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Research Report

ZC88, a novel N-type calcium channel blocker from 4-amino-piperidine derivatives state-dependent inhibits Cav2.2 calcium channels

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ABSTRACT

Small molecular inhibitors of Cav2.2 have been reported for the treatment of neuropathic pain; however, low selectivity and side effects limit their further development. In our study, a series of new compounds were designed and synthesized by optimizing the 4-amino-piperidine template. The results show that ZC88 inhibits transiently expressed Cav2.2 in state-dependent manner in oocytes with an IC50 of 0.45±0.09 μM. The steady-state inactivation relationship curve is shifted to more negative potentials for the calcium channels, suggesting that ZC88 blocks inactivated state of the channel. ZC88 does not present any remarkable effects on voltage-gated P/Q-type calcium channel currents, L-type calcium channel currents, potassium channel and sodium channel currents. Taken together, these in vitro data suggest that ZC88 is a voltage-dependent, subtype-selective Cav2.2 channel inhibitor and can achieve an improved therapeutic window over the relatively state-independent Cav2.2-selective inhibitor, which may have potential to be developed into a novel analgesic agent.

1. Introduction

There is an accumulation of evidence showing the involvement of calcium ions in nociception and antinociception, including the analgesic effects produced by opioid (Tyagarajan et al., 2011). Voltage-gated calcium channels (VGCCs) expressed in nociceptive neurons are present at the presynaptic nerve terminals in the dorsal horn of the spinal cord where they regulate transmitter release. Consistent with this, it has been suggested that Cav2.2 would be an ideal target for treating chronic pain (Vink and Alewood, 2012). Recently, there has been a dramatic increase in the number of drugs developed for the treatment of chronic pain. Ziconotide (PRIALT®), a neuroactive peptide, has been developed as a novel non-opioid treatment for severe chronic pain. However, its use in clinic is limited by intrathecal route of administration and the small therapeutic window to a subpopulation of

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pain sufferers (Miljanich, 2004). Some state dependence to ziconotide blockage is revealed at very negative potentials, since within physiological voltage ranges, ziconotide potently inhibits Cav2.2 channels regardless of whether they are in the open, closed, or inactive state (Swensen et al., 2012; Vink and Alewood, 2012). It has been demonstrated that a state-dependent Cav2.2 inhibitor, which preferentially binds to channels in an open or inactivated state may provide efficacy with an improved therapeutic window over ziconotide. This inhibitory function would be a result of enhanced activation of Cav2.2 channels in pain conditions (Snutch, 2005). Therefore, drug discovery efforts have been focused on finding small molecule state-dependent Cav2.2 channel blockers for analgesia or neuronprotection (Yamamoto and Takahara, 2009). In our previous report, C101, also a non-peptide small molecular 4-amino-piperidine derivate compound was shown to block the Cav2.2 channels (Zhang et al., 2008). Other laboratories reported effects of a series of 4-amino-piperidine derivatives on Cav2.2 channels; however, those compounds were shown to block not only Cav2.2 channels but also other voltage-dependent channels (Hu et al., 2000). In order to identify even more potential and highly selective blockers for Cav2.2 channels, a series of new compounds was designed and synthesized in our institute through optimization of the 4-amino-piperidine template. Among numerous compounds tested, ZC88, (Fig. 1) presented remarkable state-dependence suppressive effects on Cav2.2 channels. The selectivity of ZC88 was also studied by observing its effects on the voltage-gated sodium, potassium channel currents, P/Q and L-type VGCC. Our previous report had shown that ZC88 exhibited efficacy in a number of animal pain models with a therapeutic window (Meng et al., 2008). These results suggest that ZC88 may be developed as a novel oral analgesic agent.

2. Results

2.1. Concentration-dependent inhibition of ZC88 on High-voltage-activated (HVA) calcium channel currents (I_{Ba})

The cDNAs encoding for N-type calcium channel α_{1B}, $\alpha 2_{\delta}$ and β_{1b} subunits were transiently expressed in oocytes. The currents in oocytes were measured using a two-electrode voltage clamp method in the extracellular solution containing 5 mM Ba^{2+} as a charge carrier. HVA I_{Ba} was induced by a depolarization from −80 mV to +10 mV, which was inhibited by N-type VGCC specific inhibitor MVIIA effectively (3 μM) ($p<0.01$, $n=6$) (Fig. 2A). After 5-min exposure to ZC88 (0.1, 1 μM), HVA I_{Ba} was inhibited by ZC88 (0.1, 1 μM) for 5 min in a concentration-dependent manner (Fig. 2B and C). The concentration of ZC88 that induced the half-maximum current block (IC_{50}) was 0.45±0.09 μM ($n=9$) (Fig. 2D). The semilog concentration–response curve for the inhibition of HVA I_{Ba} by ZC88 in oocytes was fitted with the Hill equation $I/I_{max} = 1/\left[1 + (A/IC_{50})^{H}\right]$. IC_{50} was the concentration at which the half-maximum effect was obtained. The inhibition of ZC88 was partly reversed after a 5 min long washout with normal extracellular solutions.

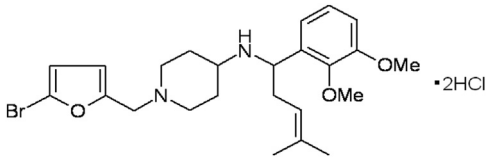

Fig. 1 – The chemical structure of ZC88. N-[1-(2,3-dimethoxyphenyl)-4-methyl-3-pentene-1-yl]-1-(5-bromo-furfuryl)-1-piperidyl-4-amine dihydrochloride (ZC88).

2.2. ZC88 inhibits HVA I_{Ba} in a voltage-dependent manner

To examine whether the inhibition of HVA I_{Ba} caused by ZC88 was voltage-dependent, the *I–V* relationship in the presence or absence of ZC88 was observed. Current–voltage (*I–V*) relationship was obtained by step depolarization between −80 mV and +100 mV in 10 mV increments. After 5-min exposure to ZC88 (0.5 μM) (Fig. 3A), the amplitude of HVA I_{Ba} holding potential was reduced from −70 mV to +60 mV, which elicited currents. The rate of inhibition was 32±2.31%, 52±3.11%, 25±4.51%, 50±1.91%, 52.7±5.01% 55±3.57%, 56±5.01% and 37±4.11% at holding potential of −30 mV, −20 mV, −10 mV, 0 mV, 10 mV, 20 mV, 30 mV and 40 mV, respectively ($n=10$, each, $p<0.05$). The threshold of activation and the reversal in potential of HVA I_{Ba} were not altered. The potential at which maximum current elicited was not altered by ZC88. Voltage steps were positive to the elicited outward currents (+50 to +100 mV), which were not inhibited by ZC88. This outward current was carried by internal K^+ flowing outward through Cav2.2 and was blocked by ω-conotoxin MVIIA (data not shown). The results suggested that ZC88-dependent current blockage is voltage-dependent. Moreover, the currents were not blocked by the application of ZC88 ranging from +70 mV to +100 mV ($n=10$, $p>0.05$, Fig. 3B).

2.3. Effect of ZC88 on voltage-dependent steady-state inactivation of HVA I_{Ba}

Voltage-dependent inactivation was studied using a conventional double-pulse protocol. Inactivation was induced by 5 s potential displacements (conditioning pulse) from −100 mV to 40 mV with increments of 10 mV immediately before 200 ms test pulse from −100 mV to 20 mV. Upon the change in conditioning potential from −100 mV to 40 mV, an increasing proportion of channels became inactivated and the amplitude of HVA I_{Ba} evoked by the test pulse was decreased. Data points were fitted with a smooth curve derived from the Boltzmann equation $I/I_{max} = 1/(1 + \exp(V_m - V_{1/2})/k)$, where V_m was prepulse potential, and $V_{1/2}$ was half-inactivation potential. The currents were normalized using the largest current from the control recorded after conditioning prepulses from −100 mV to 20 mV. The data were fitted with Boltzmann function where the half-inactivation potentials were $V_{1/2} = -59.0 \pm 2.1$ mV ($n=6$), $V_{1/2} = -66.8 \pm 3.7$ mV ($n=6$) for control and 5-min ZC88 (1 μM) exposure, respectively (Fig. 4). The one-factor ANOVA showed a significant difference between the effect of ZC88 vs. the control group ($p<0.05$, $n=6$).

2.4. Effect of ZC88 on recombinant Cav2.2 expressed in oocytes.

Previous reports showed that some Ca^{2+} channel antagonists bind with high affinity to $\alpha_2\delta$ or α_{1B} subunit (Bauer et al., 2010a; Bauer et al., 2010b). To further explore the effect of