Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Dexmedetomidine Prolongs Lidocaine Intravenous Regional Anesthesia in Rats by Blocking the Hyperpolarization-Activated Cation Current

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Purpose: Intravenous regional anesthesia (IVRA) using lidocaine provides effective localized analgesia but its duration is limited. The mechanism by which dexmedetomidine enhances lidocaine IVRA is unclear but may involve modulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels.

Materials and Methods: Lidocaine IVRA with varying dexmedetomidine concentrations was performed in the tails of Sprague-Dawley rats. Tail-flick and tail-clamping tests assessed IVRA analgesia and anesthesia efficacy and duration. Contributions of α_2 adrenergic receptors and HCN channels were evaluated by incorporating an α adrenergic receptor antagonist, the HCN channel inhibitor ZD7288, and the HCN channel agonist forskolin. Furthermore, whole-cell patch clamp electrophysiology quantified the effects of dexmedetomidine on HCN channels mediating hyperpolarization-activated cation current (I_h) in isolated dorsal root ganglion neurons.

Results: Dexmedetomidine dose-dependently extended lidocaine IVRA duration and analgesia, unaffected by α_2 receptor blockade. The HCN channel inhibitor ZD7288 also prolonged lidocaine IVRA effects, while the HCN channel activator forskolin shortened effects. In dorsal root ganglion neurons, dexmedetomidine concentration-dependently inhibited I_h amplitude and shifted the voltage-dependence of HCN channel activation.

Conclusion: Dexmedetomidine prolongs lidocaine IVRA duration by directly inhibiting HCN channel activity, independent of α_2 adrenergic receptor activation. This HCN channel inhibition represents a novel mechanism underlying the anesthetic and analgesic adjuvant effects of dexmedetomidine in IVRA.

Keywords: dexmedetomidine, hyperpolarization-activated cyclic nucleotide-gated channel, intravenous regional anesthesia, lidocaine

Introduction

Intravenous regional anesthesia (IVRA) is a widely used to rapidly induce localized anesthesia for outpatient procedures on the extremities.[1–3] Among available agents, lidocaine is the preferred option for IVRA due to its rapid onset, tourniquet compatibility, and adequate safety profile.[4,5] However, the routine application of lidocaine IVRA is limited by its relatively short duration. Incorporating adjuvants can prolong the anesthetic effect, overcoming this limitation.[6,7]

Dexmedetomidine, a highly selective α_2-adrenergic agonist, is commonly used as an adjuvant in IVRA to prolong analgesia duration.[8,9] The mechanism was initially attributed to α_2 receptor activation, leading to reduced neuronal

Received: 7 December 2023
Accepted: 23 March 2024
Published: 9 April 2024

excitability.[10,11] However, recent evidence indicates that dexmedetomidine may also act by blocking hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. A study on perineural dexmedetomidine for sciatic nerve block found that analgesia extension occurred independently of α_2 agonism, implicating HCN channel inhibition.[12] This has been corroborated by other nerve block studies using α_2 agonists.[13] HCN channels mediate hyperpolarization-activated cation current (I_h), which regulates neuronal resting membrane potential.[14,15] Inhibiting HCN channels could induce sustained hyperpolarization to extend the time of action of local anesthetics.[16] While modulation of HCN channels underlies some effects of dexmedetomidine, its specific interaction in the context of IVRA has not yet been fully elucidated.

This preclinical study explores the relatively uncharacterized effect of dexmedetomidine on HCN channels and its potential contribution to prolonged lidocaine IVRA duration in a rat model. Elucidating the interaction between dexmedetomidine and HCN channels enhances mechanistic understanding of IVRA analgesia. Additionally, this research could facilitate development of novel IVRA adjuvants that directly inhibit HCN channels to achieve extended localized anesthesia.

Material and Methods

Animals

All animal procedures were carried out in accordance with institutional ethical guidelines and the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), with efforts made to minimize suffering and limit animal numbers. The experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Fujian Medical University (Approval No. FJMU IACUC 2022–0590). Male Sprague–Dawley rats aged 8–10 weeks weighing 250–300 g were obtained from the university's animal facility. Rats were group-housed on a 12-hour light/dark cycle with free access to food and water. All experiments were conducted between 10:00–16:00 to minimize circadian effects.

Drug Solutions

We purchased lidocaine, dexmedetomidine, prazosin, yohimbine, and forskolin from MedChemExpress (Shanghai, China) and ZD7288 from Sigma–Aldrich (Shanghai, China). Lidocaine hydrochloride 2% (w/v) was prepared in 0.9% NaCl solution. Dexmedetomidine hydrochloride was diluted in 2% lidocaine solution to final concentrations of 6.8, 13.6, and 20.4 μM (approximately 3, 6, and 9 μg/kg). The α_1 antagonist prazosin (1.3 mM, ~1 mg/kg) and α_2 antagonist yohimbine (1.6 mM, ~1 mg/kg) were prepared in lidocaine. The HCN inhibitor ZD7288 (2.2 mM, ~1.14 mg/kg) and HCN activator forskolin (153.6 μM, ~110.9 μg/kg) were dissolved in 1% DMSO saline and then diluted in 2% lidocaine. All drug solutions were freshly prepared before the experiments.[12]

Intravenous Regional Anesthesia Model

The rat IVRA model developed by Luo et al[17] was employed in this study. A 24-gauge intravenous catheter was inserted into a vein in the distal third of the tail. The tail was exsanguinated using a rubber strip, and an elastic tourniquet was applied to the proximal third. The study drug solution (0.5 mL) was administered over 10 seconds. Ten minutes after administration, the tourniquet was released for subsequent evaluations.

To examine the impact of increasing concentrations of dexmedetomidine on the duration of lidocaine IVRA, we randomly assigned 40 rats to four groups (n = 10): the lidocaine group, the lidocaine + 3 μg/kg dexmedetomidine group, the lidocaine + 6 μg/kg dexmedetomidine group, and the lidocaine + 9 μg/kg dexmedetomidine group. Additionally, to determine whether the prolongation effect of dexmedetomidine on the duration of lidocaine IVRA is associated with α adrenergic receptors or HCN channels, another 80 rats were randomly divided into eight groups (n = 10): the lidocaine group, the lidocaine + dexmedetomidine group, the lidocaine + dexmedetomidine + prazosin group, the lidocaine + dexmedetomidine + yohimbine group, the lidocaine + ZD7288 group, the lidocaine + dexmedetomidine + ZD7288 group, the lidocaine + forskolin group, and the lidocaine + dexmedetomidine + forskolin group.

Tail-Flick and Tail-Clamping Tests

The tail-flick test was used to evaluate IVRA analgesia. Rats were first assessed for ordinary consciousness. Tail-flick latency was measured using a radiant heat analgesia meter (Tail-Flick Unit PL-200; Taimeng, Sichuan, China). The rat's tail was exposed to a heat source until a flick response occurred, and the latency time was recorded. The heat intensity was set at 90%, with a 10 sec cutoff to prevent tissue damage. If no tail-flick response was observed until the cutoff time, the latency was recorded as 10 seconds. The testing area for this experiment was the middle third of the tail distal to the tourniquet, while the control area was the proximal third of the tail proximal to the tourniquet. Baseline latencies were obtained before drug administration. After IVRA, tail-flick latencies were measured at 1, 3, 5, and 10 minutes and then every 5 minutes up to 120 minutes. The analgesic effect was quantified as the percentage of maximum possible effect (% MPE) calculated as %MPE = (Test Latency − Baseline Latency)/(Cutoff Time − Baseline Latency) x 100%. Analgesia onset was the time for %MPE to reach 50% after drug administration. Recovery time was the duration for %MPE to decline below 50% after tourniquet release.

The tail-clamping test was used to assess IVRA anesthetic effects. After each tail-flick measurement, the tail was clamped for 5 sec within the IVRA area using 8-inch Rochester forceps. Positive reactions such as spasms, retraction, squealing, or evasion were considered anesthesia endpoints. The onset time was defined as the interval between drug administration and the first negative clamp response. The anesthesia duration was from tourniquet release until the first positive clamp response.

Patch Clamping Recordings

Dorsal root ganglion (DRG) neurons were acutely isolated from adult male Sprague Dawley rats (250–300 g). Rats were euthanized by intraperitoneal injection of pentobarbital (150 mg/kg) following institutional animal ethics guidelines. DRGs were rapidly removed and transferred to chilled Dulbecco's modified Eagle's medium (DMEM, Gibco), which bubbled continuously with 95% O_2/5% CO_2. The ganglia were cleaned of connective tissue and digested with collagenase (1 mg/mL, Sigma) and papain (1 mg/mL, Sigma) in DMEM at 37°C for 30–45 min with gentle agitation. After enzymatic digestion, 1 mg/mL protease inhibitor (Sigma) was added to stop the reaction. The cells were mechanically dissociated with fire-polished Pasteur pipettes, and the cell suspension was filtered through a 40 μm cell strainer. The dispersed DRG neurons were plated on poly-L-lysine (0.1 mg/mL, Sigma)-coated culture dishes and kept for 3–6 hours in DMEM with 10% fetal bovine serum (Gibco) at 37°C in a humidified 5% CO_2 incubator to allow cell adhesion.

Large-diameter (> 30 μm) DRG neurons with smooth cell membranes and extensive neurite outgrowth were selected for electrophysiological experiments. The culture medium was changed to a standard external solution containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose (pH 7.4, adjusted with NaOH, ~330 mOsmol/L). Borosilicate glass electrodes (4–5 MΩ resistance) were pulled with a P-97 micropipette puller (Sutter Instruments, California, USA) and filled with an internal solution containing (in mM) 130 KCl, 1 $MgCl_2$, 10 HEPES, 10 EGTA, 3 MgATP, and 3 NaCl (pH adjusted to 7.3 with KOH, ~310 mOsmol/L). The electrode was positioned near the cell membrane using a micromanipulator (Siskiyou Corporation, OR, USA). After forming a gigaohm seal (> 1 GΩ) by gentle suction, further suction was applied to rupture the membrane for whole-cell configuration.

Electrophysiological recordings were obtained using an Axopatch 700B amplifier and pClamp 10 software (Molecular Devices, LLC, USA). The junction potential was nulled before seal formation, and the capacitance was compensated. Access resistance was monitored and maintained below 20% of initial values. Signals were low-pass filtered at 2 kHz and digitized at 10 kHz with a Digidata 1440A (Molecular Devices, LLC, USA). The I_h was elicited by 3 s hyperpolarizing voltage steps to −120 mV from a holding potential of −50 mV. The current amplitude at the end of the step was used to construct I–V relationships. Dexmedetomidine (0.1, 1, 10, and 100 μM) was applied cumulatively using a fast perfusion system. I_h was measured every 5 min following dexmedetomidine application. The results were normalized to the baseline control I_h. Concentration-response relationships were fitted with the Hill equation: $Y = 1/[1 + 10\ (\log IC_{50} - X) \times h]$, where Y represents the inhibitory ratio, X denotes the drug concentration, and h is the Hill coefficient. The voltage dependence of I_h activation was determined from tail current analysis. Tail currents were

recorded at −90 mV following 3 s steps to voltages between −50 and −140 mV in 10 mV increments. Normalized tail current amplitudes (G/Gmax) were fitted with the Boltzmann function: $G/G_{max} = 1/ [1 + \exp (V_{1/2a} - V)/k]$, where V is the test voltage, $V_{1/2a}$ is the half-maximum activation voltage, and k represents the slope factor.

Statistical Analysis

Data were analyzed using SPSS 25.0 (SPSS Inc., IL, USA). Normality was assessed with the Shapiro–Wilk test. Normally distributed data are presented as the mean (standard deviation, SD), and differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc tests. Nonnormally distributed data are shown as the median (interquartile range, IQR) and were analyzed using the Kruskal–Wallis test, followed by Dunn's multiple comparisons test. The time course of the analgesic effect was compared between groups using two-way repeated-measures ANOVA with Bonferroni posttests. Kaplan–Meier survival analysis and the Breslow-Wilcoxon test were used to compare tail-flick latencies. Spearman's rank coefficient was used to assess the correlation between dexmedetomidine concentration and IVRA duration. Electrophysiology data were analyzed by pClamp 10 software (Molecular Devices, LLC, USA) using Student's *t*-test or one-way ANOVA with Tukey's post hoc test. The results were considered statistically significant at $p < 0.05$.

A preliminary experiment was conducted to determine the duration of IVRA with lidocaine alone or lidocaine + 6 μg/kg dexmedetomidine ($n = 4$ per group). The mean (SD) duration was 17.0 ± 6.3 min in the lidocaine group and 28.5 ± 5.5 min in the lidocaine + dexmedetomidine group. A sample size of 8 per group was needed to detect the difference between groups with a power of 0.9 and alpha of 0.05 using Power Analysis and Sample Size (PASS) software (version 15; NCSS LLC, Utah, USA). Thus, the final sample size was 10 per group for 20% possible attrition.

Results

Dexmedetomidine Extended Lidocaine IVRA Analgesia and Anesthesia in a Rat Model

Compared with lidocaine alone, dexmedetomidine at 3, 6, and 9 μg/kg significantly enhanced the IVRA analgesic efficacy ($p = 0.023$, $p < 0.001$, and $p < 0.001$, respectively; Figure 1A) and increased the IVRA analgesia duration ($p = 0.019$, $p < 0.001$, and $p < 0.001$, respectively; Figure 1B). There was a positive correlation between dexmedetomidine dosage and IVRA analgesia duration (Spearman coefficient = 0.759, $p < 0.001$; Figure 1B). Similarly, compared with lidocaine alone, dexmedetomidine at 3, 6, and 9 μg/kg significantly enhanced the IVRA anesthesia efficacy ($p = 0.045$, $p < 0.001$, and $p < 0.001$, respectively; Figure 1C) and increased the IVRA anesthesia duration ($p = 0.019$, $p < 0.001$, and $p < 0.001$, respectively; Figure 1D). There was a positive correlation between dexmedetomidine dosage and IVRA anesthesia duration (Spearman coefficient = 0.719, $p < 0.001$; Figure 1D). Additionally, two rats in the lidocaine + 9 μg/kg dexmedetomidine group displayed sedation and were excluded. In summary, dexmedetomidine prolongs lidocaine IVRA analgesia and anesthesia in a dose-dependent manner.

The α-Adrenergic Blockade Did Not Alter Dexmedetomidine's Enhancement of Lidocaine IVRA

In comparison to the lidocaine + dexmedetomidine group, the Lido + dexmedetomidine + prazosin group exhibited similar IVRA analgesic effects ($p = 0.599$, Figure 2A), with comparable durations of analgesia ($p = 0.63$, Figure 2B). The lidocaine + dexmedetomidine + yohimbine group showed analogous IVRA analgesia efficacy and duration compared to the lidocaine + dexmedetomidine group ($p = 0.977$ and $p = 0.748$, respectively; Figure 2A and B). The group receiving lidocaine + dexmedetomidine + prazosin demonstrated similar IVRA anesthesia efficacy compared to the group receiving lidocaine + dexmedetomidine ($p = 0.562$, Figure 2C), as well as comparable durations of anesthesia ($p = 0.544$, Figure 2D). Similarly, no significant differences in anesthesia efficacy and duration were observed between the lidocaine + dexmedetomidine + yohimbine and lidocaine + dexmedetomidine groups ($p = 0.846$ and $p = 0.879$, respectively; Figure 2C and D). In summary, the blockade of α-adrenergic receptors did not impact the ability of dexmedetomidine to prolong lidocaine IVRA analgesia and anesthesia. This suggests that a nonadrenergic mechanism is responsible for the effects of dexmedetomidine in this context.

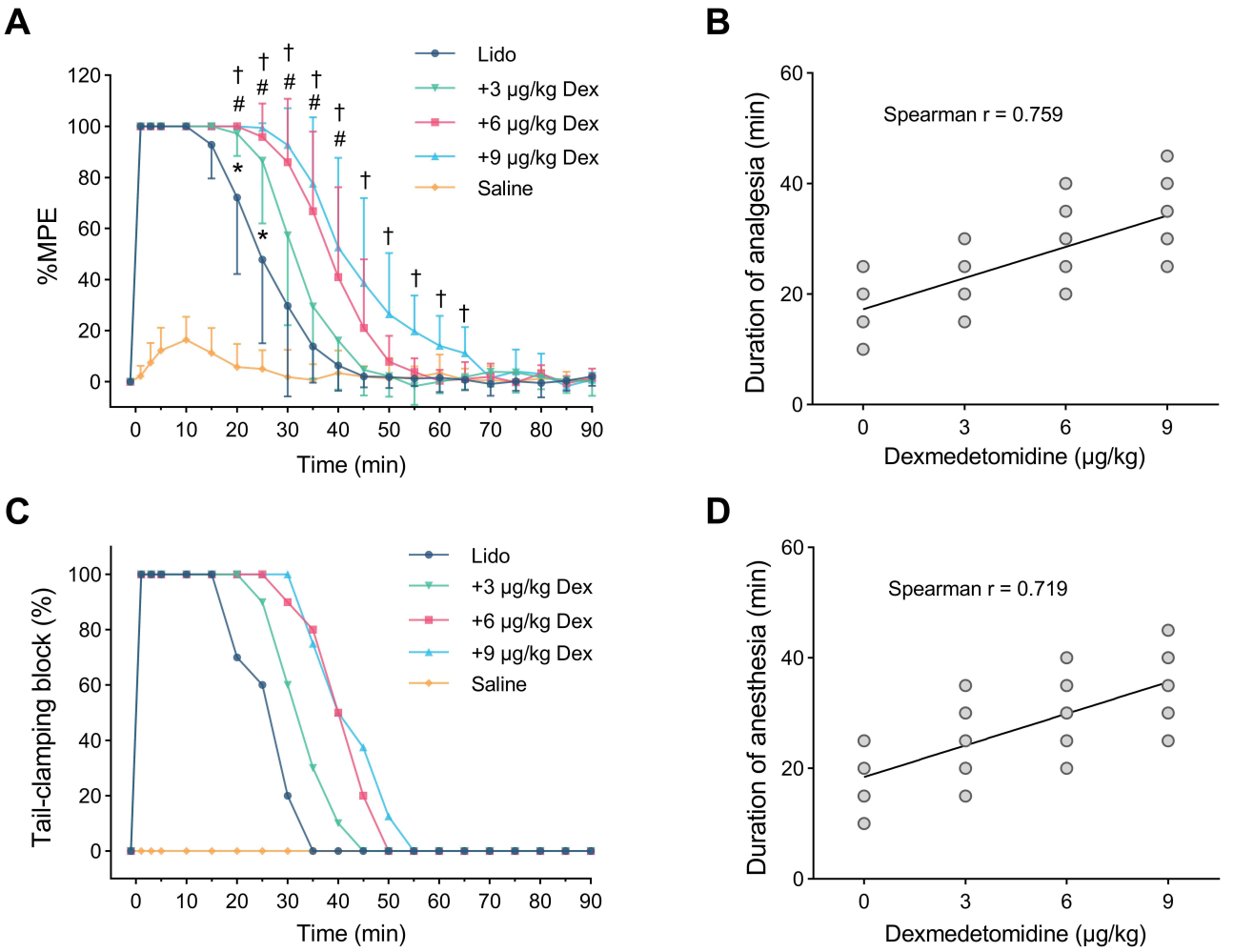

Figure 1 Dexmedetomidine prolongs lidocaine IVRA analgesia and anesthesia duration in a dose-dependent manner.
Notes: (**A**) Analgesic effect shown as %MPE over time. Dexmedetomidine at 3, 6, and 9 μg/kg enhanced analgesia compared to lidocaine alone. (**B**) Positive correlation between dexmedetomidine concentration and IVRA analgesia duration. (**C**) Anesthetic effect shown as anesthesia time. Dexmedetomidine at 3, 6, and 9 μg/kg prolonged anesthesia duration versus lidocaine alone. (**D**) Positive correlation between dexmedetomidine concentration and IVRA anesthesia duration. Data are presented as mean ± SD. *Lido + 3 μg/kg Dex group vs Lido group, $p < 0.05$; #Lido + 6 μg/kg Dex group vs Lido group, $p < 0.05$; †Lido + 9 μg/kg Dex group vs Lido group, $p < 0.05$. $n = 8$ for analysis of the Lido + 9 μg/kg Dex group; 2 rats were excluded for sedation; $n = 10$ for other groups.
Abbreviations: Dex, dexmedetomidine; IVRA, intravenous regional anesthesia; Lido, lidocaine; %MPE, percentage of maximum possible effect; SD, standard deviation.

HCN Channel Blockade with ZD7288 Prolonged Lidocaine IVRA but Did Not Further Augment Dexmedetomidine's Effects

The HCN channel blocker ZD7288 enhanced lidocaine IVRA analgesia efficacy and duration compared to lidocaine alone ($p < 0.001$ and $p = 0.002$, respectively; Figure 3A and B). Interestingly, the addition of ZD7288 (lidocaine + dexmedetomidine + ZD7288) did not lead to further improvements versus dexmedetomidine alone (lidocaine + dexmedetomidine), with no significant differences in analgesia efficacy and duration between these groups ($p = 0.503$ and $p = 0.335$, respectively; Figure 3A and B). Similarly, the lidocaine + ZD7288 group also exhibited enhanced IVRA anesthesia compared with the lidocaine group (anesthesia efficacy: $p < 0.001$, Figure 3C; anesthesia duration: $p < 0.001$, Figure 3D). The coadministration of ZD7288 with dexmedetomidine (lidocaine + dexmedetomidine + ZD7288 group) yielded IVRA anesthesia akin to that of the lidocaine + dexmedetomidine group (anesthesia efficacy: $p = 0.614$, Figure 3C; anesthesia duration: $p = 0.481$, Figure 3D). In summary, blocking HCN channels prolonged lidocaine IVRA, confirming their involvement in regulating IVRA effects. However, ZD7288 did not confer additional benefits when combined with dexmedetomidine, suggesting they act via the same mechanism to extend IVRA duration.

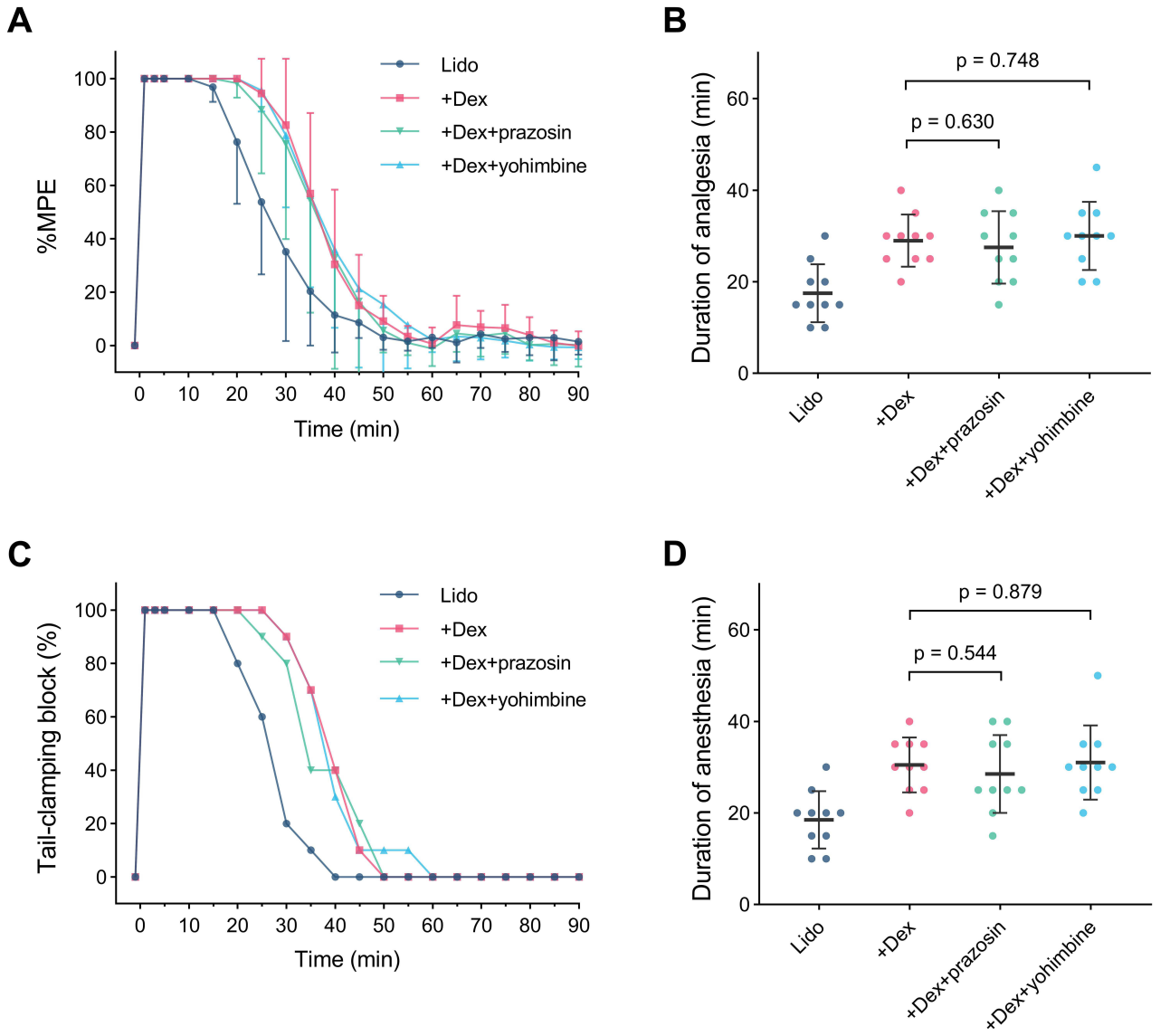

Figure 2 α-Adrenergic receptor antagonists do not alter dexmedetomidine's prolongation of lidocaine IVRA.
Notes: (**A** and **B**) The analgesic effect (%MPE) and duration were similar between the Lido + Dex + prazosin, Lido + Dex + yohimbine, and Lido + Dex groups. (**C** and **D**) The anesthetic effect and duration were analogous between the Lido + Dex + prazosin, Lido + Dex + yohimbine, and Lido + Dex groups. Data are presented as mean ± SD, *n* = 10 per group.
Abbreviations: Dex, dexmedetomidine; IVRA, intravenous regional anesthesia; Lido, lidocaine; SD, standard deviation.

HCN Channel Activation with Forskolin Reversed the Prolongation of Lidocaine IVRA by Dexmedetomidine

Compared to the lidocaine group, the lidocaine + forskolin group had reduced IVRA analgesia efficacy ($p < 0.001$, Figure 4A) and duration ($p = 0.023$, Figure 4B). The addition of forskolin also attenuated analgesia efficacy and duration in the lidocaine + dexmedetomidine + forskolin group compared to the lidocaine + dexmedetomidine group ($p = 0.012$ and $p < 0.001$, respectively; Figure 4A and B). Regarding anesthesia, the group receiving lidocaine + forskolin had reduced IVRA anesthesia efficacy ($p = 0.017$, Figure 4C) and shortened anesthesia duration ($p = 0.025$, Figure 4D) compared with the group receiving lidocaine alone. Similarly, compared with the lidocaine + dexmedetomidine group, the lidocaine + dexmedetomidine + forskolin group exhibited weakened IVRA anesthesia efficacy ($p = 0.005$, Figure 4C), along with a shortened duration of anesthesia ($p = 0.002$, Figure 4D). In summary, opening HCN channels

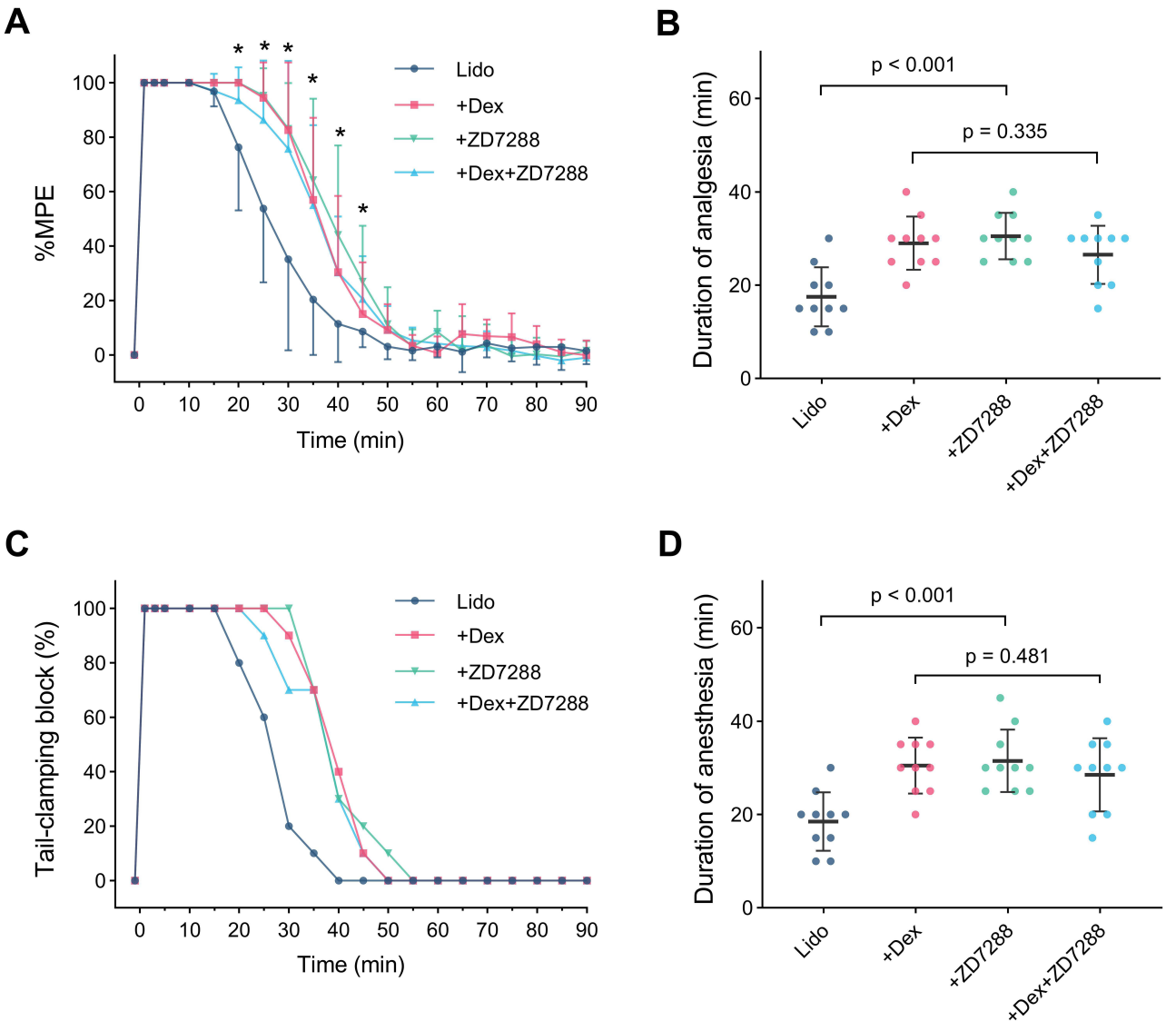

Figure 3 HCN channel blockade prolongs lidocaine IVRA but does not confer additional benefits with dexmedetomidine.
Notes: (**A** and **B**) The Lido + ZD7288 group showed enhanced analgesic effect (%MPE) and duration versus Lido alone. In contrast, the Lido + Dex + ZD7288 group showed similar analgesic effects to the Lido + Dex group, with similar analgesia durations. (**C** and **D**) Improved anesthesia effect and duration were observed with Lido + ZD7288 compared to Lido alone. Anesthesia was comparable between Lido + Dex + ZD7288 and Lido + Dex. Data are presented as mean ± SD, *n* = 10 per group. *Lido +ZD7288 group vs Lido group, $p < 0.05$.
Abbreviations: Dex, dexmedetomidine; IVRA, intravenous regional anesthesia; Lido, lidocaine; %MPE, percentage of maximum possible effect; SD, standard deviation.

with forskolin compromised lidocaine IVRA and opposed the benefits of dexmedetomidine. This provides further evidence that dexmedetomidine prolongs IVRA by inhibiting HCN channel activity.

Dexmedetomidine Inhibited I_h and Shifted HCN Channel Activation in Rat DRG Neurons

Whole-cell patch-clamp recordings in rat DRG neurons showed that dexmedetomidine dose-dependently inhibited I_h (Figure 5A and B). The inhibitory rates were 5.1 ± 2.0%, 16.1 ± 3.5%, 33.1 ± 5.7%, and 50.8 ± 7.5% for incremental dexmedetomidine concentrations (0.1, 1, 10, and 100 μM, respectively). The Hill equation fit a dose-response curve, resulting in an estimated IC_{50} value of 6.0 ± 0.8 μM. Voltage clamp steps from −50 mV to −140 mV before and after 10 μM dexmedetomidine revealed decreased I_h amplitude, significantly at −100 to −140 mV ($p < 0.05$ for all, Figure 5C). Tail current analysis showed a hyperpolarizing shift in dexmedetomidine-induced HCN channel activation ($V_{1/2a}$ −107.7

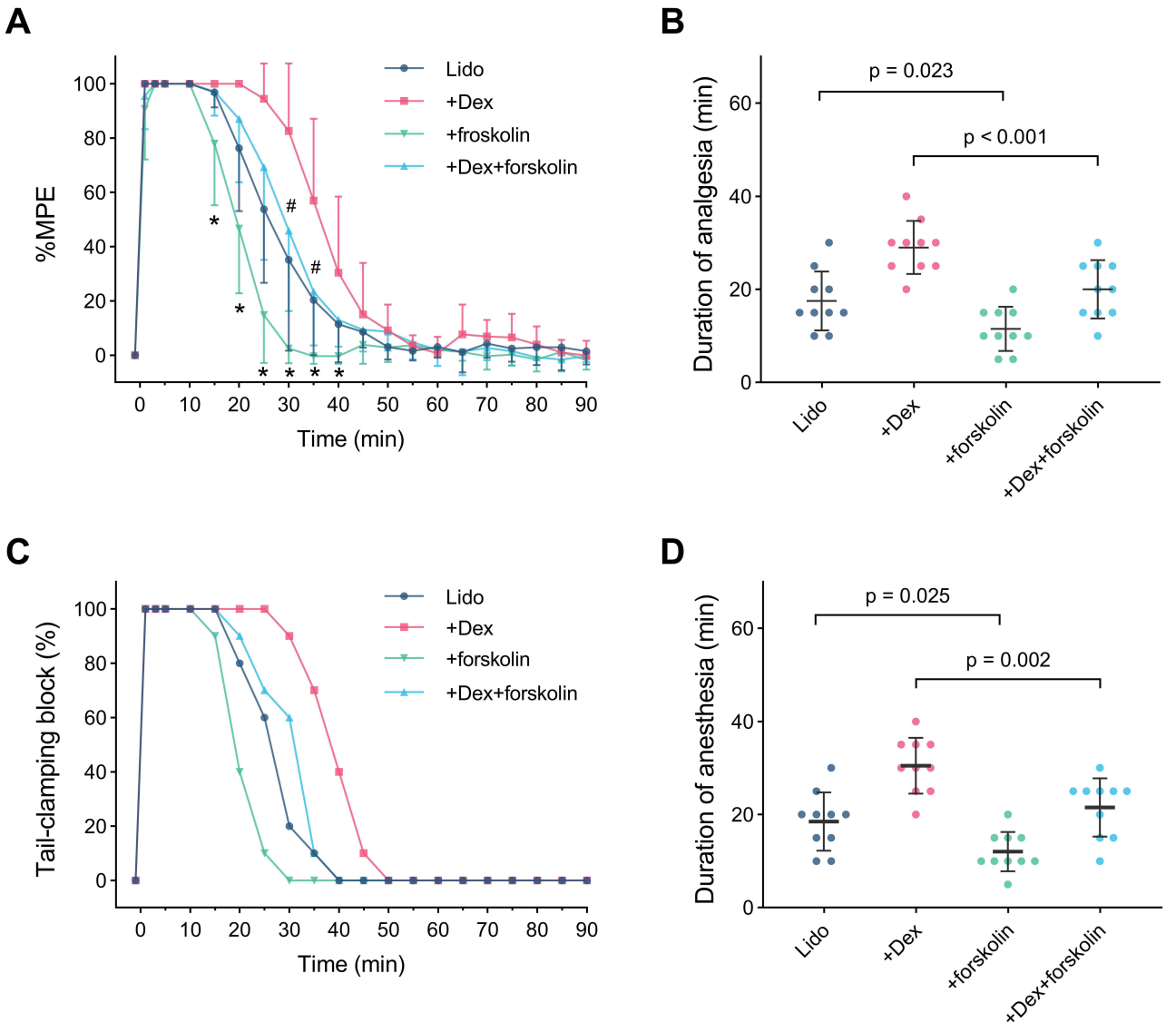

Figure 4 HCN channel activation compromises the lidocaine IVRA and opposes the enhancing effects of dexmedetomidine.
Notes: (**A** and **B**) Lido + forskolin showed reduced analgesic efficacy (%MPE) and duration versus Lido alone. Lido + Dex + forskolin had decreased analgesia compared to Lido + Dex. (**C** and **D**) Weakened anesthesia effects and a shortened duration were observed with Lido + forskolin compared to Lido alone. Lido + Dex + forskolin also exhibited diminished anesthesia versus Lido + Dex. Data are presented as mean ± SD, $n = 10$. *Lido + forskolin group vs Lido group, $p < 0.05$; #Lido + Dex + forskolin group vs Lido + Dex group, $p < 0.05$.
Abbreviations: Dex, dexmedetomidine; IVRA, intravenous regional anesthesia; Lido, lidocaine; %MPE, percentage of maximum possible effect; SD, standard deviation.

± 6.1 mV vs −96.5 ± 7.2 mV, p = 0.016, Figure 5D). In summary, dexmedetomidine acts directly on HCN channels in DRG neurons to reduce I_h in a concentration-dependent manner and alter gating through a hyperpolarized shift in voltage-dependent activation.

Discussion

The primary findings of this study reveal that dexmedetomidine enhances the duration and analgesia of lidocaine IVRA in a dose-dependent manner, independent of α_2 receptor blockade. We also found that the HCN channel inhibitor ZD7288 extends the IVRA effects of lidocaine, whereas the HCN channel activator forskolin reduces them. Moreover, our observations show that dexmedetomidine suppresses I_h current amplitude and alters the voltage dependency of HCN channel activation in DRG neurons in a concentration-dependent manner. These findings suggest that HCN channels play a significant role in the IVRA effects of lidocaine and position them as crucial mediators. This discovery not only

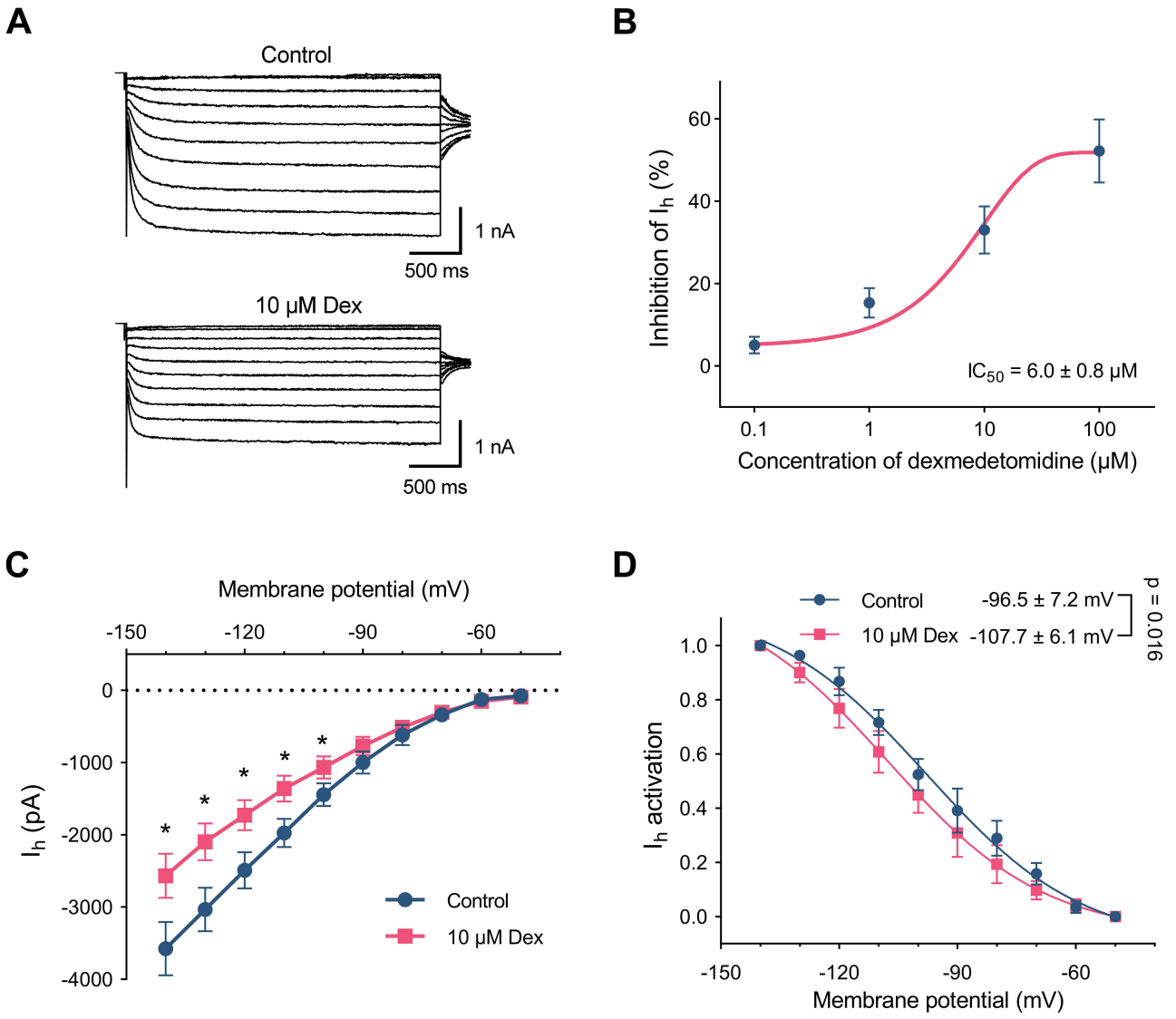

Figure 5 Dexmedetomidine inhibits I_h current amplitude and shifts the voltage dependence of HCN channels in DRG neurons.
Notes: (**A**) Representative HCN channel current (I_h) traces elicited by hyperpolarizing voltage steps, showing decreased amplitude after 10 μM dexmedetomidine. (**B**) Dexmedetomidine inhibited I_h in a concentration-dependent manner. (**C**) Hyperpolarizing shift in the HCN channel activation curve induced by 10 μM dexmedetomidine. (**D**) Significantly more hyperpolarized $V_{1/2}$ activation voltage with 10 μM dexmedetomidine. Data are presented as mean ± SD, $n = 6$. *10 μM dexmedetomidine vs control, $p < 0.05$.
Abbreviations: DRG, dorsal root ganglion; HCN, hyperpolarization-activated cyclic nucleotide-gated; SD, standard deviation.

elucidates a novel mechanism underlying regional anesthesia but also highlights HCN channels as potential targets for developing new local anesthetic agents.

Our observation that adjunct dexmedetomidine improves lidocaine IVRA aligns with clinical studies showing increased duration and reduced pain. For instance, adding dexmedetomidine enhanced intraoperative conditions and duration beyond lidocaine alone during carpal tunnel surgery.[18] Likewise, accelerated onset and prolonged lidocaine IVRA sensory blockade were reported in orthopedic patients.[8] Our controlled rat model systematically substantiates and provides mechanistic insights into these clinical reports of analgesic supplementation. By quantifying the dose-dependent magnification of lidocaine IVRA by dexmedetomidine, we confirmed and expanded upon clinical observations at the neuronal level.

Our finding that α-adrenergic receptor blockade did not affect dexmedetomidine's enhancement of lidocaine IVRA suggests an alternative, nonadrenergic mechanism of action. This finding aligns with studies reporting α_2-independent

https://doi.org/10.2147/DDDT.S450971 DovePress

analgesic effects of dexmedetomidine.[12,19] Using a lidocaine IVRA model, our work adds to the understanding of the multifaceted pharmacodynamic properties of dexmedetomidine. However, Yoshitomi et al[20] attributed the analgesic properties of dexmedetomidine to α_2-agonism, evidenced by diminished enhancement with the α_2 antagonist yohimbine. This discrepancy from our findings may arise from differences in the experimental models and anesthetic techniques used. The predominant HCN channel involvement in our IVRA model points to unique interactions within the localized tourniquet-induced environment, contrasting α_2 receptor roles. Alternatively, α_2 receptors' influence in Yoshitomi et al's intracutaneous anesthesia may relate to distinct skin distribution where these receptors critically regulate neurotransmitter release from nerve terminals - pivotal factors in peripheral analgesia.[21] Furthermore, the unchanged dexmedetomidine effect with prazosin, an α_1 antagonist, implies a nonvascular mechanism, spotlighting neural pathways as central to its anesthetic-enhancing actions. This contrast highlights the context-specific nature of the pharmacological effects of dexmedetomidine, emphasizing the diverse receptor interactions that occur in different anesthetic settings.

Our study elucidates the role of dexmedetomidine in augmenting lidocaine IVRA through HCN channel inhibition, a pathway that resonates with prior research. Brummett et al[12] suggested an HCN channel-related mechanism for dexmedetomidine's effectiveness in peripheral nerve blocks, independent of α_2-adrenergic receptor antagonism, a concept that our results corroborate substantiates. Kroin et al[13] further illuminated that I_h currents, rather than α-adrenergic pathways, are instrumental in clonidine's prolonging effect on lidocaine nerve blocks. Zhao et al[16] elucidated that HCN channel blockers significantly extended lidocaine nerve blocks, an effect counteracted by HCN channel enhancers, underlining the critical function of channels in anesthetic modulation. Additionally, genetic evidence from HCN_1 knockout mouse studies[22] directly implicates HCN channels in the anesthetic action of lidocaine, suggesting a systemic role for these channels in anesthesia that transcends the specific influence of dexmedetomidine.

HCN channels regulate neuronal excitability by generating I_h, which modulates resting potentials and input responsiveness.[23,24] Highly expressed in the nervous system, HCN channels govern action potential genesis thresholds in nociceptive neurons and contribute to rhythmic/spontaneous firing patterns underlying pain signaling.[25,26] Dexmedetomidine may dampen neuronal excitability by inhibiting HCN currents, raising firing thresholds and decreasing responsiveness to painful stimuli. This attenuates the propagation of pain signals along sensory neurons, enhancing lidocaine anesthesia and analgesia.

Our study's insights into dexmedetomidine's enhancement of lidocaine IVRA via HCN channel inhibition carry substantial clinical relevance, especially considering dexmedetomidine's frequent use as an adjuvant. Elucidating the dose-dependent mechanism is critical for fine-tuning dosages to maximize effectiveness while reducing adverse effects such as bradycardia or hypotension.[27,28] Specifying the role of HCN channels opens avenues for developing novel pharmacological agents directly targeting these channels, potentially leading to more precise and effective IVRA without the undesirable effects associated with dexmedetomidine. Additionally, a deeper understanding of dexmedetomidine's interaction with HCN channels may offer new insights into managing various pain conditions that involve modulation of neuronal excitability.[29,30]

There are still some limitations in the current study. While forskolin has been extensively employed as an enhancer of HCN channels through the elevation of cyclic AMP levels, it is noteworthy that cAMP, a second messenger, may elicit additional effects. Furthermore, forskolin induces vasodilation, potentially influencing the duration of regional anesthesia autonomously and impacting the I_h current. Another consideration is the nonselective nature of ZD7288, serving as a blocker across HCN1 and HCN2 subtypes. Consequently, the specific contributions of HCN1 and HCN2 remain challenging to assess. Electrophysiological recordings conducted on acutely isolated DRG neurons in this study raise concerns regarding their relevance to regional anesthesia. These findings underscore the need for further investigation and careful interpretation of the outcomes.

Conclusion

In summary, this study demonstrates that dexmedetomidine prolongs lidocaine IVRA in rats by inhibiting HCN channel activity in a mechanism independent of α-adrenergic receptor activation. Elucidating this novel action of dexmedetomidine on HCN channels advances our understanding of the pharmacological mechanisms underlying IVRA adjuvants. These findings may facilitate the development of improved adjuvants directly targeting HCN channel inhibition to achieve prolonged localized anesthesia.

Acknowledgments

This study was sponsored by Fujian provincial health technology project (No. 2022CXA007), Natural Science Foundation of Fujian Province (No. 2021J01378, 2023J011201), Fujian Medical University Student Innovation Training Program (No. S202310392022), and Joint Funds for the Innovation of Science and Technology, Fujian province (No. 2023Y9275).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. van Zundert A, Helmstädter A, Goerig M, Mortier E. Centennial of intravenous regional anesthesiaBier's Block (1908–2008). *Reg Anesth Pain Med*. 2008;33(5):483–489. doi:10.1097/00115550-200809000-00014
2. Nijs K, Lismont A, De Wachter G, et al. The analgesic efficacy of forearm versus upper arm intravenous regional anesthesia (Bier's block): A randomized controlled non-inferiority trial. *J Clin Anesth*. 2021;73:110329. doi:10.1016/j.jclinane.2021.110329
3. Bosdotter Enroth S, Rystedt A, Covaciu L, et al. Bilateral forearm intravenous regional anesthesia with prilocaine for botulinum toxin treatment of palmar hyperhidrosis. *J Am Acad Dermatol*. 2010;63(3):466–474. doi:10.1016/j.jaad.2009.10.034
4. Guay J. Adverse events associated with intravenous regional anesthesia (Bier block): a systematic review of complications. *J Clin Anesth*. 2009;21(8):585–594. doi:10.1016/j.jclinane.2009.01.015
5. Goh AXC, Yeo JW, Gao JF, et al. Comparative efficacy of anesthetic methods for closed reduction of pediatric forearm fractures: a systematic review. *Emerg Med J*. 2022;39(12):888–896. doi:10.1136/emermed-2021-212108
6. Bakri MH, Ismail EA, Abd-Elshafy SK. Analgesic effect of nalbuphine when added to intravenous regional anesthesia: a randomized control trial. *Pain Physician*. 2016;19(8):575–581.
7. Viscomi CM, Friend A, Parker C, Murphy T, Yarnell M. Ketamine as an adjuvant in lidocaine intravenous regional anesthesia: a randomized, double-blind, systemic control trial. *Reg Anesth Pain Med*. 2009;34(2):130–133. doi:10.1097/AAP.0b013e31819bb006
8. Memiş D, Turan A, Karamanlioğlu B, Pamukçu Z, Kurt I. Adding dexmedetomidine to lidocaine for intravenous regional anesthesia. *Anesth Analg*. 2004;98(3):835–840. doi:10.1213/01.ane.0000100680.77978.66
9. Karmaniolou I, Staikou C, Surda P. The role of dexmedetomidine as an additive to intravenous regional anesthesia: a systematic review and meta-analysis. *Balkan Med J*. 2021;38(3):156–164. doi:10.5152/balkanmedj.2021.20076
10. Weerink MAS, Struys MMRF, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine. *Clin Pharmacokinet*. 2017;56(8):893–913. doi:10.1007/s40262-017-0507-7
11. Fairbanks CA, Stone LS, Wilcox GL. Pharmacological profiles of alpha 2 adrenergic receptor agonists identified using genetically altered mice and isobolographic analysis. *Pharmacol Ther*. 2009;123(2):224–238. doi:10.1016/j.pharmthera.2009.04.001
12. Brummett CM, Hong EK, Janda AM, Amodeo FS, Lydic R. Perineural dexmedetomidine added to ropivacaine for sciatic nerve block in rats prolongs the duration of analgesia by blocking the hyperpolarization-activated cation current. *Anesthesiology*. 2011;115(4):836–843. doi:10.1097/ALN.0b013e318221fcc9
13. Kroin JS, Buvanendran A, Beck DR, Topic JE, Watts DE, Tuman KJ. Clonidine prolongation of lidocaine analgesia after sciatic nerve block in rats Is mediated via the hyperpolarization-activated cation current, not by alpha-adrenoreceptors. *Anesthesiology*. 2004;101(2):488–494. doi:10.1097/00000542-200408000-00031
14. Benarroch EE. HCN channels: function and clinical implications. *Neurology*. 2013;80(3):304–310. doi:10.1212/WNL.0b013e31827dec42
15. Combe CL, Gasparini S. Ih from synapses to networks: HCN channel functions and modulation in neurons. *Prog Biophys Mol Biol*. 2021;166:119–132. doi:10.1016/j.pbiomolbio.2021.06.002
16. Zhao W, Liang P, Liu J, et al. Capsazepine prolongation of the duration of lidocaine block of sensory transmission in mice may be mediated by modulation of HCN channel currents. *PeerJ*. 2019;7:e7111.
17. Luo WJ, Chai YF, Liu J, et al. A model of intravenous regional anesthesia in rats. *Anesth Analg*. 2010;110(4):1227–1232. doi:10.1213/ANE.0b013e3181ce1f84
18. Mizrak A, Gul R, Erkutlu I, Alptekin M, Oner U. Premedication with dexmedetomidine alone or together with 0.5% lidocaine for IVRA. *J Surg Res*. 2010;164(2):242–247. doi:10.1016/j.jss.2009.03.005
19. Yang YC, Meng QT, Pan X, Xia ZY, Chen XD. Dexmedetomidine produced analgesic effect via inhibition of HCN currents. *Eur. J Pharmacol*. 2014;740:560–564.
20. Yoshitomi T, Kohjitani A, Maeda S, Higuchi H, Shimada M, Miyawaki T. Dexmedetomidine enhances the local anesthetic action of lidocaine via an alpha-2A adrenoceptor. *Anesth Analg*. 2008;107(1):96–101. doi:10.1213/ane.0b013e318176be73
21. Yam MF, Loh YC, Tan CS, Khadijah Adam S, Abdul Manan N, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. *Int J Mol Sci*. 2018;19(8):2164. doi:10.3390/ijms19082164

22. Zhou C, Ke B, Zhao Y, et al. Hyperpolarization-activated cyclic nucleotide-gated channels may contribute to regional anesthetic effects of lidocaine. *Anesthesiology*. 2015;122(3):606–618. doi:10.1097/ALN.0000000000000557
23. Arnold EC, McMurray C, Gray R, Johnston D. Epilepsy-induced reduction in HCN channel expression contributes to an increased excitability in dorsal, but not ventral, hippocampal CA1 neurons. *eNeuro*. 2019;6(2):36. doi:10.1523/ENEURO.0036-19.2019
24. Sartiani L, Mannaioni G, Masi A, Novella Romanelli M, Cerbai E, Garland CJ. The hyperpolarization-activated cyclic nucleotide-gated channels: from biophysics to pharmacology of a unique family of ion channels. *Pharmacol Rev*. 2017;69(4):354–395. doi:10.1124/pr.117.014035
25. Cao Y, Pang J, Zhou P. HCN channel as therapeutic targets for heart failure and pain. *Curr Top Med Chem*. 2016;16(16):1855–1861. doi:10.2174/1568026616666151215104058
26. Tanaka S, Ishida T, Ishida K, et al. A randomized, double-blinded, placebo-controlled, crossover study of the HCN channel blocker ivabradine in a capsaicin-induced pain model in healthy volunteers. *Sci Rep*. 2022;12(1):17246. doi:10.1038/s41598-022-22309-7
27. Lee S. Dexmedetomidine: present and future directions. *Korean J Anesthesiol*. 2019;72(4):323–330. doi:10.4097/kja.19259
28. Buckley MS, Agarwal SK, MacLaren R, Kane-Gill SL. Adverse hemodynamic events associated with concomitant dexmedetomidine and propofol for sedation in mechanically ventilated ICU patients. *J Intensive Care Med*. 2020;35(12):1536–1545. doi:10.1177/0885066619884548
29. Lu TL, Lu TJ, Wu SN. Effectiveness in block by dexmedetomidine of hyperpolarization-activated cation current, independent of Its agonistic effect on α_2-adrenergic receptors. *Int J Mol Sci*. 2020;21(23):9110. doi:10.3390/ijms21239110
30. Chen CS, So EC, Wu SN. Modulating hyperpolarization-activated cation currents through small molecule perturbations: magnitude and gating control. *Biomedicines*. 2023;11(8):2177.