

Original Article

Metergoline inhibits the neuronal Nav1.2 voltage-dependent Na⁺ channels expressed in *Xenopus* oocytes

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Aim: Metergoline is an ergot-derived psychoactive drug that acts as a ligand for serotonin and dopamine receptors. The aim of this study was to investigate the regulatory effects of metergoline on the neuronal Nav1.2 voltage-dependent Na⁺ channels *in vitro*.

Methods: *Xenopus* oocytes were injected with cRNAs encoding rat brain Nav1.2 α and β 1 subunits. Voltage-activated Na⁺ currents were recorded using two-electrode voltage clamp technique. Drugs were applied through perfusion.

Results: Both metergoline and lidocaine reversibly and concentration-dependently inhibited the peak of Na⁺ currents with IC₅₀ values of 3.6±4.2 and 916.9±98.8 μ mol/L, respectively. Metergoline (3 μ mol/L) caused a 6.8±1.2 mV depolarizing shift of the steady-state activation curve of the Na⁺ currents, and did not alter the inactivation curve. In contrast, lidocaine (3 μ mol/L) caused a 12.7±1.2 mV hyperpolarizing shift of the inactivation curve of the Na⁺ currents without changing the steady-state activation curve. Both metergoline and lidocaine produced tonic and use-dependent inhibition on the peak of Na⁺ currents.

Conclusion: Metergoline exerts potent inhibition on the activity of neuronal Nav1.2 channels, which may contribute to its actions on the central nervous system.

Keywords: metergoline; ergot alkaloid; antidepressant; Na⁺ channels; Nav1.2; *Xenopus* oocyte; lidocaine

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Introduction

In mammals, there are nine sodium channel α subunit isoforms (Nav1.1–1.9) found in excitable tissues in the central nervous system, peripheral nervous system, muscle, and heart^[1]. Na⁺ channels are transmembrane proteins that consist of a pore-forming α subunit and auxiliary β 1– β 4 subunits^[2]. The α subunit includes four homologous domains (I–IV), each composed of six α -helical transmembrane segments (S1–S6); among them, the S4 segment acts as the voltage-sensing apparatus of the Na⁺ channel^[3]. The pore-forming α subunit is responsible for voltage-dependent increases in Na⁺-selective permeability. These changes trigger the inward Na⁺ current that initiates axonal and somatic action potentials in nerve and muscle fibers and may also be involved in axonal information

transfer during intraneuronal or interneuronal communication^[3]. Agents, such as neurotoxins and other drugs, exhibit their pharmacological effects by modulating Na⁺ channels. For example, flecainide (an anti-arrhythmic drug), lidocaine (a local anesthetic), and phenytoin (an anticonvulsant) confer their pharmacological actions by altering both Na⁺ permeation and gating properties^[4].

Ergot is a fungus found on grain and other plants since the earliest days of farming^[5], and ergot alkaloids are one of the constituents of ergot fungi. Metergoline is a representative ergot-derived drug that has been studied in a variety of clinical settings, including as a treatment for seasonal affective disorder^[6], prolactin hormone regulation^[7], premenstrual dysphoric disorder in women^[8], carbon dioxide-induced anxiety^[9], and in veterinary medicine as a pregnancy termination drug for dogs^[10]. Metergoline and ergot derivatives are used clinically for lactation inhibition in disorders associated with hyperprolactinemia and in the prophylaxis of migraine headaches^[7, 11]. The pharmacological properties of metergoline have well-known interactions with the serotonergic 5-hydroxytryptamine (5-HT) system. Metergoline acts as an

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antagonist at many of the 5-HT receptor subtypes at a relatively low concentration^[12] and has dopamine agonist properties^[13]. While metergoline is known to be an effective clinical treatment, little is known regarding the effects of metergoline on different types of receptors and ion channels.

In the present study, we examined whether metergoline could exert inhibitory effects on neuronal Na⁺ currents mediated by rat brain Nav1.2 α and β 1 subunits. The cRNAs encoding these channels were expressed in *Xenopus* oocytes, a model system that has several endogenous ion channels^[14] and allows heterologous expression of ion channels for various biochemical studies^[14, 15]. Our results revealed that metergoline inhibited Na⁺ currents in a concentration-dependent and reversible manner. In addition, the effects of metergoline were use- and state-dependent. Together, these results show that metergoline can inhibit neural Na⁺ channels and that Na⁺ channel inhibition could be one of the pharmacological effects of metergoline in the central nervous system.

Materials and methods

Materials

The cDNA for the rat brain Na⁺ channel Nav1.2 α and β 1 subunits was kindly provided by Dr AL Goldin (University of California, Irvine, CA, USA). Metergoline (Tocris Bioscience, Ellisville, MS, USA) was dissolved in dimethyl sulfoxide (DMSO) and diluted with bath medium before use. The final DMSO concentration was less than 0.05% in all cases. The other agents were purchased from Sigma (St Louis, MO, USA).

Preparation of the cRNA encoding the Na⁺ channel Nav1.2 α and β 1 subunits and microinjection

The cDNA encoding the Nav1.2 α and β 1 subunits was linearized by digestion with the appropriate restriction enzymes. The cRNA from the linearized templates was obtained using an *in vitro* transcription kit (mMessage mMachine; Ambion, Austin, TX, USA) with T7 polymerase. The cRNA was dissolved in RNase-free water at a concentration of 1 μ g/ μ L, divided into aliquots and stored at -70 °C until use. The oocytes were injected with a 1:2 mixture of the cRNA encoding the Na⁺ channel Nav1.2 α and β 1 subunits, using a Nanoject Automatic Oocyte Injector (Drummond Scientific, Broomall, PA, USA). The injection pipette was pulled from glass capillary tubing and used for recording electrodes; the tip had a diameter of 15–20 μ m^[15].

Preparation of *Xenopus* oocytes

Xenopus laevis frogs were purchased from Xenopus I (Ann Arbor, MI, USA). The care and handling of all animals used in this experiment were in accordance with the highest standards of the institutional guidelines. To isolate the oocytes, the frogs were anesthetized with an aerated solution of 3-aminobenzoic acid ethyl ester. The oocytes were then surgically removed and separated by collagenase treatment, followed by agitation for 2 h in a Ca²⁺-free medium containing 82.5 mmol/L NaCl, 2 mmol/L KCl, 1 mmol/L MgCl₂, 5 mmol/L HEPES, 2.5 mmol/L sodium pyruvate, 100 units/mL penicillin, and

100 μ g/mL streptomycin. Stage VI oocytes were then collected and stored in ND96 (96 mmol/L NaCl, 2 mmol/L KCl, 1 mmol/L MgCl₂, 1.8 mmol/L CaCl₂, and 5 mmol/L HEPES, pH 7.5) supplemented with 0.5 mmol/L theophylline and 50 μ g/mL gentamicin. This oocyte-containing solution was then maintained at 18 °C under continuous gentle shaking and changed daily. Electrophysiological experiments utilizing the oocytes were conducted within 5–6 days after isolation.

Data recording

A custom-made Plexiglas net chamber was used for the two-electrode voltage clamp recordings. The chamber was constructed by milling two concentric wells into the chamber bottom (diameter/height: upper well, 8/3 mm; lower well, 6/5 mm) and then gluing a plastic mesh (approximately 0.4 mm grid diameter) onto the bottom of the upper well. The perfusion inlet (approximately 1 mm in diameter) was then formed through the wall of the lower well, and a suction tube was placed on the edge of the upper well. Individual oocytes were placed on the net that separated the upper and lower wells, with the net grids serving to keep the oocytes in place during the electrophysiological recordings. Each oocyte was impaled with two microelectrodes filled with 3 mol/L KCl (0.2–0.7 M Ω). Recordings were performed in ND96 solution. All electrophysiological experiments were conducted at room temperature using an oocyte clamp (OC-725C; Warner Instruments, Hamden, CT, USA), and stimulation and data acquisition were controlled using a pClamp 10.2 (Axon Instruments, Union City, CA, USA).

Data analysis

To obtain the concentration response curves describing the effects of metergoline on the Na⁺ currents, the peak currents at different concentrations of metergoline were plotted. Origin Pro 7.0 software (Origin, Northampton, MA, USA) was then used to fit the plot to the Hill equation, which is as follows: $y/y_{\max} = [A]^{n_{\text{H}}} / ([A]^{n_{\text{H}}} + [IC_{50}]^{n_{\text{H}}})$, where y is the peak current at a given concentration of metergoline or lidocaine, y_{\max} is the maximal peak current, IC_{50} is the concentration of agents that produces a half-maximum effect, $[A]$ is the concentration of metergoline or lidocaine, and n_{H} is the interaction coefficient. IC_{50} values were obtained using the Origin software.

The voltage-dependence of Na⁺ channel activation was calculated by measuring the peak current at test potentials ranging from -50 mV to +10 mV evoked in 5 mV increments. The conductance (g_{Na}) was calculated according to the equation, $g_{\text{Na}} = I_{\text{Na}} / (V_{\text{g}} - V_{\text{r}})$, where I_{Na} is the peak amplitude of the Na⁺ current, V_{g} is the test potential, and V_{r} is the reversal potential for Na⁺. The conductance-voltage curves were drawn according to the equation $g_{\text{Na}} / \max g_{\text{Na}} = 1 / \{1 + \exp [(V_{\text{g}0.5} - V_{\text{g}}) / kg]\}$, where $\max g_{\text{Na}}$ is the maximum value for g_{Na} , $V_{\text{g}0.5}$ is the potential at which g_{Na} is 0.5 $\max g_{\text{Na}}$ and kg is the slope factor (potential required for an e -fold change). The voltage-dependence of Na⁺ channel inactivation was determined using 500 ms conditioning pre-pulses ranging from -90 mV to -20 mV from a holding potential of -100 mV in 5 mV increments, followed by

a test pulse of -10 mV for 5 ms.

The peak current was normalized to its respective maximum value (I_{Na}/I_{Na}^{max}) and plotted as a function of the pre-pulse potential. The steady-state inactivation curves were drawn according to the equation $I_{Na}/I_{Na}^{max} = 1 / \{1 + \exp [(V_h - V_{h0.5}) / kh]\}$, where V_h is the pre-pulse potential, $V_{h0.5}$ is the potential at which I_{Na} is 0.5 I_{Na}^{max} and kh is the slope factor. The frequency-dependent effect of metergoline was examined using a protocol in which 60 depolarizing pulses of 10 ms duration and 10 Hz frequency were applied to -10 mV from a holding potential of -100 mV. The protocol was performed in the absence and presence of metergoline or lidocaine. The current amplitude of each pulse was normalized to the peak maximal current (pulse number 1) and plotted as a function of the pulse number.

All values are presented as the mean \pm SEM. The differences between the mean values of the control and treatment values were determined using an unpaired Student's *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Effects of metergoline on neuronal Nav1.2 channel currents and the current-voltage relationship

The effect of metergoline on the currents from neuronal Nav1.2 channels expressed in *Xenopus* oocytes was examined. The Na⁺ channel current was recorded using the two-electrode voltage clamping of oocytes injected with cRNAs encoding Nav1.2 α and β 1 subunits. We first constructed the current-voltage relationship curves in the absence of metergoline. To elicit the currents, we applied voltage steps (200-ms duration) at 5-s intervals from a holding potential of -100 mV. The currents evoked were transient inward Nav1.2 channel currents that decayed rapidly. As shown in Figure 1, the addition of

metergoline resulted in a significant reduction of the peak inward current. Metergoline (10 μ mol/L) inhibited the peak currents by 77.9% \pm 8.2% in Nav1.2 channels.

Concentration-dependent and reversible inhibition of the Nav1.2 channels by metergoline

Next, oocytes were held at -100 mV, and the Na⁺ channel current was elicited by depolarization to -10 mV at a low frequency (0.2 Hz). This procedure minimized the use-dependent blockade and allowed us to evaluate whether metergoline produced tonic blockades of peak Na⁺ currents^[4, 15]. For comparison, we examined the effect of lidocaine on the peak Na⁺ currents. Application of lidocaine (300 μ mol/L) or metergoline (10 μ mol/L) inhibited the peak Na⁺ currents by 23.1% and 67.9%, respectively, indicating that both agents induced a tonic inhibition of the Na⁺ current. In Figure 2B, the representative traces depict lidocaine- or metergoline-induced inhibition of the channel current. Figure 2C and 2D show concentration-dependent responses of metergoline and lidocaine during the inhibition of Na⁺ currents. We found that metergoline inhibited peak Na⁺ currents in a concentration-dependent manner that was saturated at approximately 30 μ mol/L. The IC₅₀ range of values of metergoline was 3.6 \pm 4.2 μ mol/L ($n=10-12$ from five different frogs). The Hill coefficient was 1.1 \pm 0.2. However, the values of IC₅₀ and hill coefficient of lidocaine were 916.9 \pm 98.8 μ mol/L and 1.1 \pm 0.2, respectively.

Effects on steady-state activation and inactivation of Nav1.2 channels by metergoline

Next, we examined the effects of metergoline and lidocaine on the voltage-dependence of Na⁺ channel steady-state activation and inactivation. First, the effect of metergoline on Na⁺

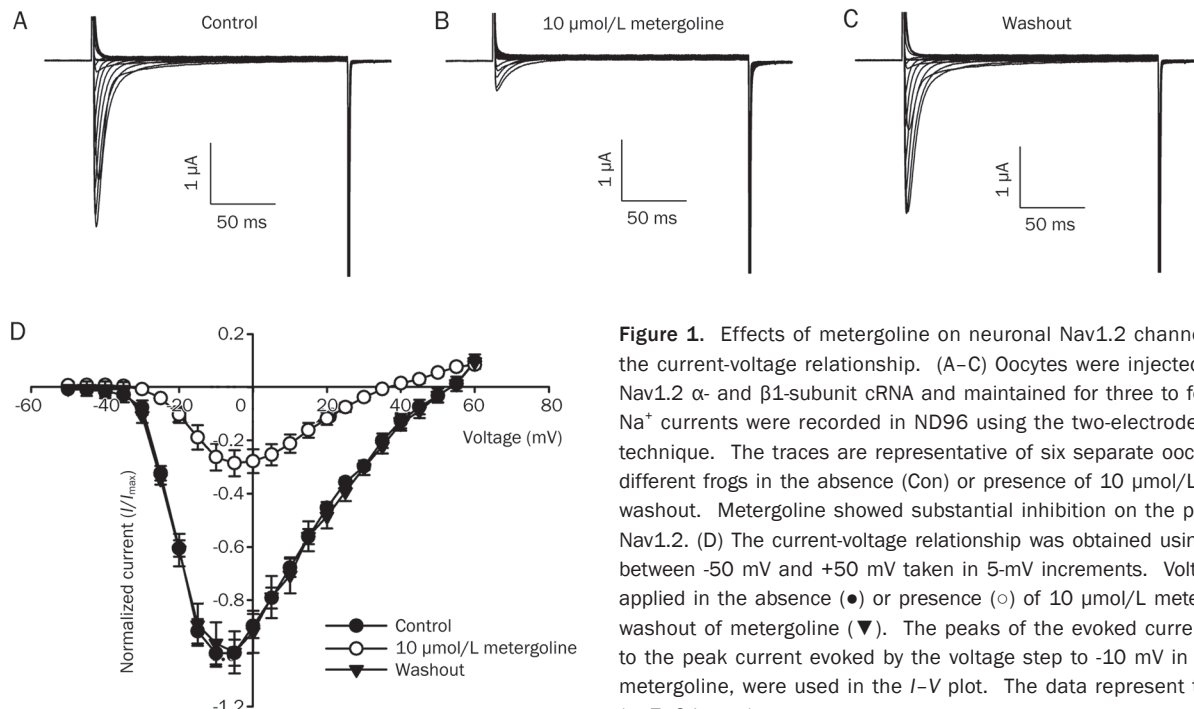


Figure 1. Effects of metergoline on neuronal Nav1.2 channel currents and the current-voltage relationship. (A–C) Oocytes were injected with wild-type Nav1.2 α - and β 1-subunit cRNA and maintained for three to four days before Na⁺ currents were recorded in ND96 using the two-electrode voltage clamp technique. The traces are representative of six separate oocytes from three different frogs in the absence (Con) or presence of 10 μ mol/L metergoline or washout. Metergoline showed substantial inhibition on the peak currents of Nav1.2. (D) The current-voltage relationship was obtained using voltage steps between -50 mV and +50 mV taken in 5-mV increments. Voltage steps were applied in the absence (\bullet) or presence (\circ) of 10 μ mol/L metergoline or after washout of metergoline (\blacktriangledown). The peaks of the evoked currents, normalized to the peak current evoked by the voltage step to -10 mV in the absence of metergoline, were used in the I - V plot. The data represent the mean \pm SEM ($n=7-9$ /group).

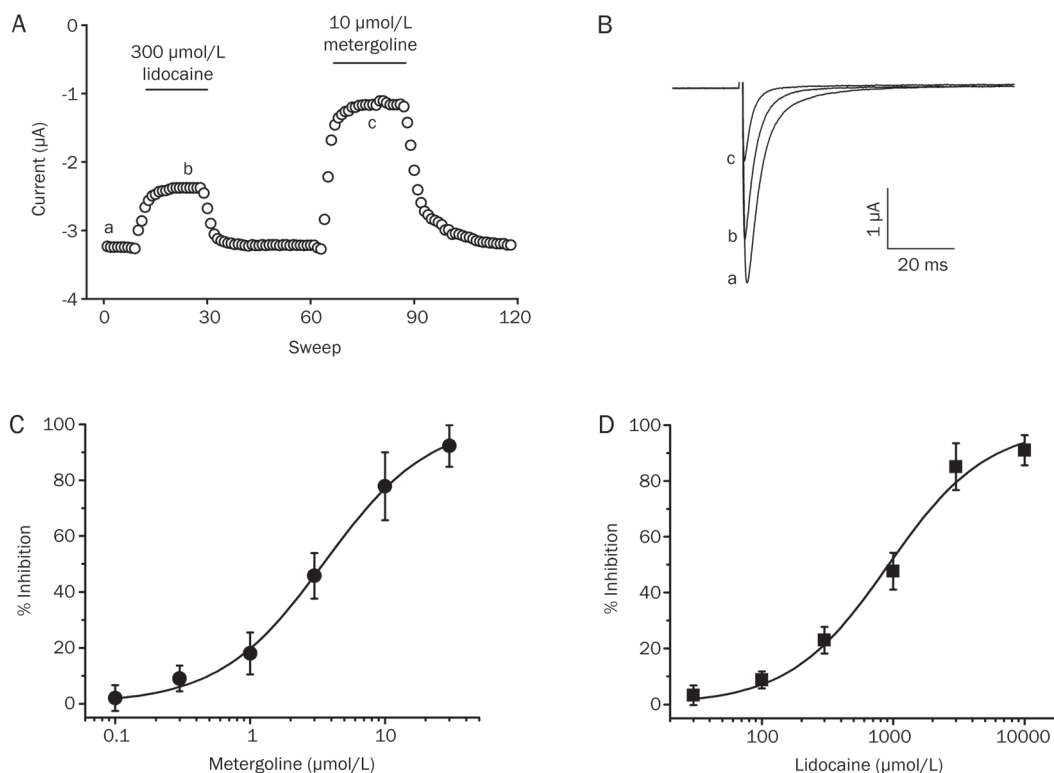


Figure 2. Tonic inhibition of Nav1.2 channel currents by metergoline and lidocaine. (A) The peak inward current amplitudes (○) elicited by 200-ms depolarizations to -10 mV from a holding potential of -100 mV, evoked every 5 s. Data were obtained in eight separate oocytes from three different frogs. Metergoline (10 μmol/L) or lidocaine (300 μmol/L) were applied during the period indicated by the solid bars. (B) Averaged traces of Nav1.2 channel currents taken at the time “a”, “b”, and “c” in (A). (C and D) Concentration-response curves of metergoline or lidocaine-mediated inhibition of the peak currents of the Nav1.2 channel. Metergoline inhibited the large inward peak currents of the Nav1.2 channel in a concentration-dependent manner. The solid lines were fit using the Hill equation, as described in the Materials and methods section. The data represent the mean±SEM ($n=12-15$ /group).

channel activation was determined by a conductance transformation of the peak current-voltage relationship (Figure 3A), with the curves representing the best data fitted using the Boltzmann function. There was a slight depolarizing shift of the half-maximal activation voltage ($V_{g0.5}$). The $V_{g0.5}$ was -29.8 ± 0.8 mV in the control experiments and -23.3 ± 0.7 mV in the metergoline-treated oocytes ($P<0.01$, compared to control, $n=10$). However, the slope factor (k_g) was not significantly different, yielding values of 5.4 ± 0.3 mV under the control conditions and 5.5 ± 0.3 mV after the metergoline treatment. This result showed that the metergoline treatment produced a 6.8 ± 1.2 mV depolarizing shift in the activation voltage. For comparison, we tested the effects of lidocaine on steady-state activation. The $V_{g0.5}$ was -28.9 ± 0.7 mV in the control experiments and -29.3 ± 0.3 mV in the lidocaine-treated oocytes (Figure 3C, $n=10$).

We then investigated the effect of metergoline on voltage-dependent Na^+ channel inactivation by plotting the normalized peak current against the conditioning pre-pulse voltage (Figure 3B) and then fitting the data to the Boltzmann function. There was no significant difference in the half-maximal inactivation voltage ($V_{h0.5}$) and the slope factor (kh) between the

control and metergoline-treated groups; $V_{h0.5}$ was -51.7 ± 0.2 mV and -52.3 ± 0.2 mV, respectively, and kh was 8.8 ± 0.5 and 9.3 ± 0.3 , respectively ($n=8-11$). However, the $V_{h0.5}$ was -51.5 ± 0.8 mV in the control experiments and -63.9 ± 1.5 mV in the lidocaine-treated oocytes. There is a hyperpolarizing shift of 12.7 ± 1.2 mV in the inactivation voltage after treatment with 1 mmol/L lidocaine. This result is consistent with a previous report^[4]. These findings indicate that metergoline affects the steady-state activation, but not the inactivation, of the Na^+ channel.

Use-dependent inhibition of Nav1.2 channels by metergoline

Many Na^+ channel blockers, such as lidocaine and other anti-depressants, exhibit use-dependent inhibition^[4, 15]; we tested whether metergoline behaved as a use-dependent inhibitor at different concentrations shown to induce tonic blockage of Na^+ currents. We first pre-applied 1 or 3 μmol/L metergoline, allowed the system to stabilize for 3 min and initiated the recordings by eliciting Na^+ currents with 10-ms pulses from -100 to -10 mV for 60 repetitions at 10 Hz (Figure 4). Each recorded peak Nav1.2 channel current was normalized to the initial pulse peak current. We observed a large reduction in the peak Na^+ currents: $54.1\%\pm 4.9\%$ and $73.6\%\pm 7.4\%$

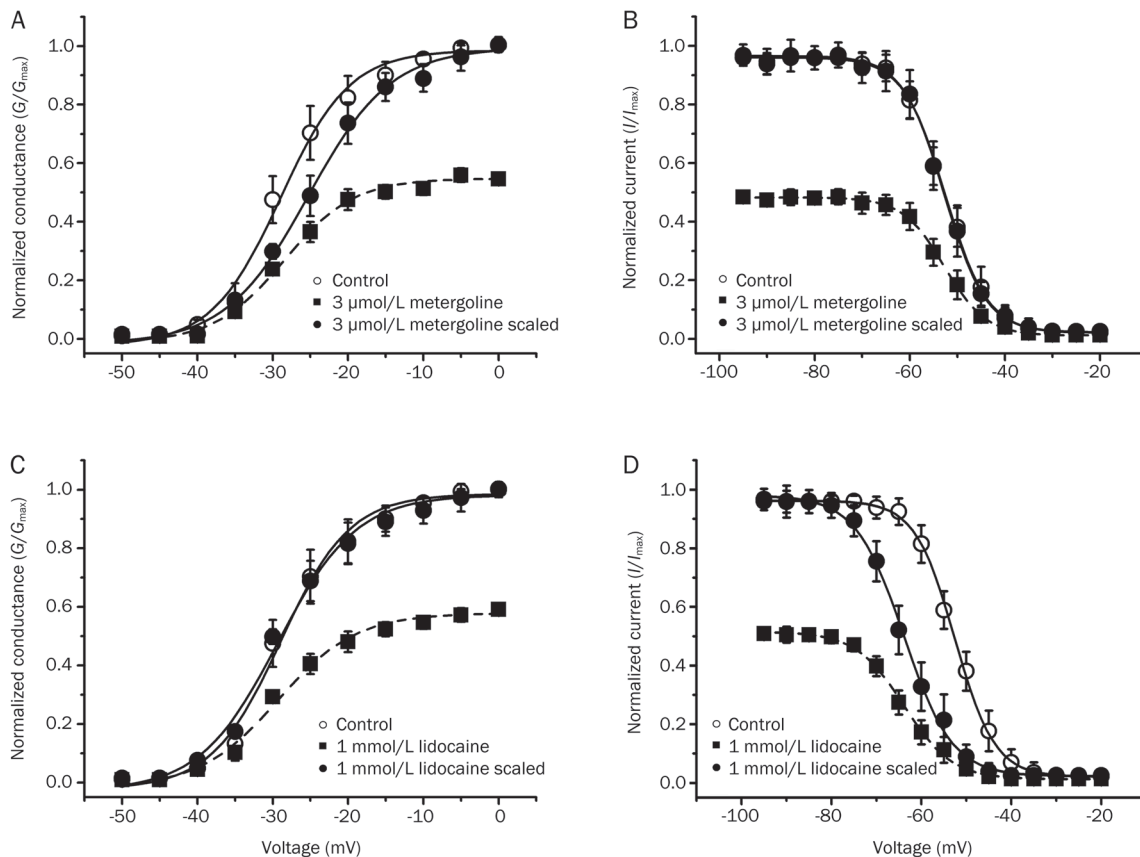


Figure 3. The effect of metergoline and lidocaine on the steady-state activation and inactivation of the Nav1.2 channel current. (A and C) Effect of metergoline or lidocaine on the steady-state activation and inactivation of Nav1.2 channel current was examined as described in a previous report^[15]. In brief, the voltage-dependence of Na⁺ channel activation was calculated by measuring the peak current at test potentials ranging from -50 mV to +10 mV evoked in 5 mV increments. The voltage-dependence of conductance was compared in the absence (○) or presence (■) of 10 μmol/L metergoline or 1 mmol/L lidocaine. The conductance curve in the presence of metergoline or lidocaine is scaled to the maximum conductance (●). Inactivation was measured using a two-pulse protocol in which oocytes were held at -100 mV and depolarized to potentials from -90 mV to -20 mV for 500 ms, followed by a test-pulse to -10 mV for 10 ms to determine channel availability. Inactivation curves are shown in the absence (○) or presence (■) of 10 μmol/L metergoline or 1 mmol/L lidocaine. Inactivation curves in the presence of metergoline or lidocaine are scaled to the maximum (●). The data represent the mean±SEM (*n* = 8–10/group). The curves represent a two-state Boltzmann function as described in the Materials and methods section.

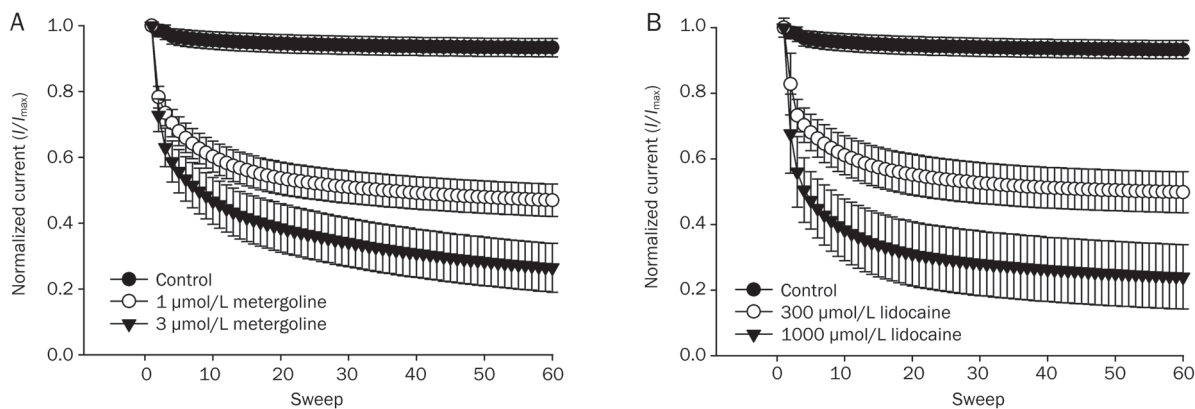


Figure 4. Use-dependent blockade of the Nav1.2 channel current by metergoline or lidocaine. Sixty 20-ms depolarizing pulses to -10 mV were applied from a holding potential of -100 mV at 10 Hz in the absence or presence of metergoline [1 μmol/L (○) and 3 μmol/L (▼) in panel A] or lidocaine [300 μmol/L (○) and 1000 μmol/L (▼) in panel B] in oocytes expressing neuronal Nav1.2 channels. The peak currents were normalized to the current during the first pulse in the presence of agents to compare control current. Treatment with metergoline and lidocaine induced use-dependent inhibition of the Nav1.2 channel. Data represent the mean±SEM (*n* = 5–7/group).

on treatment with 1 and 3 $\mu\text{mol/L}$ metergoline, respectively, at 10 Hz ($n=10$, each). Additionally, lidocaine induced a use-dependent inhibition of current values: $51.2\% \pm 8.7\%$ and $76.1\% \pm 11.5\%$ on treatment with 300 and 1000 $\mu\text{mol/L}$ lidocaine, respectively. These results indicate that metergoline and lidocaine appear to induce use-dependent inhibition of Na^+ currents.

Discussion

Metergoline is an ergot-derived drug that has been studied in a variety of clinical treatments^[5]. Converging evidence suggests that metergoline has other benefits, such as prolactin hormone regulation effects, antidepressant, anti-anxiety, and antifungal effects, and neuroprotective properties. For example, Roca *et al* showed that metergoline attenuated premenstrual dysphoric disorder symptoms, and Turner *et al* suggested that depressed patients with seasonal affective disorder showed improvement in depressive symptoms following oral administration of metergoline in a double-blind and placebo-controlled study^[6]. Graeff *et al* reported that metergoline has an anti-anxiety effect on humans^[16]. Halford and Blundell showed that metergoline reduced the antidepressant-induced suppression of food intake via inhibitory effects of 5-HT_{1/2} receptors^[17]. Kang *et al* further suggested that metergoline elicited a cell death process in *Candida krusei*, a notorious yeast species that is inherently resistant to common antifungal agents, through elevation of intracellular ROS levels and perturbation of mitochondrial homeostasis, followed by damage of the nucleus and eventual cell demise^[18]. In addition, CO₂-induced anxiety in healthy volunteers was significantly enhanced by the oral administration of metergoline^[9]. However, very little is known about the molecular and cellular mechanisms underlying the anti-anxiety and neuroprotective effects of metergoline.

In this study, we characterized the effects of metergoline on brain Na^+ channels expressed in *Xenopus* oocytes and found three major results. First, metergoline induced tonic inhibition of peak Na^+ currents in a concentration-dependent and reversible manner. Second, metergoline affected the steady-state activation voltage, but not the inactivation voltage, of the Na^+ channel. Third, metergoline produced a use-dependent blockade of the Na^+ channel following high-frequency stimulation.

Antidepressants have been used as orally administered adjuvant drugs to treat chronic pain for more than 50 years^[11], and an analgesic effect seems to occur via cross-interactions in various antidepressant signaling systems. Many reports suggest that antidepressants have multiple functions through complex pharmacological effects and interactions with various receptors and channels. Amitriptyline and imipramine show a regulatory effect on different subtypes (voltage-gated, KATP and Ca²⁺-gated) of K⁺ channels^[19] and activate alpha_{2A}-adrenoceptors^[20] in each. Gray *et al* showed that dothiepin hydrochloride and amitriptyline hydrochloride, a tricyclic antidepressant, operate via direct and indirect mechanisms on opioid receptors, with the indirect pathway involving the release of endogenous opioid peptides^[21].

In addition, the regulatory effects on the adenosine A₁ receptor by desipramine and fluoxetine^[22], the NMDA receptor by amitriptyline^[23], the AMPA receptor by fluoxetine^[24], and GABA_B receptors by imipramine^[25] have been reported. In particular, the effects of antidepressants on sodium channels have led to an increased interest in potential side effects. Many previous reports have shown that antidepressants (such as amitriptyline, duloxetine, imipramine, or desipramine) have a regulatory effect on voltage-dependent sodium channels^[26]. Metergoline is also known as an analgesic for migraine headache^[11, 16] and may improve anxiolytic effects^[16] and depressive symptoms^[6]. In our study, the pharmacological actions of metergoline on Na^+ channels are indeed very similar to those of antidepressants, which elicit strong use-dependent blockage of Nav1.2 channel currents.

In conclusion, we used neuronal Na^+ channels expressed in a *Xenopus* oocyte model system to investigate whether metergoline blocks neuronal Nav1.2 channels in this study. A detailed study of the interactions between metergoline and the Nav1.2 channel suggests that metergoline is a potent channel blocker for Nav1.2 in a concentration-, state-, and use-dependent manner. These results provide important insights regarding Na^+ channel regulation induced by metergoline and show that metergoline can be a potent inhibitor of neuronal Na^+ channels.

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Author contribution

Jun-ho LEE, Minkyu SHIN, and Hyunsu BAE designed the study; Jun-ho LEE and Jian LIU performed the molecular and electrophysiological experiments; Jun-ho LEE and Seung-yeol NAH analyzed the data; Jun-ho LEE, Moochang HONG, and Hyunsu BAE wrote the manuscript.

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