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HYP-17, a novel voltage-gated sodium channel blocker, relieves inflammatory and neuropathic pain in rats

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ABSTRACT

Clinical and experimental studies suggest that voltage-gated sodium channels (VGSCs) play a key role in the pathogenesis of neuropathic pain and that blocking agents against these channels can be potentially therapeutic. In the current study, we investigated whether a novel compound, (-)-2-Amino-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-propan-1-one(HYP-17), binds to VGSCs and evaluated its inhibitory effect on Na^+ currents of the rat dorsal root ganglia (DRG) sensory neurons and its analgesic effect on inflammatory and neuropathic pain. HYP-17 (10 μM) reduced both the tetrodotoxin-sensitive (TTX-S) and the TTX-resistant (TTX-R) currents in DRG sensory neurons. However, neither the voltage-dependent activation curves nor the steady-state inactivation curves for TTX-S and TTX-R currents were changed by HYP-17. In rats injected with 5% formalin under the plantar surface of the hind paw, HYP-17 (10 μg) significantly reduced both the early and late phase spontaneous pain behaviors. Systemic injection with HYP-17 (60 mg/kg, i.p.) also significantly relieved the mechanical, cold, and warm allodynia induced by rat tail nerve injury. Furthermore, HYP-17 (60 mg/kg, i.p.) significantly relieved the central neuropathic pain induced by spinal cord injury (SCI), and inhibited c-Fos expression in lumbar (L) 4–L5 spinal segments. Electrophysiological study showed that HYP-17 significantly attenuated the hyper-responsiveness of lumbar dorsal horn neurons. In addition, HYP-17 significantly reduced the levels of pp38MAPK and p-JNK in microglia and astrocytes, respectively, in the L4-L5 spinal dorsal horn. Therefore, our results indicate that HYP-17 has potential analgesic activities against nociceptive, inflammatory and neuropathic pain.

1. Introduction

Neuropathic pain associated with nerve injury, tissue inflammation, or diseases causing dysfunction at any level of the somatosensory system results in enhanced pain sensitivity, leading to spontaneous pain, hyperalgesia and allodynia. Neuroinflammatory responses play a key role in acute and chronic pain syndromes. After tissue injury, immune cells migrate to the injury site and pro-inflammatory cytokines and chemokines are produced, mediating inflammatory reaction. This inflammation reduces the firing thresholds of A-δ and C-fiber nociceptors, resulting in spontaneous and enhanced pain sensitivity driven by spontaneous action potential firing and hyperexcitability of primary afferent neurons (Lai et al., 2003; Waxman, 1999), which are likely to be implicated in dynamic changes in voltage-gated sodium channels (VGSCs), such as alterations of VGSC activity and subtype profile (Cummins et al., 2007; Devor, 2006; Eglen et al., 1999).

VGSCs are complex transmembrane proteins that allow the rapid influx of sodium ions and thereby mediate the generation and propagation of action potential in primary afferent neurons. Nine VGSCs, which are distinct kinetically and pharmacologically, are found in dorsal root ganglion (DRG) neurons (Roy and Narahashi, 1992; Yoshida et al., 1978). Among these nine VGSCs, Nav1.3, Nav1.7, Nav1.8, and Nav1.9 have been implicated as potential players in inflammatory and

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neuropathic pain (Hains et al., 2003b; Jarvis et al., 2007; Lai et al., 2002; Priest et al., 2005; Yeomans et al., 2005). Despite the serious side effects of currently available VGSC blockers that act on the heart and central nervous system (Amir et al., 2006), VGSCs are still considered viable targets for the development of novel analgesics (Gold, 2008). Some sodium channel blockers have been developed to block neuropathic pain. For example, pyrrolopyrimidines and spirooxindoles as an Nav1.7 sodium channel inhibitor (Chakka et al., 2012; Chowdhury et al., 2011) and A-803467 and A-887826, a potent Nav1.8 sodium channel blocker have been reported to attenuate neuropathic and/or inflammatory pain in rats (Jarvis et al., 2007; Zhang et al., 2010).

The synthesis of a HYP-1 and it's in vivo analgesic effect in rat models of inflammatory and neuropathic pain were reported recently (Kam et al., 2012; Kam et al., 2010a; Kam et al., 2010b). In the current study, we investigated whether another novel compound, (-)-2-Amino-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-propan-1-one (HYP-17), binds to VGSCs and evaluated both its inhibitory effect on Na^+ currents in DRG sensory neurons and its analgesic effect on inflammatory and neuropathic pain.

2. Materials and methods

2.1. Animals and ethics statement

Male Sprague-Dawley rats (220–230 g; Samtako, Osan, Korea) were used in this study. All animal experiments were performed in accordance with the Guidelines and Polices for Rodent Survival Surgery provided by the Animal Care Committee of the Kyung Hee University (permission number: KHUASP(SE)-15-006).

2.2. Synthesis

The synthetic procedures are consisted of two main reactions: coupling reaction and reductive amination as illustrated in Fig. 1. The coupling reaction of amine with *N*-Boc-L-alanine, followed by *N*-Bocdeprotection of the compound using HCl-dioxane gave HYP-17.

2.2.1. Synthesis of (+)-tert-Butyl 1-(4-((4-chlorophenyl)(phenyl)methyl)-piperazin-1-yl)-1-oxopropan-2-ylcarbamate (2)

In 3 ml of DMF, *N*-Boc L-alanine (2.46 mmol), (4-chlorophenyl)(phenyl)methylpiperazine1, (2.71 mmol) and PyBOP (2.71 mmol) were dissolved. Then, 4.92 mmol of DIEA was added and stirred at room temperature (RT) for 16 h. Twenty milliliter of 10% HCl was put into the reaction solution, and extracted with 30 ml of EtOAc. The organic layer was washed with 20 ml of 10% HCl, 20 ml of a saturated $NaHCO_3$ solution twice and with 20 ml of a saturated NaCl solution twice. The organic layer was collected, dried over anhydrous $MgSO_4$, and filtered. The organic solvent in the filtrate was removed under reduced pressure. The residue was recrystallized from EtOActo afford desired compound 2.

(+)-*tert*-Butyl 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-oxopropan-2-ylcarbamate (Austin and Moalem-Taylor, 2010): white solid; yield: 52%; $[\alpha]_D^{25.3}$ 7.4° (MeOH, *c* 0.31); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58–7.60 (m, 4H), 7.32–7.41 (m, 5H), 5.37 (s, 1H), 4.54 (q, J = 7.2 Hz, 1H), 3.70–3.87 (m, 4H), 2.68–2.93 (m, 4H), 1.43 (s, 9H), 1.27 (d, J = 6.8 Hz, 3H); HR-FABMS Calcd for $C_{25}H_{33}O_3N_3Cl$ $(M + H)^+$: 458.2210, Found: 458.2212.

2.2.2. Synthesis of (-)-2-Amino-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-propan-1-one (HYP-17)

Chloroform (5 ml) and 4 M HCl-dioxane (5 ml) were added to (+)-*tert*-Butyl 1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-1-oxopropan-2-ylcarbamate 2 (0.76 mmol) and was allowed to stand for 3 h. The solvent and excess acid was removed under reduced pressure.

(-)-2-Amino-1-(4-((4-chlorophenyl)(phenyl)methyl)piperazin-1-yl)-propan-1-one (HYP-17): pale yellow solid; yield: 99%; mp 220–222 °C; $[\alpha]_D^{22.7}$−1.9° (MeOH, *c* 0.79); ^{1}H NMR (D_2O, 400 MHz) δ 7.63–7.66 (m, 4H), 7.51–7.57 (m, 5H), 5.36 (s, 1H), 4.51 (q, J = 7.2 Hz, 1H), 3.80–3.90 (m, 4H), 3.29–3.35 (m, 4H), 1.50 (d, J = 6.8 Hz, 3H); HR-FABMS Calcd for $C_{20}H_{25}ON_3Cl$ $(M + H)^+$: 358.1686, Found: 358.1690.

2.3. Test on Na^+ currents

DRG neurons were obtained from adult rats. Acutely isolated DRGs were incubated in sterile complete saline solution (CSS), pH 7.2, enzymatically digested for 20 min with collagenase A (1 mg/ml; Roche, Indianapolis, IN, USA) and then with collagenase D (1 mg/ml; Roche) and papain (30 U/ml; Worthington, Lakewood, NJ, USA) in CSS at 37 °C, and gently centrifuged (100 ×*g* for 3 min). Pellets were triturated in DRG media (1:1 DMEM/F12, 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin) containing 1.5 mg/ml BSA (Fraction V; Sigma, St. Louis, MO, USA) and 1.5 mg/ml trypsin inhibitor (Sigma). Cells were plated on poly-ornithine-laminin-coated glass coverslips, flooded with DRG media after 1 h, and incubated at 37 °C (humidified 95% air–5% CO_2).

Voltage-clamp recordings of tetrodotoxin-resistant (TTX-R) or -sensitive (TTX-S) sodium currents were obtained with an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA), and data were analyzed using pClamp10.3 (Molecular Device) and OriginPro 8.5 (Microcal, Northampton, MA, USA) software. The external solution for TTX-R sodium current contained (in mM) 70 NaCl, 70 Choline-Cl, 3 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 20 TEA-Cl, 0.1 $CdCl_2$, 0.0003 TTX, 10 glucose and 10 HEPES, pH 7.3 (adjusted to 320 mOsm/l with sucrose). For TTX-S sodium current, the 140 mM NaCl was replaced with 30 mM NaCl and 110 mM Choline-Cl, and TTX was omitted in the external solution. We ensured that the TTX-S sodium currents were completely blocked by 300 nM TTX in each experiment. The internal solution contained 140 mM CsF, 10 mM NaCl, 1 mM EGTA and 10 mM HEPES, at a final pH of 7.3 (adjusted to 315 mOsm/l with sucrose). Small size DRG neurons (<30 μm in diameter) were selected for TTX-R sodium current, whereas medium size DRG neurons (30–45 μm) were used for TTX-S sodium current. A 100 mM stock solution of HYP-17 was prepared in DMSO and diluted in external solution to the final concentration.

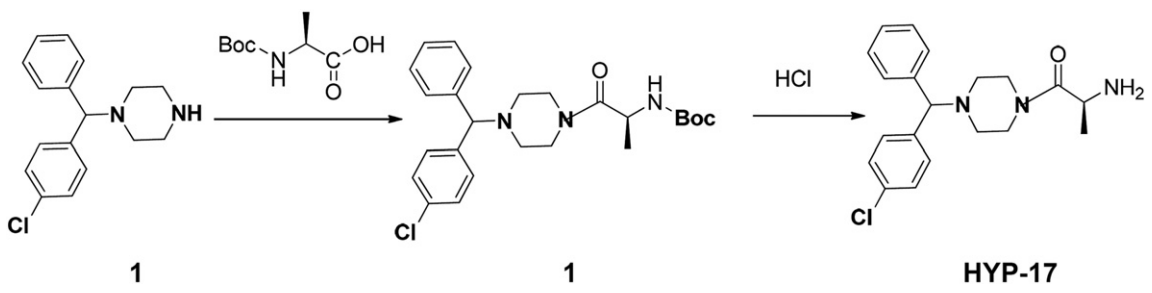

Fig. 1. Synthetic procedure for HYP-17.