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Tramadol as a Voltage-Gated Sodium Channel Blocker of Peripheral Sodium Channels $Na_v1.7$ and $Na_v1.5$

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Abstract

Tramadol is an opioid analog used to treat chronic and acute pain. Intradermal injections of tramadol at hundreds of millimoles have been shown to produce a local anesthetic effect. We used the whole-cell patch-clamp technique in this study to investigate whether tramadol blocks the sodium current in HEK293 cells, which stably express the pain threshold sodium channel $Na_v1.7$ or the cardiac sodium channel $Na_v1.5$. The half-maximal inhibitory concentration of tramadol was 0.73 mM for $Na_v1.7$ and 0.43 mM for $Na_v1.5$ at a holding potential of –100 mV. The blocking effects of tramadol were completely reversible. Tramadol shifted the steady-state inactivation curves of $Na_v1.7$ and $Na_v1.5$ toward hyperpolarization. Tramadol also slowed the recovery rate from the inactivation of $Na_v1.7$ and $Na_v1.5$ and induced stronger use-dependent inhibition. Because the mean plasma concentration of tramadol upon oral administration is lower than its mean blocking concentration of sodium channels in this study, it is unlikely that tramadol in plasma will have an analgesic effect by blocking $Na_v1.7$ or show cardiotoxicity by blocking $Na_v1.5$. However, tramadol could act as a local anesthetic when used at a concentration of several hundred millimoles by intradermal injection and as an antiarrhythmic when injected intravenously at a similar dose, as does lidocaine.

Key Words: Tramadol, Pain, Sodium channel, Local anesthetic

INTRODUCTION

As an opioid analog, tramadol is used to treat acute and chronic pain (Grond and Sablotzki, 2004). The analgesic effect of tramadol is thought to be achieved by opioid receptors and serotonin and norepinephrine reuptake inhibitors. Interestingly, intradermal injections of tramadol have been shown to have local anesthetic effects (Pang *et al*., 1998; Altunkaya *et al*., 2003). Because tramadol also blocks some ion channels such as $K_v3.1$ and $Na_v1.2$ (Haeseler *et al*., 2006; Tsai *et al*., 2006), we hypothesized that tramadol might affect pain-related ion channels. Voltage-gated sodium channels contribute to the generation of action potentials of nerves, muscles, and endocrine cells and are expressed differently depending on their function, characteristics, and location (Yu and Catterall, 2003; Catterall *et al*., 2005). $Na_v1.7$ is preferentially expressed in the sympathetic and sensory nerves and lowers the pain threshold in nociceptive neurons (Dib-Hajj and Waxman, 2019). Gain-of-function mutations in $Na_v1.7$ cause heritable pain disorders such as paroxysmal extreme pain disorder and inherited erythromelalgia. Conversely, loss-of-function mutations cause congenital indifference to pain (Cox *et al*., 2006; Waxman and Dib-Hajj, 2019), which is why they have been studied as major targets for developing analgesics. To date, whether tramadol blocks $Na_v1.7$ has not been investigated.

Mutations of the cardiac sodium channel $Na_v1.5$, which shares well-conserved sequences and structures with $Na_v1.7$, can cause hereditary heart diseases such as Brugada syndrome, atrial fibrillation, and sick sinus syndrome (Wang *et al*., 1996; Han *et al*., 2018). In this study, we investigated the effect of tramadol on $Na_v1.7$ and $Na_v1.5$ channels using the whole-cell patch-clamp technique.

MATERIALS AND METHODS

Cell preparation

Human embryonic kidney (HEK293) cells stably expressing human $Na_v1.7$ were purchased from Millipore (CYL3011; Millipore, Billerica, MA, USA). The stable cell line expressing

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human $Na_v1.5$ was established in a previous study (Choi *et al*., 2023). Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum was used to culture cells in a maintained humidified atmosphere at 37°C with 5% CO_2-enriched air. Cells were recorded after 24 h while plated on 12-mm circle glass coverslips.

Patch-clamp recordings

Whole-cell patch-clamp recordings were performed at room temperature (22-25°C) using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA, USA). Patch pipettes (0.8-1.3 MΩ) were pulled from soft glass capillaries (PG10165-4; World Precision Instruments, Sarasota, FL, USA) and polished using a microforge. In order to reduce the capacitance, we wrapped pipette tips with parafilm, which permitted a stable current recording with low-resistance pipettes during external bath solution perfusion. The cells were placed on the temperature-controlled recording chamber (RCP-10; Dagan Corporation, Minneapolis, MN, USA) and continuously perfused using the perfusion pencil (Automate Scientific, Berkeley, CA, USA) with an extracellular bath solution at 22°C. Cells were eliminated from the analysis if they had high leakage currents (holding current >0.5 nA at a holding potential of –120 mV) or an access resistance greater than 2 MΩ. The intracellular pipette solution contained 140 mM CsF, 1 mM EGTA, 10 mM NaCl, and 10 mM HEPES and was adjusted to pH 7.3 using CsOH and to 300 mOsm/L using sucrose. The external bath solution contained 140 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES and was adjusted to pH 7.3 using NaOH and to 305 mOsm/L using sucrose. Cells were recorded 5 min after establishing the whole-cell configuration to allow the currents to stabilize. Under the recording steps, the currents elicited from the holding potentials varied with each measurement process.

The sampling rate of currents was 100 kHz and they were filtered at 5 kHz. In all experiments, we minimized voltage errors using >80% series resistance compensation. Data acquisition and voltage-clamp pulses were controlled using pClamp 10.7 software (Molecular Devices) and a Digidata 1440A acquisition board (Molecular Devices).

Drug application

A 3 mM tramadol stock solution (Sigma, St Louis, MO, USA) was initially prepared by dissolving tramadol in the external bath solution and storing it at –20°C. We used the external bath solution to dilute the stock solution to obtain the desired concentration. Tramadol solutions were freshly made before the recordings and applied using a perfusion pencil (Automate Scientific) through a gravity-driven system, which allowed for rapid perfusion of the recording chamber. The cells were continuously perfused with the test solution during the recording.

Data analysis

Concentration-dependent inhibition of currents by tramadol was elicited using a 40 ms depolarizing pulse to 0 mV from holding potentials of –120 mV and –100 mV for $Na_v1.7$ at 5 s intervals. In the case of $Na_v1.5$, it was elicited from holding potentials between –120 mV and –90 mV. Data were best fitted to the logistic equation $Y=1/[1+(IC_{50}/T)^p]$ using Origin Pro 2015 software (OriginLab Corp., Northampton, MA, USA). In this equation, IC_{50} is the half-maximal inhibitory concentration; T is the tramadol concentration, and p is the Hill coefficient (n_H).

For voltage dependency of activation, whole-cell Na^+ currents were elicited by 50 ms test pulses to potentials between –80 mV and +40 mV in steps of 5 mV from a holding potential of –120 mV for $Na_v1.7$ and –100 mV for $Na_v1.5$. The peak current recorded after each voltage step was normalized into conductance (G) according to the formula $I=G(V–V_{rev})$. V_{rev}, in this formula, represents the reversal potential of the sodium current. Voltage-dependent activation curves were fitted using the Boltzmann equation $G/G_{max}=1/[1+\exp(V_{1/2}–V_m)/k]$. Here, $V_{1/2}$ is the voltage at the half-maximal conductance, V_m is the test potential, and k is the slope factor for the activation curve.

Sodium currents were elicited to 0 mV after 500 ms conditioning pulses from a holding potential of –120 mV for $Na_v1.7$ and –100 mV for $Na_v1.5$ for steady-state inactivation curves. The steady-state inactivation curves were fitted using the Boltzmann equation $I/Imax=1/[1+\exp(V_m–V_{1/2})/k]+C$. V_m is the preconditioning potential, $V_{1/2}$ is the midpoint potential, k is the slope factor of the curve, and C is the proportion of non-inactivating current.

Recovery from inactivation was measured as the peak current in response to a step to –10 mV, preceded by a 40 ms pulse to –10 mV and a recovery period with variable durations of i) 2, 5, 10, 100, 500, 1000, and a 5000 ms pulse for $Na_v1.7$ and ii) 2, 5, 10, 100, 500, and a 1000 ms pulse for $Na_v1.5$ from a holding potential of –120 mV.

Use-dependent inhibition was determined using 20 repetitive 40 ms depolarization pulses to 0 mV for $Na_v1.7$ from a holding potential of –120 mV at frequencies of 0.5, 1, 3, and 10 Hz. For $Na_v1.5$, the currents were elicited to –10 mV from a holding potential of –120 mV at frequencies of 0.5, 1, and 10 Hz.

Statistical analysis

The data were summarized as the mean ± SE. All data were analyzed using Clampfit 10.7 and Origin Pro 2015 software. Statistical analysis was performed using Student's t-test and one-way analysis of variance for comparisons of multiple groups followed by Fisher's test. Differences were considered significant at $p<0.05$.

RESULTS

Tramadol blocked the currents induced by $Na_v1.7$ or $Na_v1.5$ in a concentration-dependent manner

To examine whether tramadol (Fig. 1A) exhibits an analgesic effect by blocking a pain-threshold sodium channel, we investigated the mechanism by which tramadol blocks $Na_v1.7$. In addition, because of its structural similarity with $Na_v1.7$, the blocking effect of tramadol on $Na_v1.5$ was also investigated. Tramadol reduced the peak amplitudes of $Na_v1.7$ (Fig. 1B) and $Na_v1.5$ (Fig. 1C) currents in a concentration-dependent manner. The 50% inhibitory concentration (IC_{50}) and Hill coefficient (n_H) of tramadol for $Na_v1.7$ and $Na_v1.5$ were calculated using a logistic function (Fig. 1D, 1E). The IC_{50} values of tramadol for $Na_v1.7$ and $Na_v1.5$ were 0.98 ± 0.03 mM and 0.85 ± 0.04 mM, respectively, at a holding potential of –120 mV, and were lower at more depolarized holding potentials (Table 1). The n_H values of tramadol at all holding potentials were near 1, suggesting that the binding motif is single, and/or that the interaction of binding motifs is independent. The IC_{50} values

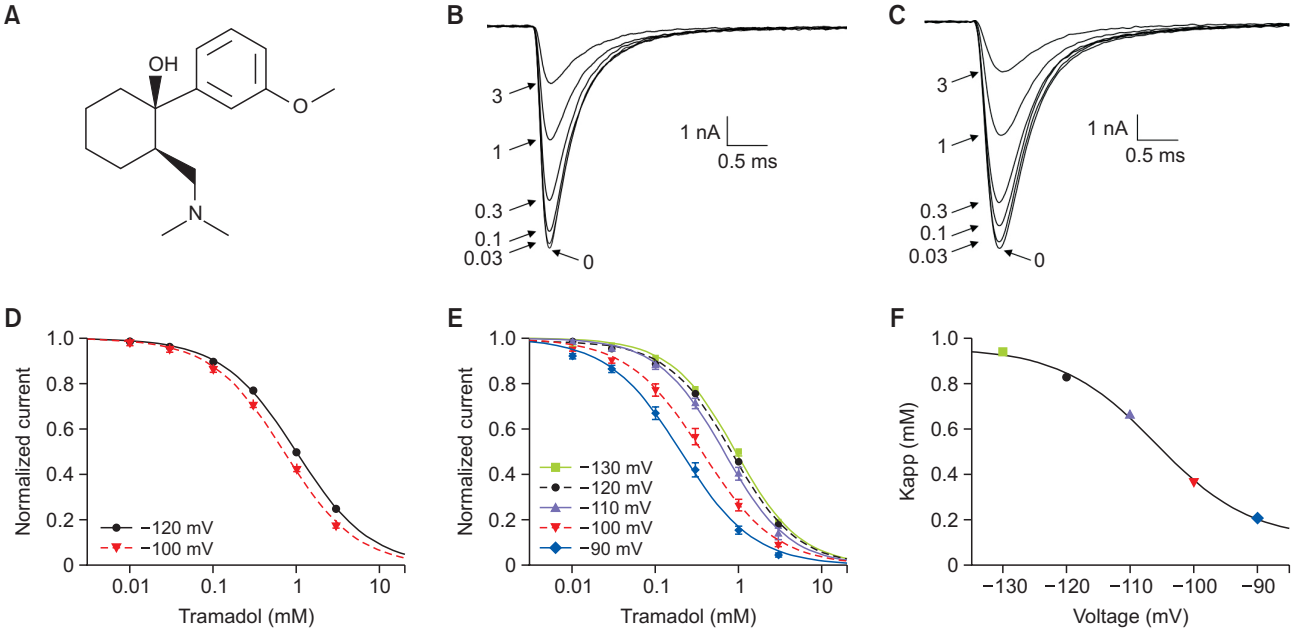

Fig. 1. Concentration-dependent block and washout of tramadol on $Na_v1.7$ and $Na_v1.5$ currents. (A) Chemical structure of tramadol. Representative traces of whole-cell currents of $Na_v1.7$ (B) and $Na_v1.5$ (C) in the absence and presence of tramadol (mM). Normalized peak currents of $Na_v1.7$ (D) and $Na_v1.5$ (E) are plotted as a function of tramadol concentrations. (F) Kapp values were calculated from IC_{50} using the equation y=1/(1+[tramadol]/Kapp). The fitted line is the best fit to the equation Kapp=1/[h/Kc+(1–h)/Ki)], where h and 1–h are the fractions of channels in the closed and inactivated states of $Na_v1.5$, respectively.

Table 1. The inhibition of $Na_v1.7$ and $Na_v1.5$ currents by tramadol

Holding potential	$Na_v1.7$	
	IC_{50} (mM)	n_H
–100 mV (n=11)	0.73 ± 0.04	1.00 ± 0.05
–120 mV (n=12)	0.98 ± 0.03	0.97 ± 0.02
Holding potential	$Na_v1.5$	
	IC_{50} (mM)	n_H
–90 mV (n=10)	0.22 ± 0.02	1.02 ± 0.04
–100 mV (n=19)	0.43 ± 0.06	1.06 ± 0.05
–110 mV (n=13)	0.73 ± 0.07	1.10 ± 0.02
–120 mV (n=10)	0.85 ± 0.04	1.03 ± 0.02
–130 mV (n=7)	1.02 ± 0.08	1.03 ± 0.03

Parameters obtained from fitting concentration-dependent curves by a logistic function. IC_{50} indicates the 50% inhibitory concentration. nH indicates the Hill coefficient.

of tramadol for $Na_v1.5$ were found to be voltage-dependent. Therefore, in order to better understand the binding of tramadol to $Na_v1.5$, we calculated the apparent affinity of tramadol binding (Kapp) as well as the affinities for binding to the closed state (Kc) and to the inactivated state (Ki) (as shown in Fig. 1F), based on the previous report (Bean *et al.*, 1983). The resulting apparent values for Kc and Ki were 0.96 mM and 0.12 mM, respectively.

The effects of tramadol on the channels are completely reversible

Next, we examined whether tramadol accumulated in the channel (Fig. 2) and found that 3 mM of tramadol resulted in the blockage of the peak current of $Na_v1.7$ by 23.61 ± 0.29% at a holding potential of –120 mV (n=4). The time constant of the onset of inhibition (t_{on}) was 1.5 ± 0.1 s. The blockage of the $Na_v1.7$ current by tramadol recovered after a brief washout, and the time constant of the offset of blockade (t_{off}) by tramadol was 3.1 ± 0.2 s. The results showed that 1 mM of tramadol blocked the peak current of $Na_v1.5$ by 32.53 ± 2.59% at a holding potential of –100 mV, and the time constant of the inhibition onset (t_{on}) was 7.1 ± 1.6 s (n=4). Blockage of $Na_v1.5$ currents by tramadol recovered after a brief washout, and the time constant of the offset of the blockade (t_{off}) by tramadol was 8.3 ± 0.7 s. Repeated application of tramadol did not result in any accumulation of its inhibitory effect in either channel. Altogether, blocking and washing out of tramadol were rapid and completely reversible.

Tramadol changed the voltage-dependent, steady-state inactivation of $Na_v1.7$ and $Na_v1.5$

We investigated the voltage-dependent activation and steady-state inactivation curves depending on the presence of tramadol using 1 mM for $Na_v1.7$ and 0.3 mM for $Na_v1.5$. The representative current traces recorded from cells expressing $Na_v1.7$ and $Na_v1.5$ channels are shown in Fig. 3A and 3B, respectively. The voltage-dependent activation curves were fitted with the Boltzmann function (Fig. 3C, 3D, Table 2). The $V_{1/2}$ value of the activation curve for $Na_v1.7$ was shifted in a significantly hyperpolarized direction in the presence of tramadol, 6.73 mV more than in the absence of tramadol. However, the

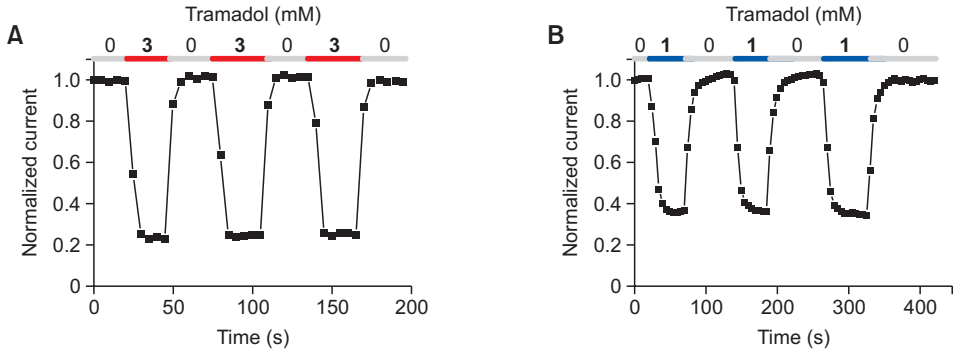

Fig. 2. Reversible inhibition of Na$_v$1.7 and Na$_v$1.5 currents by tramadol. Time course of Na$_v$1.7 (A) and Na$_v$1.5 (B) current inhibition by tramadol. The bar indicates the application time of tramadol (red or blue) and the control solution (gray).

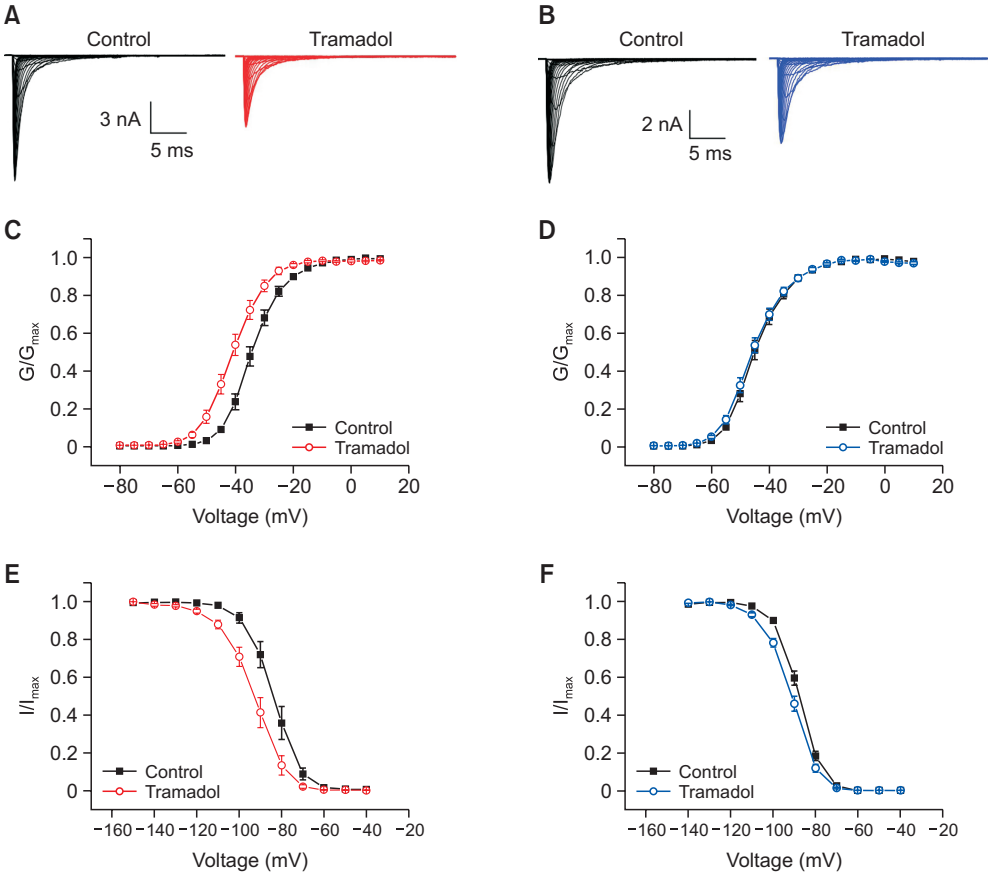

Fig. 3. Voltage-dependent block and steady-state inactivation of Na$_v$1.7 and Na$_v$1.5 in the presence and absence of tramadol. The representative traces of Na$_v$1.7 currents in the absence and presence of 1 mM tramadol (A) and of Na$_v$1.5 currents in the absence and presence of 0.3 mM tramadol (B). The voltage-dependent activation curves for Na$_v$1.7 in the absence (■) and presence (○) of 1 mM tramadol (C) and for Na$_v$1.5 in the absence and presence of 0.3 mM tramadol (D). (E, F) The steady-state inactivation curves of Na$_v$1.7 in the absence (■) and presence (○) of 1 mM tramadol (E) and those of Na$_v$1.5 in the absence and presence of 0.3 mM (F).

$V_{1/2}$ of the activation curve for Na$_v$1.5 was not changed. The slope (*k*) of the activation curve in the presence of tramadol was significantly shallower than that in the absence of tramadol for Na$_v$1.5, but the difference was only 0.43 mV. In addition, the steady-state inactivation curves of Na$_v$1.7 and Na$_v$1.5 were shifted in the direction of hyperpolarization by tramadol (Fig. 3E, 3F, Table 2). The slopes of Na$_v$1.7 and Na$_v$1.5 in the presence of tramadol were significantly shallower than in the absence of tramadol.

Tramadol decreased the rate of recovery from the inactivation of Na$_v$1.7 and Na$_v$1.5

As the steady-state inactivation of Na$_v$1.7 and Na$_v$1.5 showed a hyperpolarization shift in the presence of tramadol

Table 2. The $V_{1/2}$ and slope of the voltage-dependent activation and inactivation for $Na_v1.7$ and $Na_v1.5$ in the presence and absence of tramadol

The voltage-dependent activation curves				
	$Na_v1.7$		$Na_v1.5$	
	$V_{1/2}$ (mV)	*k* (mV)	$V_{1/2}$ (mV)	*k* (mV)
Control	–33.92 ± 1.07 (n=11)	5.02 ± 0.18	–44.33 ± 1.07 (n=7)	5.60 ± 0.27
Tramadol	–40.65 ± 1.32 (n=11)*	5.21 ± 0.16	–45.10 ± 0.94 (n=7)	6.02 ± 0.25*
The steady-state inactivation curves				
	$Na_v1.7$		$Na_v1.5$	
	$V_{1/2}$ (mV)	*k* (mV)	$V_{1/2}$ (mV)	*k* (mV)
Control	–83.87 ± 2.22 (n=6)	5.58 ± 0.09	–88.12 ± 0.89 (n=7)	5.10 ± 0.08
Tramadol	–93.08 ± 2.19 (n=6)*	7.07 ± 0.37*	–91.49 ± 1.04 (n=7) *	5.89 ± 0.12 *

*p<0.05 vs. control.

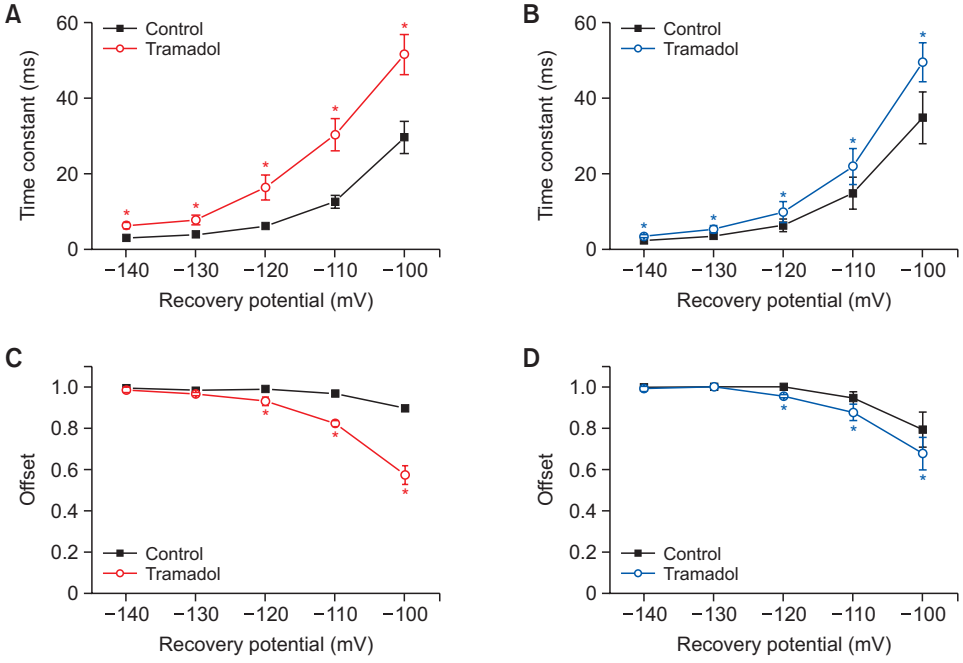

Fig. 4. Recovery from inactivation of $Na_v1.7$ and $Na_v1.5$ currents in the presence and absence of tramadol. (A, B) Voltage dependencies of time constants of recovery from inactivation were fitted by exponential function in the absence (■) and the presence (○) of tramadol. (A) The time constant of $Na_v1.7$ was compared in the absence and presence of 1 mM tramadol. (B) The time constant of $Na_v1.5$ was compared in the absence and presence of 0.3 mM tramadol. (C, D) Voltage dependencies of maximal recoveries from inactivation (offset) in the absence (■) and presence (○) of tramadol. (C) Offsets of $Na_v1.7$ compared in the absence and presence of 1 mM tramadol. (D) Offsets of $Na_v1.5$ compared in the absence and presence of 0.3 mM tramadol (*p<0.05 versus control by Student's t-test).

(Fig. 3E, 3F), we assumed that the rate of recovery from inactivation would be much slower than the rate of entry into inactivation. This would lead to more channels being in an inactivated state for a longer time. Therefore, we analyzed the recovery from inactivation to test our hypothesis. The recovery kinetics from inactivation in the presence and absence of tramadol fitted well with monoexponential functions. The time constants of recovery from the inactivation of $Na_v1.7$ and $Na_v1.5$ currents in the presence of tramadol were significantly slower than those in the absence at all tested voltages (Fig. 4A, 4B). The offset of recovery from inactivation for $Na_v1.7$ and $Na_v1.5$ in the presence of tramadol decreased significantly at recovery potentials between –120 mV and –100 mV (Fig. 4C, 4D).

Tramadol induced a use-dependent inhibition of $Na_v1.7$ and $Na_v1.5$ channels

As tramadol slowed the recovery rate from inactivation, it could induce the accumulation of inactivated states with high-frequency depolarization pulses. As expected, tramadol caused a strong use-dependent inhibition of $Na_v1.7$ (Fig. 5A) and $Na_v1.5$ (Fig. 5B). At 0.5, 1, 3, and 10 Hz, the peak current

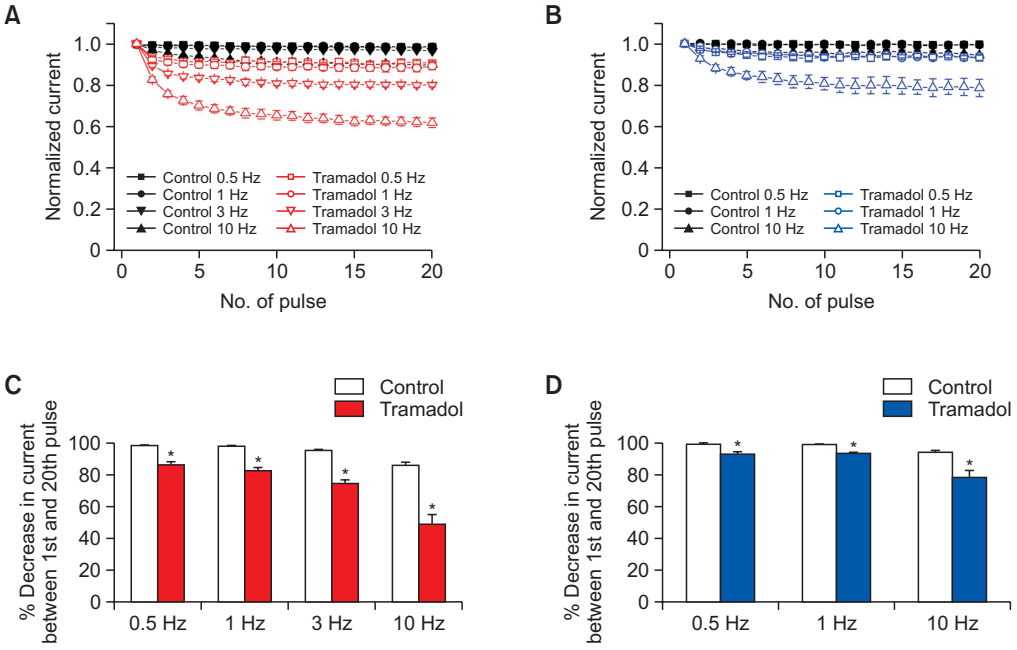

Fig. 5. Use-dependent inhibition of $Na_v1.7$ and $Na_v1.5$ in the presence and absence of tramadol. (A, B) Use-dependent fall-off of peak currents in the absence (closed symbols) and presence of tramadol (open symbols) at 0.5 (squares), 1 (circle), 3 (inverted triangles), and 10 (triangles) Hz frequency stimulation for $Na_v1.7$ (A) and $Na_v1.5$ (B). (C, D) The percentages of the peak current between the 1st and 20th pulse of each frequency stimulation protocol in the absence (black) and presence (gray) of tramadol. (C) The peak currents of $Na_v1.7$ were compared in the absence and presence of 1 mM tramadol. (D) The peak currents of $Na_v1.5$ were compared in the absence and presence of 0.3 mM tramadol (*p<0.05 versus control by Student's t-test).

amplitudes of $Na_v1.7$ decreased by 9.5, 10.8, 19.8, and 38.0%, respectively (Fig. 5C). At 0.5, 1, and 10 Hz, the peak current amplitudes of $Na_v1.5$ decreased by 6.2, 6.8, and 21.3%, respectively (Fig. 5D). Taken together, the results showed that tramadol exhibited a stronger use-dependent inhibition in proportion to the stimulation frequency.

DISCUSSION

In the present study, we investigated the effect of tramadol on $Na_v1.7$ and $Na_v1.5$ currents stably expressed in HEK293 cells. Due to the difficulty in developing isoform-specific Na_v channel blockers, newly developed, relatively non-specific, voltage-gated sodium channel blockers often cause adverse effects in patients (Mulroy, 2002; de Lera Ruiz and Kraus, 2015; Dokken and Fairley, 2021). Because some opioid analogs such as fentanyl, oxycodone, buprenorphine, meperidine, and loperamide block sodium channels (Wagner *et al*., 1999; Olschewski *et al*., 2001; Wolff *et al*., 2004; Haeseler *et al*., 2006; Leffler *et al*., 2012; Wu *et al*., 2017; Meents *et al*., 2018), we aimed to determine whether tramadol also blocks sodium channels.

Our results indicate that tramadol blocked $Na_v1.7$ and $Na_v1.5$ currents in a concentration- and use-dependent manner. Tramadol induced a greater decrease in peak amplitude of $Na_v1.7$ and $Na_v1.5$ when the channels were inactivated at more depolarized holding potentials. Therefore, tramadol may bind to a greater extent with the inactivated states rather than with the closed states of such channels. The Hill coefficients of tramadol for $Na_v1.7$ and $Na_v1.5$ were near 1 (Table 1), and the blockage of these channels by tramadol was rapidly washed out (Fig. 2A, 2B), suggesting that tramadol may noncovalently bind to the extracellular binding sites of these channels with 1:1 stoichiometry.

Tramadol has been reported to have local anesthetic effects (Pang *et al*., 1998; Altunkaya *et al*., 2003) and inhibits nerve conduction *in vivo* (Mert *et al*., 2003; Beyazova *et al*., 2011). Tramadol inhibits sodium channels in a manner similar to lidocaine-like local anesthetics in that it has a higher binding affinity for inactivated channels than for resting channels (Haeseler *et al*., 2006). The hyperpolarizing shift of the steady-state inactivation curve and the use-dependent inhibition by tramadol stabilizes the inactivation state of $Na_v1.7$ channels and modulates sodium influx. Moreover, as most $Na_v1.7$ channels are inactivated at membrane potentials between –60 mV and –70 mV (Fig. 3E), tramadol may exhibit a higher $Na_v1.7$ blocking effect than that in experimental conditions at the physiologic resting potential of neurons. In another study, tramadol showed an IC_{50} of 25 μM for the delayed rectifier K^+ (K_{DR}) channel (Tsai *et al*., 2006). Inhibition of the K_{DR} channels can inhibit the repolarization of neural action potentials, thereby increasing the proportional, inactivated state of $Na_v1.7$ by prolonging depolarized membrane potentials (Tsai *et al*., 2006). Meperidine (Wolff *et al*., 2004) and droperidol (Olschewski *et al*., 2001), both opioids with local anesthetic effects, showed this simultaneous inhibition effect on voltage-gated K^+ and Na^+ channels, resulting in decreased action potential frequencies. Therefore, this implies that tramadol may also inhibit the neuron firing frequency through multiple mechanisms that inhibit K^+ and Na^+ channels, in addition to $Na_v1.7$. These mechanisms may include inhibition of $Na_v1.8$ and $Na_v1.9$, which also

play a role in pain sensation.

Tramadol is a relatively safe opioid drug and severe cardiovascular toxicity has not been reported (Smyj *et al*., 2013). The therapeutic concentration of tramadol is 1-2 μM (Grond and Sablotzki, 2004), and in this study, the IC_{50} of tramadol for $Na_v1.7$ was 0.73 mM at a holding potential of –100 mV. However, the IC_{50} value could be lower at the resting membrane potential (~–60 mV) of dorsal root ganglions (Choi *et al*., 2007). The IC_{50} of lidocaine for $Na_v1.7$ has been found to be 0.45 mM (Chevrier *et al*., 2004), which is similar to the results of this study, suggesting that the mechanism by which tramadol acts as a local anesthetic might be similar to that of lidocaine. Interestingly, the intradermal injection of tramadol showed local anesthetic effects similar to those of lidocaine (Pang *et al*., 1998; Altunkaya *et al*., 2003).

However, at a holding potential of –90 mV, which is close to the cardiac resting membrane potential, the IC_{50} of $Na_v1.5$ was 0.22 mM. This is more than 100 times higher than the peak plasma concentration of tramadol for oral, rectal, and intramuscular use (Grond and Sablotzki, 2004). In addition, considering that the maximum plasma concentration of a 19-year-old male who abused tramadol for 6 months was ~30 μM (Faria *et al*., 2018) and the IC_{50} of tramadol for $Na_v1.5$ was higher than that concentration (Grond and Sablotzki, 2004), it would be difficult for tramadol to affect $Na_v1.5$ at a therapeutic concentration. According to a recent report (Emamhadi *et al*., 2012), patients who took tramadol presented with tachycardia and QT prolongation. In this study, tramadol slowed the rate of recovery from the inactivation of $Na_v1.5$ (Fig. 4B) and blocked repeated activated currents (Fig. 5B), suggesting that the administration of tramadol mimics the loss-of-function of $Na_v1.5$ that can lead to cardiac dysfunction (Calloe *et al*., 2013; Nakajima *et al*., 2015).

As tramadol showed a binding affinity for $Na_v1.2$ (Haeseler *et al*., 2006), we postulate that tramadol may interact with $Na_v1.7$ and $Na_v1.5$ because of the similarity between these two voltage-gated sodium channels (Yu and Catterall, 2003; Catterall *et al*., 2005). Therefore, as tramadol blocked $Na_v1.7$ and $Na_v1.5$ in this experiment, it is possible that tramadol interacts with other isoforms of voltage-gated sodium channels, such as $Na_v1.8$ and $Na_v1.9$, sodium channels considered promising targets for painkillers. The hyperpolarization of steady-state inactivation along with the concentration- and use-dependent block all are commonly observed mechanisms of tramadol for $Na_v1.2$ (Haeseler *et al*., 2006), $Na_v1.7$, and $Na_v1.5$ (Fig. 1D, 1E, 3E, 3F, 5A, 5B). If $Na_v1.8$ and $Na_v1.9$ act via these same mechanisms, we could hypothesize that there is an additional possibility of tramadol being a local anesthetic, as well as an opioid analog.

In conclusion, we showed that tramadol alters the electrophysiological properties of $Na_v1.5$ and $Na_v1.7$ channels. Although tramadol does not yet have a pharmacological application as a voltage-gated sodium channel blocker, it is possible that tramadol-induced alterations in the gating properties of Na_v channels could be exploited in novel treatments as a sodium channel blocker.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

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