Discovery of a selective, state-independent inhibitor of Na\textsubscript{V}1.7 by modification of guanidinium toxins

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The voltage-gated sodium channel isoform Na\textsubscript{V}1.7 is highly expressed in dorsal root ganglion neurons and is obligatory for nociceptive signal transmission. Genetic gain-of-function and loss-of-function Na\textsubscript{V}1.7 mutations have been identified in select individuals, and are associated with episodic extreme pain disorders and insensitivity to pain, respectively. These findings implicate Na\textsubscript{V}1.7 as a key pharmacotherapeutic target for the treatment of pain. While several small molecules targeting Na\textsubscript{V}1.7 have been advanced to clinical development, no Na\textsubscript{V}1.7-selective compound has shown convincing efficacy in clinical pain applications. Here we describe the discovery and characterization of ST-2262, a Na\textsubscript{V}1.7 inhibitor that blocks the extracellular vestibule of the channel with an IC\textsubscript{50} of 72 nM and greater than 200-fold selectivity over off-target sodium channel isoforms, Na\textsubscript{V}1.1–1.6 and Na\textsubscript{V}1.8. In contrast to other Na\textsubscript{V}1.7 inhibitors that preferentially inhibit the inactivated state of the channel, ST-2262 is equipotent in a protocol that favors the resting state of the channel, a protocol that favors the inactivated state, and a high frequency protocol. In a non-human primate study, animals treated with ST-2262 exhibited reduced sensitivity to noxious heat. These findings establish the extracellular vestibule of the sodium channel as a viable receptor site for the design of selective ligands targeting Na\textsubscript{V}1.7.

The voltage-gated sodium ion channel (Na\textsubscript{V}) isoform 1.7 has emerged as a high-interest target for the discovery of non-opioid pain therapeutics based on compelling validation from human genetics and preclinical studies\textsuperscript{1}. Na\textsubscript{V}1.7 loss-of-function mutations result in whole-body insensitivity to pain; conversely, gain-of-function variants are associated with episodic extreme pain disorders and small fiber neuropathies\textsuperscript{2–5}. Discovery of selective inhibitors of Na\textsubscript{V}1.7 has been challenging due to the structural conservation of off-target Na\textsubscript{V} isoforms (Na\textsubscript{V}1.1–1.6, Na\textsubscript{V}1.8 and Na\textsubscript{V}1.9), inhibition of which is likely to result in safety liabilities\textsuperscript{6–8}.

Na\textsubscript{v}s are integral membrane proteins expressed in excitable cells that comprise a ~260 kD pore-forming α-subunit and up to two accessory β-subunits (Fig. 1A)\textsuperscript{9}. The central pore of the α-subunit is encircled by four voltage-sensing domains (VSD I–IV). Channel gating occurs through protein conformational changes in response to membrane depolarization. At least nine discrete binding sites on the Na\textsubscript{v} α-subunit have been identified for peptides and small molecules that influence ion conductance\textsuperscript{10}. The large majority of molecules that engage Na\textsubscript{v}s bind preferentially to a specific conformational state of the channel and show use-dependent activity. Clinical Na\textsubscript{v} inhibitors (e.g., bupivacaine, lidocaine, carbamazepine) are both state- and frequency-dependent agents that lodge in the intracellular pore of the α-subunit, a site that is highly conserved between isoforms. These drugs rely on local administration to achieve a margin between the desired pharmacodynamic effect and dose-limiting side effects. Certain investigational Na\textsubscript{v} inhibitors, such as peptide toxins isolated from venomous species, interact with VSDs to alter the kinetics or voltage dependence of channel activation or inactivation\textsuperscript{11,12}. Similarly, a class of small molecule aryl and acyl sulfonamide compounds bind to an activated conformation of VSD IV and prevent...
recovery from inactivation (Fig. 1B)13-16. By contrast, cationic guanidinium toxins and peptide cone snail toxins inhibit ion conduction by sterically occluding the extracellular vestibule of the channel pore (Site 1). The former are a unique collection of small molecule natural products exemplified by saxitoxin and tetrodotoxin—high affinity, state-independent blockers against six of nine NaV subtypes (Fig. 1C)17.

In the pursuit of isoform-selective inhibitors of NaV1.7, two binding sites, the cystine knot toxin site at VSD II and the sulfonamide site at VSD IV, have been heavily interrogated. Certain cystine knot toxins that engage VSD II such as HwTx-IV, Pn3a, and ProTx-II exhibit 6-1,000× selectivity for NaV1.7 over other channel isoforms. Potency and selectivity for this target have been improved with synthetic peptide toxin derivatives22-26. Among small, Lipinski-type molecules, only the aryl and acyl sulfonamides pioneered by Icagen/Pfizer and subsequently investigated by Amgen, Chromocell, Genentech/Xenon, Merck, and others have shown evidence of significant NaV1.7 isoform selectivity7,16. Within the sulfonamide series, selectivity levels are > 1,000× over certain off-target isoforms including the cardiac isoform, NaV1.5, but generally 10-50× against NaV1.2 and NaV1.6. Many but not all sulfonamide NaV1.7 inhibitors suffer from undesirable pharmaceutical properties, including high plasma protein binding (e.g. > 99.8%), cytochrome p450 inhibition, in vitro hepatotoxicity and high unbound clearance27,28, which have hindered clinical development. Although a number of candidates have been advanced to human testing, one compound has been discontinued after a Phase 2 study likely due to limited efficacy (PF-05089771); others have been discontinued after Phase 1 trials for reasons that may be related to safety liabilities such as elevated expression of liver transaminases and hypotension (GDC-0276)29,30.

Electrophysiology studies with naturally occurring Site 1 ligands against different wild-type and mutant NaV isoforms have identified the extracellular vestibule of NaV1.7 as a promising locus for selective inhibitor design31-33. The outer mouth of the channel is formed from residues that link the S5–S6 helices (referred to as pore loops) from each of the four domains. The domain III pore loop of human NaV1.7 contains a T1398/I1399 sequence motif that is not present in other human NaV subtypes (which contain MD at equivalent positions, Suppl Table 1)31. Comparison of the amino acid sequence of the domain III pore loop across species indicates that the sequence motif in hNaV1.7 is unique to primates. The half-maximal inhibitory concentration (IC50) value for saxitoxin (STX) is markedly altered (250-fold change) depending on the presence or absence of the T1398 and I1399 residues. Against rNaV1.4, the IC50 of STX is 2.8 ± 0.1 nM compared to 702 ± 53 nM for hNaV1.731. Introduction of the alternative domain III pore loop sequence by mutagenesis restores potency (hNaV1.7 T1398M/I1399D IC50 = 2.3 ± 0.2 nM). These findings suggest that it may be possible to capitalize on structural differences in the extracellular vestibule between hNaV isoforms to design NaV1.7-selective inhibitors.

Recent advances in the de novo synthesis of guanidinium toxin analogues have enabled systemic examination of the structure–activity relationship (SAR) properties that govern hNaV1.7 potency and isoform selectivity34-37. Prior to 2016, the binding orientation of STX proposed in the literature indicated that the C11 methylene carbon was positioned proximally to domain III pore loop residues38-40. SAR and mutant cycle analysis studies posited a revised pose in which the C13 carbamate moiety abuts DIII32. This revised binding pose was recently confirmed by cryoelectron microscopy (cryo-EM) structures of STX bound to NaVPaS and hNaV1.718,41. In the present study, analogues of STX substituted at both the C11 and C13 positions were investigated to understand the requirements for selective inhibition of hNaV1.7. These efforts led to the discovery of ST-2262, a potent and selective inhibitor of NaV1.7 that reduces sensitivity to noxious heat in a preliminary study in non-human primates (NHPs).

Results
Discovery of ST-2262. ST-2262 was discovered through a rational design strategy aimed at identifying derivatives of natural bis-guanidinium toxins that preferentially inhibit hNaV1.7 over other off-target hNaV isoforms31. Mutagenesis, homology modeling, and docking studies conducted prior to 2016 suggested that bis-
guanidinium toxins orient in the outer mouth of the channel with the C11 methylene center positioned toward the domain III pore loop of NaV (Fig. 2A, Original pose) 38-40. Exploration of substitution at C11 of decarbamoyl saxitoxin (dcSTX) led to the identification of a series of analogues bearing aryl amide groups at this site. Certain compounds, as exemplified by ST-282, show excellent potency against hNaV1.7 but minimal selectivity (~1:1) over off-target isoforms such as hNaV1.4 (Fig. 2B). The finding that hNaV1.7 isoform selectivity could not be achieved by modification of the C11 substituent led us to investigate SAR at alternative positions. These studies followed evidence that the proper binding orientation of STX is rotated ~180° from earlier models, thus placing the C13 substituent in close proximity to domain III (Fig. 2A, Revised pose) 42.

Derivatives of STX bearing amide, carbamate, ester, ether, and urethane substituents at the C13 position were prepared in an effort to identify compounds with improved selectivity for hNaV1.7. Insight from studies of a naturally-occurring STX C13 acetate congener, STX-OAc, helped guide selection of compounds for synthesis (Suppl Figure 1) 32. The difference in potencies between STX and STX-OAc is striking considering that these two structures vary at a single position (NH₂ → CH₃). Following this lead, we explored substituents at C13 that could replace the hydrolytically unstable acetate group. Ultimately, the C13 succinimide was discovered as a suitable acetate isostere, which was paired with a C11 tetrahydronaphthyl amide to generate ST-2262, the focus of the present study.

**ST-2262 is a potent and selective inhibitor of hNaV1.7.** The potency of ST-2262 against hNaV1.7 stably expressed in HEK293 cells was assessed by manual patch clamp electrophysiology with a voltage-protocol that favors the resting state of the channel. Using a stimulation protocol with a holding potential of ~110 mV and a stimulus frequency of 0.33 Hz, the IC₅₀ of ST-2262 against hNaV1.7 was measured at 0.072 µM (95% confidence interval (CI) 0.064–0.082) (Fig. 3A, Suppl Table 2). Potencies against off-target sodium channel isoforms (hNaV1.1–1.6, hNaV1.8) were determined following a similar protocol. Activity against hNaV1.9 was not evaluated due to the difficulty of expressing this subtype heterologously 42. ST-2262 was determined to be >200-fold selective over off-target isoforms such as hNaV1.3 (IC₅₀ = 17.9 µM, 95% CI 14.8–22.1), >900-fold selective over hNaV1.8 (IC₅₀ = 65.3 µM, CI 62.7–68.1), and >1,000-fold selective over all other NaV isoforms tested. Similar IC₅₀ values against the eight hNaV subtypes were obtained in an independent study using the PatchXpress automated electrophysiology platform (Suppl Table 3).

Exposure of hNaV1.1 and hNaV1.2 to high concentrations of ST-2262 (10–100 µM) resulted in a reduction of the rate of fast inactivation; a similar effect was noted, albeit to a lesser degree, with hNaV1.3 and hNaV1.4 (Suppl Figure 2). Lower concentrations of ST-2262 (1–3 µM), which remain sufficiently high to achieve >90% inhibition of hNaV1.7, had no measurable effect on fast inactivation of hNaV1.1 and hNaV1.2. It is possible that elevated concentrations of ST-2262 result in a secondary mode of binding against these NaV subtypes, however, efforts have not been made to examine such a mechanism at this time. To our knowledge, changes in the rate of fast inactivation have not been observed with STX.

To investigate whether the potency of ST-2262 was dependent on the membrane holding potential or frequency of stimulus, an IC₅₀ value was measured against hNaV1.7 using a two-pulse protocol with a pre-pulse to the voltage at half-inactivation (8 s step) and with a protocol that depolarizes the cell at high frequency (30 Hz stimulus). The potency of ST-2262 was not appreciably altered using either stimulation protocol (IC₅₀ = 0.087 µM, 0.056–0.120 and IC₅₀ = 0.112 µM, 0.015–0.357, respectively; Fig. 3B, Suppl Table 4). These results indicate that ST-2262 is a selective, use-independent inhibitor of hNaV1.7.

**Species variation in potency and mutagenesis.** The potency of ST-2262 was assessed against a panel of species variants of NaV1.7, including mouse, rat, and cynomolgus monkey (Suppl Table 5). Consistent with the hypothesis that NaV1.7 potency is affected by the presence of the T1398/I1399 sequence motif in the DIII pore loop, the IC₅₀ of ST-2262 against cynoNaV1.7 (0.101 µM, 0.073–0.140) was similar to human. In contrast, ST-2262 was >50× less potent against mouse (IC₅₀ = 3.78 µM, 3.23–4.43) and rat NaV1.7 (IC₅₀ = 4.95 µM, 4.17–5.87) than the human ortholog. Affinity was restored within twofold of the hNaV1.7 potency by introduction of domain III MD-T1 mutations to mouse NaV1.7 (IC₅₀ = 0.130 µM, 0.055–0.307; Fig. 3C, Suppl Table 6). Multiple lines of evidence suggest that ST-2262 binds to the extracellular vestibule of the sodium channel (i.e., Site 1) including: (i) the structural similarity of ST-2262 to natural bis-guanidinium toxin ligands, (ii) the...
state- and frequency-independent mode of NaV inhibition that is characteristic of extracellular pore blockers, and (iii) the influence of DIII pore loop residues on potency. To gain additional support that ST-2262 binds to the outer pore of NaV1.7, we generated a point mutant of hNaV1.7, D1690N, at a position known to significantly destabilize binding of STX39. The domain IV residue D1690 forms a critical bridged hydrogen bond with the C12 hydrated ketone of STX39,41. We also measured potency against the hNaV1.7 T1398M/I1399D double mutant to directly confirm that the domain III TI sequence motif contributes to hNaV1.7 affinity31. The introduction of other point mutations to NaV1.7 was attempted (Y362S and E916A), but these variants proved challenging to express39,43. ST-2262 exhibited a > 1,000-fold loss in potency against hNaV1.7 D1690N (IC50 > 100 µM) and a ~ 48-fold loss against hNaV1.7 T1398M/I1399D (IC50 = 1.87 µM, 1.47–2.39) compared to the wild-type channel (IC50 = 0.039 µM, 0.032–0.047; Fig. 3D, Suppl Table 6). Collectively, these results indicate that ST-2262 binds to the extracellular vestibule of NaV1.7, displaying significant species variation in potency and isoform selectivity in large part due to molecular interactions with residues T1398 and I1399, which are unique to human and non-human primate NaV1.7 orthologs31,32.

Figure 3. (A) Dose–response curves for the inhibitory effect of ST-2262 on NaV1.1–NaV1.8 stably expressed in CHO or HEK293 cells using a single-pulse (resting state) protocol with a 10 ms pulse from a holding potential of ~110 mV to voltage at peak activation (~20 to +10 mV). NaV1.X IC50 (in µM, mean, 95% CI). NaV1.1: > 100; NaV1.2: > 100; NaV1.3: 65.3, 62.7–68.1; NaV1.4: 80.7, 71.1–93.3; NaV1.5: > 100; NaV1.6: 17.9, 14.8–22.1; NaV1.7: 0.072, 0.064–0.082; NaV1.8: > 100. (B) Comparison of dose–response relationship of ST-2262 inhibition against NaV1.7 using different stimulation protocols: resting state; two-pulse protocol contained an 8 s conditioning step to the voltage at half-inactivation, followed by a 20 ms step to voltage at full activation (half-inactivation protocol)45; high frequency single-pulse protocol stimulated at 30 Hz, NaV1.7 IC50 (in µM, mean, 95% CI). Resting state: 0.123, 0.104–0.145; half-inactivation: 0.087, 0.056–0.120; high frequency: 0.112, 0.015–0.357. (C) Comparison of dose–response relationship of NaV1.7 inhibition against WT mNaV1.7 and M1407T/D1408I mNaV1.7 on a resting state protocol. mNaV1.7 IC50 (in µM, 95% CI). WT: 2.57, 2.30–2.87; M1407T/D1408I: 0.130, 0.055–0.307. (D) Comparison of dose–response of ST-2262 against transiently expressed hNaV1.7 WT, hNaV1.7 D1690N, and hNav1.7 T1398M/I1399D. IC50 (in µM, mean, 95% CI). WT: 0.039, 0.032–0.047; D1690N: > 100; T1398M/I1399D: 1.87, 1.47–2.39.

ST-2262 increases withdrawal latency in a nonhuman primate model of thermal pain. Mice and humans with genetic NaV1.7 loss-of-function are profoundly insensitive to noxious heat2,3,44–46. To understand whether pharmacological block of NaV1.7 affects noxious thermal sensitivity, we conducted an initial evaluation (n = 4) of the effect of ST-2262 in a non-human primate (NHP) model of acute thermal pain. Experiments were approved by the Montana State University institutional animal care and use committee and performed in accordance with institutional, national, and international guidelines and regulations. It is not possible to study the influence of ST-2262 on acute thermal pain in rodents as this compound is > 50-fold less potent against H.47

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**Figure 3.** (A) Dose–response curves for the inhibitory effect of ST-2262 on NaV1.1–NaV1.8 stably expressed in CHO or HEK293 cells using a single-pulse (resting state) protocol with a 10 ms pulse from a holding potential of ~110 mV to voltage at peak activation (~20 to +10 mV). NaV1.X IC50 (in µM, mean, 95% CI). NaV1.1: > 100; NaV1.2: > 100; NaV1.3: 65.3, 62.7–68.1; NaV1.4: 80.7, 71.1–93.3; NaV1.5: > 100; NaV1.6: 17.9, 14.8–22.1; NaV1.7: 0.072, 0.064–0.082; NaV1.8: > 100. (B) Comparison of dose–response relationship of ST-2262 inhibition against NaV1.7 using different stimulation protocols: resting state; two-pulse protocol contained an 8 s conditioning step to the voltage at half-inactivation, followed by a 20 ms step to voltage at full activation (half-inactivation protocol)45; high frequency single-pulse protocol stimulated at 30 Hz, NaV1.7 IC50 (in µM, mean, 95% CI). Resting state: 0.123, 0.104–0.145; half-inactivation: 0.087, 0.056–0.120; high frequency: 0.112, 0.015–0.357. (C) Comparison of dose–response relationship of NaV1.7 inhibition against WT mNaV1.7 and M1407T/D1408I mNaV1.7 on a resting state protocol. mNaV1.7 IC50 (in µM, 95% CI). WT: 2.57, 2.30–2.87; M1407T/D1408I: 0.130, 0.055–0.307. (D) Comparison of dose–response of ST-2262 against transiently expressed hNaV1.7 WT, hNaV1.7 D1690N, and hNav1.7 T1398M/I1399D. IC50 (in µM, mean, 95% CI). WT: 0.039, 0.032–0.047; D1690N: > 100; T1398M/I1399D: 1.87, 1.47–2.39.

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Na_v,1.7 in species that lack the T1398/I1399 sequence motif (Suppl Table 5). A NHP model of acute thermal pain was identified that uses a heat lamp to deliver a stimulus to the dorsal surface of the hand of lightly anesthetized cynomolgus macaques and measures the time to withdrawal. Prior to advancing ST-2262 into the NHP acute thermal pain model, a standard battery of preclinical assays was completed to evaluate ADME and pharmacokinetic properties of this compound in cynomolgus macaques (Suppl Table 7). Off-target activity of ST-2262 using a commercially available radioligand binding assay panel against 68 different targets was also measured (LeadProfilingScreen, Eurofins, Taipei, Taiwan). No hits were identified on the off-target panel, defined as > 50% inhibition with 10 µM ST-2262 (Suppl Table 8).

Male cynomolgus monkeys were anesthetized with propofol to a level in which the withdrawal reflex of the hand occurred at a consistent latency of approximately 3 s, a response time that was comparable to the detection of sharp pain from Aδ fibers when tested in prior studies on human volunteers. The dorsal surface of the hand was exposed to a thermal stimulus that selectively activates Aδ nociceptors (Fig. 4A–C). The thermal stimulus was turned off at 5 s to prevent tissue damage. Heart rate was monitored throughout the study, and presentation of the noxious thermal stimuli consistently led to a transient increase in heart rate that peaked beyond the stimulus and then returned to baseline (ΔHR). Acute noxious thermal stimuli transiently increase heart rate in human subjects; the percent change in heart rate correlates with subjective pain scores.

ST-2262 hydrochloride administered IV increased the withdrawal latency to noxious thermal stimuli (Fig. 4A). Efficacy was assessed in one subject at four dose levels (0.01, 0.05, 0.25, 1.25 mg/kg), in two subjects at the three higher dose levels (0.05, 0.25, 1.25 mg/kg), and in one additional subject at the highest dose level only (1.25 mg/kg). At the highest dose of 1.25 mg/kg, all four animals showed no hand withdrawal prior to the 5 s cut-off latency (Fig. 4A), a significant increase in withdrawal latency compared to baseline values (Mixed effects model: F(3,7) = 7.468, p < 0.05; 0.01 mg/kg was not included in this analysis because only one subject received this dose). The 1.25 mg/kg dose of ST-2262 also almost completely reduced ΔHR (Fig. 4B; Mixed effects model: F(3,7) = 6.654, p < 0.05.)

Plasma samples were obtained from animals to assess the PK/PD relationship between drug exposure and thermal withdrawal latency. We found that 0.25 mg/kg ST-2262 resulted in ~ 1,400 ng/ml in plasma at the 5 min time point (n = 2), which corresponds to 7× the IC_{50} value of ST-2262 against cynoNa_v,1.7, corrected for plasma protein binding (cyno PPB = 73.5%). The unbound exposure of drug was reduced to 3.4 × cynoNa_v,1.7 IC_{50} at the 30 min time point. At a dose of 1.25 mg/kg, the total plasma concentration was ~ 7,000 ng/ml at 5 min (n = 2), which corresponds to an unbound exposure of 32 × cynoNa_v,1.7 IC_{50} and was maintained above 15× cynoNa_v,1.7 IC_{50} for over 100 min (Fig. 4C). Lumbar CSF samples collected from two animals receiving the 1.25 mg/kg dose indicated that ST-2262 was peripherally restricted, with CSF:plasma ratios < 10^{-3} (n = 2; [ST-2262] 0.8, < 0.5 ng/ml in CSF).

By adjusting radiant heat parameters, the noxious heat model can be used to selectively assess responses to cutaneous C-fiber nociceptor activation, which produces a burning pain in volunteers. The effect of ST-2262 on C-fiber induced hand withdrawal and heart rate change was investigated on two cynomolgus subjects. As with the Aδ nociceptive response, 1.25 mg/kg ST-2262 completely abolished the C-fiber-mediated hand withdrawal and ΔHR (Fig. 4D,E). Collectively, these results are consistent with the hypothesis that pharmacological block of Na_v,1.7 reduces sensitivity to noxious heat, phenotypically analogous to studies of Na_v,1.7 loss-of-function in CIP patients. In addition, analysis of the PK/PD relationship of ST-2262 in this model provides insight into the level of Na_v,1.7 target occupancy that may be necessary to achieve a pharmacodynamic effect. Recognizing the limited number of animals tested due to the challenge of working with non-human primates, additional work is warranted to further define the relationship between pharmacological inhibition of Na_v,1.7 and sensitivity to noxious thermal stimuli.

**Discussion**

The finding that humans lacking functional Na_v,1.7 exhibit an inability to experience pain raises the intriguing possibility that selective inhibitors of Na_v,1.7 may be potent analgesics. In the present study, we describe the discovery and characterization of ST-2262, a selective pore blocker of hNa_v,1.7 advanced through rational modification of a natural small molecule toxin lead, STX. In whole cell voltage clamp recordings, ST-2262 exhibited > 200-fold selectivity for hNa_v,1.7 over hNa_v,1.1–1.6 and hNa_v,1.8. The selectivity of ST-2262 was not examined against hNa_v,1.9, a channel subtype that is difficult to express in heterologously. hNa_v,1.9 contains a residue in the domain I p-loop, S360, that confers resistance to STX and lacks the domain II threonine/isoleucine sequence motif that is essential for high potency of ST-2262 against hNa_v,1.7. Thus, inhibition of hNa_v,1.9 by ST-2262 is unlikely.

The properties of ST-2262 are in contrast to other preclinical and clinical inhibitors of hNa_v,1.7, which preferentially bind to an inactivated conformation(s) of the channel. Mutagenesis experiments indicate that specific residues in the extracellular pore of Na_v,1.7, including a two amino acid sequence variation in the domain III pore loop that is unique to primates, are required for ST-2262 binding to cyno- and human Na_v,1.7. These findings establish the extracellular vestibule of Na_v,1.7 as a viable receptor site for the design of potent and selective channel inhibitors.

Whereas congenital insensitivity to pain in humans is the result of complete and permanent Na_v,1.7 loss-of-function, inhibition by small molecule agents is incomplete and transient. This difference raises several important questions regarding the pharmacology of Na_v,1.7: (1) is transient inhibition sufficient for analgesia, (2) what level of target engagement is required for efficacy, and (3) what anatomic compartment(s) must be accessed? In light of the preliminary nature of the behavioral studies conducted with ST-2262, the present study does not yield definitive answers to these questions. Nevertheless, the finding that NHPs administered ST-2262 exhibited reduced sensitivity to noxious thermal stimuli is consistent with the view that transient inhibition of Na_v,1.7...
is sufficient to produce analgesia. Furthermore, recognizing that ST-2262 is a polar small molecule with low membrane permeability and therefore unlikely to reach efficacious concentrations in the CNS (analysis of CSF samples obtained during NHP experiments gave a CSF:plasma ratio of $< 10^{-3}$), the observed effects on thermal withdrawal latency and $\Delta$HR are likely the result of peripheral inhibition. Our findings, however, do not rule out an additional role for $\mathrm{NaV}1.7$ at the central terminals of primary afferents or in dorsal horn neurons, as has been suggested.

In the present study, the effect of ST-2262 on withdrawal latency to noxious heat was measured in NHPs at doses of 0.01, 0.05, 0.25 and 1.25 mg/kg IV. Doses of 0.05, 0.25 and 1.25 mg/kg resulted in unbound plasma concentrations of ST-2262 in plasma at different doses. Assuming a unitary Hill coefficient, which is consistent with the dose–response relationship for ST-2262 in whole cell recordings against cyno-$\mathrm{NaV}1.7$, these unbound exposures correspond to 41%, 78% and 94% inhibition of $\mathrm{NaV}1.7$, respectively. Further work to understand whether a similar relationship exists between $\mathrm{NaV}1.7$ target occupancy and analgesic pharmacodynamic effects in other preclinical pain models is ongoing.
Conclusion

NaV1.7 remains a compelling target for the development of non-opioid analgesics based on evidence from human genetics and rodent knock-out studies\(^1\). A major challenge in the pursuit of safe and effective NaV1.7 inhibitors has been the identification of small molecules that are selective over off-target proteins, including other NaV isoforms, to achieve a suitable margin of safety. Prior efforts to develop high precision NaV1.7 inhibitors have largely focused on a class of aryl and acyl sulfonamides that bind preferentially to VSD IV and impede recovery from inactivation\(^2\). In the present study, we disclose ST-2262, a synthetic analogue of natural bis-guanidinium toxins that lodges in the extracellular vestibule of the channel (Site 1) and occludes ion passage. A preliminary PK/PD study involving intravenous administration of ST-2262 to four cynomolgus subjects demonstrated increased withdrawal latency to noxious heat. Collectively, our findings validate the extracellular mouth of the sodium channel as a tractable receptor site for selective ligand design and provide insight into the distribution and target occupancy requirements for drug efficacy mediated by inhibition of NaV1.7.

Data availability

Additional raw data are available from the corresponding author on reasonable request.

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Author contributions

J.V.M., J.D.B., D.C.Y., H.P., and A.D. conceived of the study. H.P., J.T.B., A.D., H.S.H., G.L., D.M., X.Z., J.L., S.A., B.D.M., D.C.Y. and J.V.M. designed and performed experiments. J.T.B. and J.V.M. drafted the manuscript. J.D.B., D.C.Y., A.D. and B.D.M. critically revised the manuscript. All authors reviewed and approved the manuscript.

Competing interests

H.P., J.T.B., A.D., G.L., D.M., X.Z. and J.V.M. are employees of and shareholders in SiteOne Therapeutics. D.C.Y. and J.D.B. are advisors to and shareholders in SiteOne Therapeutics.

Additional information

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