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ORIGINAL ARTICLE

Specific immediate early gene expression induced by high doses of salicylate in the cochlear nucleus and inferior colliculus of the rat☆

Paula Santos[a,b], **Lilian Eslaine Costa Mendes da Silva**[a], **Ricardo Maurício Leão**[b,*]

[a] *Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Faculdade de Medicina Ribeirão Preto da Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil*
[b] *Department of Physiology, Faculdade de Medicina Ribeirão Preto da Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil*

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KEYWORDS
Salicylate;
Tinnitus;
Cochlear nucleus;
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Abstract
Introduction: Salicylate at high doses induces tinnitus in humans and experimental animals. However, the mechanisms and loci of action of salicylate in inducing tinnitus are still not well known. The expression of Immediate Early Genes (IEG) is traditionally associated with long-term neuronal modifications but it is still not clear how and where IEGs are activated in animal models of tinnitus.
Objectives: Here we investigated the expression of c-fos and Egr-1, two IEGs, in the Dorsal Cochlear Nucleus (DCN), the Inferior Colliculus (IC), and the Posterior Ventral Cochlear Nucleus (pVCN) of rats.
Methods: Rats were treated with doses known to induce tinnitus in rats (300 mg/kg i.p. daily, for 3 days), and c-fos and Egr-1 protein expressions were analyzed using western blot and immunocytochemistry.
Results: After administration of salicylate, c-fos protein expression increased significantly in the DCN, pVCN and IC when assayed by western blot. Immunohistochemistry staining showed a more intense labeling of c-fos in the DCN, pVCN and IC and a significant increase in c-fos positive nuclei in the pVCN and IC. We did not detect increased Egr-1 expression in any of these areas.
Conclusion: Our data show that a high dose of salicylate activates neurons in the DCN, pVCN and IC. The expression of these genes by high doses of salicylate strongly suggests that plastic changes in these areas are involved in the genesis of tinnitus.

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* Corresponding author.
E-mail: leaor@fmrp.usp.br (R.M. Leão).

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PALAVRAS-CHAVE
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Núcleo coclear;
Colículo inferior

Expressão específica de gene precoces imediatos induzida por doses elevadas de salicilato no núcleo coclear e colículo inferior de rato

Resumo
Introdução: Salicilato em doses elevadas induz zumbido nos seres humanos e em animais experimentais. No entanto, os mecanismos e *loci* de ação do salicilato na indução de zumbido ainda não são bem conhecidos. A expressão dos genes precoces imediatos (GPIs) está tradicionalmente associada a alterações neuronais em longo prazo, mas ainda não está claro como e onde os GPIs são ativados em modelos animais de zumbido.
Objetivos: No presente estudo investigamos a expressão de c-fos e Egr-1, dois GPIs, no núcleo coclear dorsal (NCD), colículo inferior (CI) e núcleo coclear ventral posterior (NCVp) de ratos.
Métodos: Os ratos foram tratados com doses que, conhecidamente, induzem zumbido em ratos (300 mg/kg IP/dia, por três dias) e as expressões das proteínas c-fos e Egr-1 foram analisadas por meio de *Western blot* e imunoistoquímica.
Resultados: Após a administração de salicilato, a expressão da proteína c-fos aumentou significativamente no NCD, NCVp e CI, quando analisados por *Western blot*. A coloração imunoistoquímica mostrou uma marcação mais intensa de c-fos no NCD, NCVp e CI e um aumento significativo de núcleos positivos de c-fos no NCVp e CI. Não detectamos aumento da expressão de Egr-1 em nenhuma dessas áreas.
Conclusão: Nossos dados mostram que uma dose alta de salicilato ativa neurônios no NCD, NCVp e CI. A expressão desses genes por doses altas de salicilato sugere que as alterações plásticas nessas áreas estão envolvidas na gênese do zumbido.

Introduction

Tinnitus is a phantom sound sensation that can be the consequence of several factors including acoustic trauma, drugs, temporal mandibular disorders or deafness.[1] The mechanisms of tinnitus induction and maintenance are still a matter of debate, especially since tinnitus can result from several different conditions. High doses of salicylate induce tinnitus in humans, and at doses of 150–400 mg/kg it induces behavioral symptoms of tinnitus in experimental animals in less than 24 h; this effect subsides within 72 h after treatment interruption.[2]

Despite the common use of salicylate as a tinnitus-inducing agent, its mechanisms and loci of action are still obscure. In vivo imaging experiments in rats have shown that high doses of salicylate induces hyperactivity in specific auditory areas, including the Inferior Colliculus (IC), the Dorsal Cochlear Nucleus (DCN) and the Auditory Cortex (AC), but not the Ventral Cochlear Nucleus (VCN).[3,4] On the other hand, studies of expression of the immediate early gene c-fos showed less consistent results. A single dose of salicylate (350 mg/kg) increased c-fos expression only in the AC of gerbils.[5] A previous study did not show c-fos expression in the auditory brainstem after a single dose of salicylate, but only in non-auditory areas such as the locus coeruleus and periaqueductal gray area.[6] Another study observed a decrease of c-fos expression in the IC of gerbils.[7] Another study using chronic treatment with salicylate (250 mg/mL) showed increased c-fos expression only in the IC and not in the DCN.[8] Most of these data are inconsistent with the observations that tinnitus induced by salicylate activates extralemniscal auditory pathways, especially the DCN.[2,3]

The expression of Immediate Early Genes (IEGs) is considered a marker of increased brain activity in response to diverse stimuli. These genes are transcription factors that trigger the expression of other genes responsible for long-term changes in neurons. The expression of the IEG c-fos is a commonly used marker of neuronal activity and it is quickly upregulated after neuronal stimulation.[9–12] The IEG Egr-1 is activated in response to neuronal calcium influx and promotes functional and structural changes in neurons, including in the auditory system.[13,14]

In this study, we aimed to investigate the activation of c-fos and Egr-1 in the DCN and IC in auditory pathways of rats subjected to a protocol of salicylate administration, which is effective in inducing tinnitus in rats (3 daily doses of 300 mg/kg).[3] Due to its proximity to the DCN we also studied the expression of these genes in the posterior division of the Ventral Cochlear Nucleus (pVCN).

Methods

Animals and drug treatment

All experimental procedures performed on animals were approved by the institution's Animal Care and Use Committee (protocol n° 011/2013) and followed the guidelines and recommendations of the National Institutes of Health on animal care. Experiments were performed on male Wistar rats weighing 60–65 g. Rats were group-housed four to five per