Sodium salicylate reduces gamma aminobutyric acid-induced current in rat spinal dorsal horn neurons

Han Xu,1,2 Neng Gong,1,2 Lin Chen1 and Tian-Le Xu2,CA

1Department of Neurobiology and Biophysics, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China; 2Institute of Neuroscience, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences, Shanghai 200031, China

CACorresponding Author: tlxu@ion.ac.cn
Received 3 March 2005; accepted 26 March 2005

Sodium salicylate is one of the nonsteroidal antiinflammatory drugs (NSAIDs). It is believed that NSAIDs relieve pain through inhibiting cyclooxygenases (COXs), thereby reducing prostaglandin synthesis [1,2]. However, studies have indicated that SS may have other targets to counteract pain [3–6]. In addition, the side effects of SS, such as its induction of reversible tinnitus in humans [7] and tinnitus-like phantom in rats [8], are not explained by the inhibition of prostaglandin synthesis, suggesting that NSAIDs may have other neuronal targets.

The spinal cord dorsal horn is important in transmitting and processing pain sensation. Recent evidence indicates that disinhibition of GABAergic transmission plays a role in pain perception at the spinal cord level [9]. Currently, the modulatory effect of SS on GABAergic transmission in the spinal dorsal horn has not been reported. Whether γ-aminobutyric acid type A receptors (GABAARs) of the spinal dorsal horn neurons are targets of SS is not clear. To elucidate this issue, we investigated the modulatory effect of SS on γ-aminobutyric acid type A receptor (GABAA) current in cultured rat spinal dorsal horn neurons. Sodium salicylate was found to reduce GABAA current in a reversible and concentration-dependent manner, but did not change its ion selectivity. Sodium salicylate was effective only when GABA and sodium salicylate were applied together. Application of sodium salicylate immediately before, but not during, the application of GABA did not result in a significant reduction of GABAA current. Our results demonstrate that sodium salicylate reversibly attenuates the GABAA response of dorsal horn neurons, suggesting that GABAA receptors in the region are pharmacological targets of sodium salicylate. NeuroReport 16:813–816 © 2005 Lippincott Williams & Wilkins.

Key words: γ-Aminobutyric acid type A receptor; Pain regulation; Spinal dorsal horn neurons; Sodium salicylate; Whole-cell patch-clamp

INTRODUCTION

Sodium salicylate (SS) is widely used as a medicine and belongs to the family of nonsteroidal antiinflammatory drugs (NSAIDs). It is believed that NSAIDs relieve pain through inhibiting cyclooxygenases (COXs), thereby reducing prostaglandin synthesis [1,2]. However, studies have indicated that SS may have other targets to counteract pain [3–6]. In addition, the side effects of SS, such as its induction of reversible tinnitus in humans [7] and tinnitus-like phantom in rats [8], are not explained by the inhibition of prostaglandin synthesis, suggesting that NSAIDs may have other neuronal targets.

Sodium salicylate is one of the nonsteroidal antiinflammatory drugs and is clinically used for antinflammation and chronic pain relief. In the present study, we investigated the actions of sodium salicylate on γ-aminobutyric acid type A receptor (GABAAR) current in cultured rat spinal dorsal horn neurons. Sodium salicylate was found to reduce GABAAR current in a reversible and concentration-dependent manner, but did not change its ion selectivity. Sodium salicylate was effective only when GABA and sodium salicylate were applied together. Application of sodium salicylate immediately before, but not during, the application of GABA did not result in a significant reduction of GABAA current. Our results demonstrate that sodium salicylate reversibly attenuates the GABAA response of dorsal horn neurons, suggesting that GABAA receptors in the region are pharmacological targets of sodium salicylate. NeuroReport 16:813–816 © 2005 Lippincott Williams & Wilkins.

INTRODUCTION

Sodium salicylate (SS) is widely used as a medicine and belongs to the family of nonsteroidal antiinflammatory drugs (NSAIDs). It is believed that NSAIDs relieve pain through inhibiting cyclooxygenases (COXs), thereby reducing prostaglandin synthesis [1,2]. However, studies have indicated that SS may have other targets to counteract pain [3–6]. In addition, the side effects of SS, such as its induction of reversible tinnitus in humans [7] and tinnitus-like phantom in rats [8], are not explained by the inhibition of prostaglandin synthesis, suggesting that NSAIDs may have other neuronal targets.

The spinal cord dorsal horn is important in transmitting and processing pain sensation. Recent evidence indicates that disinhibition of GABAergic transmission plays a role in pain perception at the spinal cord level [9]. Currently, the modulatory effect of SS on GABAergic transmission in the spinal dorsal horn has not been reported. Whether γ-aminobutyric acid type A receptors (GABAARs) of the spinal dorsal horn neurons are targets of SS is not clear. To elucidate this issue, we investigated the modulatory effect of SS on GABA-induced current (IGABA) in cultured rat spinal dorsal horn neurons using whole-cell patch-clamp recordings. Our data demonstrate that SS reversibly reduced IGABA amplitude, which indicates that GABAARs are another target for NSAIDs in the spinal cord.

MATERIALS AND METHODS

The care and use of animals for these experiments followed the guidelines and protocols approved by the Institutional Animal Care and Use Committee of the Institute of Neuroscience, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences.

Cultured spinal dorsal horn neurons: Spinal dorsal horn neurons from 15-day-old embryonic Sprague–Dawley rats were isolated by a standard enzyme treatment protocol. Briefly, spinal dorsal horns were dissociated by trypsin and plated (1.5 × 10⁶ cell/ml) on poly-L-lysine (Collaborative Biomedical Products) coated cover glasses. The neurons were grown in Dulbecco’s modified Eagle’ medium (Gibco, USA) with L-glutamine, 10% fetal bovine serum (Gibco) and 10% F-12 nutrient mixture (Gibco) for 24 h. Then, neuron-basal medium (1.5 ml, Gibco) with 2% B27 (Gibco) was replaced every 3–4 days. Treatment with 5-fluoro-5-deoxy-uridine (20 μg/ml, Sigma, St Louis, Missouri, USA) on the fourth day after plating was used to block cell division of nonneuronal cells, which helped to stabilize the cell population. The cultures were maintained at 37°C in a 5% CO₂ humidified atmosphere. Cells were used for electrophysiological recordings 7–20 days after plating.

Solutions and drugs: The standard external solution contained (in mM): NaCl, 150; KCl, 5; MgCl₂, 1; CaCl₂, 2; glucose, 10; and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 10; adjusted to pH of 7.4 with Tris-base. The osmolality of the solutions was adjusted to 310–320 mOsm/l with sucrose. The patch pipette solution for whole-cell patch recording contained (in mM): KCl, 120; NaCl, 30; MgCl₂, 1; CaCl₂, 0.5; ethylenediaminetetraacetic
Electrophysiological recording: A patch-clamp amplifier (200B; Axon Instruments, USA) was used for whole-cell patch-clamp recordings. Data were sampled and analyzed using a Digidata 1320A interface and a computer installed with Clampex and Clampfit softwares (Version 9.0.1; Axon Instruments). Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm on a two-stage puller (PP-830, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with pipette solution and the reference electrode was 4–6 MΩ. In most experiments, 80–90% series resistance was compensated. The membrane potential was held at −60 mV throughout the experiment, except when the voltage dependence of the effect of SS on \( I_{\text{GABA}} \) was examined. All experiments were performed at room temperature (22–25°C).

Data analysis: All the data are shown as the mean ± SEM, with statistical significance assessed by Student’s \( t \)-test. Statistically significant differences were assumed as \( p < 0.05 \) for all data. \( p \) and \( n \) represent the value of significance and the number of neurons, respectively.

RESULTS

Reversible inhibition of sodium salicylate on gamma aminobutyric acid-induced current: Under the condition of voltage-clamp at a holding potential \( V_H \) of −60 mV, the application of 100 µM GABA evoked an inward current in all neurons tested. This current could be completely abolished by 10 µM bicuculline, a selective GABA_A antagonist (data not shown), suggesting that the currents were primarily mediated by GABA_A in the present preparation. SS induced no detectable current when applied alone at concentrations less than 1000 µM. When co-applied with GABA, however, SS decreased \( I_{\text{GABA}} \) amplitude in a concentration-dependent manner (Fig. 1a). This effect was reversible on washing out SS. The relationship between the amount of \( I_{\text{GABA}} \) decrease and SS concentrations is shown in Fig. 1b.

Inhibition of sodium salicylate on gamma aminobutyric acid-induced current induced at different concentrations: To understand how the inhibitory effects of SS on \( I_{\text{GABA}} \) vary with GABA concentrations, we evaluated the inhibition of \( I_{\text{GABA}} \) by 1000 µM SS at three different GABA concentrations. As shown in Fig. 2a, the efficacy of inhibition by SS of \( I_{\text{GABA}} \) gradually decreased with increasing GABA concentration. In the presence of 1000 µM SS, \( I_{\text{GABA}} \) induced at 10, 100 and 1000 µM were suppressed to 76 ± 9%, 82 ± 7% and 90 ± 10% of the control, respectively (Fig. 2b).

Modulation of sodium salicylate on gamma aminobutyric acid-induced current with different drug application modes: To further explore the mechanism by which SS inhibited \( I_{\text{GABA}} \), we applied different drug application protocols on the same neurons. In the pretreatment protocol (protocol a), neurons were pretreated with SS for 15 s and then applied with SS and GABA together; in the sequential application protocol (protocol b), neurons were applied with GABA alone immediately after 15 s perfusion of SS; in the co-application protocol (protocol c), neurons were co-applied with SS and GABA. As shown in Fig. 3a, the inhibition efficacy varied in three drug application protocols. The GABA-induced currents at 100 µM were reduced to 83 ± 7%, 96 ± 3% and 88 ± 3% in a, b and c protocols, respectively (Fig. 3b). These reductions were statistically significant when GABA and SS were applied together (protocols a and c; \( p < 0.01 \) in each case; Fig. 3b), but the reduction in \( I_{\text{GABA}} \) was not statistically significant with sequential application of SS and GABA (protocol b, \( p > 0.05 \)).

Voltage-dependence of the modulatory effect of sodium salicylate on gamma aminobutyric acid-induced current: To learn the voltage dependence of the effect of SS on \( I_{\text{GABA}} \), we
1000
inhibitory effect of SS on I\textsubscript{GABA} between SS and GABA application, it is unlikely that the applied immediately after SS pretreatment (i.e., no interval presence of 1000 \mu M SS. Besides, the effect of SS on GABA showed no voltage dependence over a range of membrane potential from -80 to +40 mV. The reversal potential of I\textsubscript{GABA} in both the absence and the presence of SS was close to the theoretic chloride equilibrium potential, indicating that SS did not change the ion selectivity of GABA\textsubscript{A}.R.

**DISCUSSION**

In our study, although SS did not activate currents in cultured spinal dorsal horn neurons by itself, it could reversibly reduce I\textsubscript{GABA}. The observed reduction in I\textsubscript{GABA} caused by SS depended on concentrations of both SS and GABA (Figs. 1a and 2). SS was more potent in inhibiting I\textsubscript{GABA} induced by low GABA concentrations (Fig. 2). The reduction of I\textsubscript{GABA} was only seen when GABA and SS were applied together (Fig. 3) and showed no dependence on the membrane potentials (Fig. 4).

In our experiment, the potency of SS inhibition on I\textsubscript{GABA} is dependent on the sequence of drug application (Fig. 3). Sequential application of SS and GABA did not result in a significant reduction of I\textsubscript{GABA} (Fig. 3ab). Because GABA was applied immediately after SS pretreatment (i.e., no interval between SS and GABA application), it is unlikely that the inhibitory effect of SS on I\textsubscript{GABA} was attributed to a metabolic process associated with second messenger production. Besides, the effect of SS on I\textsubscript{GABA} showed no voltage dependence, ruling out the possibility that SS acts as an open-channel blocker. Finally, SS did not change the ion selectivity of the GABA\textsubscript{A}.R. For the reversal potential of I\textsubscript{GABA} was close to the theoretic chloride equilibrium potential during SS application (Fig. 4), indicating that SS inhibited I\textsubscript{GABA} without changing the ion selectivity of the I\textsubscript{GABA}. On the basis of the above observations, we suggest that SS reduces I\textsubscript{GABA} via allosteric regulation of GABA\textsubscript{A}Rs. Alternatively, SS may compete with GABA for the GABA binding site on GABA\textsubscript{A}Rs.

Because NSAIDs permeate the blood–brain barrier [11], it is very likely that SS affects GABA\textsubscript{A}R of the central nervous system (CNS). In fact, chronic drinking of salicylate is reported to affect GABAergic activity in rat inferior colliculus [12]. As mentioned above, a large dose of SS is known to induce reversible tinnitus in humans [7] and tinnitus-like phantom in rats [8]; in addition, Szczepaniak and Moller [13] hypothesized that GABA-mediated dis-inhibition is involved in the processing of tinnitus-related neuronal activity. It would be interesting to know whether similar modulation of GABA\textsubscript{A}R function by SS occurs in the central auditory system. If SS reduces I\textsubscript{GABA} in a similar manner, the reduction of GABA\textsubscript{A} response may participate in the generation of tinnitus. Further experiments are required to test this possibility.

Recently, Coull et al. [9] reported that peripheral nerve injury leads to transsynaptic reduction in the expression of the K\textsuperscript{+}–Cl\textsuperscript{-} cotransporter, KCC2, and consequently disrupts
the postsynaptic Cl⁻/C₀ homeostasis in rat spinal superficial lamina I neurons; and the disruption of Cl⁻ homeostasis in lamina I neurons is sufficient to trigger neuropathic pain. As a result of the disruptive Cl⁻ homeostasis, GABA-mediated depolarizing response even triggered action potential production in these neurons [9]. Thus, it would be interesting to explore whether SS exerts its analgesic action by suppressing GABAₐ response of spinal dorsal horn neurons in the case of neuropathic pain.

**CONCLUSION**
We conclude that SS reduces GABAₐ responses in cultured rat spinal dorsal horn neurons. Thus, GABAₐR may be a potential target of SS in the CNS. The results help to provide some insights into the pharmacological role of SS in the CNS and into the mechanisms of its harmful side effects at the clinic.

**REFERENCES**

Acknowledgements: This study was supported by the National Natural Science Foundation of China (Grants 30270380, 3022802Z) and to the National Natural Science Foundation of China (Grants 3022802Z). We thank the anonymous reviewers and section editor for their comments on the first version of this paper.