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Original Article

Facilitation of spinal α-motoneuron excitability by histamine and the underlying ionic mechanisms

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Abstract: Spinal α-motoneurons directly innervate skeletal muscles and function as the final common path for movement and behavior. The processes that determine the excitability of motoneurons are critical for the execution of motor behavior. In fact, it has been noted that spinal motoneurons receive various neuromodulatory inputs, especially monoaminergic one. However, the roles of histamine and hypothalamic histaminergic innervation on spinal motoneurons and the underlying ionic mechanisms are still largely unknown. In the present study, by using the method of intracellular recording on rat spinal slices, we found that activation of either H_1 or H_2 receptor potentiated repetitive firing behavior and increased the excitability of spinal α-motoneurons. Both of blockage of K^+ channels and activation of Na^+-Ca^{2+} exchangers were involved in the H_1 receptor-mediated excitation on spinal motoneurons, whereas the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels were responsible for the H_2 receptor-mediated excitation. The results suggest that, through switching functional status of ion channels and exchangers coupled to histamine receptors, histamine effectively biases the excitability of the spinal α-motoneurons. In this way, the hypothalamospinal histaminergic innervation may directly modulate final motor outputs and actively regulate spinal motor reflexes and motor execution.

Key words: histamine; histamine receptors; motoneurons; spinal cord; K^+ channels; Na^+-Ca^{2+} exchangers; HCN channels

组胺对脊髓α-运动神经元兴奋性的易化作用及其离子机制

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摘 要：脊髓α运动神经元直接支配骨骼肌活动，是躯体运动和行为控制的最后公路(final common path)。因此，运动神经元兴奋性调控对于运动行为的执行至关重要。事实上，脊髓运动神经元接受多种神经调质的传入，尤其是单胺能神经调质。然而，组胺和下丘脑组胺能神经系统对脊髓运动神经元的调控作用及其下游离子机制至今仍不清楚。本研究采用大鼠脊髓脑片细胞内记录方法，发现组胺H_1和H_2受体的激活均能增强脊髓α运动神经元的重复放电行为和兴奋性。钾通道的关闭和钠-钙交换体的激活共同参与了H_1受体激活介导的脊髓运动神经元的兴奋，而超极化激活的环核苷酸门控(hyperpolarization-activated cyclic nucleotide-gated, HCN)通道的开放则负责H_2受体激活介导的兴奋性效应。以上结果提示，通过切换与组胺受体相耦联的离子通道和交换体的功能状态，组胺可以有效地偏置脊髓α运动神经元的兴奋性，而下丘脑组胺能神经传入很可能以这种方式直接调控最终的运动输出并主动调节脊髓运动反射和运动执行。

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关键词：组胺；组胺受体；运动神经元；脊髓；钾通道；钠-钙交换体；HCN通道
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Alpha motoneurons located in the ventral horn of the spinal cord directly innervate skeletal muscles, evoke muscles to contract and control muscle tone. They integrate descending motor commands, sensory inputs, as well as excitatory and inhibitory synaptic inputs from spinal interneurons, and comprise the final common path for initiating movements [1, 2]. It has been also noted that spinal motoneurons also receive various neuromodulatory inputs, especially monoaminergic one, from supraspinal origins [3, 4]. These neuromodulatory inputs influence intrinsic properties and excitability of spinal motoneurons and subsequently regulate their firing activities and patterns that control muscle contractions. Among the monoaminergic innervations on spinal motoneurons, functions and mechanisms of serotoninergic [5–7], dopaminergic [8] and noradrenergic [9] inputs have attracted more attention. However, role of histaminergic projections on spinal motoneurons and the underlying mechanism are still less known.

In fact, as an important general modulator for whole brain activity [10, 11], histamine has already been implicated in the regulation of somatic motor control [12–18] through its uniform excitatory actions on extensive subcortical motor structures, including the cerebellum [13, 17, 18], basal ganglia [15, 19–21], red nucleus [22] and vestibular nuclei [14, 23–28]. Interestingly, autoradiographic mapping studies have revealed presence of histamine H_1 receptor in spinal dorsal horn as well as ventral horn in cats and guinea pigs [29, 30]. Moreover, we have also reported histamine-induced excitation and potentiated repetitive firing behaviors via postsynaptic histamine H_1 and H_2 receptors on rat spinal α-motoneurons *in vitro* [31]. However, the ionic mechanisms underlying the histaminergic modulatory roles in different central motor structures are varied. Thus, in the present study, by using the method of intracellular recording on rat spinal slices, we investigate the ionic mechanisms underlying the activation of H_1 and H_2 receptors in spinal α-motoneurons. Since spinal α-motoneurons have been considered as the final common path, the finding will contribute to understanding the role of central histaminergic system in transformation of neural activity to motor behavior.

1 MATERIALS AND METHODS

1.1 *Spinal slice preparations*

Coronal slices of the lumbar spinal cord were prepared from the neonatal (12–18 days) Sprague-Dawley rats of either sex, since the histaminergic fibers reach an adult like appearance about two weeks postnatally [11]. The rats were deeply anaesthetized by sodium pentobarbital, then immediately decapitated. The spinal cord was carefully exposed by a dorsal laminectomy in a dissection dish and quickly placed into ice-cold artificial cerebrospinal fluid (ACSF, composition in mmol/L: NaCl 127, KCl 1.2, $MgSO_4$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 26, $CaCl_2$ 2.4 and *D*-glucose 10, pH 7.4) equilibrated with 95% O_2 and 5% CO_2. The spinal cord was transected at T10 and L5 and gently seperated from the vertebral column. Then, the coronal spinal cord slices (300–400 μm thick) were prepared with a vibroslicer (VT 1200 S, Leica, Germany), according to the brain atlas of rat [32]. The slices were incubated in the ACSF solution equilibrated with 95% O_2 and 5% CO_2 at (35.0 ± 0.5) °C for at least one hour and then maintained at room temperature until neuronal electrophysiological recordings. All experimental procedures were performed in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23, revised 2011), and were approved by the Experimental Animal Care and Use Committee of Nanjing University. All efforts were made to minimize the number of animals used and their suffering.

1.2 *Intracellular recordings, motoneuron identification, drug application and data acquisition*

During recording session, the slices were transferred to a submerged chamber and continuously superfused with 95% O_2 and 5% CO_2 oxygenated ACSF at a rate of 2.6 mL/min maintained at (32.0 ± 0.5)°C. Intracellular recordings were obtained from α-motoneurons in the spinal ventral horn with the blind method [31, 33]. Intracellular glass microelectrodes prepared from borosilicate glass pipettes were filled with 3 mol/L potassium acetate (impedance 80–120 MΩ). The ventral horn of the spinal slice was visually identified with the aid of a stereomicroscope (SD-30, Olympus, Japan). Combined electrophysiological and morphological identification of α-motoneurons in the spinal ventral horn following

our previous report [31] was employed in the present study. Briefly, only the neurons showing an antidromic spike potential following stimulation of the ventral rootlets, as well as typical morphological and electrical membrane properties, were identified as α-motoneurons. Intracellular recordings were acquired with an Axoclamp-2B amplifier (Axon Instruments, USA), and the signals were fed into a computer through a Digidata-1322A interface (Axon Instruments, USA) for data capture and analysis (pClamp 8.2, Axon Instruments, USA). Recordings were digitized at 10 kHz and analyzed with pClamp 8.2 (Axon Instruments, USA) and Origin software. Prior to bath application of histaminergic compounds, the steady membrane potential of the recorded spinal motoneuron was observed for at least 20 min [18, 24–26, 31]. Neurons were excluded from the study if their membrane potential was not stable.

We bathed the slices with histamine (100 μmol/L, Sigma, USA) to stimulate the recorded α-motoneurons. 2-Pyridylethylamine (2-PyEA; 30–300 μmol/L, Tocris, UK) and dimaprit (30–300 μmol/L, Sigma, USA) were applied to selectively activate histamine H_1 and H_2 receptors, respectively. To assess the underlying ionic mechanism coupled to histamine H_1 and H_2 receptors, respectively, in current-clamp recording, changes in membrane resistances of recorded motoneurons were evaluated by intracellular injection of constant-current hyperpolarizing pulses (0.2 nA, 250 ms, 0.2 Hz) and current-voltage plots (*I–V* curves) were obtained before and during drug application using a hyperpolarizing step command from −1.3 nA to 0 nA in 0.05 or 0.1 nA (1 000 ms) steps to allow for attainment of steady-state conditions. $BaCl_2$ (1 mmol/L), KB-R7943 (50 μmol/L, Tocris, UK), and ZD7288 (50 μmol/L, Tocris, UK) were applied to block the K^+ channels, Na^+-Ca^{2+} exchangers (NCXs), and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, respectively. Repetitive firing behaviors of recorded motoneurons were induced by intracellular injection of a depolarizing current step from 0 nA to 0.95 nA in 0.05 nA (800 ms) steps, and frequency-current (*F–I*) relationships were fitted by linear regression to assess the excitability of motoneurons.

1.3 *Statistics*

All data were expressed as means ± SEMs. Statistical analysis were performed with GraphPad Prism (http://www.graphpad.com/scientific-software/prism/#1). Student's paired *t*-test was employed for statistical analysis of the data, and P values of < 0.05 were considered to be significant.

2 RESULTS

2.1 Histamine H_1 and H_2 receptors mediate the histamine-induced excitation in spinal α-motoneurons

In the present study, intracellular recordings were performed on a total of 60 lumbar spinal α-motoneurons from 60 rats. These α-motoneurons were identified electrophysiologically and morphologically, and they showed a mean resting membrane potential of (−68.6 ± 0.8) mV (n = 60), time constant of (4.3 ± 0.5) ms (n = 60), input resistance of (29.3 ± 3.2) MΩ (n = 60), spike amplitude of (73.5 ± 1.3) mV (n = 60), and time in spike half-amplitude of (0.68 ± 0.01) ms (n = 60). All these characteristics are similar to our [31] and other previous reports [33–35].

Consistent with our previous report [31], we found that both histamine H_1 and H_2 receptors mediated the excitatory effect of histamine on rat spinal α-motoneurons in this study. 2-PyEA (100 μmol/L, a highly selective histamine H_1 receptor agonist) and dimaprit (100 μmol/L, a highly selective H_2 receptor agonist) both effectively mimicked the depolarization of histamine (100 μmol/L) on spinal α-motoneurons (n = 5 or 6; Fig. 1*A–D*). Moreover, these α-motoneurons (n = 6; Fig. 1*B*, *D*) exhibited a concentration-dependent excitatory response of (3.6 ± 0.8) mV, (6.2 ± 1.1) mV, and (8.9 ± 2.2) mV depolarization to 2-PyEA (30, 100, and 300 μmol/L; Fig. 1*B*), and in several cases, 300 μmol/L 2-PyEA even elicited a sufficiently strong depolarization to activate firing of the motoneurons (Fig. 1*B*). Similarly, dimaprit also depolarized the spinal α-motoneurons in a concentration-dependent manner (n = 6, Fig. 1*C*, *D*). As shown in Fig. 1*C*, 30, 100 and 300 μmol/L dimaprit evoked a depolarization of (2.9 ± 0.4) mV, (3.2 ± 0.7) mV, and (5.9 ± 1.2) mV on a recorded α-motoneuron, respectively.

2.2 Activation of H_1/H_2 receptor increases intrinsic excitability of spinal α-motoneurons

We further assessed the contribution of activation of H_1 or H_2 receptor in the histamine-induced potentiation on repetitive firing behavior of spinal α-motoneurons. We applied depolarizing current pulses of increasing amplitude to induce firing of α-motoneuron and determined the effect of H_1 receptor activation on the motoneuron firing behavior by employment of 2-PyEA. As

shown in Fig. 2*A*, the same 0.2-nA pulse stimulation can be subthreshold or superthreshold depending on the absence or presence of 2-PyEA. Selective activation of H_1 receptors by 2-PyEA decreased the threshold current, i.e., the minimal injected current that fires action potentials, to (56.55 ± 11.59)% of the control (normal ACSF) (n = 6, P < 0.05; Fig. 2*B*). Moreover, we examined the effect of H_1 receptor activation on repetitive firing behavior of α-motoneurons to the intracellular injection of a series of depolarizing current steps. As shown in Fig. 2*C*, 2-PyEA (100 μmol/L) enhanced the neuronal repetitive firing frequency at every stimulation intensity. We further constructed the relationship between neuronal repetitive firing frequency and the amplitude of injected currents (*F–I* curve) and found that 2-PyEA shifted the *F-I* relationship to the left (i.e., lower amplitude of injected current was required for stimulating the motoneuron to fire at a certain frequency), indicating that H_1 receptor activation dramatically potentiates the motoneuron excitability (Fig. 2*D*). However, 2-PyEA had no effect on the slope of the *F–I* relationship [(57.4 ± 3.6) Hz/nA in the control *vs* (54.1 ± 3.9) Hz/nA in 2-PyEA; n = 6, P = 0.112; Fig. 2*E*], indicating unchanged dynamic properties of spinal motoneurons by H_1 receptor activation.

We next examined the effect of dimaprit on the repetitive firing behavior of spinal α-motoneurons to assess the role of H_2 receptor in the histamine-induced enhancement on the motoneuron excitability. As shown in Fig. 3*F*, the tested cell was held at its resting mem-

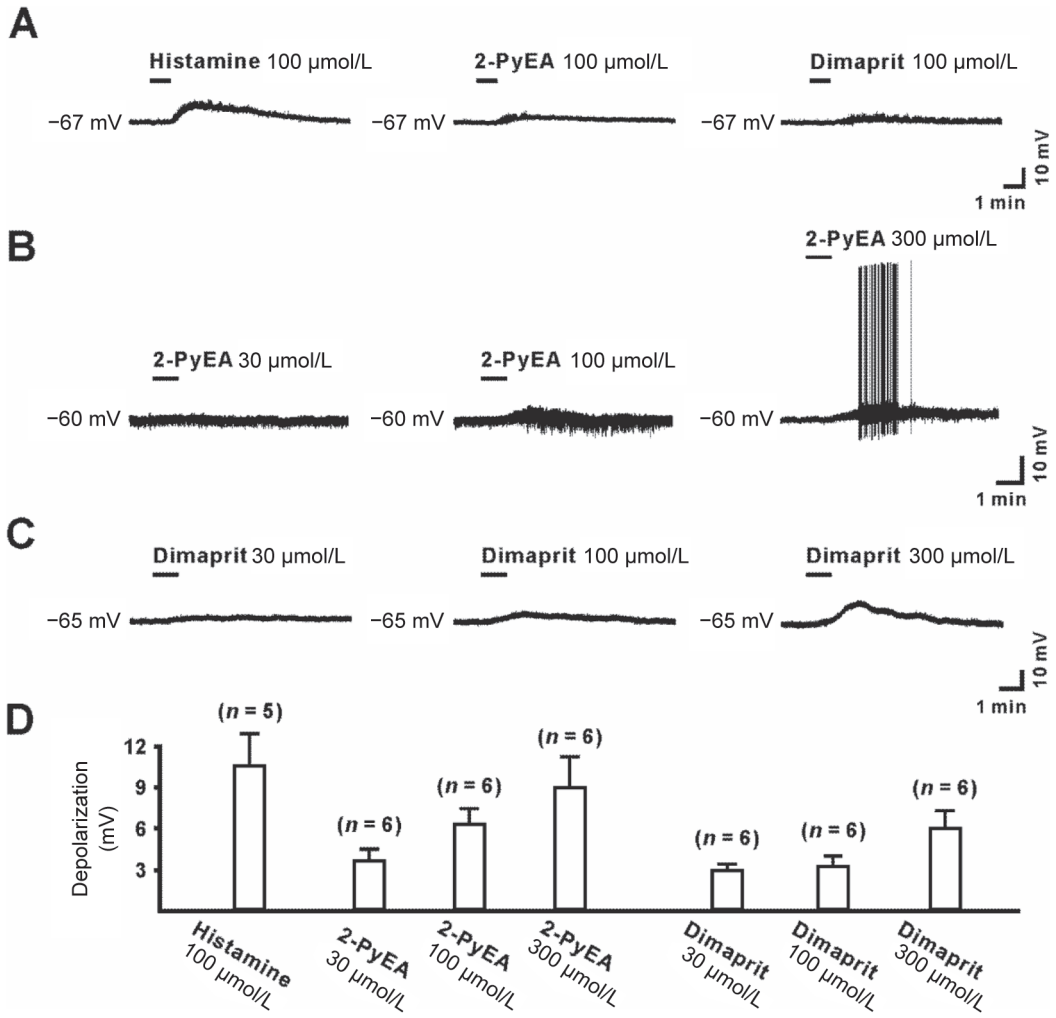

Fig. 1. H_1 and H_2 receptors mediate the histamine-induced depolarization on spinal α-motoneurons. *A–C*: The histamine-induced depolarization on the spinal motoneurons (*A*) was mimicked by 2-PyEA (a selective agonist of H_1 receptor, *B*) and dimaprit (a selective agonists of H_2 receptor, *C*), respectively. *D*: Group data of tested α-motoneurons (n = 5 for histamine and n = 6 for 2-PyEA/dimaprit, respectively). Data shown are means ± SEM.

brane potential of −65 mV, and a series of depolarizing current steps was applied before and during bath of dimaprit (100 μmol/L). Similar with 2-PyEA, dimaprit enhanced the neuronal repetitive firing behavior produced by current steps injection (Fig. 2*F*). Also, dimaprit decreased the amplitude of injected current required for stimulating the motoneuron to fire at a certain frequency and shifted the *F–I* curve to the left (Fig. 2*G*), whereas did not influence its slope [(62.0 ± 1.6) Hz/nA in control *vs* (58.4 ± 2.3) Hz/nA in dimaprit; $n = 6$, $P = 0.228$; Fig. 2*H*]. All these results suggest that activation of H_1 or H_2 receptor leads to a significant increase in intrinsic excitability of spinal motoneurons.

2.3 Activation of H_1 receptor increases membrane resistance of spinal α-motoneurons

Since the above results together with our previous report [31] show that H_1 and H_2 receptors co-mediate the effect of histamine on activity and excitability of spinal α-motoneurons, we next separately examined the ionic mechanisms coupled to histamine H_1 and H_2 receptors on the motoneurons by selective activation of histamine receptor subtypes. The change in membrane input resistance that induced by histamine H_1 receptor agonist 2-PyEA was assessed first. As shown in Fig. 3*A*, *B*, a constant, low hyperpolarizing current was injected into α-motoneurons through a recording pipette to mea-

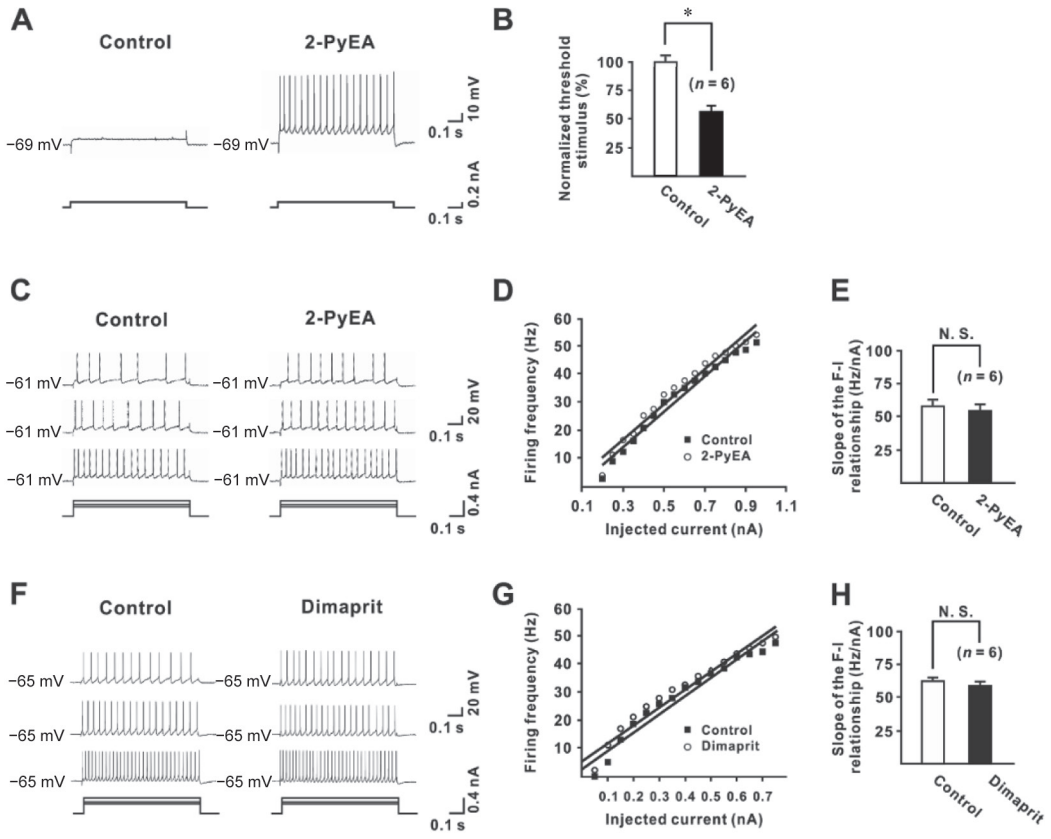

Fig. 2. H_1 and H_2 receptors mediate the histamine-induced facilitation of spinal α-motoneuron excitability. *A*: A 0.2 nA depolarizing current pulse did not evoke action potentials in the control (normal ACSF, left panel), but trigger repetitive firing on the motoneurons in the presence of 300 μmol/L 2-PyEA (right panel). *B*: Group data of normalized threshold stimulation in the absence and presence of 2-PyEA ($n = 6$). *C*, *F*: Repetitive firing evoked by 800-ms constant-step (0.2–0.95 nA) current pulse in 0.05-nA increments before and during 2-PyEA (*C*)/dimaprit (*F*) application on a recorded α-motoneuron. Three repetitive firing traces evoked by current pulse (0.25, 0.3 and 0.4 nA) are shown. *D*, *G*: Frequency-current (*F–I*) relationship for the steady-state (final 400 ms) before and during 2-PyEA (*D*)/dimaprit (*G*) application on the motoneuron respectively recorded in *C* and *F*. The slopes of *F–I* relationship for the steady-state before and during 2-PyEA application were 50.4 Hz/nA ($r = 0.987$) *vs* 50.7 Hz/nA ($r = 0.984$), respectively. The slopes of *F–I* relationship for the steady-state before and during dimaprit application were 65.94 Hz/nA ($r = 0.980$) *vs* 64.91 Hz/nA ($r = 0.980$), respectively. *E*, *H*: Group data of the tested motoneurons recorded in *D* and *G*, respectively ($n = 6$ for each group). Data shown are means ± SEM. N.S. no significant difference; $^*P < 0.05$.

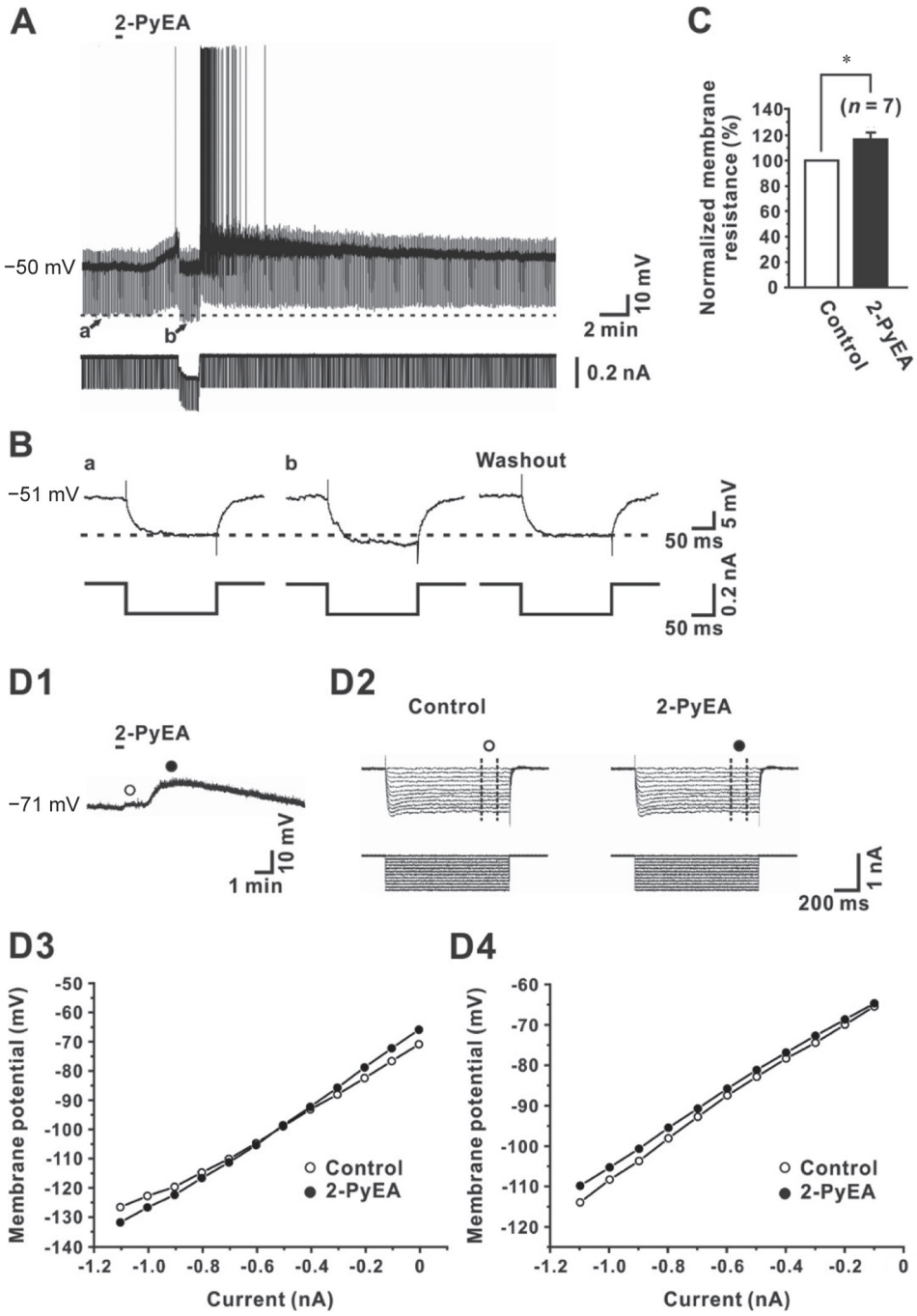

Fig. 3. Activation of H_1 receptor increases membrane resistance of spinal α-motoneurons. *A*, *B*: The 2-PyEA-induced depolarization on a recorded α-motoneuron accompanied by an increase in membrane input resistance. The membrane potential was held to its resting level (–51 mV) during the 2-PyEA-induced depolarization. Downward deflections indicate increase in electronic potentials produced by the constant-current hyperpolarizing pulse (0.3 nA, 250 ms, 0.2 Hz) passing through the recording electrode. Note that this constant low hyperpolarizing current did not activate HCN channels or induce a sag potential on the recorded motoneuron (*B*). *C*: Mean membrane input resistance of spinal α-motoneurons before and during application of 2-PyEA (n = 7). *D*1, *D*2: Hyperpolarizing step command tests (−1.1 to 0 nA, 0.1 nA steps) were employed to evaluate the *I–V* curves under resting conditions (indicated by empty circles) and at the peak of the 2-PyEA-induced depolarization (indicated by filled circles). *D*3, *D*4: Two examples respectively show two types of the 2-PyEA-induced changes of *I–V* curves on spinal α-motoneurons. In 81.8% (9/11) of these neurons tested, the reversal potential for the depolarization induced by 2-PyEA was (−96.9 ± 2.5) mV, which is near the calculated E_k (*D*3). In the remaining 18.18% (2/11) of the tested α-motoneurons, the 2-PyEA-induced inward current had a larger amplitude at –110 mV as compared with –65 mV (*D*4). Data shown are means ± SEM. $^*P < 0.05$.

sure the membrane input resistance. When the tested motoneurons were depolarized by 2-PyEA, the depolarized membrane potential was manually clamped back to its original resting level for direct comparison of the membrane input resistance before and during the 2-PyEA-induced depolarization. We found that

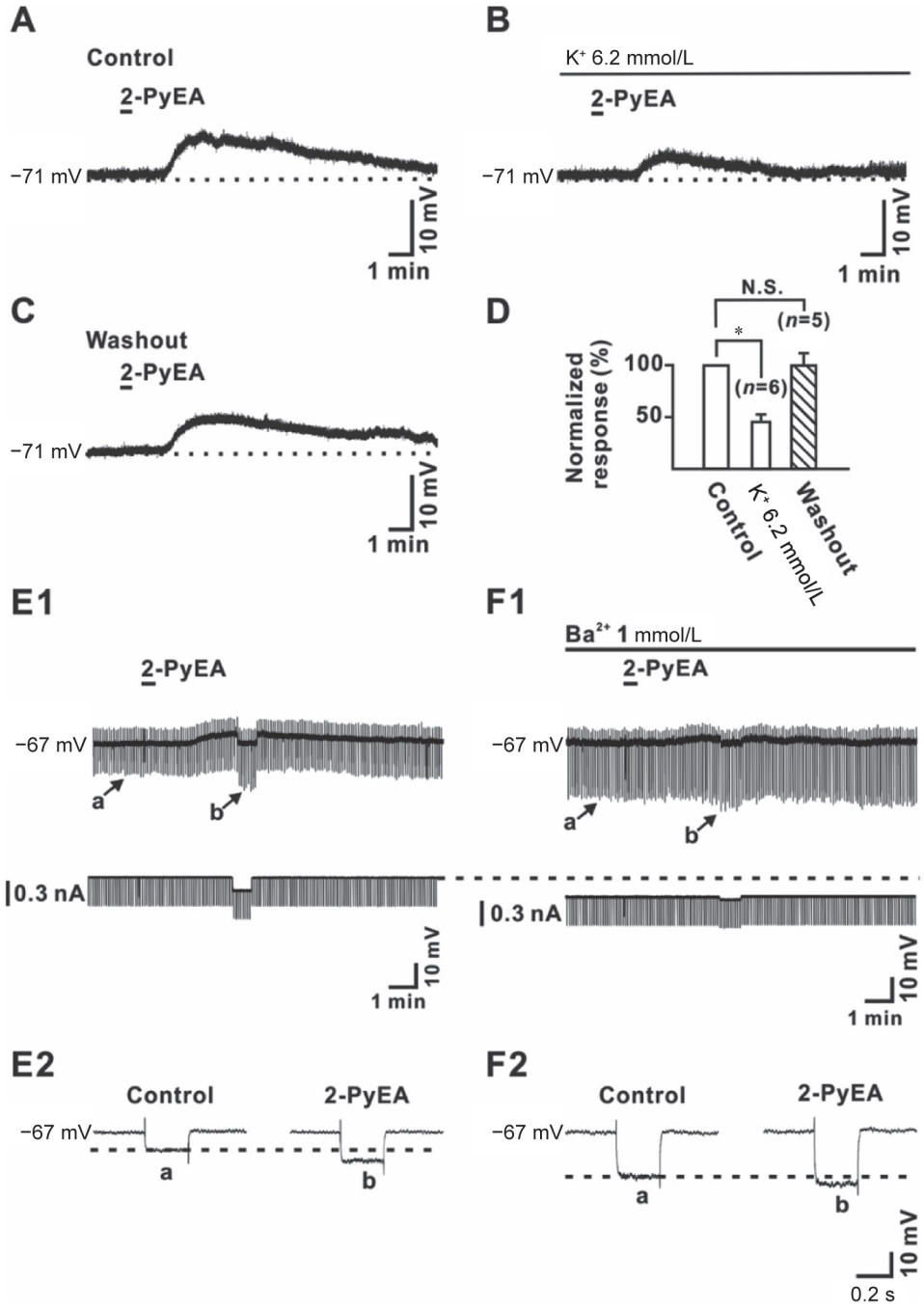

Fig. 4. Involvement of K^+ channels in the 2-PyEA-induced depolarization on spinal α-motoneurons. *A–C*: High K^+ ACSF ($[K^+]_o$ = 6.2 mmol/L) significantly decreased the depolarization induced by 2-PyEA in normal ACSF (control and washout, $[K^+]_o$ = 3.1 mmol/L). *D*: Group data of the tested motoneurons (n = 6 for high K^+ ACSF and n = 5 for washout). *E*, *F*: Effect of Ba^{2+}, a blocker for K^+ channels, on the 2-PyEA-induced increase of membrane resistance on spinal α-motoneurons. Downward deflections in *E*1 and *F*1 indicate increase in electronic potentials produced by the constant-current hyperpolarizing pulse (0.3 nA, 250 ms, 0.2 Hz) passing through the recording electrode. *E*2, *F*2: The enlargement of the hyperpolarizing pulse-induced electronic potentials during baseline (a) and 2-PyEA application (b) in *E*1 and *F*1. Data shown are means ± SEM. N.S. no significant difference; $^*P < 0.05$.

2-PyEA induced a depolarization accompanied by a significant increase in membrane input resistance [(117.37 ± 5.39)% of the control; $n = 7$, $P < 0.05$; Fig. 3*A*–*C*)], indicating that activation of H_1 receptor causes a closure of ionic channels on spinal α-motoneurons.

Next, we determined the changes of *I–V* curves in response to selective activation of H_1 receptor. We employed hyperpolarizing step command tests (−1.1 to 0 nA, 0.1 nA steps, 1 000 ms) to obtain current-voltage relationship from the recorded spinal α-motoneurons under resting conditions and at the peak of the 2-PyEA-induced depolarization (Fig. 3*D*1, *D*2). Notably, we observed two types of 2-PyEA-induced changes in the *I–V* curves from 11 spinal α-motoneurons. Within the 9 tested neurons (9/11, 81.82%), the reversal potential for the depolarization induced by 2-PyEA was (−96.9 ± 2.5) mV, which is near the calculated E_k and indicates an involvement of K^+ channels (Fig. 3*D*3). In the remaining 2 cells (2/11, 18.18%), the *I–V* curves in the absence and presence of 2-PyEA had a tendency to intersect at a potential more depolarized than −65 mV (Fig. 3*D*4). The diversity of the changes in *I–V* relationship suggests that more than one ionic component is involved in activation of H_1 receptor on spinal α-motoneurons.

2.4 Dual ionic mechanism mediates the depolarization induced by activation of H_1 receptor on spinal α-motoneurons

To examine whether K^+ channels are involved in histamine H_1 receptor-mediated excitation on spinal α-motoneurons, we elevated the concentration of extracellular K^+ from 3.1 mmol/L up to 6.2 mmol/L to observe changes of the 2-PyEA-induced depolarization of the motoneuron. As shown in Fig. 4*A–D*, the 2-PyEA-induced depolarization was diminished in the high K^+-ACSF [(44.17 ± 3.48)%; $n = 6$, $P < 0.05$] and recovered in normal ACSF [(99.82 ± 9.76)%; $n = 5$, $P = 0.877$], indicating the 2-PyEA-induced depolarization on spinal α-motoneurons changes with K^+ equilibrium potential. Moreover, we used Ba^{2+}, a blocker for K^+ channels, to assess whether the depolarization and increase in membrane resistance induced by 2-PyEA were blocked. As illustrated in Fig. 4*E*1–*F*2, 1 mmol/L Ba^{2+} significantly

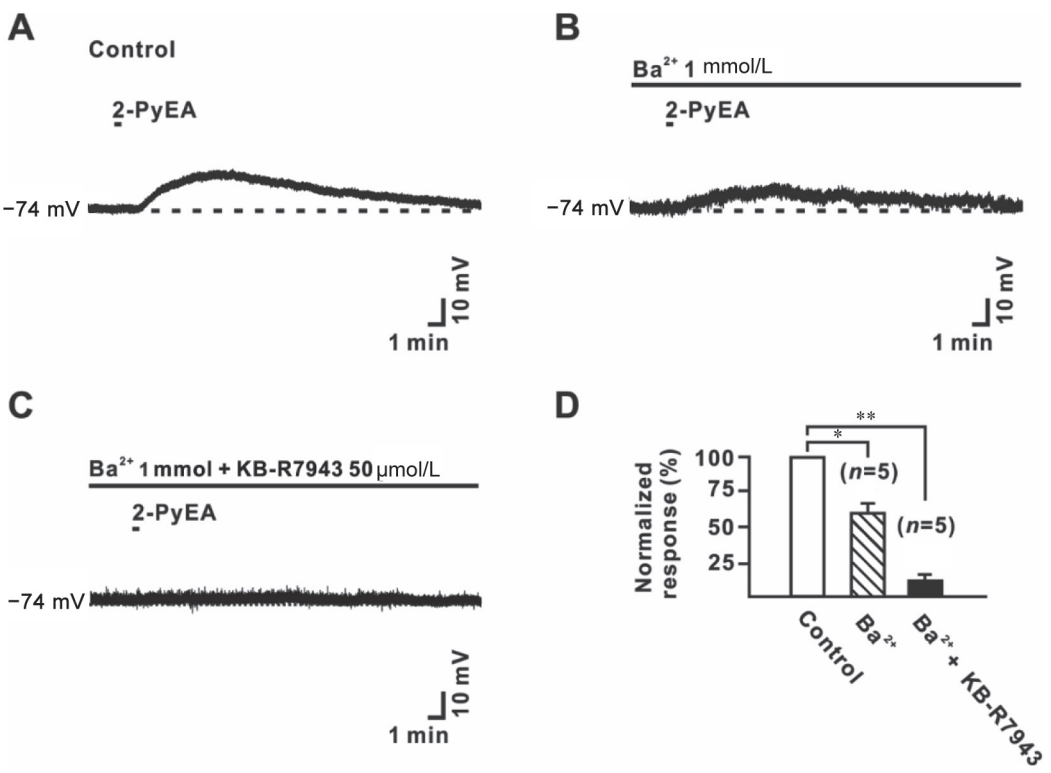

Fig. 5. Dual mechanisms including the closure of K^+ channels and activation of NCXs mediate the depolarization of spinal α-motoneurons induced by activation of H_1 receptor. *A–C*: The 2-PyEA-induced depolarization at holding potential of −74 mV on an α-motoneuron was partly suppressed by Ba^{2+}, and totally blocked by combined application of Ba^{2+} and KB-R7943 (a selective blocker for NCXs). *D*: Group data of the tested motoneurons ($n = 5$ for each group). Data shown are means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$.

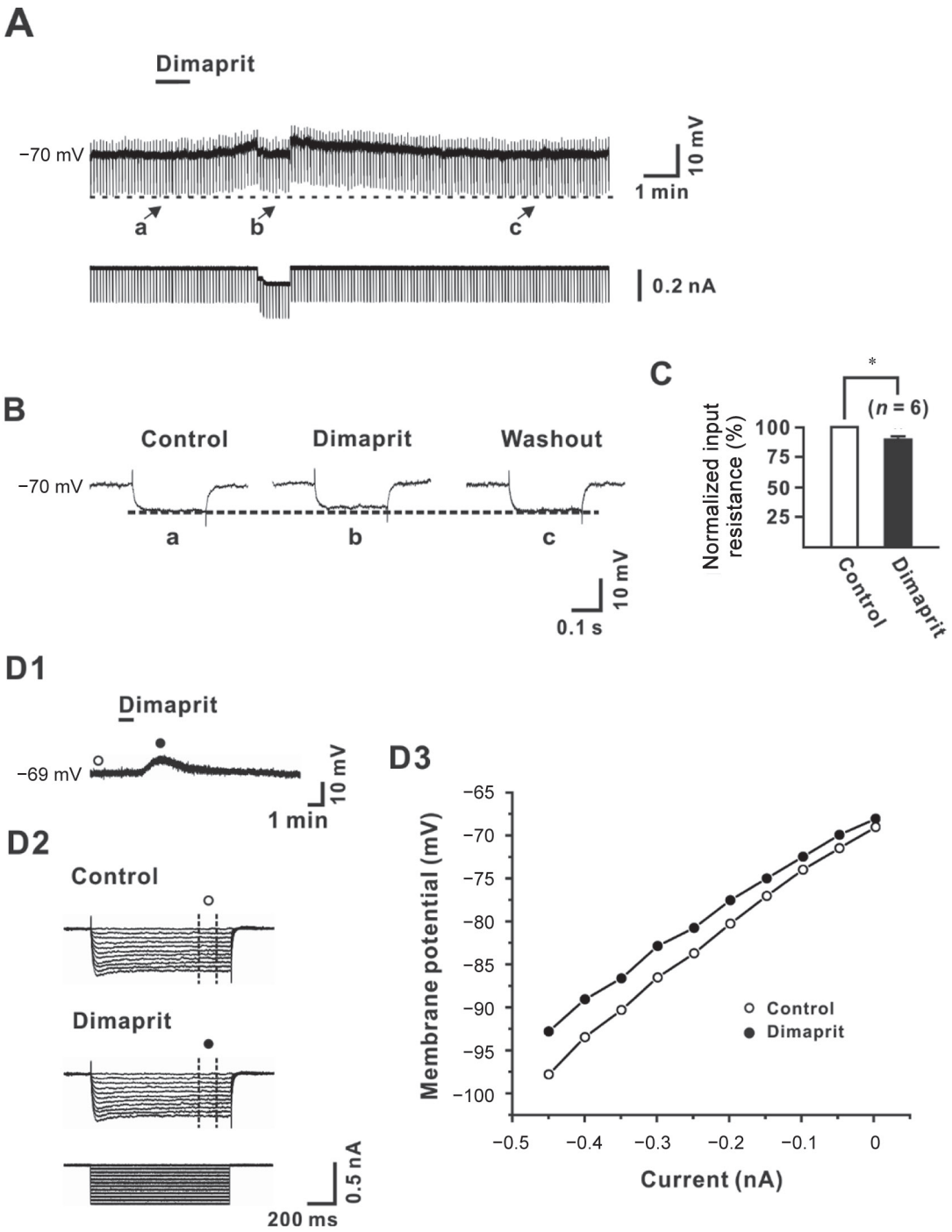

Fig. 6. Activation of H_2 receptor decreases membrane resistance of spinal α-motoneurons. *A*, *B*: The dimaprit-induced depolarization on a recorded α-motoneuron accompanied by a decrease in membrane input resistance. The membrane potential was held to its resting level, –70 mV, during the dimaprit-induced depolarization. Downward deflections indicate a decrease in electrotonic potential produced by the constant-current hyperpolarizing pulse (0.2 nA, 250 ms, 0.2 Hz) passing through the recording electrode. *B*: The enlargement of the hyperpolarizing pulse-induced electronic potentials during baseline (a), dimaprit application (b) and washout (c) in *A*. *C*: Mean membrane input resistance of spinal α-motoneurons before and during application of dimaprit ($n = 6$). *D*1, *D*2: Hyperpolarizing step command tests (−1.1 to 0 nA, 0.1 nA steps) were employed to evaluate the *I–V* curves under resting conditions (indicated by empty circles) and at the peak of the 2-PyEA-induced depolarization (indicated by filled circles). *D*3: An example of the dimaprit-induced change of *I–V* curves on spinal α-motoneurons. In all of these neurons tested (5/5, 100%), the dimaprit-induced inward current had a larger amplitude at –110 mV as compared with –65 mV. Data shown are means ± SEM. $^{*}P < 0.05$.

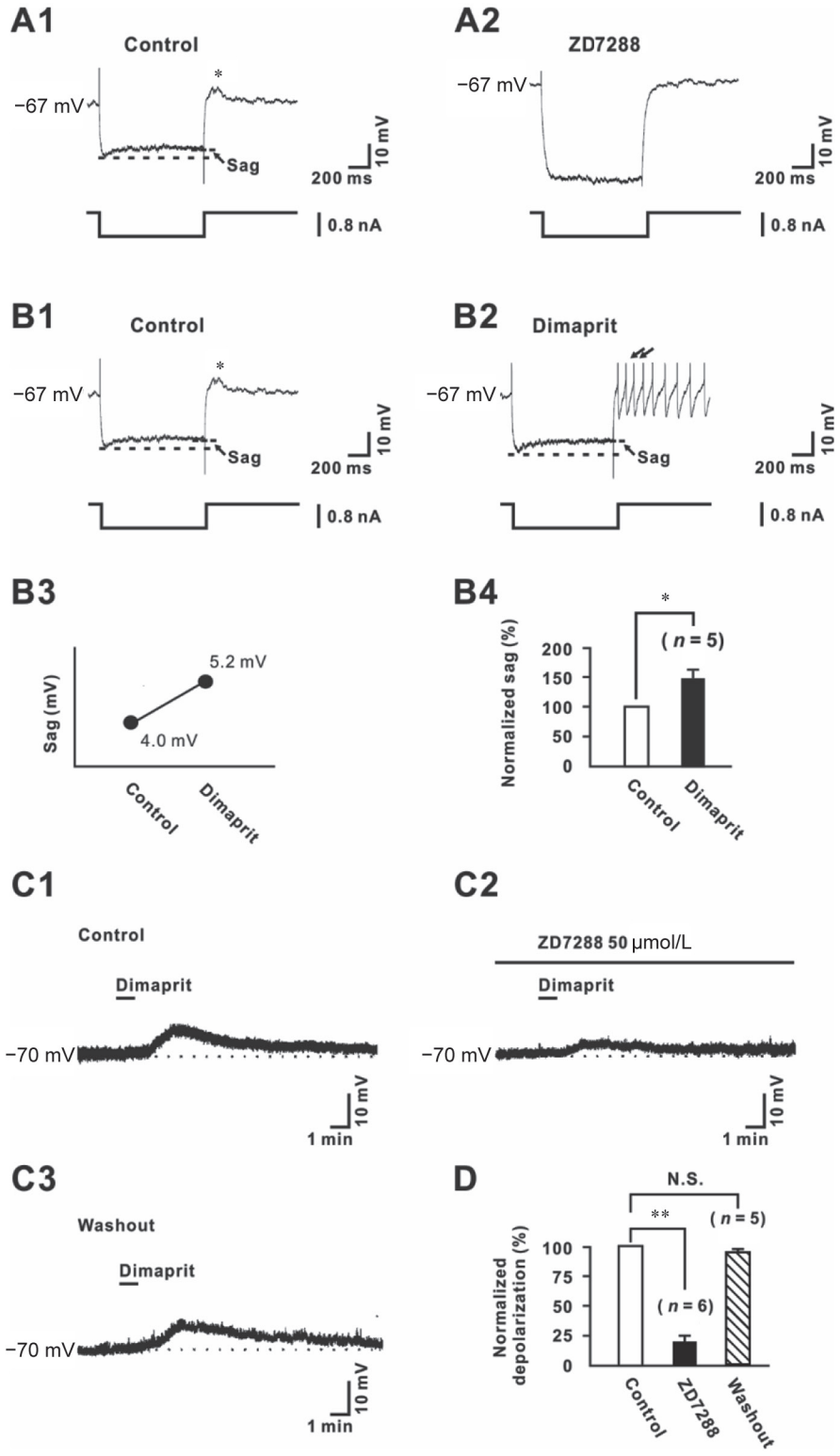

Fig. 7. HCN channels mediate the excitatory effect induced by the activation of H_2 receptor on spinal α-motoneurons. *A*1: The hyperpolarizing current step triggered a significant depolarizing voltage sag (arrow) and a rebound depolarization (asterisk) in a α-motoneuron. *A*2: The sag and rebound depolarization induced by the activation of HCN channels vanished after application of ZD7288, a selective blocker of HCN channels. *B*1–*B*3: The effect of dimaprit on the voltage sag (arrow) and rebound depolarization (asterisk) evoked by a hyperpolarizing current stimulation on a recorded motoneuron. *B*4: Group data of the tested motoneurons ($n = 5$). *C*1–*C*3: ZD7288 significantly blocked the dimaprit-induced depolarization on a motoneuron. *D*: Group data of the tested motoneurons ($n = 6$ for ZD7288 and $n = 5$ for washout). Data shown are means ± SEM. N.S. no significant difference; $^{**}P < 0.01$.

suppressed the 2-PyEA-induced depolarization [(39.80 ± 6.48)%; $n = 5$, $P < 0.05$] and remarkably attenuated the increase of membrane resistance on spinal α-motoneurons. These results suggest that the 2-PyEA-induced depolarization and increase in membrane resistance on spinal α-motoneurons were partially due to the closure of K^+ channels.

Among the various ionic mechanisms coupled to histamine H_1 receptor in the central nervous system, NCXs have a more positive reversal potential [26, 36, 37]. Considering more than one ionic component involved in activation of H_1 receptor on spinal α-motoneurons and the *I–V* curves in the absence and presence of 2-PyEA had a tendency to intersect at a more depolarized potential (Fig. 3*D*4) in some cases, we further clarified whether NCXs were involved in the 2-PyEA-induced depolarization on the motoneurons. As shown in Fig. 5, 1 mmol/L Ba^{2+} decreased the 2-PyEA-induced depolarization to (60.20 ± 6.48)% ($n = 5$, $P < 0.05$; Fig. 5*A*, *B*, *D*). Subsequently, 50 mmol/L KB-R7943, a selective blocker for NCXs, together with 1 mmol/L Ba^{2+}, almost totally blocked the 2-PyEA-induced depolarization by (89.00 ± 3.53)% ($n = 5$, $P < 0.01$; Fig. 5*A*, *C*, *D*). These results strongly demonstrate that H_1 receptor-mediated increase in excitability of spinal α-motoneurons was co-mediated by both closure of K^+ channels and activation of NCXs.

2.5 Activation of H_2 receptor decreases membrane resistance of spinal α-motoneurons

We estimated the changes in membrane resistance due to histamine H_2 receptor activation to assess the ionic mechanism coupled to H_2 receptor. During the depolarization induced by histamine H_2 receptor agonist dimaprit, we manually clamped the membrane potential of the recorded spinal α-motoneuron back to its resting level and intracellularly injected constant-current hyperpolarizing pulses (0.2 nA, 250 ms, 0.2 Hz) to measure changes in membrane resistance accompanied by H_2 receptor activation. We observed a decrease of the membrane input resistance during the dimaprit-induced depolarization on the motoneurons [(10.50 ± 0.95)%; $n = 6$, $P < 0.05$; Fig. 6*A–C*], suggesting that an opening of ionic channels underlying the excitatory effect of H_2 receptor activation. Moreover, we used hyperpolarizing step command tests (Fig. 6*D*2) to obtain the *I–V* curves in the absence and presence of dimaprit (Fig. 6*D*3). The two *I–V* curves showed a trend of intersection at a potential more depolarized than −70 mV (5/5, 100%), indicating a more positive reversal potential of the ionic channels may be coupled to H_2 receptor.

2.6 Opening of HCN channels mediates the depolarization induced by activation of H_2 receptor on spinal α-motoneurons

Previous studies have shown that the opening of HCN channels, whose reversal potential is near −30 mV, underlies H_2 receptor activation in other brain areas [10, 11, 38]. We thus examined the functional role of HCN channels in the spinal α-motoneurons. As shown in Fig. 7*A*1, a hyperpolarizing current stimulation evoked a significant depolarizing voltage sag and a rebound depolarization, the hallmarks of HCN channel activation [39], in spinal α-motoneurons. After blockage of HCN channels with ZD7288 (50 μmol/L), a selective blocker for HCN channels, the sag and rebound depolarization induced by the activation of HCN channels vanished (Fig. 7*A*2), indicating an existence of HCN channels in the spinal motoneurons. We further determined the effect of dimaprit on the activation of HCN channels, and found that dimaprit markedly increased the depolarizing sag [(45.73 ± 12.18)%; $n = 5$, $P < 0.05$; Fig. 7*B*] and enhanced the rebound depolarization to even evoke action potentials (Fig. 7*B*2), indicating a significant increase of HCN current by H_2 receptor activation in spinal α-motoneurons. Moreover, ZD7288 inhibited the dimaprit-induced depolarization ($n = 6$, $P < 0.05$; Fig. 7*C* and *D*), suggesting that HCN channels mediate facilitation of spinal α-motoneuron excitability by activation of H_2 receptor.

3 DISCUSSION

Spinal α-motoneurons provide the penultimate link between the central nervous system and motor behavior, and thus have been considered as the final common path [40]. The processes that determine the firing behavior of spinal α-motoneurons are therefore important in understanding the transformation of neural activity to motor behavior. Here, we report that histamine depolarizes spinal α-motoneurons and enhances the neuronal excitability by activation of both histamine H_1 and H_2 receptors. Moreover, K^+ channels and NCXs coupled to H_1 receptors and HCN channels linked to H_2 receptors contribute to the facilitation of histamine on the excitability of spinal α-motoneurons.

Firing activity of spinal α-motoneurons encodes skel-

etal muscle contraction and relaxation, controls muscle tone and thus holds a key position in movement generation. Suppression of spinal α-motoneuron excitability results in muscle paralysis after spinal cord injury [7] as well as surgical immobility during anesthesia [41]. Besides fast glutamatergic synaptic transmission from supraspinal and reticulospinal tracts [2, 42], firing properties of spinal α-motoneurons are also dominated by the descending monoaminergic modulations to the spinal cord [3, 4]. Noradrenergic and serotonergic (5-HTergic) inputs originating from the brainstem, as well as the descending dopaminergic pathway, have been extensively studied [3, 4]. In the present study, we reveal that the central histaminergic nervous system originating from the tuberomammillary nucleus of the hypothalamus and histamine may hold a key position in the regulation of neuronal activities of spinal α-motoneurons. Moreover, different from the complex modulation of noradrenergic and serotonergic systems which consists of both excitatory and inhibitory components depending on the targeting receptor subtypes [43], both postsynaptic H_1 and H_2 receptors activation excite spinal motoneurons and enhances their neuronal excitability. Although presence of histamine H_4 receptors on a subset of motor neurons in the ventral horn of the mouse spinal cord were reported [44], our previous [31] and present results showed that the postsynaptic excitatory effect of histamine on rat spinal motoneurons was totally blocked by combined application of histamine H_1 and H_2 receptor antagonists, indicating a co-expression of H_1 and H_2 receptors and their co-mediation in the histamine-induced excitation on spinal motoneurons. We therefore suggest that in parallel with other monoaminergic modulations on spinal cord networks, the hypothalamic histaminergic pathway provides a homogeneous excitatory drive to spinal α-motoneurons.

The activity of spinal α-motoneuron can be facilitated by various monoaminergic inputs with different ionic mechanisms. TTX-sensitive persistent Na^+ currents [5] together with L-type calcium currents [6] coupled to 5-HT_2 receptor have been reported to contribute to the 5-HT-induced facilitation of spinal α-motoneuron activity. Norepinephrine increases the motoneuron excitability via the inhibition of the inwardly rectifying K^+ current [9]. And dopamine boosts excitability in the spinal motoneurons by decreasing I_A (low-threshold, fast inactivating and 4-AP-sensitive K^+ channel) and SK (small-conductance calcium-activated K^+ channel) currents [8]. In the present study, we reveal, for the first time, that K^+ channels and NCXs coupled to H_1 receptor and HCN channels coupled to H_2 receptor co-mediate the excitatory effect of histamine on spinal α-motoneurons. NCXs, which have a highly positive reversal potential [26, 36, 37], guarantee a powerful driving force for depolarizing membrane potential. K^+ channels are quite active at rest membrane potential which play an important role in keeping rest membrane potential and modulating neuronal responsiveness to external signals [45–47]. Thus, through activation of NCXs as well as closure of K^+ channels coupled to H_1 receptor, histamine effectively depolarizes and increases the excitability of the spinal α-motoneurons. On the other hand, HCN channels, the essential pacemaker channels activated during hyperpolarization, help accelerate membrane depolarization and the generation of neuronal activity [39]. Therefore, by opening of HCN channels coupled to H_2 receptor, histamine may shape the excitability as well as modulate firing activity of spinal α-motoneurons. Considering different origins of monoaminergic modulators in the central nervous system and the diversity of receptor and ionic mechanisms, we propose that histaminergic innervation from the hypothalamus may play a unique role in modulation of spinal motoneuron excitability and precise regulation of the final motor output.

Histamine and the central histaminergic system have been revealed to actively participate in motor control [13–15, 28, 48] and to be closely related to motor diseases, such as Parkinson's disease and vestibular disorders [15, 49–51]. In addition, histamine-related drugs have traditionally been widely used in the treatment of vestibular disorders [14, 52, 53]. On the other hand, activities of ionic channels/exchangers have been implicated in the physiopathology and treatment of various movement disorders, including spinal motor disorders. In clinic, 4-AP, a blocker of rapidly activating voltage-gated K^+ channels has been used as a therapeutic agent in both multiple sclerosis and spinal cord injured patients [54]. In addition, Ca^{2+} dysregulation plays a central role in the pathophysiology of amyotrophic lateral sclerosis [55]. Electrogenic NCXs activation (3 Na^+ ions entering in exchange for 1 Ca^{2+} ion extruded from the cell) holds a key position in the maintenance of intracellular Ca^{2+} homeostasis, which may be responsible for the selective vulnerability of spinal α-motoneurons in amyotrophic lateral sclerosis [56]. Moreover, the prop-

erty of post-inhibitory rebound depolarization induced by HCN is contributed to the rhythm generation in neonatal rat spinal α-motoneurons during locomotion [57] and loss of HCN channels leads to movement dysfunction [15, 58]. Since K^+ channels, NCXs and HCN channels are ionic mechanisms underlying the modulatory effect of histamine on spinal α-motoneuron excitability, and besides anti-inflammatory profile of histamine, the histaminergic system is dysregulated in amyotrophic lateral sclerosis [59], histamine H_1 and H_2 receptors as well as their coupled ion channels and exchangers in spinal α-motoneurons may provide potential therapeutic targets for spinal motor disorders.

In conclusion, the present study reveals that histamine depolarizes and excites spinal α-motoneurons by the mediation of K^+ channels and NCXs coupled to H_1 receptor and HCN channels linked to H_2 receptor. Through switching the functional status of these ion channels and exchangers, histamine effectively biases the excitability of the spinal motoneurons. Thus, in this way, the hypothalamospinal histaminergic innervation may directly modulate final motor outputs and actively regulate spinal motor reflexes and ongoing motor execution.

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