doi: 10.4149/gpb_2013005

# 5-$HT_{1A}$ receptor-mediated activation of outward potassium current by serotonin in mouse cultured spiral ganglion neurons

Chao Yu[1], Jin Liu[2], Yanfang Chen[2] and Yuedi Tang[1]

[1] *Department of Otorhinolaryngology and Translational Neuroscience Center, West China Hospital of Sichuan University, No. 37 Guo-Xue-Xiang, Chengdu, Sichuan 610041, P. R. China*
[2] *Department of Anesthesiology and Critical Care Medicine, West China Hospital of Sichuan University, No. 37 Guo-Xue-Xiang, Chengdu, Sichuan 610041, P. R. China*

**Abstract.** Cochlear spiral ganglion neurons provide the only pathway for transmitting sound evoked activity from the hair cells to the central auditory system. Serotonin plays a role in the response properties of central auditory neurons. However, knowledge about the role of serotonin in the peripheral auditory nervous system remains limited. In the current study, we investigated the influence of serotonin on outward potassium current in mouse cultured spiral ganglion neurons using whole-cell patch clamp technique. The cell capacitance was 4.03 ± 0.18 pF ($n$ = 54). Application of serotonin caused an increase of outward potassium currents within seconds, whereas treatment with WAY100635, a selective 5-$HT_{1A}$ receptor antagonist, counteracted the increase effect of serotonin. These results suggest that serotonin increases outward potassium currents in cultured spiral ganglion neurons through the activation of 5-$HT_{1A}$ receptor. Serotonin may play an important role in sound transmission.

**Key words:** Serotonin — 5-$HT_{1A}$ receptor — Spiral ganglion neuron — Potassium channel — Whole-cell patch clamp technique

**Abbreviations:** CNS, central nervous system; SGNs, spiral ganglion neurons.

## Introduction

Spiral ganglion neurons (SGNs), the primary afferent neurons in the cochlea, are the first relay neurons in the pathway of the auditory transmission to receive precise synaptic signals encoded by hair cell receptors and convey auditory signals into the central nervous system (CNS). SGNs have been shown to express many types of neuropeptide-activated G-protein-coupled receptors, such as P2X receptors, muscarinic receptors, metabotropic glutamate receptors, purinergic receptors, GABAergic receptors, and tachykinin receptors. The expression of these receptors further emphasizes the complex regulation of the auditory signal at the afferent nerve fibers (Housley et al. 1999; Lin et al. 2000; Ito and Dulon 2002; Ito et al. 2002; Peng et al. 2004; Sun et al. 2004).

5-hydroxytryptamine (5-HT, also known as serotonin) was originally characterized as a serum vasoconstrictor in 1948 (Rapport et al. 1948). In addition to its role in vasoconstriction, serotonin may also act as a neuromodulator/neurotransmitter (Berger et al. 2009). Serotonin and its receptors are widely existed, including in the mammalian auditory system. The effects of serotonin on the response properties of auditory neurons have been measured in several auditory regions of CNS: nuclei of the superior olivary complex including medial nucleus of the trapezoid body and lateral superior olive, periolivary regions including the ventral nucleus of trapezoid body and rostral periolivary regions, auditory cortex, the dorsal cochlear nucleus, and inferior colliculus (Ebert and Ostwald 1992; Wang and Robertson 1997; Fitzgerald and Sanes 1999; Hurley and Pollak 1999; Ji and Suga 2007). The activities of serotonin are initiated *via*

Correspondence to: Yuedi Tang, Department of Otorhinolaryngology and Translational Neuroscience Center, West China Hospital of Sichuan University, No. 37 Guo-Xue-Xiang, Chengdu, Sichuan 610041, P. R. China.
E-mail: tangyd@hotmail.com

binding to its specific receptors. In auditory neurons of the CNS, 5-$HT_{1A}$ receptors open potassium channels (Vergé and Calas 2000; Monckton and McCormick 2002) and 5-$HT_{1B}$ receptors decrease the release of serotonin as autoreceptors on serotonergic neurons (Sari 2004). Furthermore, it is also established that serotonin has two main sources in the cochlea, serotonergic fibers and the blood (Vicente-Torres et al. 1998: Gil-Loyzaga et al. 2000). The function of serotonergic fibers has been suggested to be involved in the modulation of the ascending afferent system, but not the transduction of pure tones during auditory processing. Serotonin in the blood induced a biphasic reduction of blood flow, resulting in microcirculation dysfunction (Luo and Kong 2000). However, knowledge about the role of serotonin in the peripheral auditory nervous system remains limited. Previous studies provide evidence that serotonin may be synthesized in SGNs and function as a neuromodulator (Long et al. 2008). Previous study using reverse transcriptase-polymerase chain reaction (RT-PCR) analysis detected the expression of 5-HT receptor subtypes 1A, 1B, 2B, 2C, 3, 5B and 6 mRNA in mouse spiral ganglion subfractions (Oh et al. 1999). Recently, some study demonstrated the distribution of $5HT_{1A}$ receptor on the membrane of SGNs (Li et al. 2007).

As mentioned above, serotonin and its receptors were widely expressed throughout the cochlea (Vicente-Torres et al. 1998; Oh et al. 1999; Gil-Loyzaga et al. 2000; Li et al. 2007; Long et al. 2008). Outward potassium channels play fundamental roles in regulation of membrane excitability. The mechanisms of the action of serotonin on peripheral auditory nervous system remain unclear. Whether serotonin has a regulatory effect on the potassium channel currents in the cultured SGNs is still unknown. The objective of this work was to determine whether serotonin has a regulatory effect on mouse cultured SGNs. We used whole-cell patch clamp technique to examine the effect of serotonin on the potassium channels in cultured SGNs. We further tested if the effect of serotonin on the potassium channels was related to 5-$HT_{1A}$ receptor on the cell membrane. We present evidence to reveal the importance of serotonin in sound transmission.

## Materials and Methods

### *Animals and tissue culture*

Experiments were performed on the cultured SGNs of the Kunming mice. This study was approved by the Animal Care and Use Committee of the West China Medical School of Sichuan University and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Mice at day 3–8 were deeply anesthetized with Nembutal (50 mg/kg, *i.p.*) and then decapitated. Both inner ears were removed from the base of the cranium. Cochlea was extracted from the outer bony labyrinth of the inner ear. The outer ligament-stria vascularis and organ of Corti which contain the spiral ganglion were dissected from the cochlea. The ganglion sections were planted in culture dishes coated with poly-L-lysine. Cells were maintained in the following growth medium: Dulbecco's Modified Eagle Medium, supplemented with 10% fetal bovine serum, 4 mM L-glutamine, and 0.1% penicillin-streptomycin. Then neurons were kept at 37°C in a humidified incubator with 5% $CO_2$ for 7–10 days.

### *Whole-cell patch clamp recordings*

Conventional whole-cell patch clamp technique was used in this study to obtain voltage-clamp recordings from SGNs *in vitro*. In most experiments, SGNs were maintained at –90 mV. In a standard protocol, cells were stepped to a series of voltage from –60 mV to +40 mV with an increase of 10 mV for 50 ms. In order to observe the effect of drugs on the steady-state outward potassium current, SGNs were clamped at –100 mV and currents at +40 mV were continuously monitored with an interval of 30 s. The current-voltage (I/V) relationships of steady-state outward potassium currents were acquired after the monitoring curves were stable, before and after the application of drugs. Cells were placed on the stage of an inverted Olympus IX70 microscope (Olympus, Japan). Patch electrodes were pulled on a Narishige PC-10 electrode puller (Tokyo, Japan) using borosilicate glass. When filled with appropriate pipette solution, electrode resistances typically ranged from 4~6 MΩ in standard bathing solution. Data were collected using an Axopatch 200B voltage amplifier (Axon Instruments, Union City, CA). Recordings were made at room temperature. Data were digitized with a Digidata 1200Axon A/D-D/A interface, analyzed with the pCLAMP10.0 software (Axon Instruments). Each segment of data was filtered at 2 kHz, series resistances were compensated by 80%. During the recording, the change of series resistance was negligible.

### *Solutions and drugs*

The composition of the internal solution was as follows (in mM): 112 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, 11 EGTA, and 10 HEPES-KOH, pH 7.4. Neurons were exposed to the following bath solution (in mM): 1.67 $CaCl_2$, 0.98 $MgCl_2$, 5.36 KCl, 136.89 NaCl, 16.65 glucose, 50 Sucrose, and 10 HEPES-NaOH, pH 7.4. Pipette solution was frozen at –20°C and filtered prior to use. Serotonin and WAY-1000635 used in this study were both purchased from Sigma (St. Louis, USA).

*Statistical analysis*

Because our cultures contained glia as well as neurons, we limited our electrophysiological observations to cells that possessed tetrodotoxin (TTX)-sensitive, rapidly inactivating inward currents in voltage-clamp recordings. Voltage-clamp recordings which met the criteria: stable membrane potentials, low noise levels, and overshooting action potential were used for the subsequent analysis. These parameters changed when the cells were unhealthy with metabolic malfunction. The data were discarded at this situation.

Data were analyzed using Clampfit (version 10.0, Axon Instruments), SPSS16.0, and Excel (Microsoft). Data were expressed as the mean ± standard error. The paired Students'*t*-test was used to determine the effects of drugs. Increase or decrease in outward potassium currents were considered to be significant at a value of $p < 0.05$. *n* represents cell number.

## Results

*Electrophysiological characteristics of outward potassium current on SGNs*

In the present study, whole cell capacitance calculated in extracellular fluid bath solution was 4.03 ± 0.18 pF ($n = 54$). Fig. 1 shows a representative family of the whole cell currents. Outward currents were recorded by holding a cell at –90 mV, and with clamping voltage from –60 mV to +40 mV. In this range of membrane potentials, outward currents were elicited by depolarization to –60 mV for 50 ms at the holding potential of –90 mV. There were transient inward currents followed by outward currents (Fig. 1). Fig. 2A shows the current-voltage curves of the outward currents for the same SGNs in Fig. 1. The curve shows that the outward currents were activated at a holding potential of –90 mV and outwardly rectified. The amplitude of total outward currents was increased followed by the elevation of the elicited potential, but not in a proportional relationship. As shown in Fig. 2B, the outward current was strongly reduced in the presence of 20 mM tetraethylammonium (TEA), a well known blocker of potassium channels, in the external solution.

*Effect of serotonin on outward potassium current in SGNs*

Application of serotonin to SGNs caused a significant increase in the amplitude of the steady-state outward potassium current on most of the cells, whereas the remaining cells did not show a discernible change in the outward potassium current. Therefore, we further analyzed the characteristics of serotonin excitation of the outward potassium current based on the responding cells.

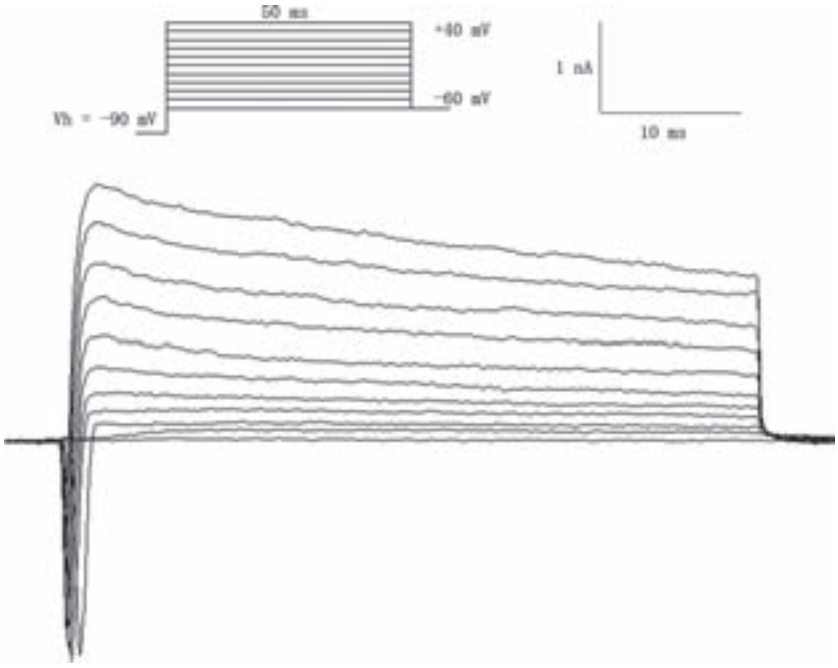


**Figure 1.** Whole-cell voltage-gated currents in a cultured mouse spiral ganglion neuron. Voltage-gated currents were evoked by a holding potential (Vh) of –90 mV for duration of 50 ms with voltage steps from –60 mV to +40 mV. There were transient inward currents followed by outward currents.

To determine the effect of serotonin on the outward potassium current, cells were held at a holding potential of –90 mV and families of the outward potassium current were activated by a series of 50 ms voltage steps from –60 mV to +40 mV in 10 mV increments. Serotonin significantly increased the outward potassium currents in the SGNs (Fig. 3A). In order to observe the effect of serotonin on the outward potassium current, currents at +40 mV were continuously monitored with an interval of 30 s. A typical effect of 0.1 μM serotonin over a period of approximately 3 min on the outward potassium current of SGNs is shown in Fig. 3B. The I/V relationships of steady-state outward potassium currents were acquired after monitoring curves were stable, before and after the application of serotonin. The results suggest that 0.1 μM serotonin induced an increase in the amplitude of the outward potassium current at +40 mV from 673.44

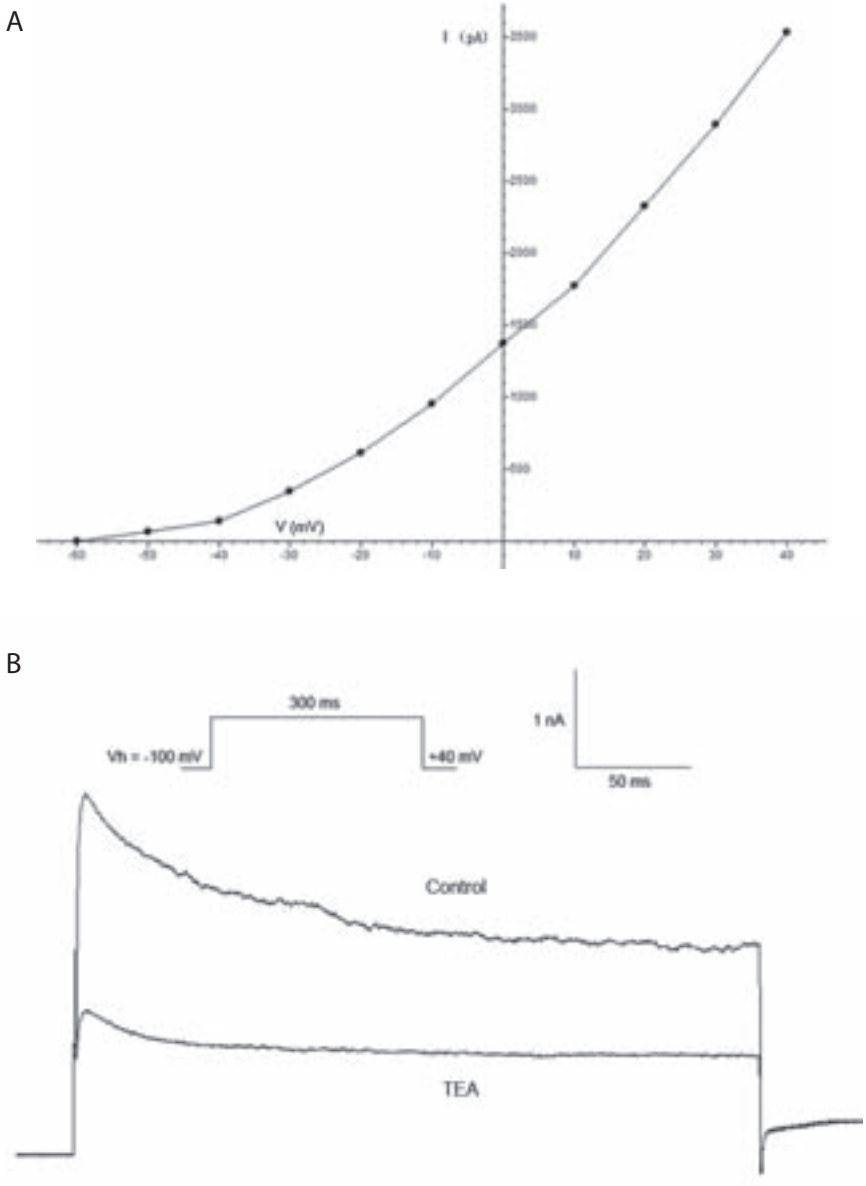


**Figure 2. A.** Current-voltage relationships for the outward currents in the same spiral ganglion neurons evoked by the voltage protocol shown in Fig. 1. The results show that steady-state outward currents were activated at a holding potential (Vh) of –90 mV and outwardly rectified. The amplitude of total outward currents was increased, followed by the elevation of the elicited potential, but not in a proportional relationship. **B**. The outward current was blocked by tetraethylammonium chloride (TEA). Outward current elicited by +40 mV for 300 ms at a holding potential of –100 mV.

± 105.13 to 787.58 ± 105.52 pA/pF ($n$ = 12, $p$ < 0.05), but without a change in the reversal potential (Fig. 3C). Using the same pulse protocol, the effect of different concentrations of serotonin on the outward potassium current was observed. Current at +40 mV was increased by 23.80 ± 7.99% ($n$ = 12, $p$ < 0.05), 30.74 ± 4.62% ($n$ = 11, $p$ < 0.05) and 38.83 ± 6.02% ($n$ = 7, $p$ < 0.05) at concentrations of 0.1, 1 and 10 μM, respectively (Fig. 4).

*Effect of 5-HT$_{1A}$ receptor antagonist on serotonin-induced activation of the outward potassium current*

Using the same protocols as described above, we used WAY100635, the selective 5-HT$_{1A}$ receptor antagonist, to preprocess the cells before application of serotonin. In the presence of 5 nM WAY100635, application of 0.1 μM serotonin didn't show any effects on the amplitude of outward potassium current at +40 mV (from 555.35 ± 38.64 to 524.09 ± 39.32 pA/pF, $p$ = 0.369, $n$ = 5). These findings suggest that serotonin increases outward potassium currents of the cultured SGNs through the activation of 5-HT$_{1A}$ receptor.

## Discussion

The nerve fibers within the mammalian cochlea are divided into two main classes: the afferent and efferent nerve fibers. The afferent nerve fibers refer to the axons from SGNs which carry messages from hair cells to the brain. Many studies reveal extensively neuromodulatory effects in SGNs which

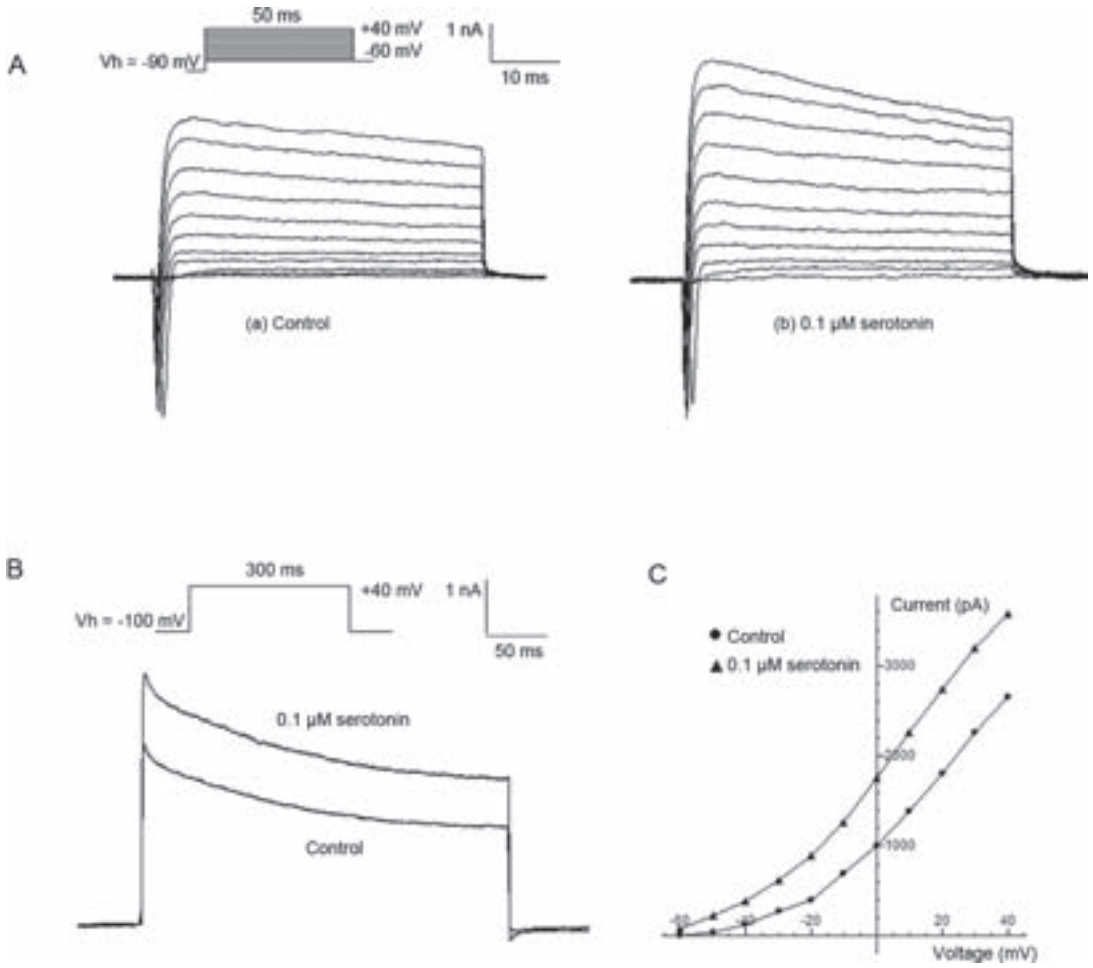


**Figure 3. A**. Effects of serotonin on steady-state outward potassium currents in a typical spiral ganglion neuron. Outward potassium currents activated by the voltage from –60 mV to +40 mV before application of serotonin (**a**). Outward potassium currents activated by the same pulse protocol after application of serotonin. Serotonin increased outward potassium currents (**b**). **B.** Typical effect of serotonin treatment over a period of 3 min on the outward potassium current of spiral ganglion neurons. **C.** The current-voltage (I-V) curves show the effect of serotonin on outward potassium current. The maximum amplitude of outward potassium currents was increased after serotonin added to the bath solution. There was no change in the reversal potential of the outward potassium current. Vh, holding potential.

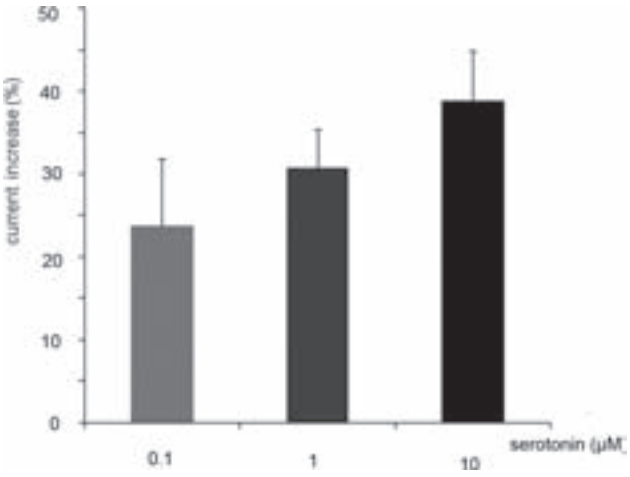


**Figure 4.** Effects of different concentrations of serotonin on outward potassium current at +40 mV (0.1 μM, $n = 12$, $p < 0.05$; 1 μM, $n = 11$, $p < 0.05$; 10 μM, $n = 7$, $p < 0.05$). Values are the mean ± SEM.

serve to optimize the signaling capabilities and allow them to carry out their critical role in the perception of sound (Housley et al. 1999; Lin et al. 2000; Ito and Dulon 2002; Ito et al. 2002; Peng et al. 2004). However, serotonin has received relatively little attention in SGNs. It has been reported that serotonin was expressed extensively in the cochlea including SGNs (Vicente-Torres et al. 1998; Gil-Loyzaga et al. 2000; Long et al. 2008). It has also been found that serotonin receptor subtype 5-$HT_{1A}$ receptor was expressed in SGNs of mice (Oh et al. 1999; Li et al. 2007). The present study demonstrated that serotonin was able to increase potassium currents in the cultured SGNs. The channels which were carrying these currents interacted with serotonin in a voltage-dependent manner. Furthermore, preprocessed by 5-$HT_{1A}$ receptor-selective antagonist WAY100635 before application of serotonin abolished the increase effect on the outward potassium currents, suggesting that response to serotonin of SGNs was functionally mediated by serotonin receptors.

Effects of serotonin on ion currents have also been reported in other cell types. Previous reports suggest that serotonin can activate inwardly rectifier potassium channels (GIRK) of the prefrontal cortex and induce outward potassium current of orexin neurons through activation of 5-$HT_{1A}$ receptor (Muraki et al. 2004; Goodfellow et al. 2009). In contrast to these results, serotonin inhibits voltage-dependent potassium channel of mesenteric arterial smooth muscle cells and $Ca^{2+}$-dependent potassium current of the rat carotid body type I cells through activation of 5-$HT_{2A}$ receptors (Zhang et al. 2003; Ko et al. 2010). Thus, there appear to be different functions of serotonin, depending on cells, receptors and channel types. Our finding is consistent with those of Vergé and Monckton (Vergé and Calas 2000; Monckton and McCormick 2002), who found serotonin can open potassium channels through activation of 5-$HT_{1A}$ receptors in the central auditory neurons, suggesting that serotonin may have alike function both in the central and peripheral auditory system through the same receptor. Although the physiological and pathological significance of the activation of potassium currents by serotonin is still unclear in the present investigation, it may play an important role in the modulation of sound signals delivery.

Evidence suggesting a role for serotonin as a neuromodulator/neurotransmitter in auditory nuclei of the CNS has accumulated over many years (Willard et al. 1984; Fitzpatrick et al. 1989; Klepper and Herbert 1991; Gil-Loyzaga et al. 1997; Kaiser and Covey 1997; Hurley and Thompson 2001; Thompson and Hurley 2004). However, the role of serotonin in the afferent pathway of auditory processing in the peripheral auditory nervous system was still unknown. The results of the present study suggest that serotonin may regulate auditory processing in the peripheral auditory nervous system by modulating the neural excitability of SGNs through activating outward potassium channels. Since serotonin positive immunoreactivity is found in the cytoplasm of SGNs (Long et al. 2008), the activation of 5-$HT_{1A}$ receptor may be in an autocrine/paracrine manner.

In this study, our results suggest that serotonin can hyperpolarize mice cultured SGNs through activation of 5-$HT_{1A}$ receptors. However, 5-$HT_{1A}$ receptor is a G-protein coupled receptor and its activation is through a G-protein-mediated mechanism (Adayev et al. 2005). The precise mechanisms of serotonin and 5-$HT_{1A}$ receptor interaction in SGNs are still unclear. Whether it is through the same signal pathway requires to be clarified by further experiment. Our results suggest that serotonin can partially increase the outward potassium currents in the cultured SGNs through the activation of 5-$HT_{1A}$ receptor. Serotonin translates information about behavioral context into changes in sensory processing *via* a diverse array of receptors. These receptors may interact to shape sensory encoding. In the inferior colliculus, serotonin has different effects on evoked responses of neurons through different receptors (Hurley 2006, 2007; Hurley et al. 2008). In the central auditory neurons, 5-$HT_{1B}$ receptors decrease the release of serotonin as autoreceptors on serotonergic neurons (Sari 2004). There are other subtypes of 5-HT receptors in the SGNs besides 5-$HT_{1A}$ receptors (Oh et al. 1999). Current study cannot exclude the role of other 5-HT receptors in modulating auditory transmission.

## Conclusions

In the cochlea, serotonin is thought to play a role as a neurotransmitter in auditory transmission. We provide evidence,

obtained using patch-clamp techniques, that serotonin influences the excitability of the cultured primary afferent auditory neurons. Furthermore, we show that the action was through its interaction with 5-$HT_{1A}$ receptor.

**Acknowledgement.** The authors thank Dr. Lili Long for her helpful assistance in data analysis. This project was supported by the National Natural Science Foundation of China, No. 30872844 and No. 30471880.

The authors report no conflicts of interest.

## References

Adayev T., Ranasinghe B., Banerjee P. (2005): Transmembrane signaling in the brain by serotonin, a key regulator of physiology and emotion. Biosci. Rep. **25,** 363–385 http://dx.doi.org/10.1007/s10540-005-2896-3

Berger M., Gray J. A., Roth B. L. (2009): The expanded biology of serotonin. Annu. Rev. Med. **60,** 355–366 http://dx.doi.org/10.1146/annurev.med.60.042307.110802

Ebert U., Ostwald J. (1992): Serotonin modulates auditory information processing in the cochlear nucleus of the rat. Neurosci. Lett. **145,** 51–54 http://dx.doi.org/10.1016/0304-3940(92)90201-H

Fitzgerald K. K., Sanes D. H. (1999): Srotonergic modulation of synapses in the developing gerbil lateral superior olive. J. Neurophysiol. **81,** 2743–2752

Fitzpatrick D., Diamond I. T., Raczkowski D. (1989): Cholinergic and monoaminergic innervations of the cat's thalamus: comparison of the lateral geniculate nucleus with other principal sensory nuclei. J. Comp. Neurol. **288,** 647–675 http://dx.doi.org/10.1002/cne.902880411

Gil-Loyzaga P., Bartolomé M. V., Vicente-Torres M. A. (1997): Serotonergic innervations of the organ of Corti of the cat cochlea. Neuroreport **8,** 3519–3522 http://dx.doi.org/10.1097/00001756-199711100-00020

Gil-Loyzaga P., Bartolomé M. V., Vicente-Torres M. A., Carricondo F. (2000): Serotonergic innervation of the organ of Corti. Acta Otolaryngol. **120,** 128–132 http://dx.doi.org/10.1080/000164800750000757

Goodfellow N. M., Beenekareddy M., Vaidya V. A., Lambe E. K. (2009): Layer II/III of the Prefrontal Cortex: Inhibition by the serotonin 5-HT1A receptor in development and stress. J. Neurosci. **29,** 10094–10103 http://dx.doi.org/10.1523/JNEUROSCI.1960-09.2009

Housley G. D., Kanjhan R., Raybould N. P., Greenwood D., Salih S. G., Järlebark L., Burton L. D., Setz V. C., Cannell M. B., Soeller C., Christie D. L., Usami S., Matsubara A., Yoshie H., Ryan A. F., Thorne P. R. (1999): Expression of the P2X2 receptor subunit of the ATP-gated ion channel in the cochlea: implications for sound transduction and auditory neurotransmission. J. Neurosci. **19,** 8377–8388

Hurley L. M., Pollak G. D. (1999): Serotonin differentially modulates responses to tones and frequency-modulated sweeps in the inferior colliculus. J. Neurosci. **19,** 8071–8082

Hurley L. M., Thompson A. M. (2001): Serotonergic innervations of the auditory brainstem of the Mexican freetailed bat, Tadarida brasiliensis. J. Comp. Neurol. **435,** 78–88 http://dx.doi.org/10.1002/cne.1194

Hurley L. M. (2006): Different serotonin receptor agonists have distinct effects on sound-evoked responses in inferior colliculus. J. Neurophysiol. **96,** 2177–2188 http://dx.doi.org/10.1152/jn.00046.2006

Hurley L. M. (2007): Activation of the serotonin 1A receptor alters the temporal characteristics of auditory responses in the inferior colliculus. Brain Res. **1181,** 21–29 http://dx.doi.org/10.1016/j.brainres.2007.08.053

Hurley L. M., Tracy J. A., Bohorquez A. (2008): Serotonin 1B receptor modulates frequency response curves and spectral integration in the inferior colliculus by reducing GABAergic inhibition. J. Neurophysiol. **100,** 1656–1667 http://dx.doi.org/10.1152/jn.90536.2008

Ito K., Dulon D. (2002): Nonselective cation conductance activated by muscarinic and purinergic receptors in rat spiral ganglion neurons. Am. J. Physiol. Cell Physiol. **282,** C1121–1136

Ito K., Rome C., Bouleau Y., Dulon D. (2002): Substance P mobilizes intracellular calcium and activates a nonselective cation conductance in rat spiral ganglion neurons. Eur. J. Neurosci. **16,** 2095–2102 http://dx.doi.org/10.1046/j.1460-9568.2002.02292.x

Ji W., Suga N. (2007): Serotonergic modulation of plasticity of the auditory cortex elicited by fear conditioning. J. Neurosci. **27,** 4910–4918 http://dx.doi.org/10.1523/JNEUROSCI.5528-06.2007

Kaiser A., Covey E. (1997): 5-HT innervations of the auditory pathway in birds and bats. In: Acoustical Signal Processing in the Central Auditory System. (Ed. J. L. Syka), pp. 71–78, Plenum, New York http://dx.doi.org/10.1007/978-1-4419-8712-9_7

Klepper A., Herbert H. (1991): Disribution and origin of noradrenergic and serotonergic fibers in the cochlear nucleus and inferior colliculus of the rat. Brain Res. **557,** 190–201 http://dx.doi.org/10.1016/0006-8993(91)90134-H

Ko E. A., Park W. S., Firth A. L., Hong da H., Choi S. W., Heo H. J., Kim M. H., Noh J. H., Ko J. H., Kim N., Earm Y. E., Song D. K., Han J. (2010): Increased sensitivity of serotonin on the voltage-dependent K+ channels in mesenteric arterial smooth muscle cells of OLETF rats. Prog. Biophys. Mol. Bio. **103,** 88–94 http://dx.doi.org/10.1016/j.pbiomolbio.2010.02.003

Li G., Tang Y. D., Chen W. B. (2007): Application of LsAB technique to identifying serotonin receptor 1A in the spiral ganglion. Journal of Audiology and Speech Pathology **15,** 235–237 (in Chinese)

Lin X., Chen S., Chen P. (2000): Activation of metabotropic GABAB receptors inhibited glutamate responses in spiral ganglion neurons of mice. Neuroreport **11,** 957–961 http://dx.doi.org/10.1097/00001756-200004070-00012

Long L., Li G., Chen W., Liu J., Tang Y. (2008): Distribution of serotonin immunoreactivity in the spiral ganglion neurons of mouse cochlea. Int. J. Pediatr. Otorhinolaryngol. **72,** 1003–1006 http://dx.doi.org/10.1016/j.ijporl.2008.03.008

Luo Z. Q., Kong W. J. (2000): The influence of serotonin on cochlear microcirculation. J. Clin. Otorhinolaryngol. **14,** 81–82

Monckton J. E., McCormick D. A. (2002): Neuromodulatory role of serotonin in the ferret thalamus. J. Neurophysiol. **87,** 2124–2136

Muraki Y., Yamanaka A., Tsujino N., Kilduff T. S., Goto K., Sakurai T. (2004): Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. J. Neurosci. **24,** 7159–7166
http://dx.doi.org/10.1523/JNEUROSCI.1027-04.2004

Oh C. K., Drescher M. J., Hatfield J. S., Drescher D. G. (1999): Selective expression of serotonin receptor transcripts in the mammalian cochlea and its subdivisions. Brain Res. Mol. Brain Res. **70,** 135–140
http://dx.doi.org/10.1016/S0169-328X(99)00110-2

Peng B. G., Li Q. X., Ren T. Y., Ahmad S., Chen S. P., Chen P., Lin X. (2004): Group I metabotropic glutamate receptors in spiral ganglion neurons contribute to excitatory neurotransmissions in the cochlea. Neuroscience **123,** 221–230
http://dx.doi.org/10.1016/j.neuroscience.2003.09.010

Rapport M. M, Green A. A., Page I. H. (1948): Serum vasoconstrictor (serotonin) IV. Isolation and characterization. J. Biol. Chem. **176,** 1237–1241

Sari Y. (2004): Serotonin1B receptors: from protein to physiological function and behavior. Neurosci. Biobehav. Rev. **28,** 565–582
http://dx.doi.org/10.1016/j.neubiorev.2004.08.008

Sun W., Ding D. L., Wang P., Sun J., Jin X., Salvi R. J. (2004): Substance P inhibits potassium and calcium currents in inner ear spiral ganglion neurons. Brain Res. **1012,** 82–92
http://dx.doi.org/10.1016/j.brainres.2004.03.051

Thompson A. M., Hurley L. M. (2004): Dense serotonergic innervations of principal of the superior olivary complex in mouse. Neurosci. Lett. **356,** 179–182
http://dx.doi.org/10.1016/j.neulet.2003.11.052

Vergé D., Calas A. (2000): Serotoninergic neurons and serotonin receptors: gains from cytochemical approaches. J. Chem. Neuroanat. **18,** 41–56
http://dx.doi.org/10.1016/S0891-0618(99)00050-2

Vicente-Torres A., Bartolomé M. V., Carricondo F., Esquifino A., Gil-Loyzaga P. (1998): HPLC detection of serotonin within the cochlea of the rat. Neuroreport **9,** 3699–3701
http://dx.doi.org/10.1097/00001756-199811160-00024

Wang X., Robertson D. (1997): Effects of bioamines and peptides on neurons in the ventral nucleus of trapezoid body and rostral periolivary regions of the rat superior olivary complex: an in vitro investigation. Hear. Res. **106,** 20–28
http://dx.doi.org/10.1016/S0378-5955(96)00211-0

Willard F. H., Ho R. H., Martin G. F. (1984): The neuronal types and the distribution of 5-hydroxytryptamine and enkephalin-like immunoreactive fibers in the dorsal cochlear nucleus of the North American opossum. Brain Res. Bull. **12,** 253–266
http://dx.doi.org/10.1016/0361-9230(84)90053-4

Zhang M., Fearon I. M., Zhong H., Nurse C. A. (2003): Presynaptic modulation of rat arterial chemoreceptor function by 5-HT: role of K+ channel inhibition via protein Kinase C. J. Physiol. **511,** 825–842
http://dx.doi.org/10.1113/jphysiol.2002.038489

Received: April 17, 2012
Final version accepted: July 3, 2012