Acta Neurol Belg (2012) 112:367–374
DOI 10.1007/s13760-012-0090-5

ORIGINAL ARTICLE

Effect of salicylate on the large GABAergic neurons in the inferior colliculus of rats

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Received: 14 March 2012 / Accepted: 15 May 2012 / Published online: 30 May 2012

Abstract Salicylate, the anti-inflammatory component of aspirin, induces transient tinnitus and hearing loss in clinical and animal experiments. However, the affected sites and mechanisms of generation remain unclear. Recently, down-regulation of inhibitory transmission mediated by γ-aminobutyric acid type A receptors was suggested to be crucial in generating tinnitus. However, the cell-specific pathways involved in this process were far from being understood. Here, we describe changes of inhibitory neurotransmitter, receptor, and glutamatergic axosomatic terminals in certain large GABAergic neurons (LGNs) in the inferior colliculus of rats treated with high doses of salicylate. Based on these results, we suggest that salicylate may affect inhibitory projection pathways from the inferior colliculus to the auditory cortex and lead to neural hyperactivity, perhaps by affecting the function of the LGNs.

Keywords Salicylate · GABAergic neurons · VGLUT2 · Inhibitory projection

Introduction

Tinnitus is the perception of phantom sounds in the absence of acoustic stimuli. High doses of salicylate reliably induce transient tinnitus in humans and animals [1, 2]. Current thinking on the mechanism of tinnitus is that salicylate-induced neural changes upset the balance between excitatory and inhibitory brain processes, resulting in neural hyperactivity. Recently, Sun et al. [3] indicated that high doses of salicylate increased the gain of the central auditory system, presumably by down-regulating inhibition mediated by γ-aminobutyric acid type A ($GABA_A$) receptors. However, the cell-specific pathways involved in this process are still far from being understood.

The ascending auditory pathway is from the cochlea through tonotopically organized nuclei in the brainstem, the inferior colliculus (IC) of the midbrain, and the medial geniculate body (MGB) of the thalamus, ascending eventually to the neocortex. Several studies suggest that the IC is a potential candidate as the locus of tinnitus generation [4, 5]. Winer et al. [6] affirmed the existence of inhibitory projection from GABAergic neurons in the IC to the MGB. Recently, Ito et al. [7] reported that the large GABAergic neurons (LGNs), which are densely covered with vesicular glutamate transporter 2 $(VGLUT2)^+$ glutamatergic axosomatic endings, may be the primary source of GABAergic tectothalamic inputs to the MGB. In our preliminary immunohistochemistry studies, we found that intense $GABA_A$ receptor α1 subunit ($GABA_A\alpha1$) immunoreactions appeared in a certain type of cells in the IC of rats. The size and distributed sites were both similar to that of the LGNs. Thus, in the present study, we first used real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis to determine if any changes in the $GABA_A$ subunit and glutamic acid decarboxylase (GAD) mRNA expression could be found after treatment with high doses of salicylate. Then, laser confocal microscopy was used to determine if similar changes could be found in the LGNs. We hypothesized that specific changes could occur in the LGNs after administration of salicylate, which may further

Electronic supplementary material The online version of this article (doi:10.1007/s13760-012-0090-5) contains supplementary material, which is available to authorized users.

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affect the inhibitory projection from the IC to the MGB and contribute to hyperactivity in the IC in rats.

Methods

Animals and operative procedures

Twenty-six adult SPF Sprague–Dawley rats, 14–15 weeks of age and weighing 250–350 g (Institute of Animal, China Medical University, Shen Yang, China; certified by the Department of Science and Technology of Liaoning Province, certificate NO.SCXK [Liao] 2008–0005), were randomly divided into experimental and control groups. All animals were fed under the same conditions at the Institute of Animal. Sodium salicylate (Sigma-Aldrich, St. Louis, MO, USA) at an intraperitoneal dose of 350 mg/kg (20 mg/mL solution) and an equal volume of 0.9 % saline were administered to rats in the experimental group and the control group, respectively, for 5 consecutive days. The concentration of salicylate and the injection time course had been confirmed to reliably induce tinnitus in rats [2]. On the sixth day, all rats were deeply anesthetized with 4 % chloral hydrate (3.5 mL/kg). The ICs from 20 rats (10 controls, 10 salicylate-treated) were removed within 5 min to avoid destruction of the protein and mRNA. The samples were immediately stored at −80 °C until real-time RT-PCR was performed. The remaining six rats (3 controls, 3 salicylate-treated) were transcardially perfused with fixative (4 % paraformaldehyde, buffered with 0.1 M phosphate buffer [PB] to pH 7.4). Brains were removed and post-fixed in the same fixative until sectioning for immunohistochemistry examination. The experimental procedures were performed in accordance with the principles of animal care outlined by the Chinese Academy of Sciences. Efforts were made to minimize animal use and suffering.

Total RNA isolation, reverse transcription, and real-time RT-PCR

Total RNA of the ICs from ten rats in each group was isolated using the Qiagen RNeasy kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. cDNA was prepared from total RNA using oligo primers and superscript reverse transcriptase according to the accompanying protocol (Clontech, Palo Alto, CA, USA). The specific primers were for rat β-actin and $GABA_A\alpha1$, $\beta2$ and $\gamma2$ subunits, and GAD65 and GAD67 (Table 1). Quantitative real-time PCR was performed in the Quantitect SYBR Green PCR kit (Rotorgene 2000, Corbett Research, Sydney, Australia). The amplification of β-actin RNA was used as an internal control. The data were analyzed by the comparative Ct method. After each real-time RT-PCR, a melting profile and agarose gel electrophoresis of each sample were performed to rule out nonspecific PCR products and primer dimers.

Immunohistochemistry procedure

After post-fixation for 4 h, the samples were cryoprotected with 30 % sucrose in phosphate-buffered saline (PBS) overnight at 4 °C. When the samples all sank to the bottom of the container, serial coronal sections were cut at a thickness of 20 μm using a freezing microtome; every fifth section was used for histology. Sections were incubated with 12 % normal donkey serum for 1 h at room temperature in PBS, followed by incubation with the primary antibodies overnight in the same buffer. The primary antibodies used were monoclonal mouse anti-VGLUT2 (1:200; 8G9.2, Abcam, MA, USA), polyclonal goat anti-$GABA_A\alpha1$ (1:200, Santa Cruz Biotechnology, CA, USA), polyclonal rabbit anti-GAD67 (1:200; ab97739, Abcam). All antibodies were diluted in incubation buffer (1 % bovine serum albumin, PBS, and 0.3 % Triton X-100). The specificities of the VGLUT2 and GAD67

Table 1 Primers used for real-time RT-PCR analysis

Gene	Sequence (forward and reverse primer)	NCBI Gene ID
$GABA_A\alpha_1$	5′-AAG GAC CCA TGA CAG TGC TC-3′	29,705
	5′-GGC TCC CTT GTC CAC TCA TA-3′	
GABAAβ2	5′-GTGCCTGACACCTACTTCCTG-3′	25,451
	5′-TAGCCATAGCTTTCGATCTCC-3′	
GABAAγ2	5′-GACGATGACCACTCTCAGCA-3′	29,709
	5′-CAGTCCTTGCCATCCAAAC-3′	
GAD65	5′-GTACGCCATGCTCATTGCCC-3′	24,379
	5′-AGAGAGGATCAAAAGCCCCG-3′	
GAD67	5′-GCTGGAAGGCATGGAAGGTTTTA-3′	24,380
	5′-ACGGGTGCAATTTCATATGTGAACATA-3′	
β-Actin	5′-TGT GAT GGT GGG AAT GGG TCA G-3′	81,822
	5′-TTT GAT GTC ACG CAC GAT TTC C-3′	

antisera were demonstrated by Western blotting (according to the manufacturer's datasheet). The specificity of the $GABA_A\alpha1$ antiserum was verified by Western blotting as described previously [8]. The next day, sections were washed and incubated with secondary antibodies that were raised from donkey and conjugated with fluorescein isothiocyanate (FITC), texas red (TR) and dylight 405 (1:200, Jackson, PA, USA). Controls were routinely carried out by replacing the primary antisera with normal sera. Sections were mounted on coated slides, air dried, rehydrated, and cover-slipped with 1,4- diazabicyclo [2.2.2]octane (DABCO).

Image acquisition

All the triple-labeled images were captured on an FV1000S-SIM/IX81 laser confocal scanning microscope, LCSM (Olympus, Tokyo, Japan) in *X Y Z* plane using a 100× oil immersion objective. To minimize the error produced in this process, we invited two experienced pathologists to assist and adopted a double-blind method. For each animal, we analyzed 4–6 alternate sections. Only the cells labeled with GAD67 and covered with $VGLUT2^+$ axosomatic terminals were the targeted LGNs. All the LGNs were selected from the central nucleus. No more than three LGNs per section that were intact morphologically were carefully traced and scanned using confocal microscopy. Finally, we obtained 20 satisfactory neurons in either group. To quantify the $GABA_A\alpha1$ and GAD67 protein levels in the LGNs, the Z-stacks (18–22 optical slices) were obtained at a thickness of 0.7 μm optical sections. Within each selected field of view, the 5th and 15th optical slices were analyzed, respectively. The cell body and the most proximal parts of the processes were outlined for digitalization of area and of fluorescence intensity for immunoreactivities with the laser scanning microscope LSM 510 version 2.5 software. For quantifying the VGLUT2 in the axosomatic terminals apposing GABAergic neurons, we chose five neurons whose long axes were parallel to the section plane for statistical analysis in either group among the 20 neurons. The Z-stacks (38–42 optical slices) were obtained at a thickness of 0.2 μm optical sections. The Z-stacks were then converted into a three-dimensional (3D) projection using Image J (1.43u). The contrast adjustment of the 3D projection images was consistent for all selected neurons to clearly show the profile of the neurons. For the Z-stack of every GABAergic neuron we chose, the 8th, 16th, 24th, and 32nd optical slices were analyzed. The surfaces of somata and dendrites were outlined for area and fluorescence intensity analysis. The parameters for digitizing photomicrographs were determined in a pilot study with optimal contrast between labeling and background and were then kept constant across conditions and animals.

Statistical analysis

The graphs and statistical analyses were performed using SPSS v13.0. All results were expressed as mean ± SE (*n*), where *n* refers to the number of samples. Data were normal distribution in both groups, we performed the two-tailed Student *t* test. The Levene test was used for equality of variances. The significance level was set at $P < 0.05$.

Results

mRNA expression

The mRNA levels of the $GABA_A\alpha1$, $\beta2$, and $\gamma2$ subunits and GAD65 and GAD67 in normal and salicylate-treated rats were analyzed by real-time RT-PCR. Data from the salicylate-treated group are expressed as fold changes compared with controls. The data shown in Fig. 1 demonstrate that mRNA expression of $GABA_A\alpha1$ and GAD67 in salicylate-treated animals were significantly lower (0.43- and 0.40-fold, respectively) than levels in the control animals ($P < 0.05$, $P < 0.01$); no such differences in $GABA_A$ $\beta2$ and $\gamma2$ and GAD65 were found between the two groups (0.89-, 1.56-, and 0.84-fold, respectively; $P > 0.05$). The results indicate that high doses of salicylate significantly down-regulated the mRNA expression of $GABA_A\alpha1$ and GAD67, but had no such effect on the mRNA expression of $GABA_A\beta2$ and $\gamma2$ or GAD65.

Protein expression of $GABA_A\alpha1$ and GAD67 in the LGNs

The distribution and expression of $GABA_A\alpha1$ and GAD67 protein in the LGNs of normal and salicylate-treated rats

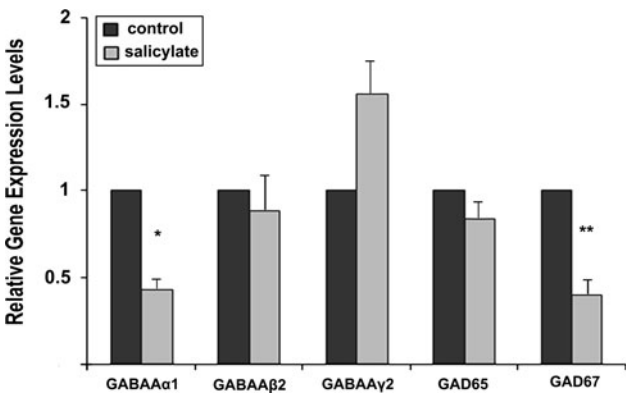

Fig. 1 Quantitative gene expression profile of the $GABA_A\alpha_1$, $\beta2$, and $\gamma2$ subunits and GAD65 and GAD67 from IC of the salicylate-treated group and the control group by real-time RT-PCR. Data from the salicylate-treated group are expressed as fold changes compared with controls ($^*P < 0.05$, $^{**}P < 0.01$)

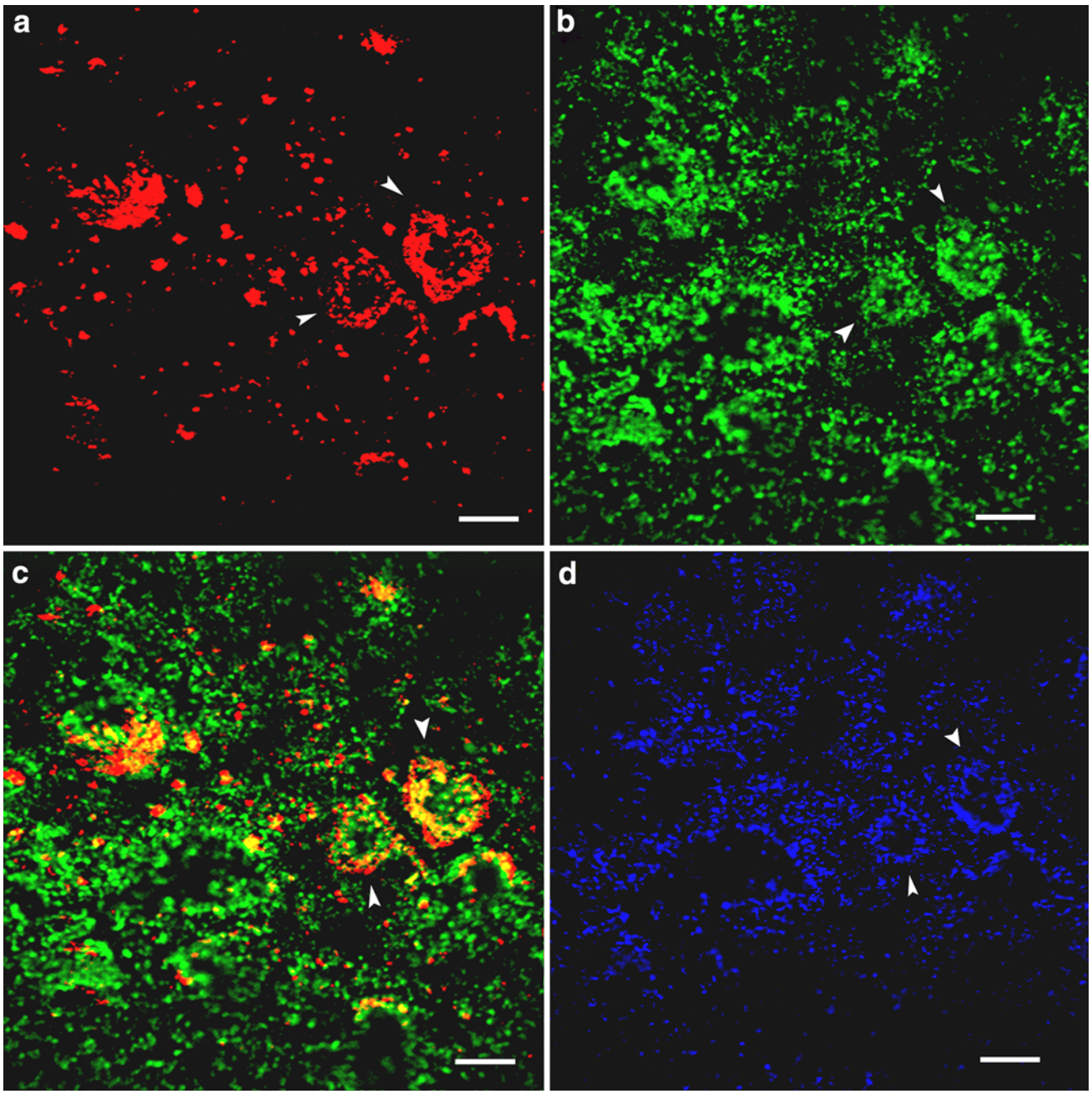

Fig. 2 Laser confocal image of the IC section, stained for VGLUT2 (*red*), GAD67 (*green*) and $GABA_A\alpha_1$ (*blue*). **a–d** Examples from control animals. **e–h** Examples from salicylate-treated animals. **c**, **g** The results of merging from *red* and *green* channels. *Arrows* point to the LGNs, which are determined by labeling with GAD67 in the cell bodies and dentrites and covered with $VGLUT2^+$ axosomatic terminals (×1000, *scale bars* 10 μm) (color figure online)

were examined with laser confocal microscopy from sections triple-stained with GAD67, VGLUT2 and $GABA_A\alpha1$ markers. As shown in Fig. 2, in the LGNs, which were determined by GAD67 staining in the cell body and dendrites and covered with $VGLUT2^+$ axosomatic terminals (Fig. 2c, g), $GABA_A\alpha1$ and GAD67 proteins were both present in the neuronal cytoplasm and cytomembrane as well as protrusions. Because the nuclei are unlabeled, the center of the neurons seemed to be relatively dark. Fluorescence intensity for immunoreactivities was used to determine the levels of protein expression. Data are shown in Fig. 3. Specifically, the mean fluorescence intensity (MFI) of GAD67 and $GABA_A\alpha1$ in the LGNs of control animals was 96.2 ± 16.7 and 72.2 ± 9.9, respectively, whereas values were 78.3 ± 13.3 and 47.5 ± 8.4, respectively, in the salicylate-treated animals. The statistical results indicate that in the LGNs, of

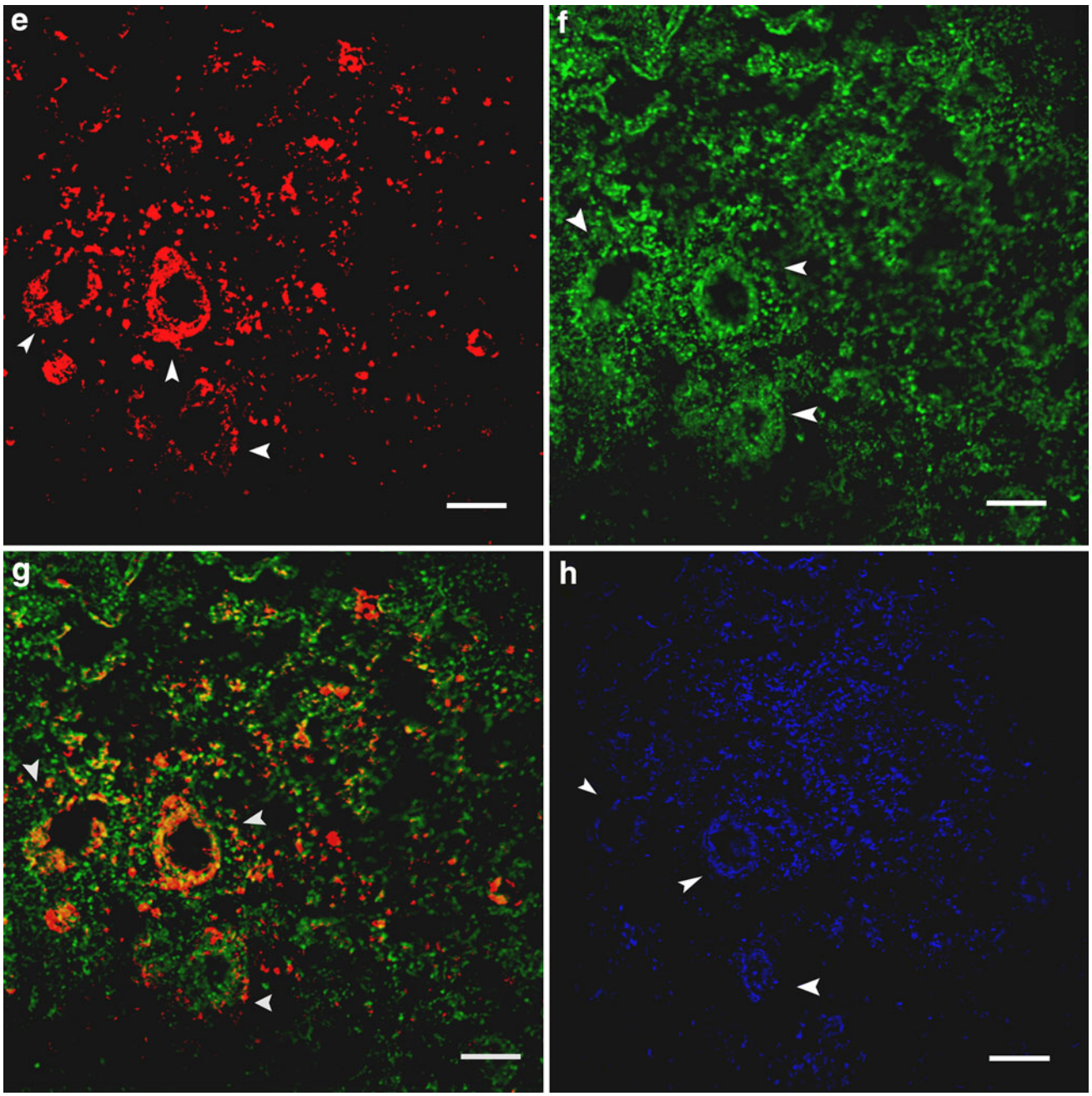

Fig. 2 continued

all 20 neurons in either group, the MFI value of GAD67 and $GABA_A\alpha1$ were both significantly decreased after salicylate treatment ($P < 0.05$).

VGLUT2$^+$ glutamatergic axosomatic terminals

As shown in Fig. 2a, e, VGLUT2 staining created a punctate staining pattern, and the VGLUT2$^+$ axosomatic terminals directly apposed the somata and dendrites of LGNs we traced. To determine the profile of the LGNs, ten LGNs were scanned, encompassing the whole cell body and dendrite, and converted to 3D projections as shown in Fig. 4a. The VGLUT2$^+$ axosomatic terminals were unevenly distributed on the cytomembrane surface of the LGNs. According to the profiles offered by 3D projection, we determined the area and fluorescence intensity of VGLUT2 in each selected neuron for analysis (Fig. 4b). Data are shown in Fig. 3. The MFI of VGLUT2 was 114.4 ± 19.0 in control animals and 102.4 ± 22.8 in salicylate-treated animals, not a significant difference,

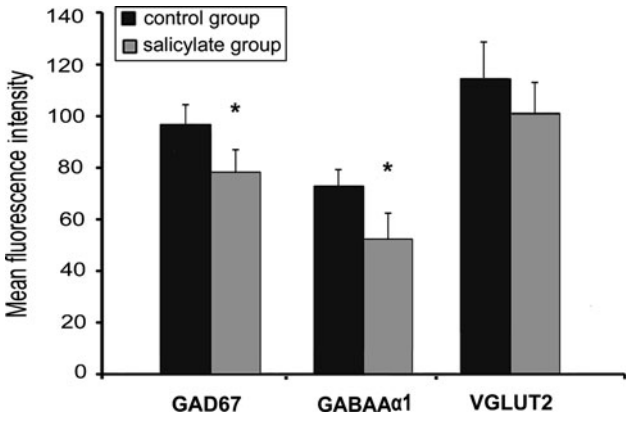

Fig. 3 Semi-quantitative analysis of MFI in either group. The MFIs of GAD67 and $GABA_A\alpha1$ in the LGNs of control animals are both significantly higher than those in the salicylate-treated animals. $^*P < 0.05$, $n = 20$; but no significant changes occurred in the MFI of VGLUT2 between the two groups. $P > 0.05$, $n = 5$

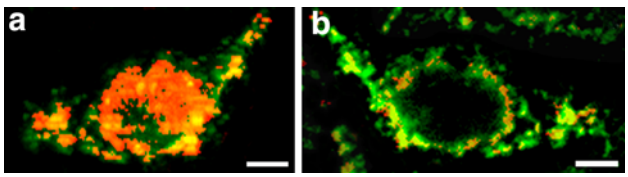

Fig. 4 3D projection immunofluorescence images of the LGN. **a** Example of a selected LGN image from 42 0.2-μm thick Z-stacks. The LGN (*green*) is densely covered with unevenly distributed $VGLUT2^+$ glutamatergic axosomatic endings (*red*). The profile of the neurons is clearly demonstrated (for animated projections, see Supplemental Movie 1) (*scale bars* 5 μm). **b** Image of the 24th optical slice from this Z-stacks. The surface of somata and dendrites is clearly shown and was outlined for area and fluorescence intensity analysis (*scale bars* 5 μm) (color figure online)

indicating that no significant changes occurred in VGLUT2 protein expression in the axosomatic terminals after salicylate treatment ($P > 0.05$).

Discussion

Activity dependence of $GABA_A\alpha1$ and GAD67 transcription

As we showed, high doses of salicylate significantly decreased $GABA_A\alpha1$ and GAD67 mRNA expression, but had no effect on the $\beta2$ and $\gamma2$ subunit protein expression during this process. We know that the main $GABA_A$ subunits transcribed in the IC of rats are the $\alpha1$, $\beta2$, and $\gamma2$ subunits, but the $\alpha1$ subunit is much more transcribed than the other two [9]. Several lines of evidence suggest that expression of the $\alpha1$ subunit is activity dependent [10]. Various mechanisms are likely to be involved in a complex system of regulatory control for the transcription of $GABA_A\alpha1$, for example, Hu et al. [11] supposed that activation of protein kinase C and protein kinase A could regulate the cell surface expression of α_1 subunit. GABA is synthesized by two GADs, GAD67 and GAD65 (encoded by the *Gad1* and *Gad2* genes, respectively), and they show a striking difference in their gene regulation [12]. The major step in the physiologic regulation of GAD67 activity is Gad1 transcription, which is dynamically regulated during development and by neural activity [13]. In this case, the $\alpha1$ subunit and GAD67 mRNA expression in the IC of salicylate-treated rats may have been altered by these manipulations.

Levels of $GABA_A\alpha1$ and GAD67 may affect the function of LGNs

GABA synthesis is the rate-limiting step in GABA metabolism, which readily influences the cellular and vesicular GABA content [14]. GAD67 catalyzes the synthesis of GABA from glutamate and is responsible for >90 % of basal GABA synthesis. Knockdown of GAD67 in a single GABAergic interneuron results in a profound cell autonomous defect in cortical organotypic cultures [15]. Down-regulation of the GAD67 level in the LGNs may result in decrease of the intracellular GABA pool for release. Neurons communicate through the exocytotic release of transmitters from presynaptic axon terminals and the ensuing activation of postsynaptic receptors. Exocytotic transmitter release is modulated by a variety of mechanisms. The GABA released by the LGNs may be modulated by the $GABA_A$ receptors in the LGN, because several studies attested that $GABA_A$ receptors could control GABA release via presynaptic modulations [16]. But the exact effect is uncertain in the LGNs, because the actions are various at different sites of brain [17, 18].

$GABA_A$ receptors are heteropentameric membrane proteins constituting the GABA-gated chloride channels. Binding of GABA to its receptor opens the ionophore and increases chloride conductance, which leads to neuronal inhibition [19]. We have known that the $\alpha1$ subunit participates in the formation of the functional benzodiazepine-binding site and neurosteroid-binding sites in $GABA_A$ receptors [20, 21]. In electrophysiological studies, it produces fast current decay and confers sensitivity to imidazopyridine, zolpidem [18]. So significantly decreased levels of the $\alpha1$ subunit protein may seriously affect the function of the $GABA_A$ receptors in the LGNs. The efficacy of inhibitory neurotransmission strongly depends on the kinetics of the postsynaptic receptor channels. Previous study with cultured rat hippocampal neurons showed that salicylate reduced the GABAergic transmission mainly by

suppressing $GABA_A$ receptor-mediated responses [22]. And several studies demonstrated that the kinetics of IPSCs varied with the α1 subunit [18]. So the decrease of $GABA_A$α1 in the present study was likely to cause decrease of inhibition possibly through affecting the function of the $GABA_A$ receptor. The LGNs were proposed to be the primary source of GABAergic tectothalamic inputs to the MGB and could produce monosynaptic inhibitory postsynaptic potentials (IPSPs) in thalamocortical relay neurons there [7, 23]. In this manner, the LGNs provide strong inhibitory GABAergic inputs to the MGB and further delivery to the auditory cortex. However, GABAergic and glutamatergic neurons both constitute the MGB projection [6, 23], and their axons both pass through the brachium of the IC (BIC). When the BIC is stimulated electrically, IPSPs appear in the MGB before the excitatory postsynaptic potentials (EPSPs) [23]. Down-regulation of the GAD67 and $GABA_A$α1 protein expression in the LGNs will lead to inadequate GABA and $GABA_A$ receptor synthesis, which may affected the inhibitory transmission mediated by the LGNs and destroy the balance between excitation and inhibition, probably by slowing down the conducted velocity of IPSPs from the IC to the MGB.

The $VGLUT2^+$ axosomatic terminals were relatively constant to the salicylate treatment

The VGLUT2 proteins are members of the solute carrier family Slc17a6-8, act as vesicular glutamate transporters, and mediate the packaging of the excitatory neurotransmitter glutamate into synaptic vesicles [24]. The presence of vesicular glutamate transporters has been a useful immunohistochemical marker for glutamatergic terminals. In the present study, we did not count the number of the $VGLUT2^+$ axosomatic terminals apposing the neurons as previous studies reported [25], because VGLUT immunoreactivity is correlated with quantal release of glutamate [26], and the fluorescence intensity of VGLUT2 immunoreactivity should be an indication of the amount of glutamatergic transmission inputs to the LGNs. In contrast, the number of $VGLUT2^+$ terminals is not equal to the quantity of the neurotransmitter in the terminals. Several studies demonstrated that the LGNs received $VGLUT2^+$ glutamatergic input through axosomatic terminals in the IC of rats, but little was known about the origin and function of these terminals. One report suggested that it helped the LGNs fire more quickly and made IPSPs arrive in the auditory thalamus before the EPSPs [7]. In the present study, we found no difference in the VGLUT2 protein expression in the axosomatic terminals after salicylate treatment, which means the amount of glutamatergic transmission was relatively constant with salicylate treatment. Perhaps the axosomatic synapses offer little to the neural hyperactivity caused by salicylate treatment.

Conclusion

In the present study, we found a significant decrease of $GABA_A$α1 and GAD67 in the IC and in the LGNs of rats after salicylate treatment, which may affect the GABAergic transmission from the IC to the MGB mediated by the LGNs. However, although the salicylate model of tinnitus used in the present study is an established model, it uses doses of salicylate greater than physiologic doses. This high dose of salicylate will maybe induce unwanted consequences such as hypernatremia or hyperacidosis. Although no evidence indicated that the side effects of salicylate had any effects on the proteins examined in the present study, efforts will be made in the future to reduce these possible influences using high doses of salicylate.

Acknowledgments This work was supported by Grant 30700938 from the National Natural Science Foundation of China; Grant L2010564 from the Foundation of Liaoning Province education office. We wish to thank GAO Jian and XU Hong-Tao for assistance with image analysis.

Conflict of interest None.

References

1. Brien JA (1993) Ototoxicity associated with salicylates. A brief review. Drug Saf 9:143–148
2. Jastreboff PJ, Brennan JF, Sasaki CT (1988) An animal model for tinnitus. Laryngoscope 98:280–286
3. Sun W, Lu J, Stolzberg D et al (2009) Salicylate increases the gain of the central auditory system. Neuroscience 159:325–334
4. Basta D, Ernst A (2004) Effects of salicylate on spontaneous activity in inferior colliculus brain slices. Neurosci Res 50:237–243
5. Bauer CA, Brozoski TJ, Holder TM, Caspary DM (2000) Effects of chronic salicylate on GABAergic activity in rat inferior colliculus. Hear Res 147:175–182
6. Winer JA, Saint Marie RL, Larue DT, Oliver DL (1996) GABAergic feedforward projections from the inferior colliculus to the medial geniculate body. Proc Natl Acad Sci USA 93:8005–8010
7. Ito T, Bishop DC, Oliver DL (2009) Two classes of GABAergic neurons in the inferior colliculus. J Neurosci 29:13860–13869
8. Milligan CJ, Buckley NJ, Garret M, Deuchars J, Deuchars SA (2004) Evidence for inhibition mediated by coassembly of GABAA and GABAC receptor subunits in native central neurons. J Neurosci 24:7241–7250
9. Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 GABA, receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 12:1040–1062
10. Harris BT, Charlton ME, Costa E, Grayson DR (1994) Quantitative changes in alpha 1 and alpha 5 gamma-aminobutyric acid

type A receptor subunit mRNAs and proteins after a single treatment of cerebellar granule neurons with *N*-methyl-D-aspartate. Mol Pharmacol 45:637–648
11. Hu Y, Lund IV, Gravielle MC, Farb DH, Brooks-Kayal AR, Russek SJ (2008) Surface expression of $GABA_A$ receptors is transcriptionally controlled by the interplay of cAMP—response element-binding protein and its binding partner inducible cAMP early repressor. J Biol Chem 283:9328–9340
12. Pinal CS, Tobin AJ (1998) Uniqueness and redundancy in GABA production. Perspect Dev Neurobiol 5:109–118
13. Kinney JW, Davis CN, Tabarean I, Conti B, Bartfai T, Behrens MM (2006) A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. J Neurosci 26:1604–1615
14. Engel D, Pahner I, Schulze K et al (2001) Plasticity of rat central inhibitory synapses through GABA metabolism. J Physiol 535:473–482
15. Ji F, Obata K (1999) Development of the GABA system in organotypic culture of hippocampal and cerebellar slices from a 67-kDa isoform of glutamic acid decarboxylase (GAD67)-deficient mice. Neurosci Res 33:233–237
16. Farrant M, Nusser Z (2005) Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. Nat Rev Neurosci 6:215–229
17. Okada M, Onodera K, Van Renterghem C, Sieghart W, Takahashi T (2000) Functional correlation of GABAA receptor α subunits expression with the properties of IPSCs in the developing thalamus. J Neurosci 20:2202–2208
18. Vicini S, Ferguson C, Prybylowski K, Kralic J, Morrow AL, Homanics GE (2001) GABA(A) receptor alpha1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. Neuroscience 21:3009–3016
19. Hamill OP, Bormann J, Sakmann B (1983) Activation of multiple-conductance state chloride channels in spinal neurones by glycine and GABA. Nature 305:805–808
20. Boileau AJ, Czajkowski C (1999) Identification of transduction elements for benzodiazepine modulation of the GABA(A) receptor: three residues are required for allosteric coupling. J Neurosci 19:10213–10220
21. Hosie AM, Wilkins ME, da Silva HM, Smart TG (2006) Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites. Nature 444:486–489
22. Gong N, Zhang M, Zhang XB, Chen L, Sun GC, Xu TL (2008) The aspirin metabolite salicylate enhances neuronal excitation in rat hippocampal CA1 area through reducing GABAergic inhibition. Neuropharmacology 54:454–463
23. Peruzzi D, Bartlett E, Smith PH, Oliver DL (1997) A monosynaptic GABAergic input from the inferior colliculus to the medial geniculate body in rat. J Neurosci 17:3766–3777
24. Reimer RJ, Edwards RH (2004) Organic anion transport is the primary function of the SLC17/type I phosphate transporter family. Pflugers Arch 447:629–635
25. Khan M, De Sevilla L, Mahesh VB, Brann DW (2010) Enhanced glutamatergic and decreased GABAergic synaptic appositions to GnRH neurons on proestrus in the rat: modulatory effect of aging. PLoS ONE 5:e10172
26. Moechars D, Weston MC, Leo S et al (2006) Vesicular glutamate transporter VGLUT2 expression levels control quantal size and neuropathic pain. J Neurosci 26:12055–12066