Dexmedetomidine Inhibits ASIC Activity via Activation of \( \alpha_{2A} \) Adrenergic Receptors in Rat Dorsal Root Ganglion Neurons

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Dexmedetomidine (DEX), a selective \( \alpha_{2} \) adrenergic receptor (\( \alpha_{2}-AR \)) agonist, has been shown to have peripheral analgesic effects in a variety of pain conditions. However, the precise molecular mechanisms have not yet been fully elucidated. Acid sensing ion channels (ASICs) are the major player in pain associated with tissue acidosis. Given that both \( \alpha_{2A}-ARs \) and ASICs exist in dorsal root ganglia (DRG) neurons, we therefore investigated the effects of DEX on the functional activity of ASICs. Herein, whole-cell patch-clamp recordings demonstrated that DEX suppressed ASIC-mediated and acid-evoked currents and action potentials in dissociated rat DRG neurons. DEX shifted downwards concentration-response curve to protons, with a decrease of 35.83 ± 3.91% in the maximal current response to pH 4.5. DEX-induced inhibition of ASIC currents was blocked by the \( \alpha_{2A}-AR \) antagonist BRL44408 in DRG neurons. DEX also inhibited ASIC3 currents in CHO cells co-expressing ASIC3 and \( \alpha_{2A}-ARs \), but not in ASIC3 transfected CHO cells without \( \alpha_{2A}-ARs \) expression. DEX-induced inhibition of ASIC currents was mimicked by the protein kinase A inhibitor H-89, and blocked by intracellular application of the Gi/o protein inhibitor pertussis toxin and the cAMP analog 8-Br-cAMP. In addition, peripherally administration of DEX dose-dependently relieved nociceptive responses to intraplantar injection of acetic acid in rats through local \( \alpha_{2A}-ARs \). Our results indicated that DEX inhibited the functional activity of ASICs via \( \alpha_{2A}-ARs \) and intracellular Gi/o proteins and cAMP/protein kinase A signaling pathway in rat DRG neurons, which was a novel potential mechanism that probably mediated peripheral analgesia of DEX.

Keywords: dexmedetomidine, \( \alpha_{2} \) adrenergic receptor, acid-sensing ion channel, electrophysiology, dorsal root ganglion neuron, nociceptive behavior

Abbreviations: \( \alpha_{2}-AR \), \( \alpha_{2} \) adrenergic receptor; AC, adenylate cyclase; ANOVA, analysis of variance; APs, action potentials; ASIC, acid-sensing ion channels; CHO, Chinese hamster ovary; DEX, dexmedetomidine; DRG, dorsal root ganglion; IpH, proton-gated current; PKA, protein kinase A; PTX, pertussis toxin; TRPV1, transient receptor potential vanilloid receptor-1.
INTRODUCTION

Dexmedetomidine (DEX) is a potent highly selective α₂ adrenergic receptor (α₂-AR) agonist. It is approved as an analgesic agent since the early 1970s (Kamibayashi and Maze, 2000). DEX action is related to the wide distribution of α₂-ARs in the pain signaling pathway, including in primary afferents and spinal dorsal horn (Gold et al., 1997; Stone et al., 1998; Shi et al., 2000; Nicholson et al., 2005). DEX has shown potential analgesic effects at the supraspinal, spinal and peripheral levels in various pain conditions. Intrathecal or systemic administration of DEX produces analgesic effects in animals and humans (Aho et al., 1991; Kingery et al., 2000; Kimura et al., 2012). Peripheral DEX also shows anti-nociceptive effects since peripherally restricted α₂-AR antagonist could block the effect of systemic DEX on neuropathic pain (Poree et al., 1998; Lee et al., 2013). Moreover, DEX has stronger analgesic effects following peripheral nerve injury via α₂-ARs (Poree et al., 1998; Malmberg et al., 2001). Studies with α₂-AR knock-out animal models suggest that α₂-AR plays a major role in DEX analgesia (Hunter et al., 1997; Stone et al., 1997; Malmberg et al., 2001). In addition to neuropathic pain, studies have also confirmed that DEX has analgesic effects on pain under postoperative, acute and chronic inflammatory conditions (Mahmoud and Mason, 2015; Zhao et al., 2020). Clinically, DEX has been used to relieve acute postoperative pain, ischemic pain, refractory cancer pain (Hoy and Keating, 2011).

Apart from α₂-ARs, a number of ligand-gated and voltage-gated ion channels are also expressed in primary sensory neurons, including dorsal root ganglion (DRG) neurons, which can convert nocuous stimulation into ascending nociceptive signals (McCleskey and Gold, 1999). Thus, modulation of these ion channels may be a potential mechanism underlying peripheral anti-nociceptive effects of DEX. For example, DEX has been shown to inhibit voltage-gated sodium channels via α₂-ARs in DRG and trigeminal ganglion neurons (Gu et al., 2015; Im et al., 2018). DEX also inhibits the activity of TRPV1 in DRG neurons depending on the activation of α₂-ARs followed by the inhibition of the adenylate cyclase/cAMP/protein kinase A (PKA) pathway (Lee et al., 2020).

To detect changes in pH, primary sensory neurons also express several acid sensors, such as acid sensitive ion channels (ASICs), TRPV1, proton-sensing GPCRs, and certain K2P channels (Holzer, 2009; Pattison et al., 2019). Among these, ASICs and TRPV1 have been most thoroughly studied. ASICs, as pH sensors, are expressed in both DRG cell bodies and sensory terminals, where they contribute to proton-evoked nociceptive signaling (Alvarez De La Rosa et al., 2002; Benson et al., 2002). To date, there are seven different ASIC subunits encoded by five different genes (Wemmie et al., 2013). Among these ASIC subunits, ASIC3 subunit is the most abundant in DRG and has emerged as a critical pH sensor (Deval et al., 2008; Lee et al., 2013). Proton, a canonical ligand for ASICs, is released and causes tissue acidosis under multiple pathological conditions such as inflammation, tissue injury, ischemic stroke and cancer (Deval et al., 2011; Deval and Lenguiglia, 2015). Amiloride, a non-selective ASIC inhibitor, can significantly alleviate the pain caused by moderate (up to pH 6.0) pH, suggesting that ASICs, rather than TRPV1 or other acid sensors, mainly mediate the pain sensation (Ugawa et al., 2002; Deval et al., 2008). ASICs, especially ASIC3, are the major player in pain associated with tissue acidosis and represent novel potential targets for development of analgesics (Reeh and Steen, 1996; Wemmie et al., 2013; Li and Xu, 2015; Dulai et al., 2020).

Given that both α₂-ARs and ASICs exist in DRG neurons, the aim of this study was to investigate whether ASICs are also modulatory targets of DEX. We observed that DEX inhibited the electrophysiological activity of ASICs through activation of α₂-ARs and an intracellular cAMP and PKA signaling pathway in rat DRG neurons. DEX also relieved ASIC-mediated nociceptive behaviors in rats by activating peripheral α₂-ARs.

MATERIALS AND METHODS

Preparation of DRG Neurons

All experimental protocols were approved by the animal research ethics committee of Hubei University of Science and Technology. Sprague-Dawley male rats (5–6 weeks old) were anesthetized and then killed. The DRGs were removed and chopped with thin spring scissors. The minced ganglia were transferred to a test tube containing Dulbecco’s modified Eagle’s medium (DMEM, Sigma) and incubated in a shaking for 25–30 min at 35°C. Incubation solution contained 1.0 mg/ml collagenase (type I-A, Sigma), 0.5 mg/ml trypsin (type II-S, Sigma) and 0.1 mg/ml DNase (type IV, Sigma). Tryptsin digestion was terminated by adding 1.25 mg/ml Soybean trypsin inhibitor (type II-S, Sigma). The cells were cultured in DMEM supplemented with 10% fetal bovine serum and 100 ng/ml never growth factor (NGF) for 12–24 h at 37°C in a water saturated atmosphere with 95% O₂ and 5% CO₂.

Electrophysiological Recordings

Electrophysiological experiments were carried out as described previously (Wei et al., 2021). Whole-cell patch clamp recordings were carried out at room temperature (22–25°C) using EPC-10 patch clamp amplifier and PULSE software (HEKA Electronic, Lambrecht, Germany). The isolated DRG neurons were transferred to a 35 mm culture dish and kept in normal external solution for at least 60 min before electrophysiological recordings. The external solution contained the following (in mM): 150 NaCl, 5 KCl, 2 MgCl₂, 2.5 CaCl₂, 10 HEPES, 10 d-glucose. Its pH and osmolality was adjusted to 7.4 with NaOH and 330 mOsm/L with sucrose, separately. Recording pipettes were pulled using a Sutter P-97 puller (Sutter Instruments, CA, United States) and its resistance was in the range of 3–6 MΩ. The micropipettes solution contained (in mM): 140 KCl, 2 MgCl₂, 11 EGTA, 10 HEPES, 4 ATP, and 0.3 Na₂GTP. Its pH and osmolality was adjusted to 7.2 with KOH and 310 mOsm/L with sucrose, separately. After whole-cell configuration established, 70–80% series resistance and membrane capacitance current were compensated. The recording currents were sampled at 10 kHz and filtered at 2 kHz. Data detection and analysis were performed using the
pCLAMP 10 software (Axon Instruments, CA, United States). The neurons selected for electrophysiological recordings were 15–35 μm in diameter, which are thought to be nociceptive neurons. The membrane voltage was maintained at −60 mV in all voltage-clamp experiments. Current-clamp recordings were obtained by switching to current-clamp mode after a stable whole-cell configuration was formed in voltage-clamp mode. Only cells with a stable resting membrane potential (more negative than −50 mV) were used in the study.

**Cell Culture and Transfection**

Rat ASIC3 and human α2A-AR cDNAs were used for heterologous expression in Chinese hamster ovary (CHO) cells as described previously (Wu et al., 2017). In brief, CHO cells were cultured at 37°C in a humidified atmosphere of 5% CO2 and 95% O2 and passaged twice a week. Transient transfection of CHO cells was performed using a HilyMax liposome transfection reagent (Dojindo Laboratories). CHO cells were maintained in F-12 nutrient mixture (added 1.176 g of NaHCO3/L medium) supplemented with 10% fetal bovine serum and 1% glutamaxTM-1 (×100; Invitrogen). When ASIC3 and α2A-AR cDNA were co-transfected, the ratio was maintained at 1:1. All plasmids contained, in addition to the desired ASIC3 cDNA, the coding sequence for enhanced green fluorescent protein to aid in the identification of transfected cells. Electrophysiological measurements were performed 24–48 h after transfection.

**Drug Application**

In the experiment, drugs included hydrochloric acid, dexamethasone, clonidine, yohimbine, BRL44408, 8-Br-cAMP, H-89, amiloride, APETx2, capsaicin, and AMG 9810. They were obtained from Sigma Chemical Co. (St. Louis, MO, United States). Different pH values were configured with hydrochloric acid and external solution. The working concentration of drugs was freshly prepared in normal external solution and their pH was adjusted to 7.4 with NaOH. Each working drug was stored in a series of independent reservoirs and applied by gravity. The distance was ~30 μm between drug exit and recorded neurons. To block G-protein and intracellular signal, some antagonists or blockers were dissolved in the internal solution and applied for intracellular dialysis through recording patch pipettes as described previously (Liu et al., 2021). To ensure that dialysis drugs are infused into the cell interior, there is at least a 30 min interval between the establishment of the whole cell pathway and the current measurement. To functionally characterize ASIC activity, we used AMG9810 (5 μM) to block TRPV1 in the extracellular solution.

**Nociceptive Behavior Induced by Acetic Acid in Rats**

Behavior experiments were carried out as described previously (Wei et al., 2021). Male rats were allowed to habituate for at least 30 min before nociceptive behavior experiments in a Plexiglas chamber. Separate groups of rats were coded and pretreated with 50 μl AMG 9810 (10 μM) together with vehicle, different dose (10, 30 and 100 ng) of DEX, 150 ng BRL44408 + 100 ng DEX in ipsilateral hindpaw using a 30-gauge needle connected to a 100 μl Hamilton syringe. After 5 min, another experimenter subcutaneously administered acetic acid solution (1%, 50 μl) into the hindpaw and tested nociceptive behavior. In one group, acetic acid was injected into one hindpaw and DEX (100 ng) was injected into contralateral hindpaw. Nociceptive behavior (that is, number of flinches) was counted over a 5 min period starting immediately after the injection (Deval et al., 2008; Omori et al., 2008).

**RESULTS**

**DEX Inhibits ASIC Currents in Rat DRG Neurons**

In the majority of DRG neurons tested (76.9%, 10/13), a perfusion of pH 5.0 acid solution to DRG neurons for 5 s evoked a rapid inward current (IpH5.0), even though blockade of proton-induced TRPV1 activation by addition of AMG9810 (5 μM) in external solution (Figure 1A). Most (70.0%, 7/10) of IpH5.0 were characterized by a fast inactivated inward current, followed by a smaller and non-desensitizing sustained current in ten DRG neurons sensitive to pH 5.0 acid stimuli. All seven IpH5.0 currents with this characteristic could be completely blocked by the broad-spectrum ASIC channel blocker amiloride (10 μM) and ASIC3 blocker APETx2 (2 μM). In the presence of AMG9810, capsaicin (100 nM) failed to evoke any membrane currents in all DRG neurons tested. However, capsaicin (100 nM) evoked an inward current in the majority of DRG neurons sensitive to pH 5.0 acid stimuli (71.4%, 5/7) after washout of AMG9810. Thus, these proton-induced currents were considered to be ASIC currents or ASIC3 currents after TRPV1 activation was blocked by AMG9810. In the following study, we mainly observed the ASIC3-like currents with the above characteristics.

In some DRG neurons sensitive to pH 5.0 acid stimuli (52.9%, 9/17), pre-application of α2-AR agonist DEX (3 μM) for 2 min decreased the peak amplitude of the ASIC currents (Figure 1B). In all nine cells response to DEX, pre-application of clonidine (10 μM) for 2 min had also similar inhibitory effects on the ASIC currents (Figure 1B). In addition, DEX shortened the inactivation time constant of ASICs from 1,413 ± 184.64 to 1,094.44 ± 146.38 msec ($p < 0.05$, paired t-test, n = 9). The inhibition of IpH5.0 was dependent upon the concentration of DEX (Figures 1C,D). In a representative DRG neuron, the peak amplitude of IpH5.0 progressively decreased as concentration of pre-treated DEX increased from 0.1 to 10 μM (Figure 1B). Figure 1C shows concentration-effect curve of DEX on IpH5.0 with an IC50 (half-maximal effective concentration) value of 1.18 ± 0.11 μM. The results indicated that DEX concentration-dependently inhibited ASIC currents in rat DRG neurons.
We then investigated the effect of DEX on concentration-response curve to protons. Currents were measured by applying a range of different low pH values before and after treatment with DEX.

**Figure 2A** shows that pre-application of DEX (3 μM for 2 min) decreased all peak amplitudes of IpH6.5, IpH5.5 and IpH4.5. **Figure 2B** shows concentration-response to protons in the absence and presence of DEX (3 μM). First, the maximal current response (IpH4.5) of curve decreased 35.83 ± 3.91% after DEX was treated to DRG neurons. Second, the Hill coefficient or slope of two curves had not significant difference (pH: n = 1.23 ± 0.16; DEX + pH: n = 1.16 ± 0.21; p > 0.1, post hoc Bonferroni’s test). Third, the pH0.5 (pH for half-maximal activation) values of two curves had also no statistical difference (pH: pH0.5 = 5.97 ± 0.17; DEX + pH: pH0.5 = 5.89 ± 0.20; p > 0.1, post hoc Bonferroni’s test). These results indicated that the maximum response to protons was inhibited by DEX, but that no shift in proton sensitivity.

**α₂-ARs Mediate DEX-Induced Inhibition of ASIC Currents**

DEX is a selective α₂-AR agonist. To address whether inhibition of ASIC currents by DEX application was mediated by α₂-ARs, we examined the effect of yohimbine, an α₂-AR antagonist, on inhibitory effects of DEX on ASIC currents. As shown in **Figures 3A,B**, DEX-induced suppression of IpH5.0 was significantly blocked by yohimbine. The amplitude of IpH5.0 decreased 38.48 ± 4.25% by 3 μM DEX pre-treatment alone. In contrast, the amplitude of IpH5.0 decreased only 3.73 ± 3.23% in DRG neurons pre-treated with both 3 μM yohimbine and 3 μM DEX (p < 0.01, compared with DEX pretreatment alone, one-way ANOVA followed by post hoc Bonferroni’s test, n = 7). Moreover, we also examined the effect of BRL44408, an α₂A-AR antagonist, on DEX-induced inhibition of ASIC currents. Pre-incubation of BRL44408 (3 μM) alone did not affect the amplitude of IpH5.0, but significantly blocked DEX-induced suppression of IpH5.0 (**Figures 3A,B**). The results indicated that DEX inhibited ASIC currents mainly through α₂A-ARs in DRG neurons.

To further verify whether α₂A-ARs mediate DEX-induced inhibition of acid-evoked currents, α₂A-ARs were co-expressed with ASIC3 in CHO cells. We recorded ASIC3-mediated acid currents, since they were completely blocked by APETx2 (2 μM). Similar to that observed in DRG neurons, the ASIC3 currents were inhibited by the pre-application of DEX (3 μM) in CHO cells co-expressing ASIC3 and α₂A-ARs (**Figures 3C,D**). The DEX-induced inhibition of ASIC3 currents was also blocked by pre-incubation of 3 μM BRL44408, an α₂A-AR antagonist (**Figures 3C,D**). In contrast, DEX had no effect on ASIC3 currents at a concentration of 3 μM in CHO cells expressing ASIC3 and α₂A-ARs.

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**FIGURE 1** | DEX concentration-dependently inhibits proton-gated currents in rat DRG neurons. (A) Representative current traces were evoked by application of a pH 5.0 acidic solution for 5 s in a tested DRG neuron in the presence of AMG9810 (0.1 μM). The proton-gated current (IpH5.0) could be blocked by broad-spectrum ASIC channel blocker amiloride (Amil, 10 μM) and ASIC3 blocker APETx2 (2 μM). Capsaicin (Cap, 100 nM) failed to evoke any membrane currents in the presence of AMG9810 (0.1 μM). However, capsaicin (100 nM) evoked an inward current after washout of AMG9810 in the same neuron. All membrane potentials were clamped at −60 mV. (B) In the same DRG cell, pre-application of α₂-AR agonist DEX or clonidine caused an inhibitory effect on IpH5.0. (C) The sequential current traces illustrate that IpH5.0 amplitude was inhibited by pre-application of different concentration of DEX (for 2 min) in a representative DRG neuron. (D) The graph shows concentration-effect curve of DEX on IpH5.0 with an IC50 of 1.18 ± 0.11 μM. Each point represents the mean ± SEM of 6–10 cells.
ASIC3 alone, but not expressing α2A-ARs (Figures 3E,F). The pH 5.0-induced ASIC3 channel activation were completely blocked by APETx2 (2 μM) in CHO cells expressing ASIC3 alone. Guanabenz, another α2A-AR agonist, is recently found to activate ASIC3 at neutral pH (Callejo et al., 2020). However, we did not observe that DEX had such an effect when applied in CHO cells expressing ASIC3 alone. DEX (3 or 500 μM) failed to induce any membrane currents at neutral pH (n = 8, data not shown). These results further indicated that α2A-ARs mediated DEX-induced inhibition of ASIC3 currents.

Guv/o-Protein and PKA Signaling Participate in the Inhibition of ASIC Currents by DEX

α2A-ARs belong to Gα/o protein-coupled receptor family, which leads to a cascade of events, such as inhibition of adenylate cyclase (AC) activity and the cAMP/PKA pathway (Wu et al., 1988). Therefore, we first examined whether DEX-induced inhibition of ASIC currents occurs via Gα/o proteins. Pertussis toxin (PTX, 1 μg/ml), an inhibitor of Gα/o proteins, was applied internally to DRG neurons and significantly prevented the decrease of IpH5.0 amplitude induced by DEX (Figures 4A,B). To further explore intracellular signal transduction mechanisms underlying suppression of ASIC currents by DEX, we observed the effect of 8-Br-cAMP, a membrane permeable cAMP analog, on DEX suppression of ASIC currents. When seven DRG neurons were pre-treated with 8-Br-cAMP (1 mM) for 5 min, the relative amplitudes of IpH5.0 were increased to 142.46 ± 13.64% of control. In the presence of 8-Br-cAMP (1 mM), pre-application of DEX (3 μM for 2 min) produced only decreases of 6.25 ± 4.38% on IpH5.0 (p > 0.1, compared with 8-Br-cAMP treatment alone, one-way ANOVA followed by post hoc Bonferroni’s test, n = 7; Figures 4C,D). We then observed the effect of H-89, a membrane-permeable inhibitor of PKA, on DEX suppression of ASIC currents. When DRG neurons were pre-treated with H-89 (0.3 μM) for 5 min, the relative amplitudes of IpH5.0 were reduced to 51.21 ± 7.95% of control (n = 7), suggesting that H-89 mimicked the action of DEX on IpH5.0 (Figures 4E,F). In the presence of H-89 (0.3 μM), pre-application of DEX (3 μM for 2 min) no longer further inhibited IpH5.0 (p > 0.1, compared with H-89 treatment alone, one-way ANOVA followed by post hoc Bonferroni’s test, n = 7; Figures 4E,F). Together, the results indicated that the suppression of ASIC currents by DEX was dependent upon Gα/o proteins and intracellular cAMP/PKA signaling pathway.

DEX Suppresses Acid-Evoked Action Potentials in Rat DRG Neurons

ASICs are trimeric cation-permeable channels. Once activation of ASICs by protons causes cation influx (largely Na+) and membrane potential depolarization, resulting in bursts of action potentials if the proton-induced depolarization is large enough to activate Na+, subunits (Pattison et al., 2019). We further observed whether DEX had effects on acid-evoked action potentials of rat DRG neurons. Although proton-induced TRPV1 activation was blocked in the presence of 5-μM AMG9810, we observed that an acid stimulus of pH 5.0 could trigger bursts of action potentials (APs) in DRG neurons under current-clamp conditions (Figures 5A,C). Consistent with that observed under voltage-clamp conditions, DEX also decreased the number of APs evoked by acidic stimuli of pH 5.0 in DRG neurons (Figures 5A,B). In seven DRG neurons with DEX (3 μM for 2 min) pre-treatment, the number of APs evoked by acidic stimuli of pH 5.0 significantly decreased (p < 0.01, paired t-test, n = 7, Figure 5B). However, the number of APs had not changed in other seven DRG neurons co-treated with both BRL44408 (BRL, 3 μM) and DEX (3 μM) (p > 0.1, paired t-test; n = 7, Figures 5C,D). These results indicated that DEX also suppressed acid-evoked action potentials in rat DRG neurons through α2A-ARs.

DEX Relieves Acid-Induced Nociceptive Behaviors in Rats

Above results demonstrated that DEX inhibited ASIC activity in vitro. We further ascertained whether DEX had effect on ASIC-
mediated nociceptive behaviors through interacting with ASICs in vivo. After acetic acid was injected into rat hind paws, rats displayed an intense flinch/shaking response even if AMG 9810 (10 μM) blocked the activation of TRPV1. We found that pretreatment with DEX (10, 30, and 100 ng) dose-dependently relieved the acid-induced nociceptive behaviors \((p < 0.05\) and \(0.01\), one-way ANOVA followed by post hoc Bonferroni’s test, \(n = 10\); Figure 6). However, Co-injection of BRL44408 (BRL, 150 ng) blocked the relieving effect of 100 ng DEX on acid-induced nociceptive behaviors. The mean number of flinches in these rats significantly increased, compared with that observed in rats pretreated with 100 ng DEX alone \((p < 0.05\) and 0.01, one-way ANOVA followed by post hoc Bonferroni’s test, \(n = 10\); Figure 6), and were no different from control rats without DEX pretreatment. In addition, injection of 100 ng DEX into the contralateral paws did not relieved acid-induced nociceptive behaviors. These results indicated that DEX relieved acid-induced nociceptive behaviors in rats through activation of \(\alpha_2A\)-ARs localized in the injected hindpaw.

**DISCUSSION**

The present data demonstrated that selective \(\alpha_2\)-AR agonist DEX inhibited the electrophysiological activity of ASICs through \(\alpha_2A\)-ARs. DEX reduced ASIC-mediated and acid-evoked currents and action potentials in dissociated rat DRG neurons. PTX-sensitive \(G_{i/o}\) proteins and cAMP/PKA signaling cascade was involved in the inhibition of ASICs by DEX. Behaviorally, DEX also relieved acid-induced nociceptive responses in rats by activating peripheral \(\alpha_2A\)-ARs.

It has been found that six ASIC subunits are expressed in neuron cell bodies in DRG, and ASIC3 subunit is the most abundant (Deval et al., 2008). In the presence of AMG9810, the low pH-evoked currents were mediated by ASICs in rat DRG cells, since they were completely blocked by amiloride and APETx2. The present study showed that DEX, a selective \(\alpha_2\)-AR agonist, inhibited ASIC currents and the number of action potentials evoked by low pH stimulation. Moreover, DEX accelerated the inactivation of ASICs, which also helps to reduce the number of action potentials evoked by acidic stimuli. DEX inhibited the electrophysiological activity of ASICs in rat DRG neurons through \(\alpha_2\)-ARs. First, another \(\alpha_2\)-AR agonist clonidine has also been shown to effectively inhibit ASIC currents. Second, DEX-induced inhibition was completely blocked by the \(\alpha_2A\)-AR antagonist BRL44408. Last, DEX could also inhibit acid-evoked currents mediated by ASIC3 in CHO cells co-expressing \(\alpha_2A\)-ARs and ASIC3, but not in CHO cells only expressed ASIC3 and lacked \(\alpha_2A\)-AR expression, further suggesting that the inhibition of ASIC3 currents by DEX required...
the co-expression of α2A-ARs. In addition, only some, but not all, ASIC-like responses were inhibited by DEX/clonidine, which may be related to the degree of the co-expression of α2A-ARs and ASIC3 in rat DRG neurons. Thus, we speculated that DEX-induced inhibition of ASIC currents maybe also occur only in DRG neurons co-expressing α2A-ARs and ASICs, although the direct evidence needs to further determine. It has been shown that three subtypes of the α2-ARs (α2A-, α2B- and α2C-ARs) are expressed in the rat DRGs (Shi et al., 2000). But α2B-AR subtype is found only in a very small population (Chung et al., 1993; Shi et al., 2000). The expression of α2A and α2C-AR subtypes is regulated by peripheral nerve injury. Proteins and mRNA levels of α2A and α2C-AR subtypes are regulated by peripheral nerve injury. Proteins and mRNA levels of α2A and α2C-AR subtypes are increased and decreased, respectively, in DRG neurons after chronic constriction injury of sciatic nerve (Cho et al., 1997; Shi et al., 2000). In addition to ASICs, other cation channels, such as Nav1.8, TRPV1 and TRPM8 are also inhibited via α2A-AR subtype in DRG neurons (Bavencoffe et al., 2010; Gu et al., 2015; Lee et al., 2020). In addition, α2A-AR agonist is recently found to activate ASIC3 at neutral pH and potentiate acid-gated currents (Callejo et al., 2020). However, we observed that DEX failed to induce any membrane currents at neutral pH when applied in CHO cells expressing ASIC3 alone. The lack of such a role of DEX may be due to the difference of the molecular backbone between DEX and guanabenz, although both of them are α2A-AR agonists.

α2A-AR is a Gβγ protein-coupled receptor that inhibits AC and reduces CAMP production upon activation (Wu et al., 1988). The present data demonstrated that Gβγ proteins and intracellular CAMP/PKA signal transduction were involved in DEX-induced inhibition of ASIC currents in rat DRG neurons. First, preventing Gβγ recruitment by PTX treatment blocked DEX-induced inhibition of ASIC currents. Second, DEX-induced inhibition was lack after DRG neurons treated with the cAMP analog 8Br-cAMP. Last, inhibition of intracellular PKA with H-89 mimicked the inhibiting effect of DEX on ASIC currents. Then on the base of these, DEX no longer further inhibited ASIC currents. These findings were consistent with the general notion that stimulation of α2A-ARs by DEX brings about Gβγ-mediated inhibition of AC and reduction of intracellular CAMP levels, suggesting that DEX inhibited ASIC currents through PTX-sensitive Gβγ proteins and classical AC/CAMP/PKA signaling pathway. Previous studies show that ASICs are modulated by intracellular CAMP/PKA signaling. For example, inhibition of PKA phosphorylation of
ASICs decreases the amplitude of ASIC currents in cortical neurons (Chai et al., 2007). It has been shown that cAMP-dependent signaling pathway involves in the inhibition of ASIC-mediated functional activity by sumatriptan, a Gi/o protein-coupled 5-HT1D receptor agonist, in rat trigeminal ganglion neurons (Guo et al., 2018). Our previous studies found that activation of μ-opioid receptors or CB1 cannabinoid receptors inhibits ASICs in DRG neurons in a cAMP/PKA-dependent manner (Liu et al., 2012; Cai et al., 2014). These studies provide further support that the suppression of ASIC currents by DEX was dependent upon intracellular cAMP and PKA signaling pathway.

Intraplantar injection of low pH solution produces an intense flinch/shaking response in rats by activating ASICs (Deval et al., 2008; Omori et al., 2008). In the presence of AMG9810, ASICs are activated by injected protons, causing cation influx (largely Na+) and membrane depolarization in nociceptors, where nociception will occur if the depolarization is large enough to activate Na+ subunits, resulting in generation of action potential that transmit nociceptive signals (Pattison et al., 2019). The present data show that peripheral pretreatment of the DEX relieved the ASIC-mediated nociceptive behaviors in a dose-dependent manner. The DEX’ actions occurred locally rather than systematically, since the low pH-evoked nociceptive behaviors did not changed when DEX was injected into contralateral hindpaws. Local application of BRL44408, a specific α2A-AR antagonist, significantly blocked the effect of DEX on ASIC-mediated nociceptive behaviors, suggesting that the DEX exerted its analgesic effect by acting directly on α2A-AR subtypes localized on nociceptors.

Protons are released and result in a decrease in pH value in some pathological pain conditions, such as postoperative pain. The extracellular decreased pH is sustained for a few days after incision (Woo et al., 2004). Peripheral ASIC3-containing channels have been found to detect local changes in pH and contribute to postoperative pain (Deval et al., 2011). Intraoperative application of an ASIC3 inhibitor has a potent analgesic effect on postoperative pain (Deval et al., 2011). Clinically, intra-operative DEX decreases requirements for postoperative analgesics of various surgical patients, improves pain management in post-anesthesia care unit, and has no obvious side effects (Alhashemi and Kaki, 2004;
The present results that DEX-induced inhibition of ASICs in primary sensory neurons provided a clue that ASICs may be therapeutic targets for peripheral DEX to relieve some types of pain, such as postoperative pain.

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Arain et al., 2004). The present results that DEX-induced inhibition of ASICs in primary sensory neurons provided a clue that ASICs may be therapeutic targets for peripheral DEX to relieve some types of pain, such as postoperative pain.

**CONCLUSION**

In summary, our results suggested DEX-induced inhibition of ASIC-mediated the electrophysiological activity and pain, revealing a novel peripheral mechanism underlying DEX analgesia. It is clinically important that local application of DEX can not only effectively relieve pain by inhibiting ASICs in primary sensory neurons, but also this route of administration would avoid side-effects that accompany systemic activation of α2A-ARs.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the animal research ethics committee of Hubei University of Science and Technology. Written informed consent was obtained from the owners for the participation of their animals in this study.

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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