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Excitability of auditory brainstem neurons, in vivo, is increased by cyclic-AMP

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Abstract

Physiological control of auditory neural responses is critical for accurate representation of acoustic information, such as sound source localization and speech perception. Central auditory neural responses are almost certainly regulated by a range of mechanisms, including second messenger systems, such as the cAMP pathway. An increase in spontaneous neural discharge is known to accompany cochlear insults. Here we report that an increase in spontaneous as well as tone-evoked discharge can also be induced by pressure application of forskolin, a pharmacological agent that elevates intracellular cAMP level by activating adenyl cyclase. The forskolin induced increase in superior olivary complex (SOC) brainstem neurons is specific, dose-dependent, and reversible, whereas application of artificial cerebrospinal fluid (aCSF, the vehicle) does not alter activity. Forskolin-application also has a relatively greater effect on spontaneous activity compared to tone evoked responses. Blockade of the hyperpolarization-activated current, I_h, by ZD7288, consistently reversed the effects of forskolin. Based on these findings, we propose that the second messenger, cAMP, can significantly modulate neural excitability and spontaneous discharge in SOC neurons, principally by shifting the activation of I_h channels.

1. Introduction

The mechanisms underlying detrimental or pathological changes in individual auditory neuron excitability, such as increased (e.g., tinnitus) or decreased (e.g., sluggish neurons in aged animals) activity, have not been elucidated. It is well accepted that hearing impairment resulting from intense sound is often accompanied by tinnitus, and hyperacusis (Jastreboff, 1990; Axelsson and Barrenas, 1992; Kaltenbach et al., 2004). Cochlear damage due to intense sound exposure (Willott and Lu, 1982; Kaltenbach et al., 1996, 2000, 2004; Kaltenbach and Afman, 2000; Brozoski et al., 2002), ototoxic agents (Chen and Jastreboff, 1995; Salvi et al., 1990, 2000; Rachel et al., 2002), or cochlear ablation (Gerken, 1979) is also associated with elevated rates of spontaneous neuronal discharge, and persistently enhanced excit-

Abbreviations: cAMP, cyclic-AMP, cyclic-adenosine monophosphate; SOC, superior olivary complex; aCSF, artificial cerebrospinal fluid; I_h, hyperpolarization activated, mixed, cationic conductances; SA, spontaneous activity; ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; CNS, central nervous system; PDE4, phosphodiesterase E4; PKA, phosphokinase A; WGA-HRP, wheat-germ agglutinin horseradish peroxidase; dB SPL, decibel sound pressure level; PSTH, post-stimulus time histogram; EI, neuron excitated by ipsilateral but inhibited by contralateral auditory stimulus; TMB/GOD, tetramethhylbenzidine/glucose oxidase; LSO, lateral superior olive; BF, best-frequency; SD, standard deviation; HCN, hyperpolarization activated cyclic nucleotide gated

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ability of auditory neurons. Several types of plasticities in the central auditory pathway following cochlear damage may affect excitability. Morphological changes after cochlear ablation or intense sound exposure include degeneration of auditory nerve fibers followed by growth and rearrangement of new synaptic contacts (Benson et al., 1997; Bilak et al., 1997; Potashner and Suneja, 1999; Muly et al., 2002, 2004). Changes in transmitter release (Potashner et al., 1997; Suneja et al., 1998a), and glycine and AMPA receptors (Suneja et al., 1998b, 2000) in the cochlear nuclei have been shown following cochlear ablation. AMPA receptor binding in ventral cochlear nuclei is also affected after intense sound exposure (Muly et al., 2004). Recently, cochlear ablation has been shown to activate extracellular signal-regulated kinase (ERK) and the stress-activated protein kinase (SAPK) signal transduction pathways within central auditory neurons (Suneja and Potashner, 2003). The ERK pathway alters genetic expression and likely contributes to plastic changes in the CNS (Xia et al., 1996; Fields et al., 1997; Grewal et al., 1999; Pearson et al., 2001; Impey et al., 1999; Mazzucchelli and Brambilla, 2000; Sweatt, 2001). In addition, ERK enhances inhibition of phosphodisterase E4 (PDE4), an enzyme that hydrolyzes intracellular cAMP, and therefore, should be associated with elevated intracellular cAMP concentrations. In this study, the role of cAMP on auditory neuron excitability was examined.

Intracellular cAMP levels can directly or indirectly (e.g., via protein kinase A (PKA) dependent pathways) alter the activity of many ionic conductances and transmitter systems. Recently, it has been recognized that transmitters, affecting adenyl-cyclases, induce membrane depolarization and increase excitability in many neurons, due to elevations in cAMP acting principally on the hyperpolarization-activated mixed cationic conductance (I_h) channel (Saitow and Konishi, 2000; Chapin and Andrade, 2001; Bickmeyer et al., 2002; Sun et al., 2003; Pisani et al., 2003). An intracellular cAMP-binding site (Santoro et al., 2000) on these channels inhibits I_h activation in the absence of cAMP (Shin et al., 2001; Wainger et al., 2001). We have found that blockade of I_h channels significantly reduces tone-evoked and SA in superior olivary complex (SOC) neurons, and the level of reduction in SA was highly positively correlated with resting firing levels (Shaikh and Finlayson, 2003). In addition, cyclic-AMP significantly modulates I_h channels and thereby intrinsic membrane properties in most auditory neurons (e.g., in the medial nucleus of trapezoid body, Banks et al., 1993; Cuttle et al., 2001; spiral ganglion, Mo and Davis, 1997; cochlear nucleus, Bal and Oertel, 2000). Therefore, cAMP levels can directly and rapidly increase I_h activation, which we predict will increase auditory neural excitability in vivo.

Neural excitability can also be controlled by many PKA dependent processes, which are activated by cAMP (Pedarzani and Storm, 1995; Santoro et al., 2000). Increased intracellular concentration of cAMP may affect neuronal excitability not only by PKA dependent phosphorylation of ion channels (such as voltage gated calcium channels and voltage gated sodium channels), but also by increasing intracellular Ca^{2+} concentration and thereby enhancing transmitter release (Qu et al., 1995; Klugbauer et al., 1999; Monteil et al., 2000; Cribbs et al., 1998; Lee et al., 1999; Pemberton et al., 2000; Kaczmarek and Strumwasser, 1984; Hampson et al., 1995; Enyeart et al., 2000; Hoffman and Johnston, 1998; Starodub and Wood, 2000; Yao and Wu, 2001). Therefore, altered levels of intracellular cAMP could affect neuronal physiology via several mechanisms. Plastic changes, such as increased ERK levels, which follow the cochlear insults should increase intracellular cAMP levels and elevate spontaneous neural discharge, and excitability.

We examined the effects of forskolin-application on tone-evoked and spontaneous firing rate of SOC neurons. Forskolin, an activator of adenyl cyclase, increases intracellular cAMP concentrations and is a suitable tool to manipulate intracellular cAMP levels in vivo. As cAMP can affect a range of mechanisms, which modulate neural excitability, we also investigated the possibility that cAMP-elevation (by forskolin) activate I_h channels and thereby increase excitability of SOC neurons, by sequentially applying forskolin, and the I_h channel blocker, ZD7288.

2. Materials and methods

2.1. Animal preparation and surgery

The Animal Investigation Committee at Wayne State University approved the care and use of animals reported in this study. Anesthesia of young adult male Long Evans rats (3–6 months in age) weighing 499 to 600 g was induced with a combination of ketamine (85 mg/kg) and xylazine (3 mg/kg), and followed 2–5 min later by an initial dose of 44 mg/kg sodium pentobarbital. Supplemental doses of anesthetics were administered as required every 2–3 h, indicated by the presence of toe pinch and eye blink reflex, alternating between ketamine (40 mg/kg) and sodium pentobarbital (25 mg/kg). The animal's rectal temperature was maintained at 37 °C by a thermostatically controlled DC heating pad.

Following surgical exposure and examination of the external auditory canals, the animal was placed in the stereotaxic plane of Paxinos and Watson (1982) using ear bars. Following fixation of the skull with a custom-made head holder, ear bars were removed, and earphones sealed within the external auditory canals to produce closed auditory stimulus delivery paths. Using