ASICs Alteration by pH Change in Trigeminal Ganglion Neurons

Uggrit Junsre, Saknan Bongsebandhu-phubhakdi

Abstract

The inflammatory events associated with the pathophysiology of headache, which involve afferents of trigeminal ganglion (TG) neurons, lead to pH drop and cause a local tissue acidosis. It is known that acid-sensing ion channels (ASICs) play a major role of sensor in response to pH change, which can activate the nociceptor in the local tissue acidosis at pH 7.0. However, how ASICs can activate TG neurons when pH change is still unclear. Thus, we investigated the effect of pH change (from pH 7.4 to pH 7.0) to small-and-medium-sized (15-80 pF) and large-sized (> 80 pF) TG neurons, which were grouped as nociceptive and non-nociceptive neurons, respectively. Primary-cultured TG neurons were subjected to whole-cell recording by patch clamp technique that injected depolarizing current steps in long periods. The electrophysiological properties of TG neurons were compared in the condition of pH 7.0 and pH 7.4. The results revealed that resting membrane potential (RMP) of small-and-medium-sized TG neurons was significantly depolarized in pH 7.0. Moreover, threshold hyperpolarization and threshold current reduction were observed. In contrast to small-and-medium-sized TG neurons, large-sized neurons did not response to pH change. These finding suggested that pH change can modulate the excitability of nociceptive neurons resulting in the increase of pain perception in trigeminal pathway.

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Trigeminal ganglion (TG) contains primary sensory neurons with afferent fibers specifically innervating the intracranial and extracranial tissues, muscles and joints that convey nociceptive information from these tissues of the head and face.[1,2] The inflammatory events associate with the pathophysiology of headache that lead to a local tissue acidosis, and activation of acid-sensing ion channels (ASICs) and transient receptor potential vanilloid type 1 (TRPV1) by extracellular protons (H^+).[3-6] In the aspect of sensitivity to pH change, ASICs are more sensitive than TRPV1. ASICs are activated by pH change from 7.4 to pH 7.0, while TRPV1 requires pH change from 7.4 to pH 6.0. Thus, ASICs are main sensor to detect acidic noxious environment in tissue acidosis.[7,8]

ASICs, which are mainly permeable to sodium ions (Na^+), are trimeric voltage-independent ligand-gated cation channels and classified into ASIC1 (ASIC1a and 1b), ASIC2 (2a and 2b), ASIC3, and ASIC4, of which ASIC2b does not function as a H^+-sensitive homotrimeric channel, but can associate with the other ASICs (except ASIC4) to form active heterotrimeric channels, whereas ASIC4 does neither.[9,10] ASICs are expressed in both the central and peripheral nervous system (CNS and PNS). In the PNS, ASIC1a and 3 are expressed in small-and-medium-sized neurons of the TG and dorsal root ganglion (DRG) which respond to noxious stimuli. ASIC3 is also expressed in large-sized neurons of the TG and DRG which have mechanoreceptor and proprioceptor properties.[11-14] The pH change (from pH 7.4 to pH 7.0) is sufficient to activate ASICs, which are expressed on nociceptive TG neurons, and elicit depolarization as well as elicit firing of action potentials (APs) in vagal pulmonary afferent of nodose ganglion, cardiac and plantar muscle afferent of DRG, and dural afferents of TG.[15-19]

However, the effect of pH change on eliciting action potential in nociceptive and non-nociceptive TG neurons has still been unclear. We investigated the electrophysiological parameters of small-and-medium-sized and large-sized TG neurons in the change of pH.

Materials and Methods

Animals

Adult male Wistar rats were purchased from the National Laboratory Animal Center (Mahidol University, Salaya, Nakhon Pathom, Thailand). Rats were maintained in a ventilated room on a 12 h dark/light cycle with free access to food and water in accordance with procedures approved by the Animal Care and Use Committee (Faculty of Medicine,

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From the Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Corresponding author:
Saknan Bongsebandhu-phubhakdi, Ph.D.
Department of Physiology, Faculty of Medicine,
Chulalongkorn University, Bangkok, Thailand
E-mail: saknan@live.jp

Chulalongkorn University, Bangkok, Thailand).

Primary cell culture

TG neurons were obtained from 4- to 8-week-old rats using a previously described dissociation protocol[20] with minor modifications, as follows: Rats were euthanized by intraperitoneal injection of an overdose of sodium pentobarbital (Ceva Sante Animale, Libourne, France) and decapitated. Trigeminal ganglia were excised and transferred to a petri dish containing ice-cold Hank's balanced salt solution without Ca^{2+}/Mg^{2+} (HBSS; Gibco, Grand Island, NY) and chopped into small pieces. After digestion in HBSS containing 10 mg/ml collagenase type IV (Invitrogen, Carlsbad, CA) and 18.2 U/ml dispase type II (Invitrogen, Carlsbad, CA) for 20 minutes at 37°C followed by HBSS containing 1.5 mg papain (Sigma, St. Louis, MO) for 20 minutes at 37°C, pieces of ganglia were triturated by passing them through sterilized fire-polished Pasteur pipette in L-15 medium (Gibco, Grand Island, NY) containing 4.8% fetal bovine serum (FBS; Gibco, Grand Island, NY), 10,000 units/ml of penicillin and 10,000 µg/ml of streptomycin (Gibco, Grand Island, NY) and 1 M HEPES (Sigma, St. Louis, MO). Dissociated neurons were spun at 1,000 rpm to sediment the neurons. The neuronal cell pellet was re-suspended in Ham's F-12 (Invitrogen) containing 10% FBS, 10,000 units/ml of penicillin and 10,000 µg/ml of streptomycin, and Glutamax (Gibco, Grand Island, NY), and then plated onto 35 mm petri dishes pre-coated with poly-D-lysine (PDL; Sigma, St. Louis, MO) and laminin (Sigma, St. Louis, MO). Neurons were maintained in an incubator at 37°C under an atmosphere of 5% CO_2 and used within 24-48 h after plating.

Neurons were identified by morphological basis that appears smooth and round shape, as well as actually brighter and larger than non-neuronal cells. Soma size was classified into small-and-medium-sized (diameter < 40 µm; capacitance 15-80 pF) and large-sized (> 40 µm; > 80 pF) (Figure 1); neuron's diameter was measured using a calibrated graticule (Pyser, Kent, UK) before whole-cell patch-clamp recordings.

Electrophysiological recording

Neurons were superfused at 2 ml/min flow rate with an extracellular solution containing 145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4 (adjusted with NaOH) and whole-cell patch-clamp recording was performed using an Axopatch 200B amplifier (Axon instruments, Foster City, CA) and pClamp 10 acquisition software (Axon instrument, Sunnyvale, CA). Borosilicate glass pipettes (1.5 mm OD × 0.86 mm ID; Sutter Instruments, Navato, CA) were pulled by using a Flaming–Brown micropipette puller P-97 (Sutter Instruments, Navato, CA), had a resistance of 2-5 MΩ when polished with a microforge (MF-830, Narishige, Japan) and filled with an internal solution

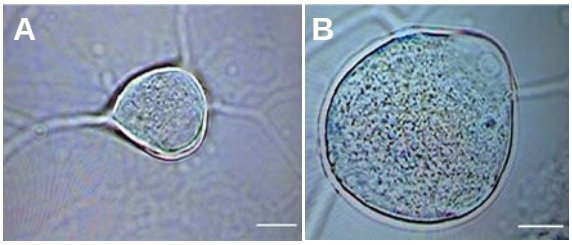

Figure 1 Representative neurons: small-sized (17.43 pF, A) and large-sized neurons (87.61 pF, B). These neurons were measured using a 40x objective lens. Scale bars are 10 µm.

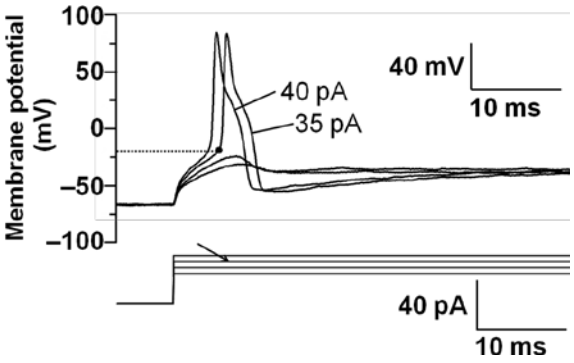

Figure 2 Measurement of threshold and threshold current. *Upper*: Threshold was measured at the black dot (-21.63 mV), while threshold current was taken at the first spike (35 pA). *Lower*: Depolarizing current steps were injected to evoke action potentials; the first spike was evoked by the 11th step with a value of 35 pA (arrow).

containing 140 mM K-gluconate, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, 0.3 mM Na-GTP, and 10 mM Mg-ATP, pH 7.3 (adjusted with KOH). Neurons were voltage clamped at -60 mV and the signal was filtered at 5 kHz (using a low-pass filter) and sampled at 25 kHz by using a Digidata 1440 series interface (Axon instruments, Sunnyvale, CA). After whole-cell voltage clamp was established, the clamp was switched to current clamp mode, which measured the resting membrane potential (RMP) with no applied current. Following and adapting a protocol described previously,[21] long (500 ms) depolarizing current pulses with -65 mV of holding potential (HP) was applied to assess the excitability of neurons. The depolarizing current pulses were stepped from -15 pA to 85 pA, each of steps was increased by 5-pA increment. After recording in the extracellular solution at physiological pH (pH 7.4), to evaluate the effect of low pH, the extracellular solution was changed to pH 7.0. The time to change the solution was approximately 10 minutes.

Action potential parameter assessments

When applying the 500 ms of current to define the excitability of neuron, threshold and threshold current were measured. Threshold (voltage threshold) was considered as the depolarizing potential that triggered the first action potential (AP) while the threshold current was the lowest injecting current that produced an AP (Figure 2). Total spikes was the number of

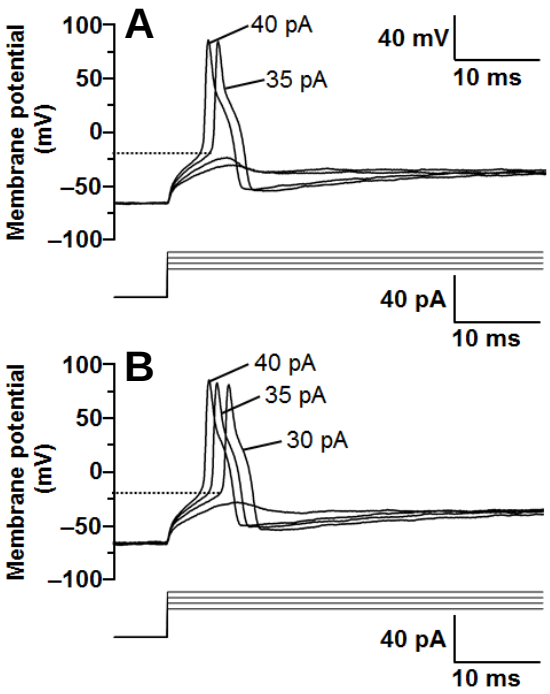

Figure 3 Representative traces showing excitability of a small-and-medium-sized neuron in response to physiological pH (7.4) and pH 7.0. *A*, At pH 7.4, the threshold and threshold current of the first spike were -21.63 mV and 35 pA, respectively; *B*, at pH 7.0 , the threshold and threshold current of the first spike were -23.01 mV and 30 pA, respectively.

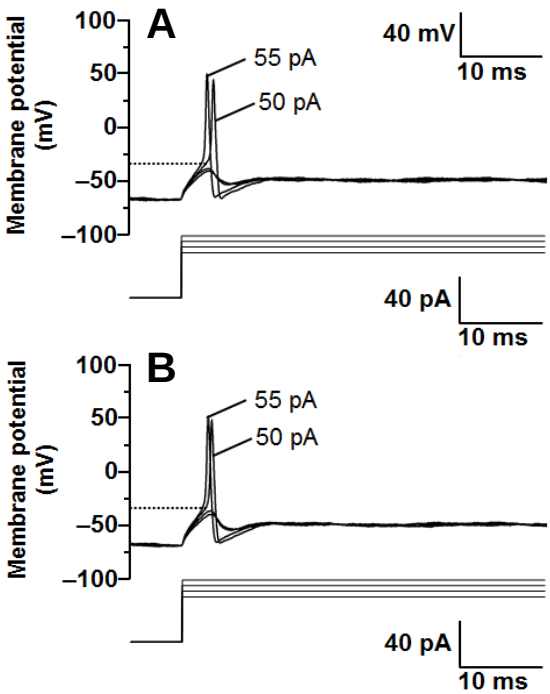

Figure 4 Representative traces showing excitability of a large-sized neuron in response to physiological pH (7.4) and pH 7.0. *A*, At pH 7.4, the threshold and threshold current of the first spike were -33.48 mV and 50 pA, respectively; *B*, at pH 7.0 , the threshold and threshold current of the first spike were -33.42 mV and 50 pA, respectively.

spikes triggered by depolarizing step currents from 0 to 85 pA, increasing 5 pA per step.

Data analysis

All data are presented as mean ± standard error of mean (SEM). Statistical analysis was performed using Student's *t* test (Sigma Plot version 10.0, San Jose, CA, USA). $P < 0.05$ was accepted as statistically significant.

Results

Effect of pH on resting membrane potential

In the resting state, there was no currents to apply to neurons before and after decreasing the pH from physiological (pH 7.4) to pH 7.0. The resting membrane potential (RMP) of small-and-medium-sized neurons (< 40 µm; n=10) was significantly depolarized at pH 7.0, while the RMP of large-sized neurons (> 40 µm, n=4) was not significantly different at physiological pH and pH 7.0 (Table 1).

Effect of pH on membrane excitability

Depolarizing current pulses were applied to neurons before and after decreasing the pH from physiological (pH 7.4) to pH 7.0.

After applying 500 ms depolarizing current pulses, the responding small-and-medium-sized neurons (n=10) tended to produce more spikes, but this change was not significant. Neurons were excited in response to pH. Their threshold was significantly hyperpolarized at pH 7.0 for a significantly decreased threshold current (Table 1). A representative trace is shown in Figure 3A-B.

The responding large-sized neurons (n= 4) were not significantly more excitable at pH 7.0. Moreover, the threshold was not changed at pH 7.0, and the threshold current was also unchanged at pH 7.0 (Table 1). A representative trace is shown in Figure 4A-B.

Table 1 Effect of pH 7.0 on ac variables of small-to-medium and large-sized neurons

Variables	Small-and-medium-sized neurons (n=10)		Large-sized neurons (n=4)	
	pH 7.4	pH 7.0	pH 7.4	pH 7.0
RMP (mV)	-57.72 ± 1.65	-51.31 ± 3.05*	-63.68 ± 0.31	-64.30 ± 1.40
Spike (spikes)	9.60 ± 1.75	16.40 ± 6.84	9.25 ± 0.48	9.25 ± 1.03
Threshold (mV)	-20.16 ± 2.28	-25.61 ± 1.29*	-35.05 ± 0.96	-34.37 ± 1.80
Threshold current (pA)	45.50 ± 6.97	40.00 ± 6.01*	43.75 ± 2.39	43.75 ± 5.15

Neuronal properties of small-and-medium-sized and large-sized neurons before and after lowering pH from 7.4 to 7.0. *$P <$ 0.05, comparing with physiological pH (pH 7.4). Each variable is shown as mean ± SEM.

Discussion

The pH range that can cause an inflammatory event associated with headache depends on the sensitivity of receptor. For example, TRPV1 can be activated by pH less than 6.0, while ASICs can be activated by pH at least pH 7.0. Yan *et al* (2011) investigated ASIC3 in dural afferent of trigeminal ganglion (TG) neurons by varying pH as 7.2, 7.1, 7.0, 6.9, 6.8, and 6.0,[19] and found that pH 7.0, 6.9, 6.8, and 6.0 can excite the trigeminal ganglion neurons in a proton concentration-dependent manner.

The present study demonstrates the effect of pH change on ASICs in trigeminal ganglion neurons. In small-and-medium-sized neurons, pH change from 7.4 to 7.0 activates ASICs that resulted in RMP depolarization, threshold hyperpolarization, and threshold current reduction. However, pH 7.0 did not have any effect on large-sized neurons in all parameters.

RMP may be shifted more positive or depolarization by a few mechanisms. The RMPs of small-and-medium-sized neurons could be depolarized by extracellular H^+ that activated ASICs[22] and also inhibited TASK-3 (TWIK-related acid sensitive K^+ channel) and TRESK (TWIK-related spinal cord K^+ channel) which are the subtypes of two-pore potassium channel (K2P).[23,24] The activation of ASICs by the extracellular proton resulted in the openning of ASICs and Na^+ influx which depolarized the membrane potential.[22,25] Meanwhile, TASK-3 and TRESK which generates the efflux of K^+ ions to stabilize the RMP under physiological conditions were inhibited by the extracellular H^+ that resulted in cellular depolarization.[23,24,26]

Our next findings, threshold hyperpolarization and threshold current reduction, are consistent with the characteristics of polymodal nociceptors whose threshold is hyperpolarized by noxious stimuli.[27] These findings implicate voltage-gated sodium channels 1.9 ($Na_V1.9$) which is tetrodotoxin (TTX)-resistant and produces persistent current in small-sized DRG and TG neurons.[28,29] Under inflammatory conditions in small-sized DRG neurons, $Na_V1.9$ was up-regulated by PGE_2 via G-protein signaling which accounted for threshold hyperpolarization and threshold current reduction.[30,31] Moreover, the relationship between pH and action potential (AP) threshold in small-sized DRG neurons[32] demonstrated that a drastic pH change (from 7.4 to 6.0) produces AP, while Yan *et al*[19] showed that a modest pH change (from 7.4 to 7.0) elicits APs in small-and-medium-sized TG neurons. Thus, pH 7.0 may possibly modulate $Na_V1.9$ to increase its current and up-regulate its expression via ASICs and signaling pathway. In addition to the result of the inflammation mediators modulating $Na_V1.9$, another cause could be a consequence of GTP, which was a component of the intracellular solution in our experiment. Previous studies demonstrated that the internal dialysis of GTP hyperpolarized threshold potential and reduced threshold current for triggering AP as well as increased the persistent current.[29,33] Moreover, GTP affects only on $Na_V1.9$, while the others ($Na_V1.7$ and $Na_V1.8$) are not affected.[33] The mechanism responsible for the effect of GTP internal dialysis on the hyperpolarization has been investigated.[34] These lines of evidence support our result. Interestingly, we changed pH approximately 10 minutes, during which GTP would internally dialyzed. Thus, GTP in the intracellular solution may activate G-protein signaling pathway and up-regulate $Na_V1.9$, resulting in threshold hyperpolarization and threshold current reduction. Moreover, $Na_V1.7$ mutation reduced threshold current, but does not have any effect on threshold potential.[35]

RMP depolarization and threshold hyperpolarization contribute directly to AP production. In inflammatory conditions, $Na_V1.3$ and $Na_V1.7$ are involved in the initiation of AP.[36,37] These channels induce Na^+ influx that increases sub-threshold depolarization to the threshold potential of $Na_V1.8$.[38] Similarly, $Na_V1.8$ is up-regulated under the inflammatory conditions in small-sized DRG neurons.[36,39] Depolarization close to threshold by $Na_V1.8$ produces upstroke and maintain the AP.[40]

In contrast, pH change did not alter the excitability in large-sized neurons. A previous study by Connor *et al* showed that the trigeminal afferents from mesencephalic neurons, which are mostly large-diameter neurons and located on mesencephalic nucleus, required at least pH 6.5 to activate ASICs.[6] Moreover, Liu and Simon[41] indicated that pH 5.9 activated large-sized TG neurons, while Molliver *et al*[14] suggested that pH 6.8 could activate large-sized DRG neurons to increase excitability. Therefore, large-sized neurons may be activated in pH 6.8. Next, Pollema-Mays *et al*[42] showed that the expression of TASK-3 is limited to small-sized DRG neurons, while Lafreniere *et al*[43] demonstrated that TRESK is expressed on small-and-medium-sized TG neurons. Moreover, Bautista *et al* showed that TASK-3 and TRESK are expressed only in small-and-medium-sized TG neurons.[26] Thus, large-sized TG neurons do not express these subtypes to respond to extracellular H^+. These support our data that the pH change under our experimental conditions did not activate ASICs in large-sized neurons.

Conclusion

Our study indicated that pH change from 7.4 to 7.0 can increase the excitability of small-and-medium-sized TG neurons probably and presumably by ASICs activation as well as TASK-3 and TRESK inhibition. Since in neuropathic pain-induced headache, pro-inflammatory mediators released from satellite glial cells can cause an increase in extracellular proton that activates CGRP system, triggering headache, the results of this study suggest

that the pH change may modulate cell excitability by threshold hyperpolarization and threshold current reduction.

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Conflict of interest

None to declare.

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