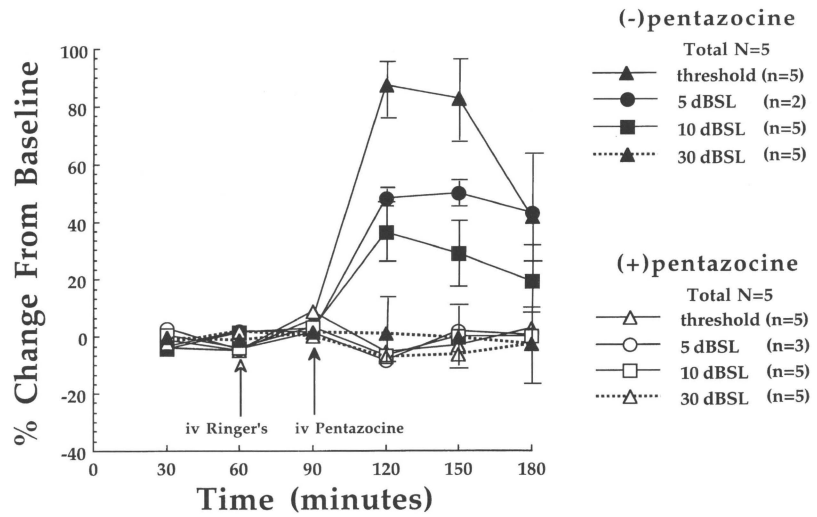
Finally, although neural excitation is possible at all three opioid receptor types, the observed ligand effects at μ - and at δ -receptors most often are reported to be inhibitory, whereas ligand effects at κ -receptors most often are reported to be excitatory [48,73,74]. The results obtained from auditory electrophysiological investigations employing κ -receptor drug agonists has indicated that the putative κ -receptor actions of dynorphins in the cochlea also may be excitatory [19,79,80].

INDIRECT EVIDENCE FOR EXCITATORY κ -RECEPTOR ACTIVITY IN THE COCHLEA

As indicated, dynorphin- and enkephalin-like immunoreactivities have been found in the terminal varicosities of lateral efferent neurons in some species, indicating the presence of these neuroactive peptides in mammalian cochleas [27]. The production of opioid μ -, δ -, and κ -receptors has been demonstrated in the rat brainstem superior olivary complex [81], and some limited evidence has existed for the presence of opioid receptors in the cochlea [82]. Nevertheless, specific receptor subtypes that bind endogenous enkephalins and dynorphins (δ and κ , respectively) have yet to be identified in the cochlea of any mammalian species.

Intravenous (IV) administration of the opioid κ -receptor drug agonist (-) pentazocine produces an enhancement in auditory sensitivity and intensity-dependent amplitude changes in the auditory nerve compound action potential (CAP) in chinchillas [83]. In that investigation, the nonopioid (+) pentazocine, administered at an equivalent IV dose and concentration, was without effect (Fig. 1). Furthermore, results of a subsequent investigation [79] indicated that large auditory neural amplitude changes at twice the dose were naloxone-reversible and occurred with no corresponding changes in the hair cell-generated cochlear microphonic potential (Fig. 2). Such powerful IV μ -receptor agonists as fentanyl and morphine are without effect on the same auditory measures [84-87]. From these investigations, some have argued that the observed auditory neural changes after IV (-) pentazocine reflect drug actions within the cochlea [79,80,83]. This finding is suggested by (-) pentazocine's relatively high tissue solubility [88] and relatively low molecular weight [89,90], factors that should permit an easy passage of (-) pentazocine across the blood-labyrinthine barrier.

Figure 1. Amplitude changes (8 mg/kg IV) after administration of the κ -receptor drug agonist (-) pentazocine (n = 5) or the non-opioid drug agonist (+) pentazocine (n = 5) on the auditory nerve compound action potential (N₁) recorded at the four stimulus intensities indicated. Each plotted point represents the mean percentage of amplitude change relative to a grand baseline amplitude mean (\pm SE) obtained during each of six 30-minute recording periods. Open arrow indicates time of intravenous Ringer's administration. Filled arrow indicates time of intravenous pentazocine administration. [Reprinted with permission from TL Sahley, RH Nodar, Improvement in auditory function following pentazocine suggests a role for dynorphins in auditory sensitivity. *Ear Hear* 15(6):422-431, 1994.]

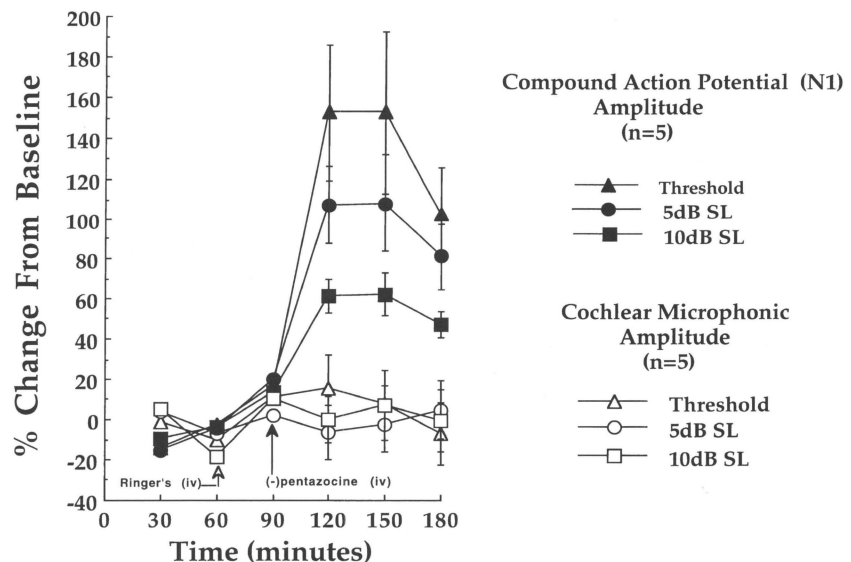


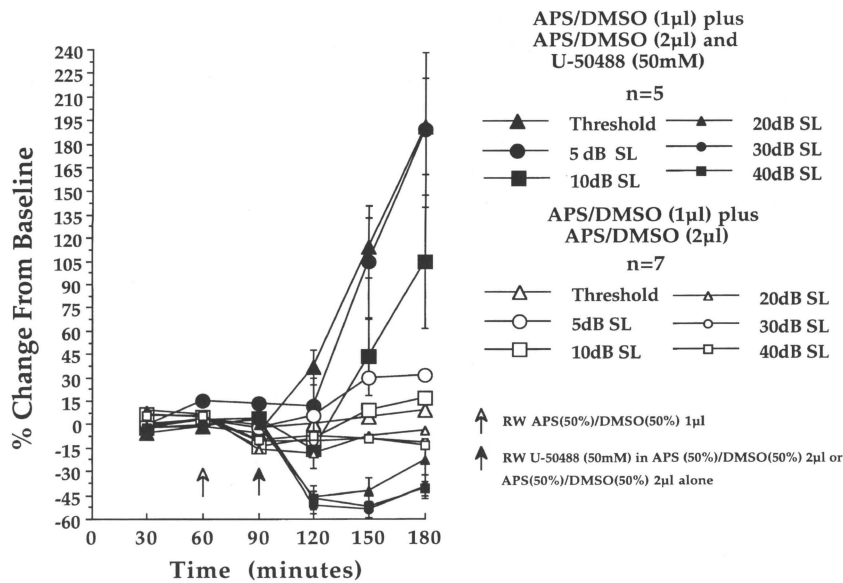
Naloxone HCl (Narcan), the antagonist drug of choice for opioid intoxication, also is known to pass easily through blood-endothelial barriers [59]. Alternatively, the observed auditory neural changes subsequent to IV administration of (-) pentazocine or naloxone may have been, at least in part, an indirect reflection of the actions of both drugs in the brainstem, leading to the subsequent release-suppression of dynorphins, or of some other neurotransmitter-modulator at the lateral efferent-type I auditory synapse. Whether and to what extent such effects occurred from indirect drug actions in the brainstem or from direct actions in the cochlea cannot be evaluated at present. Still unknown is whether κ -receptors exist in the chinchilla brainstem and whether IV (-) pentazocine effects are eliminated

in animals, maintaining ablations to the lateral efferent pathways.

Notwithstanding the foregoing limitations, the existence of functional opioid κ -receptors in the chinchilla cochlea has been suggested indirectly by the demonstration that the highly selective [91,92] κ -opioid receptor antagonist norbinaltorphimine significantly reduces the neural effects of IV (-) pentazocine when it is delivered intracochlearly via surface application to the round window (RW) membrane [80]. Additional evidence supporting the existence of functional κ -receptors in the cochlea is provided by the more recent preliminary report that either (-) pentazocine or the potent κ -receptor-selective opioid agonist U-50488H (Fig. 3) produces increased auditory sensitivity and

Figure 2. Contrasting amplitude changes after administration of the κ -receptor drug agonist (-) pentazocine (16 mg/kg IV) on the auditory nerve compound action potential (N₁) and cochlear microphonic (CM), recorded simultaneously in five animals at the three stimulus intensities indicated. Each plotted point represents the mean percentage of amplitude change relative to a grand baseline amplitude mean (\pm SE) for the two dependent measures, obtained during each of six 30-minute recording periods. Open arrow indicates time of intravenous Ringer's administration. Filled arrow indicates time of intravenous (-) pentazocine administration. [Reprinted with permission from TL Sahley, FE Musiek, RH Nodar, Naloxone blockade of (-) pentazocine-induced changes in auditory function. *Ear Hear* 17(4):341-353, 1996.]





response following cochlear administration of U-50488H and (–) pentazocine. In *Abstracts of the Twenty-Second Midwinter Meeting of the Association for Research in Otolaryngology*, 836:209–210, 1999; and TL Sahley, RH Nodar, *The Efferent Auditory System and Tinnitus. Proceedings of the Second International Tinnitus Think Tank: The Neurochemistry of Tinnitus*. Presented at the Martha Entenmann Tinnitus Research Center and the State University of New York SUNY Health Sciences Center at Brooklyn. November 5–6, 1998.]

intensity-dependent amplitude changes in the auditory nerve CAP when administered intracochlearly [93,19]. The relatively low molecular weights (<1,000 daltons) alone of either drug were predicted to assure a fairly rapid entry (<20 min) into the cochlea [94,95]. Additionally, this action was likely to have been facilitated by our use of the universal solvent dimethyl sulfoxide (DMSO), which made either drug soluble at a 7.4 pH in the artificial perilymph solution. U-50488H exhibits a 120–1,300 times greater binding affinity for opioid κ -receptors relative to its much weaker binding affinity at μ -receptors and is devoid of opioid δ -receptor activity [96,97]. (–) Pentazocine is a potent κ -receptor drug agonist and a weak μ -receptor antagonist. It also is devoid of opioid δ -receptor activity and exhibits the lowest affinity for the opioid μ -receptor of all the benzomorphans [59,97,98]. Therefore, U-50488H and (–) pentazocine each possess pharmacological properties at κ -receptors that very well could mimic the actions of endogenous dynorphins released at the cochlear axodendritic synapse. Hence, endogenous neuroactive dynorphins may play a significant role in modulating auditory neural sensitivity and, perhaps, spontaneous discharge at the cochlear lateral efferent-type I auditory synapse. Though the cochlear mechanism of action of endogenous dynorphins remains unknown, evidence suggests that opioid peptides modulate the actions of putative excitatory (nonopioid) neurotransmitters at their receptors.

Figure 3. Biphasic intensity-dependent amplitude changes after administration of the κ -receptor drug agonist U-50488H (50 mM) delivered to the cochlear round window (RW) in five animals. Each plotted point represents the mean percentage of amplitude change relative to a grand baseline amplitude mean (\pm SE) obtained during each of six 30-minute recording periods recorded at the six stimulus intensities indicated. Open arrow indicates time of administration to the RW of an artificial perilymph (APS; 50%)/dimethyl sulfoxide (DMSO; 50%) solution (1 μ l; pH 7.4) in 12 animals. Filled arrow indicates time of administration to the RW of the 50% APS/50% DMSO solution (2 μ l; pH 7.4) given alone in seven (control) animals or combined with U-50488H in five (experimental) animals. [Reprinted with permission from TL Sahley, RH Nodar, FE Musiek, Changes in the auditory neural

RELEVANCE OF COCHLEAR N-METHYL-D-ASPARTATE RECEPTORS

The initiation of auditory signaling in the mammalian cochlea is highly dependent on the release of excitatory neurotransmitter from the IHCs. Though some uncertainty still exists [99], many investigators have argued that glutamate is the afferent neurotransmitter in the mammalian cochlea [27,100–104]. Adding to the complexity is the fact of even less certainty regarding the receptor type activated by putative excitatory afferent neurotransmitters in the cochlea [99]. Considerable evidence supports the existence of excitatory non-N-methyl-D-aspartate (NMDA) receptors in the normal adult mammalian cochlea [27,99]. In postnatal rats, glutamate-sensitive NMDA receptor subtypes have been reported to appear only transiently in the developing cochlea prior to the onset of hearing [105].

However, evidence supports the gene expression of NMDA receptor subunits in the adult guinea pig cochlea [106]. In addition, alpha-amino-3 hydroxy-5-methyl-isoxazol-propionic acid-induced excitotoxicity and degeneration of cochlear spiral ganglion neurons is accompanied by a neurotropic NMDA receptor action of glutamate in this species [100]. Furthermore, a significant body of pharmacological evidence supports a functional auditory role for glutamate-sensitive NMDA receptors in the adult guinea pig cochlea [101–104,

107–109]. Cochlear NMDA receptors may, however, be restricted to the type I auditory dendrites innervating the modiolar side, as opposed to the pillar side, of the IHCs [109].

Significance of Modiolar-Oriented Type I Auditory Dendrites

Approximately 40% of the radial type I afferent dendrites form two morphologically and functionally distinct groups that contact the modiolar-oriented surface of each IHC. Here, the overall synaptic density of afferents generally is greater as compared to the density of dendrites on the pillar surfaces of the IHCs. In general, the modiolar-oriented units are characterized by relatively lower spontaneous discharge rates (SDRs), higher auditory thresholds, smaller fiber diameters, and lower mitochondrial densities [33,110–112]. In approximately 70% of these units (25–30% of the total), the auditory dendrites may be innervated by a range of 1–32 lateral efferent axons (mean, 10). The remaining 33% of the modiolar-oriented units (10–15% of the total) are extremely mitochondria-poor, exhibit very low SDRs, and have the highest auditory thresholds of all the type I auditory units. In fact, this smaller subset of modiolar-oriented units exhibits thresholds that are 20–80 dB poorer than are the thresholds of the more numerous type I units innervating the IHC pillar side [33,110,111,113]. The dendrites of each of these very low SDR–very high threshold units are innervated by 7–32 lateral efferent axons (mean, 20), an amount twice the number of lateral efferent axons terminating on each of the pillar-oriented type I auditory dendrites [33]. This anatomical array suggests that the lateral efferent system exerts relatively more influence on type I auditory neurons characterized by very low rates of spontaneous discharge and very high thresholds.

NMDA Receptors: Pain and Tinnitus

As is well-known, peripheral tissue inflammation produces functional changes in neurons of the spinal cord dorsal horn. These changes consist of alterations in SDR, a lowering of thresholds, and an enlargement of receptive fields, producing an increased sensitivity to both noxious and innocuous stimuli [73,114–116]. Commonly, this condition is called *hyperalgesia* [74,114,117]. The enlargement of receptive fields and the ensuing hyperalgesia occur via the facilitation of excitatory amino acid effects at NMDA receptors [73,114]. In addition, chronic (prolonged) pain has been attributed to a gradual increase in action potential discharge and excitability in high-threshold spinal cord

neurons in response to repeated peripheral stimulation. This temporal summation of slow synaptic potentials is termed *wind-up*. The wind-up phenomenon depends on the activation of NMDA receptors [118]. NMDA receptor–linked and non–NMDA receptor–linked neurotoxic effects of glutamate or aspartate have been implicated also in a number of severe pathophysiological neurodegenerative disorders [119–121], in auditory neural excitotoxicity [15], in acute ischemia of radial auditory dendrites [27,109] and, of course, in tinnitus [10,11,15,101,122,123].

Recently, a pathophysiological analogy was constructed between chronic nociception (pain) and chronic tinnitus [124]. More specifically, it was constructed between the wind-up phenomena and the associated hyperalgesia observed in chronic pain, and the auditory symptoms of hyperacusis and chronic tinnitus [125]. According to Moller [125], both pain and tinnitus involve neural sensitization that leads to a reduction in threshold or increased levels of neural excitability (or both). For instance, intense sounds are reported to exacerbate tinnitus [126]. Moreover, patients with severe tinnitus report discomfort from intense auditory stimuli, and repeated exposure to such stimuli actually lowers their tolerance to all sounds [125–127]. The wind-up phenomenon associated with pain depends on NMDA receptor activation in high-threshold neurons [118]. Cochlear NMDA receptors also are activated primarily by the presentation of repetitive or relatively intense auditory stimuli [27,108]. As indicated, cochlear NMDA receptors appear to be restricted to the much higher threshold, modiolar-oriented type I auditory dendrites [109]. Finally, sodium salicylate, well-known for its antinociceptive properties and its capacity to produce transient tinnitus, also increases intercellular Ca^{++} levels in neurons [128] and in cochlear outer hair cells (OHCs) [129]. Perhaps more important, however, was the recent demonstration that sodium salicylate potentiates the excitatory effects of glutamate at NMDA receptors in the mammalian inner ear [20], an action consistent with the observations and conclusions made by previous tinnitus investigators [3,130,131].

Cochlear Neuroprotection

Overstimulation of NMDA receptors constitutes a major mechanism in glutamate-induced neurotoxicity; for this reason, NMDA receptor antagonists have been used as neuroprotectants in the prevention of excitotoxic cell injury [120,121]. Noteworthy is that non–NMDA receptors also can mediate neurotoxicity, especially during prolonged or widespread insults [120,132]. Characteristically, NMDA receptor–gated activity produces an influx of extracellular Ca^{++} , which in

excess activates a variety of potentially destructive processes [120,121]. Recently, inner ear damage from overstimulation was shown to be prevented by the cochlear administration of leupeptin, an inhibitor of intracellular Ca^{++} -activated cytosolic proteases (calpains) [15,133]. Though clearly no single drug panacea exists, neuroprotective drug therapy has been proposed also for the alleviation of tinnitus [15,122,123]. Some suggested pharmacological therapies have included anti-anxiolytics, antioxidants, antihistamines, antidepressants, anticonvulsants, antiepileptogenics, diuretics, Ca^{++} channel blockers, dexamethasone, lidocaine, and (of course) glutamate-receptor antagonists [11,12,15]. Blockade of glutamate at cochlear NMDA/non-NMDA receptors with the spasmolytic drug caroverine, has been reported to result in a significant amount of therapeutic success in the treatment of "cochlear-synaptic" tinnitus [10,11,101]. Though such evidence argues that glutamate activation of cochlear NMDA receptors plays a substantial role in inner ear neuroexcitotoxicity and in the etiology, maintenance, or exacerbation of peripheral neural generators of tinnitus, what has become clear also is that stress-related events are more powerful triggers for tinnitus than are strictly auditory-related events [6,7,15,16].

STRESS AND ENDOGENOUS DYNORPHINS

Higher-than-normal levels of reactive anxiety have been reported in tinnitus sufferers, and some speculate that certain individuals may have a greater psychological predisposition or susceptibility to tinnitus than do others [134,135]. A reasonable assumption is that constitutional differences exist with respect to individual reactivities to environmental and physical stress. Equally reasonable is the expectation of individual differences with regard to the degree to which certain endogenous biochemical systems are likely to become activated during stressful events. As with pain, what is becoming clear is that tinnitus must consist of both central and peripheral components; certainly, the response of an organism to stress surely involves the entire organism, both its peripheral and central nervous systems. As has been emphasized, endogenous opioid peptides play a role in the fine-tuning and filtering of sensory information and are released from neural systems in response to stress [53,57]. Emotional or physical stress is known also to induce potent analgesic effects [53,117,136], and "stress-induced analgesia" is likely to involve multiple opioid systems [65,137]. Therefore, an important component of an organism's response to a real or perceived life-threatening emergency is a reduction in responsiveness to pain. This reaction creates little diffi-

culty in envisioning that in meeting the behavioral demands prompted by such exposure, an animal's normal reactions to pain could prove deleterious [117]. Consequently, during threatening conditions, reactions to pain would be suppressed in favor of more adaptive behaviors, enabling an organism to escape or defend itself (fight or flight).

However, an important note is that endogenous opioid μ - or δ -receptor activation by the potent hormone β -endorphin or by endogenous enkephalins produces not only antinociception but also an emotional state of well-being (euphoria). The opioid effects of κ -receptor ligands, however, often are accompanied by emotional states of dysphoria, disorientation, or depersonalization [59,98,138], and whether dynorphins are capable of producing any form of antinociception is questionable [74,139]. Also endogenous dynorphins have been demonstrated to potentiate, rather than to suppress, behavioral changes induced by stressful events [63]. Furthermore, evidence suggests that the aversive motivational properties of endogenous and exogenous opioids are mediated through peripheral κ -opioid receptors [140, 141].

Therefore, how might endogenous dynorphins be used in the auditory periphery to modulate or maintain the aversive qualities of excessive (or even abnormally synchronous) auditory neural activity? Does any evidence support that a neuropharmacological link exists between endogenous κ -receptor-sensitive dynorphins (or enkephalins) and neural excitability?

Enkephalin Interactions with NMDA Receptors in the Cochlea

Type I afferent dendrites innervating cochlear IHCs are themselves innervated by lateral efferent axons [31,32] and, as indicated, some evidence suggests that the lateral efferent system modulates auditory neural sensitivity [36,37]. The preceding review seems to clarify that only a few studies have investigated the potential effects of specific opioid receptor agonist-antagonist substances on the discharge of the auditory nerve. The results of those investigations indirectly have supported the existence of opioid receptor subtypes in the cochlea. As indicated, enkephalin-like opioid peptides are codistributed within lateral efferent terminal varicosities in the cochlea of species (e.g., the guinea pig and rat) [25,27]. Iontophoretic application of the δ -opioid receptor ligand [Met⁵]-enkephalin produces a naloxone-reversible reduction in excitatory amino acid-induced auditory neural excitation in the guinea pig cochlea. The δ -receptor effect, however, occurs at both NMDA and glutamate-sensitive non-NMDA receptors, though enkephalin appears to exert a stronger inhibi-

tory effect on non-NMDA receptor-induced auditory neural activity [142]. Such evidence is consistent with the widely observed inhibitory properties of enkephalins, and indicates that endogenous enkephalins acting through δ -receptors modulate excitatory neural activity at glutamate-sensitive NMDA and non-NMDA receptors in the mammalian cochlea. Also fairly likely is that such effects normally are initiated via the lateral efferent system.

Dynorphin Interactions with NMDA Receptors

As indicated, the reported inhibitory effects at opioid δ - and μ -receptors contrast sharply with the effects observed after the administration of κ -receptor ligands, effects that most often are excitatory [48]. Endogenous dynorphins have been reported to interact with glutamate-sensitive NMDA receptors in rat cortical neurons [143], though the precise mechanism is not yet understood [144]. Unlike previously reported δ -receptor ligand effects [142], dynorphins do not appear to interact with excitatory non-NMDA receptors [143], though potential interactions between these substances and glutamate-sensitive NMDA and non-NMDA receptors have yet to be demonstrated in the mammalian cochlea. Nevertheless, dynorphins have been proposed to play a significant role in pathologies of central and peripheral inflammation [73,115,144,145]. For example, in the spinal cord (dorsal horn), dynorphins induce an NMDA receptor antagonist-reversible enlargement of neural receptive fields. The receptive field enlargement results in a greater number of stimulated neurons and produces hyperalgesia [73,115].

Peripheral inflammation also is associated with peripheral (and central) receptive field enlargement [116]. In addition, peripheral inflammation leads to a large increase in prodynorphin gene expression (increased mRNA) in dorsal horn neurons, and this central nervous system increase parallels the development of hyperalgesia [74,146]. Dynorphins have been implicated also in the wind-up-like activation of NMDA receptors, leading ultimately to neurotoxicity [147]. For example, dynorphins produce neurotoxic effects in the spinal cord [139], and the κ -receptor-induced neurotoxicity and neurological dysfunction occur through an enhanced excitotoxic activity at NMDA receptors [73,144,148]. The excitotoxic and neurotoxic actions of dynorphins at the NMDA receptor arise through a κ -receptor-mediated facilitation of NMDA receptor sensitivity to glutamate or to aspartate [114,147]. Moreover, the existence of an activity-dependent neuronal plasticity in response to tissue injury has been hypothesized to account for the increased sensitivity and spontaneous pain associated with enhanced dorsal horn excitability at

NMDA receptors by endogenous dynorphin peptides [114,73]. Finally, the κ -receptor antagonist norbinaltorphimine has been found to be a potent antagonist of NMDA receptor activity, and a neuroprotective role has been suggested for this agent in the treatment of NMDA-induced convulsions and neurotoxicity [144, 149]. Such evidence, taken together with more recent data [19], is consistent with the widely observed excitatory properties of dynorphins, further suggesting the possibility that endogenous dynorphins, acting through κ -receptors, facilitate the excitatory neural actions of glutamate in the inner ear.

Intracochlear perfusion of glutamate or aspartate at high doses has been reported to reduce the discharge of auditory units during the presentation of sound [150]. Such effects have been attributed to auditory neural overstimulation [27]. A tempting speculation is to envision the changes that high concentrations of cochlearly administered κ -receptor drug agonists would produce on auditory neural discharge as an auditory stimulus is progressively increased in intensity from threshold. As the stimulus level is increased, glutamate concentrations in the inner ear also would increase but would be potentiated abnormally at each level of increase as a consequence of a κ -receptor facilitation of glutamate at NMDA receptors. Therefore different—even opposite—effects on neural amplitude would be predicted at each progressive increase in stimulus level from auditory neural threshold.

Consistent with this hypothesis are the intensity-dependent biphasic changes in neural amplitude recently observed after a cochlear RW administration of the κ -receptor ligands U-50488H (see Fig. 3) and (–) pentazocine [19,93]. At threshold and at relatively lower stimulus levels, these substances each promoted or enhanced neural excitation. At higher stimulus levels (≥ 20 dB above threshold), each produced neural suppression.

Similar intensity-dependent changes in neural amplitude have been observed consistently after IV administrations of (–) pentazocine [79,80,83]. However, in these investigations, neural suppression never was observed at higher stimulus levels. Whether these changes reflect actions at glutamate-sensitive NMDA, non-NMDA, or both receptor types, or at some other nonopioid receptor requires further investigation.

The precise mechanism of dynorphin interaction at the NMDA receptor is unknown. Endogenous opioids have been suggested to modulate excitation at NMDA receptors [147] by (1) increasing the affinity of the ligand for the receptor (2) decreasing the degree of dissociation of the ligand from the receptor, or (3) by producing a change in the second messenger response to receptor activation.

POSSIBLE ROLE OF DYNORPHINS IN TINNITUS

As indicated, some 80% of tinnitus sufferers have problems that can be traced to, or associated with, changes occurring in the inner ear [1]. However, as with all perceptions (and especially those that involve aversion), the experience of tinnitus certainly must involve central auditory pathways and cortical regions and nonauditory cortical-subcortical regions. Nevertheless, a peripherally generated tinnitus can result from any number of insults to the inner ear, ranging from advancing age to excessive noise exposure [2,151]. The OHCs appear to be the inner ear structures most susceptible to damage in general and to noise damage in particular [152–155], and often tinnitus is associated with OHC dysfunction [3,156,157]. Abnormally elevated spontaneous discharge rates within single auditory neurons often are observed when cochlear noise damage is confined to the OHCs [153,154,158]. Normally, OHCs serve to damp activity in unstimulated regions of the cochlear partition, thereby decreasing the auditory sensitivity at (irrelevant) frequencies corresponding to those regions [153,159–161]. This property of OHCs contributes greatly to the sharp tuning of the cochlear partition [161–164]. One popular theory [157,165] is that damaged or dysfunctional OHCs decouple from the tectorial membrane, owing to a loss in their hydrostatic pressure [166] or to stereocilia damage [153]. The corresponding loss in their normal damping properties leads to an increased production of thermal noise within specified regions of the cochlear partition. This added shift in the neural discharge spectra then is interpreted erroneously by the central auditory system as a genuine signal [3,4].

Tinnitus may, in some cases, be the immediate source of a stressful experience, though many individuals report that they begin to notice their tinnitus *only* during periods of high stress [167]. According to one view, tinnitus results from the stress-induced central enhancement and magnification of abnormal but weak triggering signals passing from the auditory periphery to the auditory cortex, where they are perceived [1]. Our position however, is that during stressful episodes, these abnormal signals (triggers) are not weak but are in fact abnormally amplified in the periphery. Though signal enhancement or increased synchronicity (or both) may occur at any location in the auditory system during extreme episodes of anxiety or stress [1], the initial neural changes involved in most forms of tinnitus begin in the auditory periphery [127].

Results from an early investigation of cochlear neurotransmitter candidates in guinea pig perilymph (though excluding an analysis of perilymphatic dynorphin-like neuropeptides) indicated that exposure to intense and

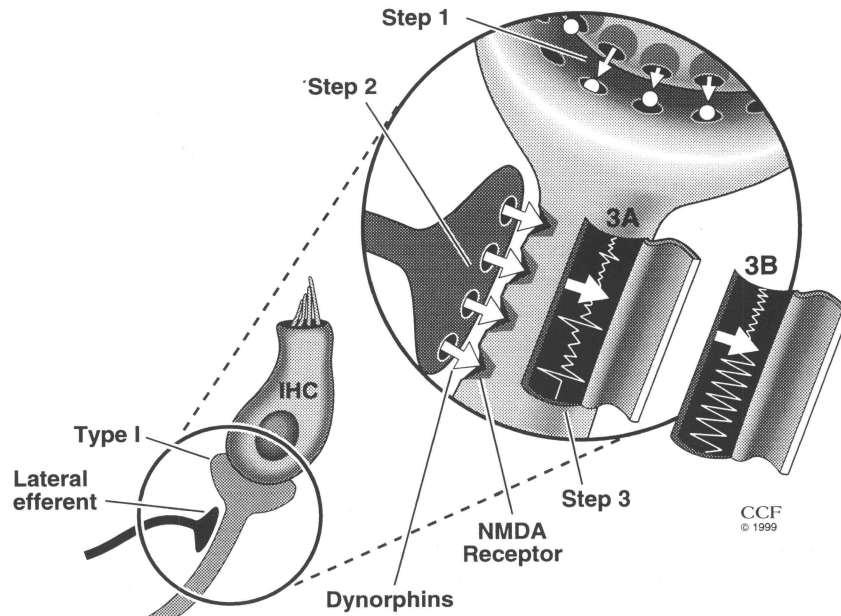
presumably stressful wide-band noise (80–115 dB SPL) significantly elevates levels of [Met⁵]-enkephalin-like opioid peptides, relative to control values obtained in quiet [168,169]. Intense emotional stress has been reported also to improve auditory thresholds temporarily in guinea pigs [170], and we have observed improved auditory thresholds after the cochlear administration of opioid κ -receptor ligands [19]. Therefore, we are proposing that a stress-induced release of neuroactive dynorphin-like opioid peptides from the lateral efferent terminals located beneath the IHCs contributes to an increase in auditory neural sensitivity and to spectral alterations in the ensemble spontaneous discharge activity of the auditory nerve.

What possible biological significance could be found in such a mechanism? The amplitude enhancement of weak auditory patterns characterizing tinnitus and hyperacusis has been suggested to be part of an evolutionary survival reflex [171]. This position is consistent with our view. Therefore, as part of an overall response to stress during actual or perceived life-threatening situations (as in extreme anxiety), endogenous neuroactive dynorphin peptides are released into the mammalian cochlea at axodendritic synapses beneath the IHCs and are released throughout the central nervous system. The result is to advance an affected organism to a heightened state of auditory vigilance [40], which may be adaptive in the short-term when a rapid fight-or-flight response is required.

A physiological shift of the auditory periphery into a temporary state of vigilance is, in our view, synonymous with the production of a “hyperacoustic” state, whereby most auditory stimuli are amplified. Individual constitutional differences naturally would dictate the degree to which peripheral (and central) dynorphin systems are activated (and consequently the severity of the subjective hyperacusis). However, those auditory (type I) neurons innervating a cochlea having sustained weakened or damaged OHCs (and therefore preordained to generate aberrant spectral discharge patterns) would be additionally susceptible to stress. The release of dynorphins and perhaps the subsequent enhanced neural sensitivity to excitatory neurotransmitter activation [73, 148] would result in amplified spectral alterations in the ensemble spontaneous discharge activity associated with tinnitus [4], thereby exacerbating or revealing the preexisting symptom.

Alternatively, depending on NMDA receptor sensitivity (or susceptibility) or on the reactivity of the lateral efferent system to the stressor, dynorphins released into an uncompromised cochlea nevertheless could result in the generation of spectral alterations in the ensemble spontaneous discharge (Fig. 4). Therefore, under certain stressful conditions, dynorphins alone may

Figure 4. Neurochemical model of peripheral tinnitus. Step 1: Excitatory neurotransmitter (glutamate) is released by the inner hair cell (A) during the presentation of a stimulus or (B) spontaneously during periods of quiet and binds to glutamate-sensitive (possibly *N*-methyl-D-aspartate) receptors located postsynaptically on the type I auditory dendrites. Step 2: Endogenous dynorphin-related opioid peptides released from lateral efferent terminals tonically, or during physical or emotional stress, interact with *N*-methyl-D-aspartate receptors located postsynaptically on the type I auditory dendrites, to potentiate the excitatory properties of glutamate. Step 3A: Neural responses to relatively low levels of an auditory stimulus are enhanced [19]. Alternatively, spectral anomalies in the ensemble spontaneous discharge associated with tinnitus may be amplified or generated, as illustrated in step 3B. (Reprinted with permission of The Cleveland Clinic Foundation.)



serve as a trigger for tinnitus. This event might occur in a manner that perhaps is similar to that described after receipt of sodium salicylate [4,5,21], shown recently to potentiate glutamate effects at NMDA receptors in the mammalian cochlea (20).

At higher levels of the neuraxis, of course, response selection would become advantageously limited or narrowed, as all the enhanced signals from the auditory periphery, including the aberrant activity interpreted as tinnitus, are assigned a significant though equal motivational saliency. In the long term, the consequence of this prolonged vigilant state is expected to produce a vicious feed-forward cycle. This hypothesized feed-forward cycle is similar conceptually to the wind-up phenomenon, which could serve to advance the organism to a state of peripheral and central auditory neural excitotoxicity and neurotoxicity.

Opioid Modulation at Central Levels

A detailed account of the NMDA receptor–dynorphin peptide distribution in the central nervous system is beyond the scope and intent of this article. Investigations designed to determine the distribution of κ -receptors and dynorphins in specific brainstem auditory pathways and in cell layers of critical auditory nuclei have yet to be performed. However, an important note is that though the focus of this article has been the auditory periphery, the possibility also exists for concurrent interactions of endogenous dynorphins with NMDA receptors at locations within the central auditory system.

First is the evidence supporting the existence of lateral efferent projections to the cochlear nuclear complex [31]. Opioid prodynorphin (and proenkephalin) derivatives have been detected throughout the medullary brainstem, in regions that have included the separate divisions of this nuclear complex [54,172,173]. Furthermore, both pharmacological [174,175] and in situ hybridization investigations [176,177] have provided evidence for the existence of NMDA and non-NMDA receptors in the mammalian cochlear nuclear complex. Additionally, a considerable number of prodynorphin-positive neurons have been detected within the inferior colliculus [173].

Finally, a likely certainty is that the mechanisms involved in the generation and maintenance of tinnitus are represented neither simply nor narrowly in central auditory pathways or in the auditory periphery (or in both). For instance, the release of excitatory amino acids (and therefore the observed hyperexcitability at NMDA receptors in neurons of the spinal cord dorsal horn) is facilitated by the release of the calcitonin gene-related peptide (CGRP) [73]. We have found CGRP immunoreactivity in a significant number of cells in the dorsal cochlear nucleus and in neurons of the ventromedial region of the lateral superior olive (LSO) [178]. Presently unknown is whether this group of LSO neurons projects specifically to the chinchilla cochlea as part of the lateral efferent system. In the rat, however, the medial limb of the LSO contains a large percentage of efferent neurons [179], suggesting that the CGRP-positive neurons observed in the chinchilla

may be part of the lateral efferent system of neurons projecting to the cochlea.

In a pilot study involving cochlear whole-mount preparations, we have observed dense immunohistochemical staining for CGRP in the IHC region of the organ of Corti in this species. Such evidence raises the possibility that a dynorphin modulation of neural excitability through, perhaps, an enhancement of glutamate effects at NMDA receptors may occur at serial locations along the auditory pathway. This possibility and the precise relationship of κ -receptor ligands to NMDA receptor-activated neural discharge in the inner ear will be the focus of future investigations.

SUMMARY

We have presented a biochemical model to explain one possible cause of the phenomenon called *tinnitus*. Recognizing that this symptom represents a broad spectrum of auditory experiences, we acknowledge the possibility of an equally broad spectrum of auditory generators. However, we are compelled to conclude that when a human organism is confronted with any number of stressful events, conditions are optimal for the overall release of neuroactive opioid substances and the generation and perception of tinnitus. Indeed, the model lends itself as well to an explanation of hyperacusis.

ACKNOWLEDGMENT

This study was supported by the NIDCD, National Institutes of Health, Bethesda, MD. R03-DC03360

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