Acta Physiologica Sinica, August 25, 2009, 61 (4): 305-316
http://www.actaps.com.cn

Research Paper

Modulation of leak K^+ channel in hypoglossal motoneurons of rats by serotonin and/or variation of pH value

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Abstract: The cloned TWIK-related acid-sensitive K^+ channel (TASK-1) is sensitive to the pH changes within physiological pH range (pK~7.4). Recently, the native TASK-1-like channel was suggested to be the main contributor to the background (or leak) K^+ conductance in the motoneurons of the brain stem. Serotonin (5-HT) and variation of pH value in perfused solution could modulate these currents. Here we aimed to examine the properties and modulation of the currents by serotonin or variation of pH value in hypoglossal motoneurons of rats. Transverse slices were prepared from the brainstem of neonatal Sprague-Dawley rats (postnatal days 7-8). Hypoglossal motoneurons were used for the study. The leak K^+ current (TASK-1-like current) and hyperpolarization-activated cationic current (I_h) were recorded with the whole-cell patch-clamp technique. The results showed that these currents were inhibited by acidified artificial cerebrospinal fluid (ACSF, pH 6.0) and activated by alkalized ACSF (pH 8.5). 5-HT (10 μmol/L) significantly inhibited both leak K^+ current and I_h with depolarization of membrane potential and the occurrence of oscillation and/or spikes. Bath application of Ketanserine, an antagonist of 5-HT_2 receptor, reversed or reduced the inhibitory effect of acidified solution on leak K^+ current and I_h. The results suggest that 5-HT_2 receptors mediate the effects of acidified media on leak K^+ current and I_h in hypoglossal motoneurons.

Key words: potassium channel, leak; hyperpolarization-activated current; patch clamp techniques; motoneurons; serotonin

5-羟色胺和/或pH值的变化对大鼠舌下神经元漏钾电流的调制作用

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摘 要：克隆的TWIK相关酸敏感型钾通道1 (TWIK-related acid-sensitive K^+ channel, TASK-1)对生理范围的pH值变化较为敏感(pK~7.4)。最近在多种脑干运动神经元上发现了TASK-1样通道，主要功能为编码背景性漏钾电流(TASK-1-like current)。本文旨在研究舌下神经元漏钾电流的特性，以及5-羟色胺(5-HT)和/或灌流液pH值的变化对该电流的影响及其可能的机制。以出生后7~8 d的Sprague-Dawley (SD)大鼠为研究对象，应用活脑片技术获取大鼠舌下神经核细胞，利用脑片全细胞膜片钳技术记录并分析运动神经元漏钾电流和超极化激活的阳离子电流(hyperpolarization-activated cationic current, I_h)。结果显示，漏钾电流和I_h可以被pH 6.0的酸性人工脑脊液(artificial cerebrospinal fluid, ACSF)所抑制，亦可被pH 8.5的碱性ACSF所激活。10 μmol/L 5-HT可显著抑制漏钾电流和I_h，并可使舌下神经元去极化，诱发阈下震荡和/或动作电位。给予5-HT_2受体拮抗剂Ketanserine，可以拮抗酸性ACSF对漏钾电流和I_h的抑制作用。以上结果表明，酸性ACSF对漏钾电流和I_h的调制作用可能是通过5-HT_2受体介导的。

关键词：漏钾电流；超极化激活电流；膜片钳技术；运动神经元；5-羟色胺
中图分类号：Q421；Q426；R56；R332.3

Received 2009-04-10 Accepted 2009-05-20

This work was supported by the National Natural Science Foundation of China (No. 30670920) and the Natural Science Foundation of Beijing Municipality, China (No. 7072079).

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TWIK-related acid-sensitive K^+ channel (TASK-1) is a typical two-pore domain K^+ channel that generates a pH-sensitive, weakly-rectifying K^+ current[1-4]. The native TASK-like currents have been identified in many regions throughout the nervous system according to their unique electrophysiological and pharmacological properties. They contribute prominent leak conductance in rat cerebellar granule cells[5,6], hypoglossal motoneurons, locus coeruleus, serotoninergic raphe neurons[7-10] and arterial chemoreceptor cells[11] .

Currently, Wang *et al*.[10] in our laboratory has reported the expression of TASK-1 in brainstem of rats, such as locus coeruleus, hypoglossal nucleus (XII) and other respiratory related neurons. We found that both the total spontaneous apnea index (TSAI) and spontaneous apnea index in NREM sleep (NSAI) were positively correlated with TASK-1 protein contents, so TASK-1 channels may function as central chemoreceptors that play a role in spontaneous sleep apneas in rats.

Hypoglossal motoneurons innervate genioglossus (GG) muscle of the tongue which contributes to pulmonary ventilation by maintaining an open pharyngeal airspace. The upper airway would be prone to collapse if the excitability of hypoglossal motoneurons is impaired and may further result in sleep apnea. Serotonin (5-HT) from medullary raphe neurons depolarizes and increases the excitability of hypoglossal motoneurons *in vitro*[12] and increases hypoglossal motor output to GG muscle both in decerebrate[13] and freely behaving animals *in vivo*[14]. Since TASK-1 mRNAs distributed in the hypoglossal motoneurons, we assume that 5-HT may modulate excitability of motoneurons via TASK-1 current.

In this study, we intended to examine their physiological functions: (1) to further investigate the relationship between the TASK-1-like current (I_h as well) in rat hypoglossal motoneurons and 5-HT and/or pH value variation in bath solution; (2) to distinguish which subtype of 5-HT receptors contributes to the sensitivity of acidity; and (3) to discuss whether there would be some intrinsic relationship between leak K^+ current and sleep apnea syndrome (SAS).

1 MATERIALS AND METHODS

1.1 *Animals*

Neonatal male or female Sprague-Dawley rats (postnatal days 7-8), provided by Department of Laboratory Animal Science of Peking University Health Science Center, were used for this study. Animal protocols were reviewed and approved by the Medical Ethics Committee of Experimental Animals of Peking University First Hospital.

1.2 *Slice preparation and maintenance*

Brainstem slices were obtained from Sprague-Dawley rats. Animals were decapitated under terminal anesthesia (halothane) and the brainstem was removed. Transverse slices (about 200 μm thick) were cut around the obex level with a vibratome (Zhejiang Xiangshan Scientific Precision Instrument Factory, China) in ice-cold, oxygenized (95% O_2 and 5% CO_2) sucrose artificial cerebrospinal fluid (ACSF) (in mmol/L: 260 sucrose, 3 KCl, 5 $MgCl_2$, 1 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 1 kynurenic acid). After cutting, slices were incubated for at least 40 min in pre-warmed [(34.5±0.5) °C], oxygenized (95% O_2 and 5% CO_2) modified ACSF (in mmol/L: 130 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose) at pH 7.30-7.35, and were subsequently maintained at room temperature (24 °C) in ACSF for about 30 min.

1.3 *Electrophysiological recordings from hypoglossal motoneurons*

The slices were continuously superfused at room temperature with a solution that was gassed continuously with 95% O_2/5% CO_2, and HEPES-buffered ACSF (in mmol/L: 130 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 25 HEPES, 10 glucose; the pH was adjusted to the desired level using NaOH) perfused at a rate of 3-4 mL/min.

Patch pipettes were pulled from thin-walled borosilicate capillaries (4-6 MΩ; WPI, USA) with a vertical puller (Narishige, Japan), and polished on a microforge (Narishige, Japan). Electrodes were filled with an internal solution of the following composition (in mmol/L): 120 KCH_3SO_3, 4 NaCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 HEPES, 10 EGTA, 3 MgATP, 0.3 GTP-Tris, pH 7.2 (in some cases, we increased HEPES concentration in the pipette solution to 25 mmol/L with a corresponding decrease in KCH_3SO_3 concentration in order to minimize the effect of intracellular variation of pH value on I_h).

Slices were visualized using a ×40 water-immersion lens mounted on an upright microscope fitted with infrared differential interference (DIC) optics (Olympus, Japan). Hypoglossal motoneurons (Fig. 1*A*) were identified by their large cell body (10-30 μm, diameter) and anatomical location ventral to the central canal. Cells near to the slice surface were chosen in order to minimize the effect of endogenous pH buffering within the slice[15] and maximize the effect of exogenous agents. Whole-cell recordings were performed in both voltage-clamp and current-clamp mode using a PC-IIC amplifier (Yi Bo Life Scientific Instrument

Limited Company of Huazhong University of Science and Technology, China) and data were sampled with p-Clamp software (Axon Instruments). Cells were held at a membrane potential of -60 mV and currents evoked by a variety of protocols as follows: Currents were evoked by a series of hyperpolarizing voltage steps (Δ-10 mV, range: -70 to -140 mV; 500 ms); currents were evoked by a series of depolarizing voltage steps (Δ+10 mV, range: -70 to +50 mV; 500 ms); in some experiments, currents were also evoked by ramp depolarizing voltage changes (from -140 to +40 mV; 900 ms) or recorded continuously in gap free mode. Data were acquired at a sampling rate of 10 kHz, filtered at 2 kHz. Analysis was performed with Clampfit 10.0. Statistical comparisons were carried out using paired *t* test, and $P<0.05$ was regarded as statistically significant. Results were given as mean ± SEM, with "*n*" as the number of experiments.

1.4 *Pharmacological agents*

Kynurenic acid, Ketanserine, WAY-100635, and 5-HT were all obtained from Sigma (Sigma, USA). All drugs were added directly to the ACSF.

2 RESULTS

2.1 Hypoglossal motoneurons express a current that is very sensitive to the pH variation

In order to characterize the pH-sensitive conductance in hypoglossal motoneurons, cells were held at -60 mV in a control extracellular solution of pH 7.3 and undergone voltage-clamp gap free recording. Without exception ($n=20$), acidification of the external solution resulted in an inward shift in holding current, whereas alkalization induced a corresponding change in the outward direction (Fig. 1). As it can be seen, compared with the controlled

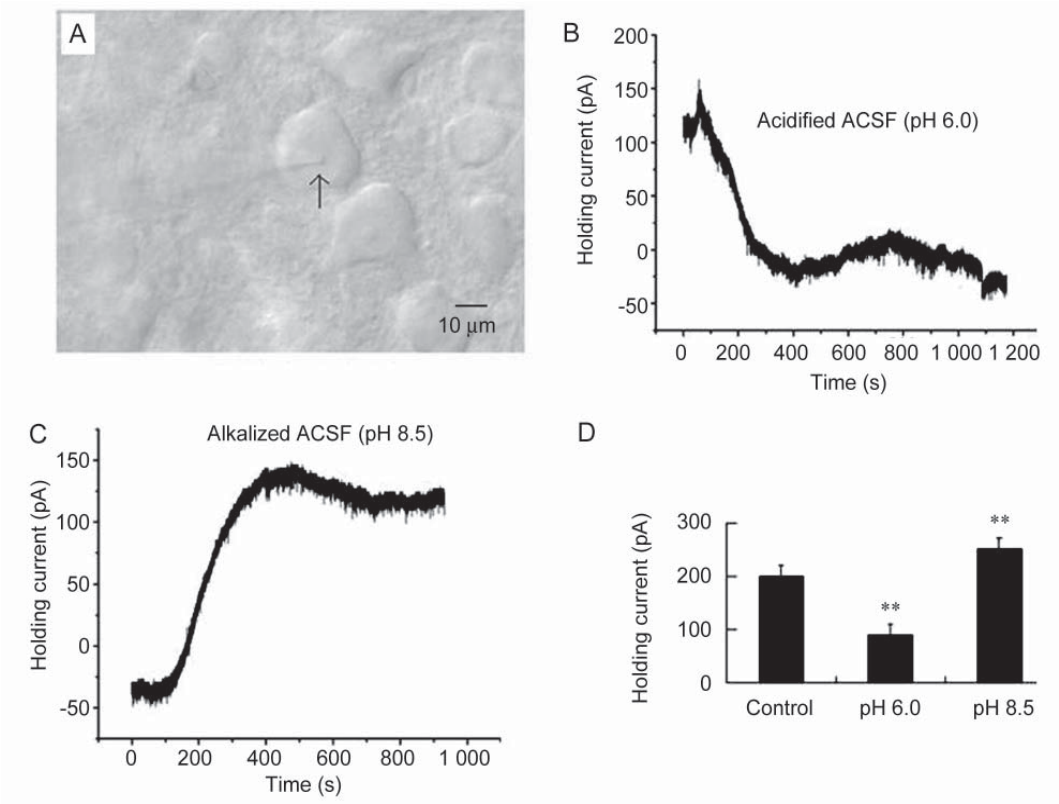

Fig. 1. The variation of extracellular pH value can significantly affect the holding currents of hypoglossal motoneurons. *A*: Infrared differential interference (DIC) image of live hypoglossal motoneurons in a slice of brainstem used for electrophysiological recording. Scale bar, 10 μm. The arrowhead shows the electrodes approaching to the hypoglossal motoneuron. *B*: Holding current measurements of a representative neuron voltage clamped at -60 mV and subjected to acidified ACSF (pH 6.0). During perfusion of acidified ACSF, there was an inward shift of the holding current. *C*: There was an outward shift of the holding current after the motoneuron perfused with alkalized ACSF (pH 8.5). *Y*-axis: holding current; *X*-axis: time course of recording; "0": the onset of variation of pH value. *D*: Histograms: Changes of holding current with variation of extracellular pH value. Acidified ACSF notably shifted the holding current inwardly (via inhibiting leak K^+ channel) by (116±52) pA. Conversely, alkalized surrounding significantly shifted the holding current outwardly (via activating leak K^+ channel) by (61±27) pA. mean ± SEM, $n=20$. $^{**}P<0.01$ *vs* control.

neutral ACSF (pH 7.3), acidified ACSF (pH 6.0) inwardly shifted the holding current from +100 pA to -50 pA (Fig. 1*B*), which suggested the leak potassium current of the hypoglossal motoneurons was significantly inhibited. On the other hand, when the same cell was superfused with alkalized ACSF (pH 8.5), the holding current shifted outwardly from -50 pA to +150 pA (Fig. 1*C*), indicating that the leak K^+ conductance was apparently activated. The average changes (n=20) of holding current resulting from variation of pH value were shown in Fig. 1*D*.

2.2 The pH-sensitive current in hypoglossal motoneurons has voltage- and time-dependent properties similar to TASK-1

To determine the voltage- and time-dependence of the pH-sensitive conductance in hypoglossal motoneurons, current-voltage (*I-V*) relationships were obtained at the peak in response to extracellular acidification (pH 6.0) and alkalization (pH 8.5). Current traces recorded from a representative cell under these conditions are shown in Fig. 2*A* and *B*, respectively. The neuron was held at -60 mV and the responses were evoked by a set of hyperpolarizing voltage steps (Δ-10 mV, range: from -70 to -140 mV; 500 ms) either in alkalized ACSF (Fig. 2*A*) or in acidified ACSF (Fig. 2*B*). It could be seen that each current trace comprises 3 parts: the capacitive transient; instantaneous outward current and slow inward rectification time-dependent current (I_h). The instantaneous component (leak K^+ current, as the arrowheads show) of each trace was measured in a time window between the settling of the transient capacitive current and the onset of the time-

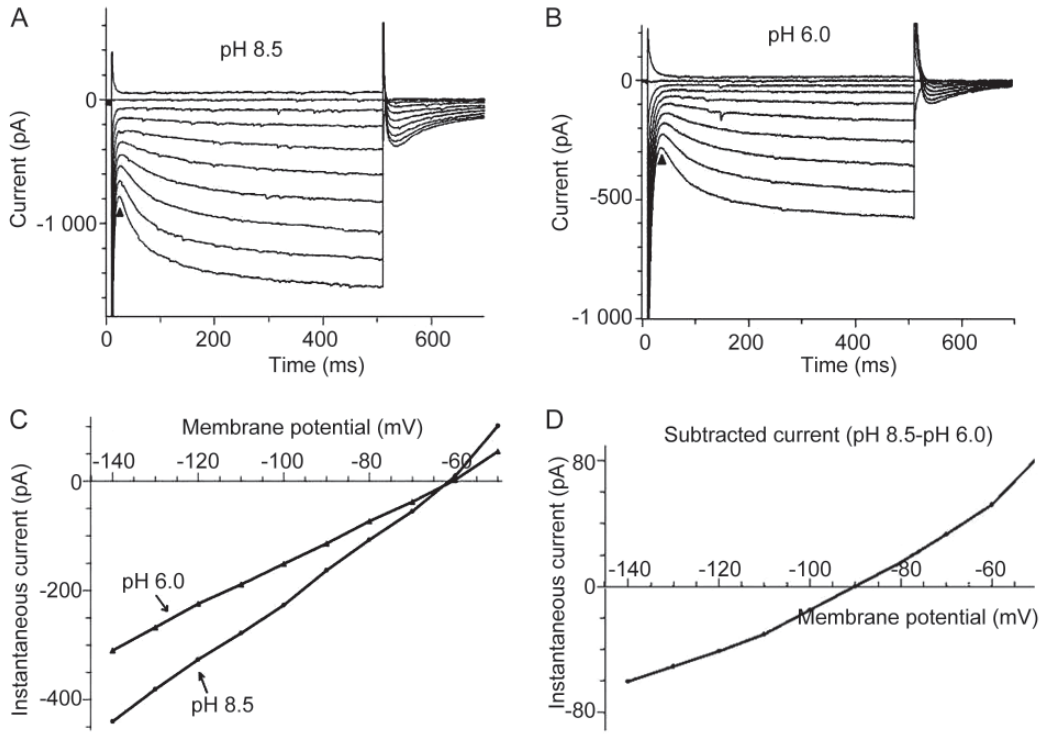

Fig. 2. Hypoglossal motoneurons express a leak K^+ conductance with pH-sensitive and voltage-dependent properties similar to those of TASK-1. *A* and *B*: Current traces from hyperpolarizing voltage steps (increment: -10 mV, range: from -70 to -140 mV) applied to the hypoglossal neuron (the neuron was held at -60 mV) subjected to alkalization (pH 8.5, *A*) or acidification (pH 6.0, *B*) of the extracellular medium. The instantaneous outwardly component of the current response was measured in a time window (~10 ms after the onset of the step, as the arrows show)[7] after the settling of the capacitive transient and prior to the onset of the time-dependent inward current (I_h). *C*: The traces of the instantaneous current responses shown by arrows in *A* and *B* were plotted as a function of membrane potential. The resulting current-voltage (*I-V*) relationships reveal a decreased conductance at pH 6.0 (by inhibiting the pH-sensitive leak K^+ current) and a reversal potential which is at about -60 mV and far away from potassium equilibrium potential (E_K) predicted for K^+, suggesting contributions from currents other than those carried by K^+. It suggests that additional current might be the hyperpolarization-activated cationic current (I_h). *D*: The resulting current-voltage (*I-V*) relationships were subtracted (pH 8.5 minus pH 6.0) to reveal that the motoneuronal pH-sensitive current was weakly rectifying one and reversed at predicted E_K (-90 mV). These data were well fitted using the Goldman-Hodgkin-Katz (GHK) current equation, indicating it must be the current of an open-rectifier K^+ conductance.

dependent current[7] (about 10-15 ms after the onset of the step). It showed a little time-dependence and the effects of pH were apparent even at the earliest time points in the voltage step, indicating that the pH-sensitive current was essentially instantaneous. The instantaneous outward current (arrowheads) was notably inhibited by acidified conditions (pH 6.0) compared to that obtained in the alkalized ACSF (pH 8.5). The corresponding *I-V* relationships of these instantaneous currents are shown in Fig. 2*C*. We could find that the current responses in alkalized ACSF (pH 8.5) were notably larger than that in acidified ACSF (pH 6.0), resulting in a steeper slope of the *I-V* relationship and indicating a larger current at the higher pH value; and further indicating that acidified solution can inhibit the leak K^+ current and increase membrane input resistance as well. By subtracting *I-V* curves obtained at alkalized pH level (pH 8.5) where the conductance was strongly activated, from those obtained at acidified pH levels (pH 6.0) where the conductance was inhibited, we can get the voltage-dependent properties of the instantaneous pH-sensitive current more purely (Fig. 2*D*). It demonstrated that the reverse potential of pH-sensitive current is very close to -90 mV, the predicted value for K^+ equilibrium potential (E_K). Furthermore, the motoneuronal *I-V* relationship were well fitted with the Goldman-Hodgkin-Katz equation (GHK), which again indicated the pH-sensitive current was a weakly outwardly leak K^+ current with a reversal potential at the predicted E_K. All of these voltage- and time-dependence properties of pH-sensitive current in hypoglossal motoneurons were very similar to those of cloned TASK-1 current[1,2], thus we term the pH-sensitive current in hypoglossal motoneurons as TASK-1-like current.

2.3 5-HT significantly inhibits the leak K^+ current and I_h as well as causes an inward change in holding current of hypoglossal motoneurons

Motoneurons of brainstem slice were perfused with serotonin (10 μmol/L; bath application) under control condition (pH 7.3). Serotonin notably inhibited the leak K^+ current in hypoglossal motoneurons [(156±98) pA, n=40]. A representative current-time profile (Fig. 3*A*) showed that 5-HT (10 μmol/L) significantly shifted the holding current inwardly from +50 pA to -150 pA. In addition, when the standard ACSF (pH 7.3) was replaced by acidified ACSF (pH 6.0) to perfuse the slice, the holding current also shifted inwardly. However, the change induced by 5-HT in the holding current was substantially larger than that induced by extracellular acidification (Fig. 3*B*). In this case, when the motoneuron was first superfused with standard acidified solution (pH 6.0), the holing current shifted from 25 pA to -100 pA. After application of 5-HT (10 μmol/L) into the acidified solution, the holding current of the same motoneuron further decreased to -200 pA. So it suggested that 5-HT could not only inhibit the pH-sensitive potassium current, but also the pH-insensitive current, which may be termed as "residual current". Given that neurotransmitters modulate multiple conductances in motoneurons, it is

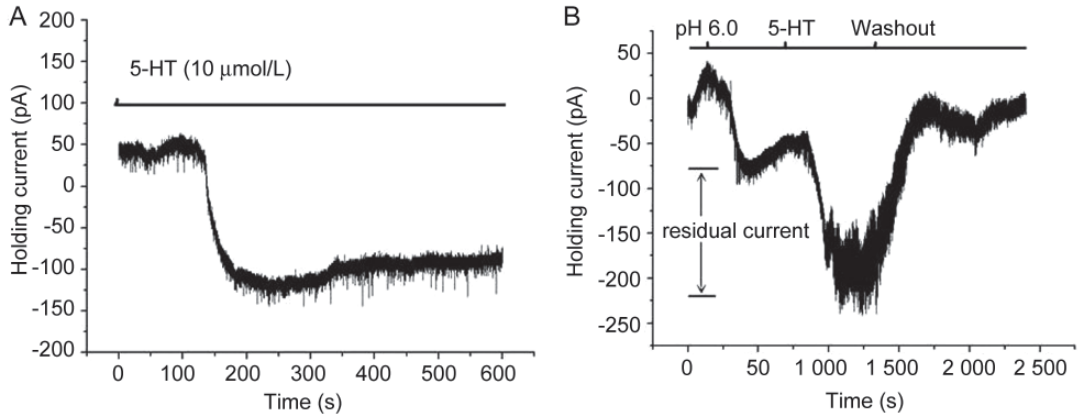

Fig. 3. Serotonin significantly inhibits the leak K^+ current and causes an inward shift in holding current. The neuron was held at -60 mV and the changes of current were measured at the voltage-clamp gap-free mode. 5-HT (10 μmol/L, 15 min; bath application) shifted the holding current from +50 pA to -100 pA (panel *A*) and if the bath solution was replaced with acidified solution (pH 6.0), the holding current of the same motoneuron also shifted inwardly. Furthermore, after application of 5-HT (10 μmol/L) into the acidified solution, the holding current of the same motoneuron further decreased to -200 pA (panel *B*). This indicates that 5-HT can inhibit not only pH-sensitive current but also pH-insensitive current (residual current, between two arrows showed). *Y*-axis: holding current, *X*-axis: time course of recording; "0" point: the onset of 5-HT application.

expected that the residual 5-HT current which appeared after inhibition of the pH-sensitive K^+ current contains the hyperpolarization-activated cationic current (I_h) similar to that has been confirmed in other motoneurons[16].

Hypoglossal motoneurons show a biphasic voltage response to hyperpolarization, which is the instantaneous and steady state response (Fig. 2). To determine whether 5-HT and acidified solution block the same leak conductance in hypoglossal neuron, other experiments were performed. Figure 4 shows a representative example of such experiments. In standard ACSF (Fig. 4*A*, curve 2), the effects of 5-HT on *I-V* relationship of the instantaneous current demonstrated the usual decrease of leak K^+ current (Fig. 4*A*, curve 1). After washing out 5-HT with the standard ACSF for 10 min, the bath solution was exchanged to acidified solution (pH 6.0), and the *I-V* relationship in response to a series of step pulses was presented in Fig. 4*B* (curve 2). When the same neuron bathed in pH 6.0 solution underwent subsequent application of 5-HT (10 μmol/L), it produced a further significant decrease of the leak K^+ current (Fig. 4*B*, curve 1). Likewise, 5-HT and acidified solution blocked the I_h in hypoglossal neuron, as can be seen in Fig. 4*C* and *D*, suggesting the serotonin blocked not only the acidified solution-sensitive I_h but also the insensitive.

So, taken together, serotonin could result in decreases in both leak K^+ current and I_h amplitude. For quantification, pooled data showed that the maximal amplitude of the instantaneous (leak K^+) current at -140 mV test potential decreased from (1 287.5±486.3) pA in control to (957.5±382.3) pA in 5-HT (P=0.01, n=4). When the pH value of bath ACSF was adjusted to pH 6.0, the mean amplitude of maximal current deceased from (1 002.3±423.9) pA to (696.5±266.8) pA (P<0.05, n=4) after adding 5-HT into the acidified ACSF. In respect to the steady-state current, 5-HT could also decrease I_h. The maximal I_h amplitude was defined as steady state current at -140 mV test potential. Two sets of data showed that 5-HT could reduce the steady-state current (I_h) in both conditions [30.4% decrease of I_h amplitude, (1 705.0±575.6) pA in control *vs* (1 186.3±345.3) pA in 5-HT group, P<0.05, n=4; and 36.2% decrease of I_h amplitude, (1 378.8±337.6) pA in pH 6.0 ACSF *vs* (880.0±293.5) pA in pH 6.0 ACSF with application of 10 μmol/L 5-HT, P<0.005, n=4]. Thus, both serotonin and acidified bath solution could modulate the two distinct currents (i.e. the instantaneous leak K^+ and I_h currents) in hypoglossal motoneurons. These data also showed that the 5-HT-sensitive currents might be composed of at least two components, i.e. one is the acidified solution-sensitive component and the other is acidified solution-insensitive.

Therefore, the data described above show that both 5-HT and acidified ACSF can block the same 5-HT-sensitive component of instantaneous leak K^+ current and I_h. It is well known that there are some subtypes of 5-HT receptors expressing in motoneurons[17], and acidified solution might block some subtypes of 5-HT conductance. In order to determine which subtype of 5-HT conductance is sensitive to the acidified solution, we use Ketaserine, a 5-HT_2 antagonist, to block the 5-HT_2 receptor subtype. Note that the acidified ACSF decreased the leak K^+ current and I_h (Fig. 4, curves 2 in panels *E* and *F*), however, application of Ketanserine (1 μmol/L) in acidified ACSF caused increase of both the leak K^+ current and I_h (Fig. 4*E*, *F*, curves 3), so the curves 3 and 1 get each other closely. The mean decrease of the leak K^+ current for all four neurons in response to adding Ketanserine (1 μmol/L) in acidified ACSF was (9.8±8.0)% [(-911.5 ±190.8) pA to (-824.0±183.2) pA, P>0.05, n=4), compared with (21.2±5.4)% [(-911.5±190.8) pA to (-709.2±90.2) pA, P<0.05, n=4] in acidified ACSF alone. Meanwhile, the mean I_h decreased by (3.8±2.3)% [(-1 083.0±143.9) pA to (-1 045.3±172.7) pA, P>0.05, n=4] and (12.2±9.4)% [(-1 083.0±143.9) pA to (-959.7±146.9) pA, P>0.05, n=4], respectively. These results indicated that 5-HT_2 receptor might mediate the effects of acidified ACSF on hypoglossal neurons. In contrast, application of WAY-100635, an antagonist of 5-HT_{1A} receptor[18], in acidified ACSF did not result in any significant increase of both the leak K^+ current and I_h (Fig. 4*G* and *H*, curves 3); so in this condition, curves 2 and 3 (in Fig. 4*G* and *H*) get closely, i.e. application of WAY-100635 in acidified ACSF had no effects on the decrease of both currents by acidified ACSF alone.

2.4 Serotonin depolarizes hypoglossal motoneurons and induces oscillation and/or spikes

Under current-clamp gap free conditions, 5-HT (10 μmol/L; bath application) caused a membrane depolarization of hypoglossal motoneurons, and subthreshold oscillations occurred, which gradually reached the threshold for repetitive sodium action potential discharge (spike); and these effects restored reversibly and completely. Figure 5*A* was a representative hypoglossal motoneuron showing effects of serotonin on membrane potential. It can be seen that before application of 5-HT, the membrane potential was at about -70 mV level, however, after 5-HT was added in bath solution, the membrane potential depolarized to -50

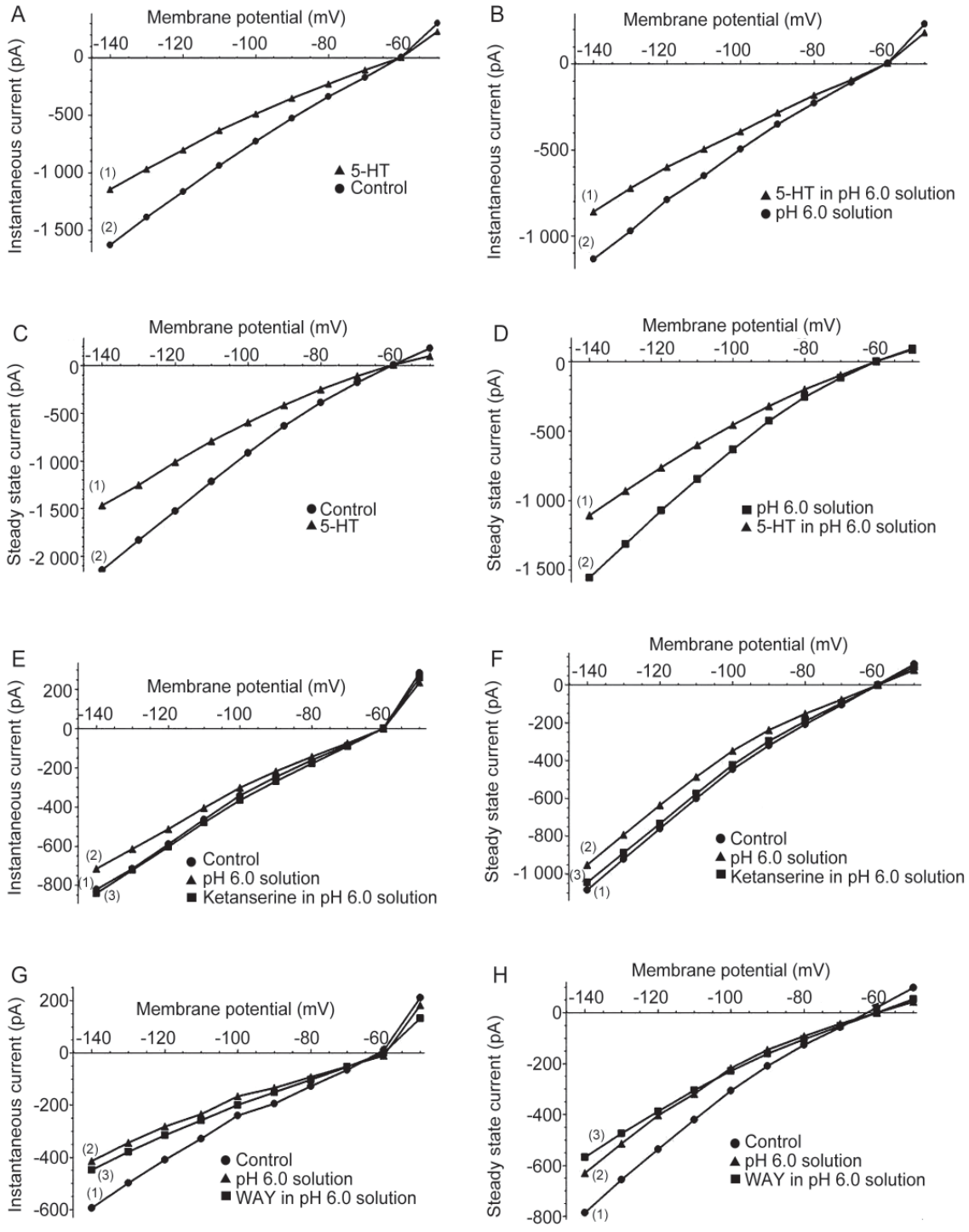

Fig. 4. Effects of 5-HT, acidified condition and antagonists (Ketanserine, WAY-100635) on *I-V* relationship of the instantaneous leak K^+ and steady-state currents (I_h). *A*, *B*, *E* and *G*: *I-V* plots of the instantaneous leak K^+ current. *C*, *D*, *F* and *H*: *I-V* plots of the hyperpolarization-activated cationic (steady-state) current. The neurons were held at -60 mV via DC current injection and the responses were evoked by a set of hyperpolarizing voltage steps (Δ-10 mV, range: from -70 to -140 mV; 500 ms) either in standard bath ACSF (control, *A* and *C*) or in acidified bath ACSF (*B* and *D*) with (curve 1) or without (curve 2) 5-HT (10 μmol/L). Panels *E* and *F*: in control (curve 1), in acidified bath ACSF (curve 2) or in acidified bath ACSF with 5-HT_2 antagonist (Ketaserine, curve 3). Panels *G* and *H*: in control (curve 1), in acidified bath ACSF (curve 2) or in acidified bath ACSF with 5-HT_{1A} antagonist (WAY-100635, curve 3). The maximal hyperpolarization-activated cationic current (I_h) amplitude defined as the difference between instantaneous and steady-state currents at -140 mV test potential. The *I-V* curves in penal *A-D* are derived from a representative neuron and those in panel *E-H* from the average data of four neurons.

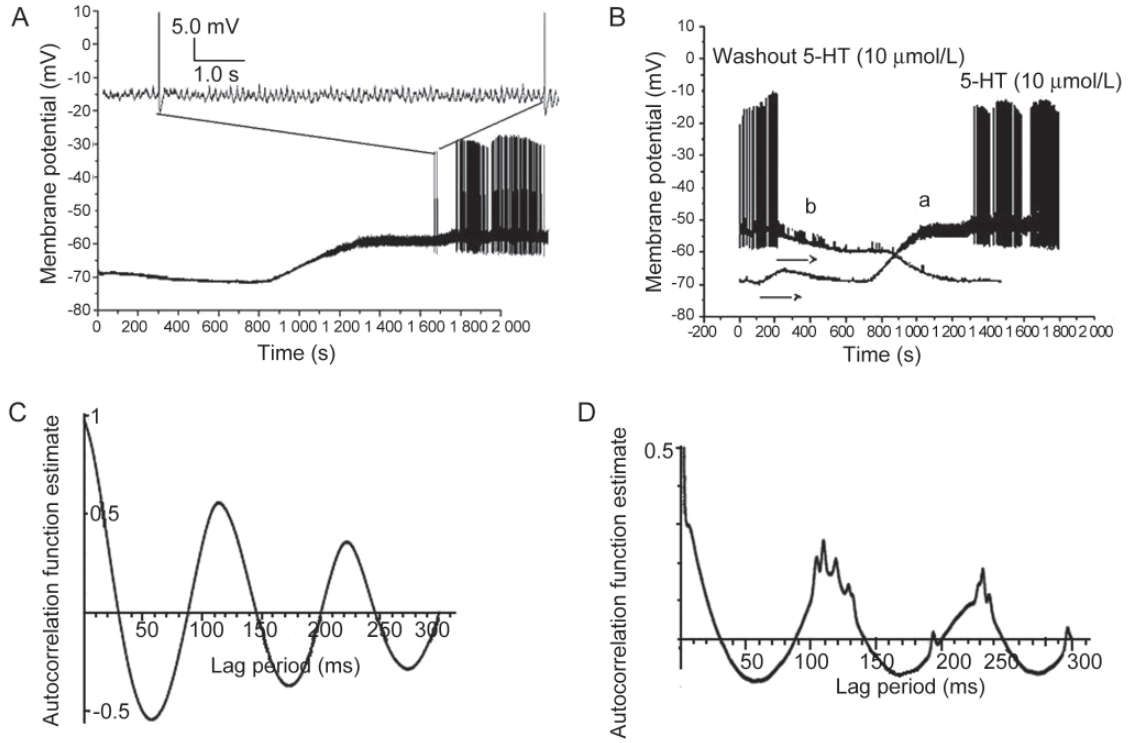

Fig. 5. Serotonin (10 μmol/L, bath application) depolarized reversibly membrane potential. *A*: When 5-HT (10 μmol/L) was added at time point "0", the membrane potential of the neuron was depolarized gradually by 10-15 mV and subthreshold oscillations were induced (in the inset, the time scale expanded to make the subthreshold oscillations clearer). After a while, the subthreshold oscillations transferred to robust spike. The neuron was held at -60 mV in neutral media (pH 7.3) and the variation of membrane potential was measured at the current-clamp mode. *B*: This panel shows the holding potential (excitability) variation (trace b) superimposed on the trace a (the same as in panel *A*). During washing out 5-HT with normal ACSF, the tendency of excitability changed similarly to trace a but in opposite direction, i.e. spikes disappeared firstly, then subthreshold oscillations abolished and finally the membrane potential returned back to the original level. *C*: Corresponding autocorrelogram of subthreshold oscillations shows a first peak at 112 ms (i.e. the dominant frequency at ~8.93 Hz). The oscillatory activity was considerably higher. *D*: Autocorrelogram of spikes shows a first peak at 110 ms (i.e. the dominant frequency at ~9.09 Hz). It is very close to the frequency of subthreshold oscillations.

mV. As a consequence of the depolarization, this normally quiescent neuron showed subthreshold oscillatory activities and suprathreshold firing discharges (action potentials or spikes). Analysis of autocorrelation for both subthreshold oscillations and spike showed high rhythmicity (Fig. 5*C* and *D*) and had frequencies of 8.93 and 9.09 Hz, respectively. The two frequencies were almost identical. Using standard ACSF (pH 7.3) to remove 5-HT, the spontaneous spikes disappeared, followed by the abolition of subthreshold oscillations, and finally the membrane potential restored to the initial level (Fig. 5*B*), suggesting that these effects of serotonin on hypoglossal neuron excitability are significant and reversible.

When hypoglossal motoneurons were held at -60 mV in current-clamp mode, majority (75 of 115 tested, 65.2%) of the neurons were quiet (data not shown) in response to a set of depolarized voltage step pulses in the control conditions (pH 7.3, without 5-HT). However, application of 5-HT (10 μmol/L) in the same bath solution could remarkably induce electrical activities, including subthreshold rebound potentials (or oscillations) and/or spikes as well as both together (Fig. 6). The number of spiking neurons also increased from 21(28.4%) in control condition to 65 (87.8%) of 74 tested neurons in the presence of 5-HT (10 μmol/L). In some neurons, only the higher depolarization pulses could elicit an entire train of action potentials (Fig. 6*A*) and some trains of the events were composed of action potentials and rebound potentials (Fig. 6*A*). In a few of neurons, depolarization pulses could mainly induce the subthreshold oscillations or rebound potentials (Fig. 6*B*). The frequency of subthreshold oscillatory activity induced by various depolarization pulses is constant basically. It

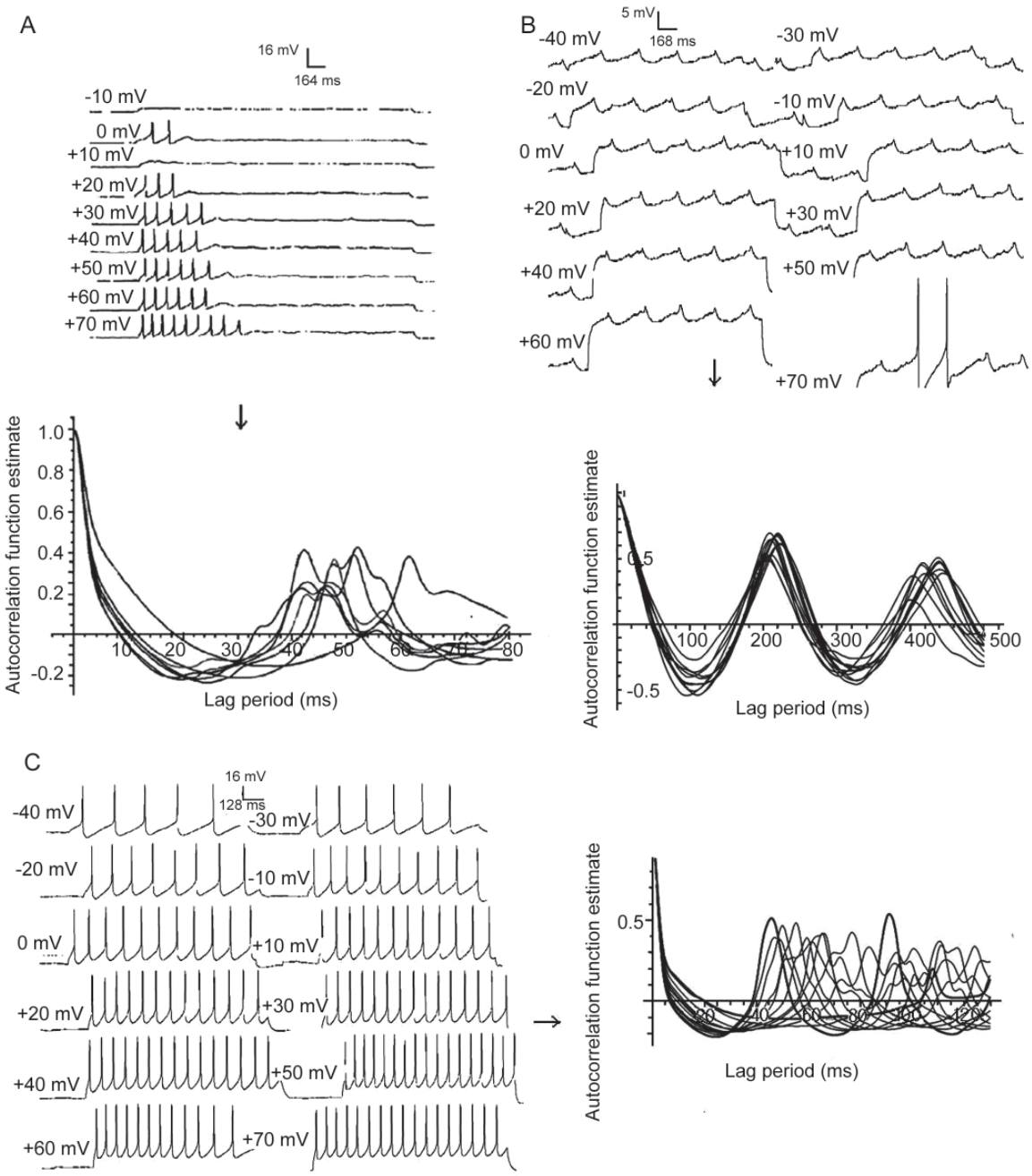

Fig. 6. Application of 5-HT (10 μmol/L) in the bath solution induced subthreshold responses (rebound potentials or oscillations) and/or spikes in current-clamp mode in some original quieter neurons by depolarization step pulses in 5-HT solution. *A*: Some trains of the events were composed of action potentials and rebound potentials induced by depolarization step pulses. Under the traces the inset shows the response frequencies are in narrow range between 16.3 and 23.8 Hz induced by various depolarization pulses. *B*: Depolarization pulses could mainly induce the subthreshold oscillations or rebound potentials in 5-HT bath solution in a few of neurons. The autocorrelograms show that the first peaks of all traces induced by various depolarization pulses are concentrated almost at the same frequency [(4.66±0.13) Hz] of the subthreshold oscillations. Note that only the deepest depolarization pulse (at +70 mV) elicited two spikes and the rhythmic spikes were phase-locked to the subthreshold oscillations of the membrane potentials. *C*: The spikes (without rebound potential) could be elicited entirely by depolarization voltage step pulses in most neurons bathed in 5-HT containing solution. The frequency of spikes increased with the level of depolarization pulse. However, the amplitudes of spikes were independent on the level of depolarization voltage. These rhythmic responses (oscillations or spikes) were induced by a set of depolaring step pulse (Δ+10 mV, range: from -40 to +70 mV). The number on the left of each trace indicates the depolarizing membrane potential. Insets under (or on the right of) each panel are the corresponding autocorrelograms.

can be seen that the rhythmic spikes were phase-locked to the subthreshold oscillations of the membrane potentials (Fig. 6*B*, bottom trace). In the case of this neuron, the mean frequency of rebound potentials was (4.66±0.13) Hz (range from 4.55 to 4.88 Hz) and the autocorrelogram indicated high rhythmicity (Fig. 6*B* inset). In spiking neurons, the spikes (without rebound potential) could be elicited entirely by depolarization voltage step pulses in 5-HT containing solution (Fig. 6*C*). The frequency of spiking was variable and dependent on the value of depolarization voltage (Fig. 6*C*, inset). However, the amplitudes of both rebound potentials and spikes were independent on the level of depolarization voltage. These data also suggested that hypoglossal motoneurons would become more excitable and easier to induce oscillations or to burst out action potentials when they were superfused with 5-HT containing solution.

As we well know, 5-HT can notably inhibit the leak K^+ current, so the depolarization in hypoglossal motoneurons might be driven, at least in part, by the inhibition of pH-sensitive K^+ conductance.

3 DISCUSSION

The brain-slice technique[19,20] has greatly facilitated the investigation of the electrical properties of neurons and recently the combination of the brain-slice technique with the patch clamp recording technique offers many advantages[21]. The biological activity of the slice is a big concern that can be affected by many factors, such as the damages caused by slicing process. Whittingham *et al.*[22] have demonstrated rapid reduction in ATP and other high-energy phosphates following decapitation and the recovery time depended on the duration of anoxia. In general, the brains of younger animals are easier to dissect free, and the slices survive better than those from adult animals[23]. So we select the neonatal Sprague-Dawley rats to assure the viability of the slices and cells. However, there are changes during postnatal development in morphology and electrophysiological properties associated with hypoglossal motoneurons[24-26], therefore we would like to use adult Sprague-Dawley rats in the afterward experiments to further explore the properties of TASK-1-like channel. This would be linked to the whole body plethysmograph recording technique and may investigate the relationship between TASK-1 and sleep apnea.

Obstructive sleep apnea (OSA) is characterized by cyclic closure and opening of the pharynx during sleep, resulting in obstructed breathing. Upper airway muscles include the genioglossus, the major extrinsic tongue protrusor muscle that is innervated by neurons in the hypoglossal nucleus. Serotonin has an excitatory effect on upper airway motoneurons. This effect is reduced considerably during sleep, presumably due to a reduction in the activity of brainstem serotonergic neurons.

The neurotransmitter 5-HT exerts either inhibitory or excitatory effects on a variety of central neurons. This diversity of action can be attributed to a large number of distinct post- and/or pre-synaptically located 5-HT receptors. 5-HT-mediated regulation of motoneuronal excitability is a major component of the facilitatory system. Serotonin and its receptor system are available in hypoglossal motoneurons. In this study, 10 μmol/L serotonin depolarized membrane potential and resulted in subthreshold oscillations and/or spikes, thus 5-HT is likely to be an important state-dependent modulator of motor activity. The tonic level of activity of upper airway motoneurons, hypoglossal (XII) inspiratory motoneurons depends mainly on the presence of an excitatory drive mediated by 5-HT[27]. This drive is likely to be reduced or withdrawn during sleep when raphe neurons are silenced, that may, in predisposed individuals, lead to sleep-related airway obstructions.

Endogenous TASK-1-like potassium channels have now been reported in many native cell types including cerebellar granule neurons[5,6,28,29], motoneurons[7,30], serotonergic neurons[31], locus coeruleus neurons[8] and many other peripheral tissues such as vascular smooth muscles, adrenal glomerulosa cells, carotid body[11,32-34]. The main contribution of these native TASK-1-like potassium channels is encoding a background or leak current and setting membrane potential. They are modulated by many neurotransmitters, such as 5-HT, TRH, substance P, NE[8] through corresponding receptors. They are sensitive to the surrounding changes of pH value. We used bath solutions with pH values at the extreme of the response curve in order to maximize current amplitudes, and to verify the pH-sensitive properties of TASK-1-like current. However, it is important to realize that functionally important effects would be expected with less severe change in pH. It is also necessary to emphasize that the precise surrounding pH at the recorded neurons cannot be known exactly, because of the pH-buffering phenomenon within slices *in vitro*. However, we could try our best to minimize such influences by choosing neurons very close to the surface of the tissue and the slices were superfused with a fast flow (2-4 mL/min). Under such conditions, it has been found that deviations in extracellular pH near the surface of neuronal tissue are relatively minor[35,36].

Synchronized neural oscillations in neurons seem to be implicated in essential functions of the nervous system, such as awareness and attention, perception, motor performance, learning and sleep[37]. The ability to generate subthreshold membrane potential oscillations has been attributed to the electrical properties of these neurons, as well as to the properties of the network[38,39]. Both the delayed K^+ current and I_h may make important contributions to subthreshold oscillations[40]. Placantonakis *et al*.[41] also stated that the calcium dependent potassium conductance, the low threshold calcium conductance and the hyperpolarization activated cationic conductance enabled olivary neurons to generate oscillatory behavior.

In our studies, we found no spontaneous subthreshold oscillation in majority of the hypoglossal motoneurons. However, when the slice was perfused with serotonin, some of the non-oscillating neurons showed induced subthreshold oscillation with a basically constant frequency at about (4.66±0.13) Hz. These subthreshold oscillations could sometimes elicit further spiking and the rhythmic spikes were phase-locked to them as seen in Fig. 6*B*. We might imagine that subthreshold oscillations enhance firing by adding to the baseline membrane depolarization and bringing the cell closer to threshold of sodium action potential. By this interpretation, spikes locked near at the peak of oscillations because the peak was the first point to reach spike threshold.

It was reported that rat facial motoneurones, spinal motoneurons, globus pallidus and many other central neurones express a prominent I_h[42]. Activation of I_h contributes to inward rectification of the membrane in response to changes in voltage and has been functionally implicated in maintaining membrane potential, the generation of oscillatory activity as we have described above, and the temporal and spatial integration of synaptic inputs. I_h can be blocked by external caesium (Cs^+) ions, however, the most selective and irreversible pharmacological blocker is ZD-7288[42]. I_h can be modulated by many neurotransmitters, such as 5-HT. 5-HT_{1A}, 5-HT_2, 5-HT_4 and 5-HT_7 receptors have previously been reported to modulate I_h in various brain areas[43]. These receptors can either facilitate or inhibit I_h via different mechanisms.

In our studies, we also found the I_h on hypoglossal motoneurons could be evoked by hyperpolarizing voltage commands (as shown in Fig. 2*A* and 2*B*). We have demonstrated that I_h can be inhibited by both acid conditions and 5-HT (Fig. 2 and Fig. 4). Inhibition of I_h implied a hyperpolarization of cell membrane and a decrease of cell excitability.

Recently, increasing lines of evidence reveal that instability of ventilation control is involved in the pathogenesis of occurrence of both central and OSA. Central respiratory chemoreceptors (CCRs) play a crucial role in sensing the alterations in brain pH and/or p_{CO_2}. TASK channels are pH-sensitive and are expressed in many neuronal types in those respiratory network known to be chemosensitive[8, 31] . So they may contribute to central respiratory chemoreception and regulate the control of breathing peripherally in response to changes in pH and p_{CO_2}.

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