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SHORT COMMUNICATION

New index of pain triggered by spinal activation of voltage-dependent sodium channels

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Abstract Voltage-dependent sodium channels (VDSCs) are crucial for pain generation. Here, to develop a new behavioral index of pain induced by spinal VDSC activation, we examined whether intrathecal veratridine injection produced nociceptive behavior. Intrathecal injection of the VDSC opener veratridine in mice dose-dependently induced nociceptive responses, with response times subsequently reduced by administration of morphine or pregabalin. Systemic administration of lidocaine and mexiletine, but not amitriptyline, also decreased this response time. Taken together, these results demonstrated that response time of nociceptive behavior induced by intrathecal veratridine injection is a quantitative index of pain triggered by spinal VDSC activation.

Keywords Veratridine · Nociceptive responses · Sodium channel blocker · Morphine · Pregabalin

Voltage-dependent sodium channels (VDSCs) are known to be crucial determinants of action potential generation in sensory neurons and play a key role in pain sensation [1]. However, although the molecular, biophysical, and pharmacological properties of VDSCs in peripheral neurons have been extensively studied, these properties have not yet been fully clarified in the spinal nervous system.

Veratridine, a lipid-soluble neurotoxin derived from the rhizomes of *Veratrum album*, targets the type 2 receptor site of VDSCs [2] and is commonly used as a VDSC opener in cell-based assays [3]. A general consensus has been reached that this neurotoxin binds selectively to VDSCs and locks them in an open conformation [4]. Veratridine infusion to the dorsal spinal cord has been reported to increase extracellular concentrations of glutamate, a pain-related substance [5]. However, whether intrathecal (i.t.) veratridine injection produces nociceptive behavior remains unclear. We therefore developed a new behavioral index of pain induced by i.t. injection of veratridine. Further, to determine analgesic effects of drugs that have been reported to inhibit veratridine-induced sodium influx in cell-based assays [3], we examined the effects of lidocaine, mexiletine, and amitriptyline in the present study.

This study was approved by the Committee for Animal Experiments of Astellas Pharma. Male ICR mice (23–26 g; SLC, Shizuoka, Japan) were housed in an air-conditioned room (room temperature 23 ± 2 °C, humidity 55 ± 10 %) with ad libitum access to food and water under a 12-h light–dark cycle. Veratridine (Sigma Chemical, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) and then diluted 250 fold with sterile artificial cerebrospinal fluid (CSF) [NaCl 142 mM, KCl 5.0 mM, $MgCl_2$ 2.0 mM, $CaCl_2$ 2.0 mM, NaH_2PO_4 1.25 mM, L-glucose 5.0 mM, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid 5.0 mM, 0.05 % bovine serum albumin, pH 7.4]. The i.t. injection was performed via the method described by Hylden and Wilcox [6]. A 5-μl volume was injected using 30-gauge needles between L5 and L6 of unanesthetized mice, with vehicle control animals receiving 0.4 % DMSO dissolved in CSF. Behavioral responses were observed after adapting each mouse to its personal transparent cage (22.0 × 15.0 × 12.5 cm) for approximately 60 min before veratridine injection (i.t.). Time spent exhibiting nociceptive behavior (head movements directed at the hindlimbs

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for biting, licking, or scratching episodes, or flinching episodes of the hind paw) was measured at 5-min intervals for 30 min immediately after veratridine injection in the time-course study and over a period of 15–20 min after veratridine injection in the pharmacological inhibition study. Morphine hydrochloride (Takeda Chemical) dissolved in saline (10 ml/kg) was administered intraperitoneally (i.p.) 45 min before behavioral observation, and pregabalin (prepared by Astellas Pharma) dissolved in distilled water (10 ml/kg) was administered orally (p.o.) 135 min before behavioral observation. Lidocaine (Sigma Chemical) dissolved in 1 % DMSO saline (10 ml/kg) was administered intravenously (i.v.) 10 min before behavioral observation, mexiletine hydrochloride (Sigma Chemical) dissolved in water (10 ml/kg) was administered (p.o.) 75 min before behavioral observation, and amitriptyline hydrochloride (Sigma Chemical) dissolved in saline (10 ml/kg) was administered (i.p.) 60 min before behavioral observation. Data values are presented as the mean ± SEM. Data were analyzed using two-way repeated-measures analysis of variance (ANOVA) and Dunnett's test. A significant difference was defined as $p < 0.05$.

Intrathecal injection of veratridine induced nociceptive responses, with no such response noted in vehicle control mice (two-way repeated-measures ANOVA: treatment, $p < 0.001$; time, $p < 0.001$; treatment × time, $p < 0.001$; Fig. 1). Given the present findings for peak timing in veratridine groups receiving 0.06 and 0.08 μg, we set the observation timing as between 15 and 20 min after veratridine injection for our pharmacological inhibition study. We also selected a veratridine dose of 0.10 μg for our subsequent studies, as nociceptive responses were stable throughout the entire observation period.

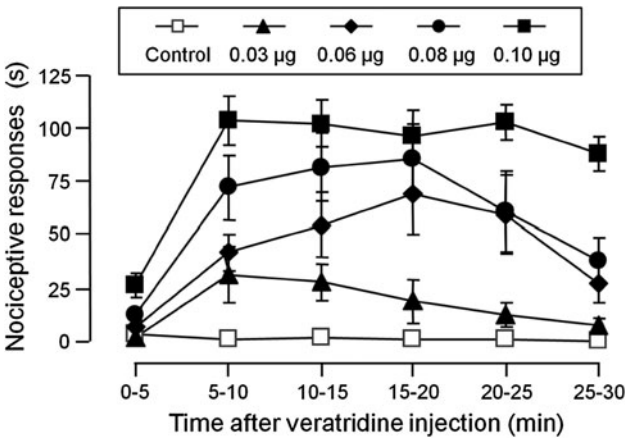

Fig. 1 Algesic effect of veratridine (sodium channel opener). Nociceptive responses (mean ± SEM; $n = 5$) are plotted against the time course after intrathecal injection of veratridine (0.03–0.1 μg)

Veratridine infusion to the dorsal spinal cord has been reported to increase extracellular concentrations of glutamate, a pain-related substance [5]. In this study, we showed that i.t. veratridine injection induced nociceptive responses (Fig. 1). Further, these nociceptive responses were shown to be inhibited by morphine and pregabalin, medicines used to treat pain (Table 1). Taken together, these findings suggest that veratridine activates the pain pathway. Future studies should investigate the changes in levels of various pain-related substances following i.t. veratridine injection.

The VDSC blocker lidocaine dose-dependently attenuated nociceptive responses in mice (Table 1), with a significantly lower response time following i.v. administration at 10 mg/kg than that of vehicle group. Lidocaine at 4 mg/kg (i.v.) is reported to inhibit spinal neuronal activity [7], and the lidocaine plasma concentration range (1–4 μg/ml) from 5 to 15 min after 10 mg/kg i.v. administration in rodents has been found to be markedly similar to the effective plasma level (0.62–5.0 μg/ml) for treating patients with pain [8, 9]. We also showed that mexiletine, a lidocaine-like sodium channel blocker, dose-dependently reduced nociceptive responses (Table 1). A significant effect was observed at dosages of 30 and 100 mg/kg. These findings obtained with two VDSC blockers indicated that

Table 1 Inhibitory effects of drugs on veratridine-induced nociceptive responses

Drug	Dose (mg/kg)	Nociceptive responses (s)
Morphine	0	100.3 ± 6.3
	1	93.2 ± 10.9
	3	64.2 ± 11.8*
	10	35.7 ± 3.9***
Pregabalin	0	93.8 ± 8.8
	10	85.2 ± 8.4
	30	63.7 ± 8.8
	100	26.3 ± 9.2***
Lidocaine	0	94.3 ± 3.5
	1	107.3 ± 10.9
	3	66.8 ± 8.0
	10	44.5 ± 8.4***
Mexiletine	0	95.3 ± 11.8
	10	76.8 ± 6.6
	30	34.0 ± 4.8***
	100	11.0 ± 1.8***
Amitriptyline	0	87.7 ± 6.2
	3	84.7 ± 8.8
	10	81.5 ± 11.6
	30	73.3 ± 5.8

Values are mean ± SEM ($n = 6$)

* $p < 0.05$, *** $p < 0.001$ versus veratridine-injected mice treated with vehicle

nociceptive responses induced by i.t. veratridine injection are a reasonable index for assessing the effects of VDSC blockers.

Amitriptyline is a VDSC-blocking agent that inhibits synaptosomal veratridine-induced sodium influx [3]. Amitriptyline, however, had no effect on veratridine-induced nociceptive responses in the present study (Table 1), suggesting that amitriptyline (3–30 mg/kg i.p.) does not inhibit spinal sodium channel activation. In contrast, many reports using rodents have demonstrated that amitriptyline inhibits nociception or hypersensitivity after inflammation and nerve injury [10, 11]. Amitriptyline is also a serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor that exerts an analgesic effect [12]. The binding efficacy for 5-HT and NE transporters [13] is much greater than inhibitory efficacy on veratridine-induced sodium influx [3]. Therefore, a higher dose of amitriptyline may be required to inhibit veratridine-induced nociceptive responses than the nociception or hypersensitivity after inflammation and nerve injury. Further, levels of both 5-HT and NE in rat brain have been reported to be affected at 30 mg/kg amitriptyline [11], suggesting that 5-HT and NE reuptake inhibition does not attenuate nociceptive responses induced by i.t. veratridine injection.

Several limitations to the present investigation warrant mention. Although analgesics and sodium channel blockers have been demonstrated to suppress the veratridine-induced nociceptive response, we cannot exclude the possibility that this response is also suppressed by drugs with other mechanisms of action. Indeed, our laboratory previously reported that the prostaglandin E_2-induced pain response was suppressed by duloxetine, 5-HT, and NE reuptake inhibitor [14]. Future efforts to characterize this model should involve testing a number of drugs.

In conclusion, we proposed a new behavioral index of pain induced by intrathecal injection of veratridine. The measurement of this response time, a quantitative index, is useful for investigating the inhibitory effect of drugs. Further studies using drugs with different mechanisms of action will enable clarification of the downstream signaling of spinal VDSCs in pain sensation.

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