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Research paper

Effects of salicylate application on the spontaneous activity in brain slices of the mouse cochlear nucleus, medial geniculate body and primary auditory cortex

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The paper is dedicated to Prof. Aage Moller, a pioneer in tinnitus research with everlasting inspirations.

Abstract

Salicylate is a well-known substance to produce reversible tinnitus in animals and humans as well. It has been shown that systemic application of salicylate changes the neuronal spontaneous activity in several parts of the auditory pathway. The effects observed in central auditory structures *in vivo* could be based upon the changed afferent cochlear input to the central auditory system or in addition by a direct action of salicylate onto neurons within the auditory pathway.

A direct influence of local salicylate application on spontaneous activity of central auditory neurons has already been described for the inferior colliculus (IC) in brain slice preparations. As spontaneous activity within all key structures of the central auditory pathway could play an important role in tinnitus generation, the present study investigated direct effects of salicylate superfusion on the spontaneous activity of the deafferented cochlear nucleus (CN), medial geniculate body (MGB), and auditory cortex (AC) in brain slices.

Out of 72 neurons, 73.4% responded statistically significantly to the superfusate by changing their firing rates. 48.4% of them increased and 51.6% decreased their firing rates, respectively. The mean change of firing rate upon salicylate superfusion was 24.4%. All responses were not significantly different between the brain areas.

The amount of neurons which responded to salicylate and the mean change of firing rate was much higher in the IC than in the CN, MGB and AC. This contributes to the hypothesis that salicylate-induced tinnitus is a phantom auditory perception mainly related to hyperexcitability of IC neurons.

However, the present results suggest that the individual, specific salicylate sensitivity of CN, MGB and AC neurons can modulate the salicylate-induced generation of tinnitus.

Keywords: Tinnitus; Sodium salicylate; Cochlear nucleus; Medial geniculate body; Auditory cortex; Spontaneous activity; Brain slice

1. Introduction

A current model to investigate the generation of tinnitus is the systemic application of salicylate in high doses. Salicylate is known to produce reversible tinnitus in humans (Sée, 1877; Jager and Alway, 1946; Mongan et al., 1973; Halla and Hardin, 1988; Day et al., 1989; Halla et al., 1991; Hicks and Bacon, 1999) and animals as well (Jastreboff et al., 1988; Bauer et al., 1999; Ruttiger et al., 2003).

Abbreviations: AC, primary auditory cortex; cACSF, carbogenized artificial cerebrospinal fluid; cACSF-S, carbogenized artificial cerebrospinal fluid with sodium salicylate; CN, cochlear nucleus; IC, inferior colliculus; MGB, medial geniculate body; PO/AH, preoptic anterior hypothalamic area

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Earlier studies showed that sodium salicylate reduces markedly the cochlear blood flow by vasoconstriction (Didier et al., 1993), activates cochlear NMDA-receptors (Guitton et al., 2003), changes the outward potassium current and thus the resting potential in outer hair cells (Liang and Zhong, 2002). Further salicylate impairs electromotility of cochlear outer hair cells and leads to subsequent functional and structural changes, i.e., a reduction of electromotile length changes (Shehata et al., 1991) and lateral wall stiffness (Russel and Schauz, 1995; Lue and Brownell, 1999). Besides these cochlear effects some studies investigated the consequences of systemic salicylate application on central auditory structures. It was demonstrated that salicylate increase c-fos expression in the dorsal cochlear nucleus as well as in the inferior colliculus (Wu et al., 2003). Further, the neurotransmitter expression changed markedly in the inferior colliculus (GABA decrease, glutamate increase) during salicylate application (Yin et al., 2006) and the serotonergic activity was increased in the inferior colliculus and auditory cortex (Liu et al., 2003).

The main common feature of systemic salicylate application is the modulation of neuronal spontaneous activity (e.g., of the auditory nerve – Evans et al., 1981; Evans and Borerwe, 1982; Schreiner and Snyder, 1987; Kumagi, 1992; Muller et al., 2003, e.g., of the inferior colliculus – Jastreboff and Sasaki, 1986; Chen and Jastreboff, 1995; Manabe et al., 1997, e.g., of the auditory cortex – Ochi and Eggermont, 1996; Kenmochi and Eggermont, 1997; Eggermont and Kenmochi, 1998; Yang et al., 2006).

Hence, tinnitus can be regarded as a perceptual correlate of altered spontaneous electrical activity in the central auditory pathway.

It is unclear as yet to what extent peripheral or central salicylate effects contribute to the generation of tinnitus. One major obstacle to solve this problem is a methodological bias. The systemic salicylate application is frequently used in experimental studies. On one hand, activity changes within the central auditory pathway after salicylate application *in vivo* can result from an alteration of the cochlear input and – on the other hand – by an additional direct action of salicylate on central auditory neurons. A direct action of salicylate application on central auditory neurons was described for the inferior colliculus (IC) (Basta and Ernst, 2004). Further the mechanisms of such a direct drug action on central neurons were investigated in detail for the IC and the auditory cortex. These recordings were done in brain slice preparations or with dissociated neurons. The results showed that the direct action of salicylate on central neurons is based on the depression of the delayed rectifier potassium current (Liu and Li, 2004a) and the blockage of voltage-gated sodium channels which shift the inactivation curve into the hyperpolarization range (Liu and Li, 2004b). It could also be demonstrated that salicylate reduces inhibitory postsynaptic currents in neurons of the auditory cortex (Wang et al., 2006).

However, all these studies were not carried out in spontaneously active neurons. The spontaneous activity seems to play an essential role in tinnitus generation (Møller, 1995) so that the other deafferented key structures of the central auditory pathway – apart from the IC (Basta and Ernst, 2004) – should be investigated as well by direct salicylate application. It is therefore the aim of the present study to investigate the direct influence of salicylate superfusion on the spontaneous neuronal firing rate in brain slices of the cochlear nucleus (CN), medial geniculate body (MGB) and primary auditory cortex (AC).

2. Materials and methods

The experiments were carried out in 17 young, healthy mice of both sexes (*Mus musculus*, NMRI strain) after the onset of hearing (day 20–45). The study protocol was in accordance with the European Communities' Council Directive and Institutional Animal Care Guidelines. All efforts were made to minimize pain or discomfort to the animals. On the day of experiment, the animal was decapitated and the brain was carefully taken out. Using a vibrating microtome (Vibroslice 752, WPI, Aston, UK), 200 μm thick, frontal slices including the CN, MGB or AC were microdissected. After 2 h of incubation in carbogenized (95% O_2 – 5% CO_2) artificial cerebrospinal fluid (cACSF) at 35 °C, one slice was transferred to a submerged-type recording chamber. The recording chamber was continuously perfused (4.5 ml/min) with heated (37 °C) cACSF (buffered at pH 7.4, osmolarity adjusted at 298 mOsm). The cACSF contained the following concentrations (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.8 $MgSO_4$, 1.6 $CaCl_2$, 10 glucose, 26 $NaHCO_3$.

The temperature of the bath solution was measured with a thermistor probe in the recording chamber and maintained within a small range (±0.2 °C) by a temperature controller (npi-electronics, Germany). The heating equipment was connected with the cACSF reservoir and a second bottle which contained cACSF with sodium salicylate (Sigma, Germany) (buffered at pH 7.4, osmolarity adjusted at 298 mOsm) at a concentration of 1.4 mM (cACSF-S). This plasma concentration (1.4 mM salicylate = 22.4 mg/dl) is known to induce tinnitus in rats (Bauer et al., 1999) and humans (Myers and Bernstein, 1965; Jardini et al., 1978; McFadden and Plattsmier, 1984). During the experiment, the inflow could be switched between the two solutions. In additional experiments the effect of different salicylate concentrations (1.4, 0.7, 0.35 mM) on the neuronal activity was investigated. Further the responsibility of a non-auditory brain area (preoptic anterior hypothalamic area (PO/AH)) to salicylate was determined.

Extracellular single unit recordings from spontaneously active neurons within the CN (dorsal part), MGB (dorsal and ventral part) or AC were performed with glass electrodes (Science Products, Germany). The electrodes were pulled on a P87 horizontal puller (Sutter Instruments, Novato, CA, USA) and filled with a sodium chloride solution (154 mM). The resulting electrode resistance was approximately 3 MΩ.