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Electrophysiological properties of thermosensitive neurons in slices of rat lateral parabrachial nucleus

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A B S T R A C T

Both warm- and cold-sensitive neurons are found in the lateral parabrachial nucleus (LPB), a crucial relay for skin temperature information from the spinal cord to the preoptic area. The aims of this study were to investigate the electrophysiological properties of temperature-sensitive and -insensitive neurons in brain slices, and elucidate the basic mechanisms underlying the thermosensitivity of rat LPB neurons. In warm-sensitive neurons, temperature exerted no significant effects on resting membrane potential (RMP), threshold potential, and amplitude of the afterhyperpolarizing potential. However, warming significantly increased the prepotential rates of depolarization and the inactivation rates of potassium A current (I_A) in warm-sensitive neurons, which in turn shortened their interspike interval and elevated the firing rate. In contrast, temperature had no significant effects on the depolarizing prepotentials and inactivation rate of I_A in temperature-insensitive neurons. Besides, in cold-sensitive neurons, cooling and warming produced membrane depolarization and hyperpolarization, respectively, and there was a strong correlation between firing rate and membrane potential thermosensitivity. Nevertheless, temperature exhibited no significant effect on the depolarizing prepotential of cold-sensitive neurons. These results suggest that LPB neuronal warm sensitivity may reside in the temperature-dependent prepotentials and I_A, while neuronal cold sensitivity might be mainly due to heat-induced changes in RMP.

1. Introduction

Previous studies have identified the presence of local thermosensitive neurons in the external lateral subnucleus (el), central subnucleus (c) and dorsal subnucleus (d) of lateral parabrachial nucleus (LPB) (Xue et al., 2016). These three subnuclei play crucial roles during the transmission of cutaneous thermosensory signals that drive autonomous and behavioral thermoregulatory responses to environmental challenges (Nakamura and Morrison, 2008, 2010; Geerling et al., 2016; Yahiro et al., 2017; Nakamura, 2018). However, the basic mechanism by which LPB neurons sense the changes in local temperature has not been addressed. Several other studies have investigated the mechanisms underlying neuronal thermosensitivity in the preoptic area (POA, a thermoregulatory command center), and two different hypotheses have been proposed. Some researches (Kiyohara et al., 1990; Kobayashi and Takahashi, 1993; Hori et al., 1999) suggest that heat-activated inward cationic currents may contribute to a slow depolarization of a neuron's resting membrane potential (RMP), resulting in neuronal warm sensitivity. A recent study has suggested that transient receptor potential channel M2 and V4 can participate in regulation of body temperature as a part of the thermoreceptor in the POA (Song et al., 2016; Yadav et al., 2017). Nevertheless, this hypothesis is opposed by another research group who indicates that RMP is a less significant factor for the thermosensitivity of preoptic neurons. Instead,

Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarizing potential; 4-AP, 4-aminopyridine; I_A, potassium A current; LPB, lateral parabrachial nucleus; LPBc, central subnucleus of LPB; LPBd, dorsal subnucleus of LPB; LPBel, external lateral subnucleus of LPB; POA, preoptic area; RMP, resting membrane potential; SCP, superior cerebellar peduncle

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these studies demonstrate that temperature affects depolarizing prepotential, which is the slow depolarization that occurs prior to the membrane potential reaching threshold (Griffin and Boulant, 1995; Griffin et al., 1996).

Warming elevates the prepotential rates of depolarization of preoptic warm-sensitive neurons, which in turn reduces the intervals among consecutive action potentials, leading to increased firing rates, and the temperature-dependent inactivation of a potassium A current (I_A), which shows a transient activation, followed by inactivation during depolarization, is recognized as an essential determinant of the increased rate of prepotential (Griffin et al., 1996). Therefore, it is suggested that preoptic warm sensitivity is dependent on prepotential stage, as opposed to peripheral thermoresponsiveness that relies on conductance changes for the regulation of membrane potential (Griffin, 2004).

This study aimed to elucidate the basic mechanisms underlying the thermosensitivity of rat LPB neurons. Whole-cell intracellular recordings were used to measure the activities of warm- and cold-sensitive as well as temperature-insensitive LPB neurons. The thermal effects on RMPs, transient potentials [e.g. depolarizing prepotentials and after-hyperpolarizing potentials (AHPs)] and I_A were compared between different types of LPB neurons.

2. Materials and methods

2.1. Brain slice preparation

Sprague–Dawley rats (male, 80–150 g) were used in this study. All procedures were approved by the National Institutes of Health and Chengdu Medical College Laboratory Animal Care and Use Committee. According to previously reported procedures (Xue et al., 2016), a coronal brainstem slice consisting of LPB was prepared. In brief, each rat was anesthetized with pentobarbital and quickly decapitated. A 0.5-cm section of the brainstem containing the LPB was prepared and was then sliced into 300-μm thick transverse slices with a Vibratome VT1200 tissue slicer (Leica, Germany). The slices were perfused continuously with 300 mOsm/kgH_2O artificial cerebrospinal fluid (ACSF; pH 7.4) containing NaCl (124 mM), $NaHCO_3$ (26 mM), glucose (10 mM), KCl (5 mM), $CaCl_2$ (2.4 mM), $MgSO_4$ (1.3 mM) and KH_2PO_4 (1.24 mM) at 1.2 ml/min. The ACSF was equilibrated with carbogen (95% O_2 and 5% CO_2) and warmed to 36–37 °C using a Peltier thermoelectric device (SC-20, Warner Instruments Inc., USA).

2.2. Electrophysiological recordings

The firing activities of neurons located in the LPB were recorded under a whole-cell current clamp mode. The whole-cell recordings were performed with 4–7 MΩ patch pipettes filled with 295 mOsm/kgH_2O intracellular solution (pH 7.2) containing potassium gluconate (130 mM), EGTA (10 mM), HEPES (10 mM), MgATP (2 mM), Na_2GTP (2 mM) and $CaCl_2$ (1 mM) as described previously (Xue et al., 2016). A liquid junction potential of approximately 12 mV has been determined for this solution (Griffin and Boulant, 1995), and this value was subtracted from all reported potentials. The LPB was identified visually as a crescent-shaped lucent region at the dorsolateral surface of the pons that was bordered dorsally by the ventral spinocerebellar tract and ventrally by the superior cerebellar peduncle (SCP) (Hayward and Felder, 1999; Xue et al., 2016). The three LPB subnuclei were defined by their relationship to the SCP (Fulwiler and Saper, 1984), which were visualized and photographed by the infrared differential interference contrast videomicroscopy. The locations of the recorded neurons were summarized in Fig. 1. Recordings were carried out using an EPC10 (HEKA Elektronik, Lambrecht/Pfalz, Germany), which filtered and digitized at 2 and 10 kHz, respectively. The PatchMaster software (HEKA) was used for data acquisition and analysis. An action potential of ≥55 mV as well as a stable membrane potential recording were applied for the recordings of spontaneous activity at 36–37 °C (Burgoon and Boulant, 2001).

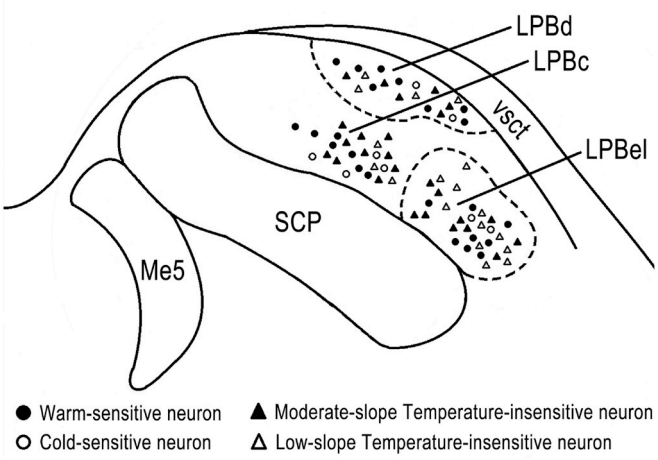

Fig. 1. Locations of the recorded warm-sensitive, cold-sensitive, moderate-slope temperature-insensitive and low-slope temperature-insensitive neurons in the coronal LPB tissue slices. LPBc = central subnucleus of LPB; LPBd = dorsal subnucleus of LPB; LPBel = external lateral subnucleus of LPB; Me5 = mesencephalic trigeminal nucleus; SCP = superior cerebellar peduncle; and vsct = ventral spinocerebellar tract.

In some experiments, voltage clamp recordings were used to assess the temperature dependence of potassium conductances in different types of LPB neurons. After evaluating neuronal thermosensitivity, the recordings were performed with 0.5 μM tetrodotoxin, and specific stimulation protocol was used to elicit I_A currents. Subsequently, temperature-dependent changes in I_A and its rate of inactivation were determined. In certain experiments, 1 mM 4-aminopyridine (4-AP) was administered for blocking I_A currents.

2.3. Data analysis

Neuronal thermosensitivity [impulses(imp)/s/°C] was determined by a linear regression slope (or known as thermal coefficient m) generated by plotting firing rates as a function of temperature and assessed over a temperature range of at least 3 °C, where the neuron was most thermosensitive. As similar to POA criteria (Wright et al., 2008; Tang et al., 2012), the thermal coefficient of cold-sensitive neurons was ≤–0.6 imp/s/°C, while that of warm-sensitive neurons was ≥0.8 imp/s/°C. Besides, the thermal coefficients of low-slope and moderate-slope temperature-insensitive neurons were –0.2 to 0.2 imp/s/°C and 0.2 to 0.8 imp/s/°C, respectively.

Neuronal firing rate activity, intrinsic membrane properties and I_A currents were measured and analyzed with regard to different temperature ranges. According to previous published protocols (Griffin and Boulant, 1995; Griffin et al., 1996), RMPs were measured through the filtration of all rapidly changing potentials (including action potentials) using a Grass filter in the program package pCLAMP 10.1. The membrane potential thermosensitivity (mV/°C) was determined by the slope generated by plotting membrane potentials as a function of temperature. A threshold potential was characterized by the inflection points produced during spike initiation (Graham et al., 2008). The differences between RMP and its maximum positive peak were used to measure the amplitudes of action potential, while the duration of action potential was examined at one-half of the peak amplitude. The differences between the threshold potential and maximum negative peak after the falling phase of action potential were used to measure the amplitudes of AHP. To minimize the influence of AHP on the recorded prepotential, the increase in rate of depolarizing prepotential was calculated from the slope generated during 4-20 ms immediately preceding the action potential (Griffin et al., 1996; Burgoon and Boulant, 2001).

Thermosensitivity of rate of rise of the prepotential or inactivation of I_A (X) was indicated as Q_{10} (proportionate increase in rate per 10 °C rise in temperature). The values of Q_{10} were calculated as follows: $Q_{10} = (X2/X1)^{10/(temperature2–temperature1)}$ (Griffin et al., 1996).

All data were presented as mean ± SE. Multivariate analysis of variance with repeated measures was employed to compare neuronal responses as a cell classification factor and different temperatures (cool = 32–33 °C, neutral = 36–37 °C and warm = 39–40 °C). When appropriate, the variables between/across different types of neurons were compared using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. Differences were considered significant for probability values (P) less than 0.05.

3. Results

3.1. Effects of temperature on firing rates and membrane potentials

To explore the basic mechanisms underlying LPB neuronal thermosensitivity, the intracellular spontaneous activities of 75 neurons in 67 rat tissue slices were recorded for at least one cyclic temperature change. These included 31 neurons located in LPBel, 24 in LPBc, and 20 in LPBd (Fig. 1). Among the 75 neurons, 23 were warm-sensitive, 8 were cold-sensitive, 26 were moderate-slope temperature-insensitive and 18 were low-slope temperature-insensitive, representing 31, 11, 34 and 24%, respectively.

Fig. 2 A–B reveals the impacts of temperature on the integrated membrane potentials and firing rates of thermosensitive neurons. The thermal coefficients of warm- and cold-sensitive neurons were 1.02 imp/s/°C and –0.74 imp/s/°C, respectively. As shown in Fig. 2A, the firing rates of warm-sensitive neuron were decreased and increased during cooling and warming, respectively. However, RMP was not associated with the changes in firing rates, due to low membrane potential thermosensitivity (i.e., 0.08 mV/°C). In contrast, the firing rates of cold-sensitive neuron were reduced to 0 imp/s at approximately 37 °C during warming, and began to increase upon cooling to 35 °C in the falling phase (Fig. 2B). Notably, RMP might contribute to these firing rate changes, as warming produced membrane hyperpolarization and cooling produced membrane depolarization. Furthermore, the membrane potential thermosensitivity (i.e., –1.08 mV/°C) was relatively close to firing rate thermosensitivity.

The membrane potential thermosensitivity of cold-sensitive neurons (–0.87 ± 0.08 mV/°C; $n = 8$) was significantly lower than that of other types of neurons, and was almost identical to firing rate thermosensitivity (–0.79 ± 0.06 imp/s/°C; n = 8). However, no significant differences were observed among the membrane potential thermosensitivities of warm-sensitive (0.08 ± 0.07 mV/°C; $n = 23$), moderate-slope temperature-insensitive (0.07 ± 0.06 mV/°C; $n = 26$), and low-slope temperature-insensitive neurons (–0.02 ± 0.05 mV/°C; $n = 18$). Fig. 2C shows the plots of the membrane potential thermosensitivity of each cold-sensitive neuron as a function of neuronal firing rate thermosensitivity. The linear relationship of this plot indicated a strong correlation ($r = 0.76$) between firing rates and membrane potential thermosensitivities, suggesting that neuronal cold sensitivity might be mainly due to heat-induced changes in RMP. The low correlation coefficient ($r = 0.09$) of the plot indicated no association between membrane potentials and firing rate thermosensitivities in the other three types of neurons (Fig. 2D), suggesting that neuronal warm sensitivity may not be attributed to heat-induced changes in RMP.

3.2. Effects of temperature on the prepotential rates of depolarization

The majority of LPB neurons exhibited depolarizing prepotential that brought the membrane potential to threshold to generate action potential. Fig. 3 shows the superimposed computer-averaged prepotentials of the different types of LPB neurons. Fig. 3 indicates that temperature had different effects on the prepotentials of the four types of neurons. Temperature did not affect the prepotential rates of depolarization in the low-slope temperature-insensitive neuron in Fig. 3A. Hence, the interspike intervals of this neuron remained constant over a range of 32–39 °C (Fig. 3B). Moreover, for low-slope temperature-insensitive neuronal population ($n = 18$), a Q_{10} of 1.02 ± 0.05 for the rate of rise indicated that temperature exhibited very little impact on the depolarizing prepotential. In addition, temperature exerted a slight effect on the average prepotential of moderate-slope temperature-insensitive neurons (Fig. 3), and the rate of rise of this population ($n = 25$) had a Q_{10} of 1.24 ± 0.05. Besides, temperature caused noticeable differences in the average prepotential of warm-sensitive neurons (Fig. 3). Warming to 39 °C tended to increase the prepotential rates of depolarization, reduce interspike intervals and elevate firing rates. On the contrary, cooling to 32 °C tended to decrease the prepotential rates of depolarization, prolong interspike intervals and reduce firing rates. For warm-sensitive neuronal population ($n = 21$), the rate of rise of the prepotential exhibited a Q_{10} of 1.80 ± 0.09, and this rate was higher at hyperthermic temperatures compared to normothermic and hypothermic temperatures, as well as at normothermic temperatures compared to hypothermic temperatures (Fig. 3C). In addition, warm-sensitive neurons exerted a significantly greater thermal effect on the average prepotentials and a higher Q_{10} value for the rate of rise compared to the other three types of neurons ($P < 0.01$). Although temperature could influence the interspike interval of the cold-sensitive neuron (Fig. 3), their prepotential rate of depolarization was not significantly affected by temperature. The rate of rise for this population ($n = 8$) displayed a Q_{10} of 1.16 ± 0.09. In the normothermic temperature range, a rapid firing, warm-sensitive neuron showed greater depolarization rate than a slow firing, low-slope temperature-insensitive neuron; however, the rate of rise of the prepotential did not differ among the other three types of neurons (Fig. 3C).

3.3. Effects of temperature on I_A currents

Considering the influence of I_A currents on depolarizing prepotential (Griffin et al., 1996), voltage clamp experiments were performed to characterize the thermosensitivity of I_A currents, and a signal subtraction approach was carried out to isolate I_A from the total K^+ currents (Fig. 4A–C). I_A represented the peak of the subtracted currents, which was sensitive to 4-AP. During voltage clamp experiments, I_A was found in 5 (out of 5) warm-sensitive neurons, 2 (out of 2) moderate-slope temperature-insensitive neurons and 5 (out of 7) low-slope temperature-insensitive neurons, but not in 5 (out of 5) cold-sensitive neurons. As shown in Fig. 4D and E, temperature exerted differential effects on the I_A of the different types of neurons. Warming increased I_A and its inactivation rate in the warm-sensitive neurons (Fig. 4D). The mean I_A of warm-sensitive neurons was significantly increased at hyperthermic temperatures compared to hypothermic temperatures at +40 mV (Table 1), with a Q_{10} of 1.60 ± 0.28 ($n = 5$). In these same warm-sensitive neurons, the rate of I_A inactivation was measured, and the hyperthermic rates were significantly higher than the hypothermic rates (Table 1), and the inactivation rate displayed a Q_{10} of 2.40 ± 0.63 ($n = 5$). This Q_{10} value appeared to be slightly higher, but was relatively similar to that of depolarizing prepotential (i.e., 1.80) in warm-sensitive neurons, suggesting that I_A currents may contribute to the temperature-dependent prepotentials of warm-sensitive LPB neurons. In contrast, the I_A and its inactivation rate of the temperature-insensitive neurons remained unaffected during warming (Fig. 4E). Moreover, the Q_{10} values for I_A amplitude and I_A inactivation rate were 1.05 ± 0.14 and 1.24 ± 0.25, respectively, in temperature-insensitive neuronal population ($n = 7$), indicating that temperature exhibits little effect on I_A currents. It was noted that under the same temperature range, the amplitudes and inactivation rate of I_A were greater in warm-sensitive neurons than those in temperature-insensitive neurons (Table 1).

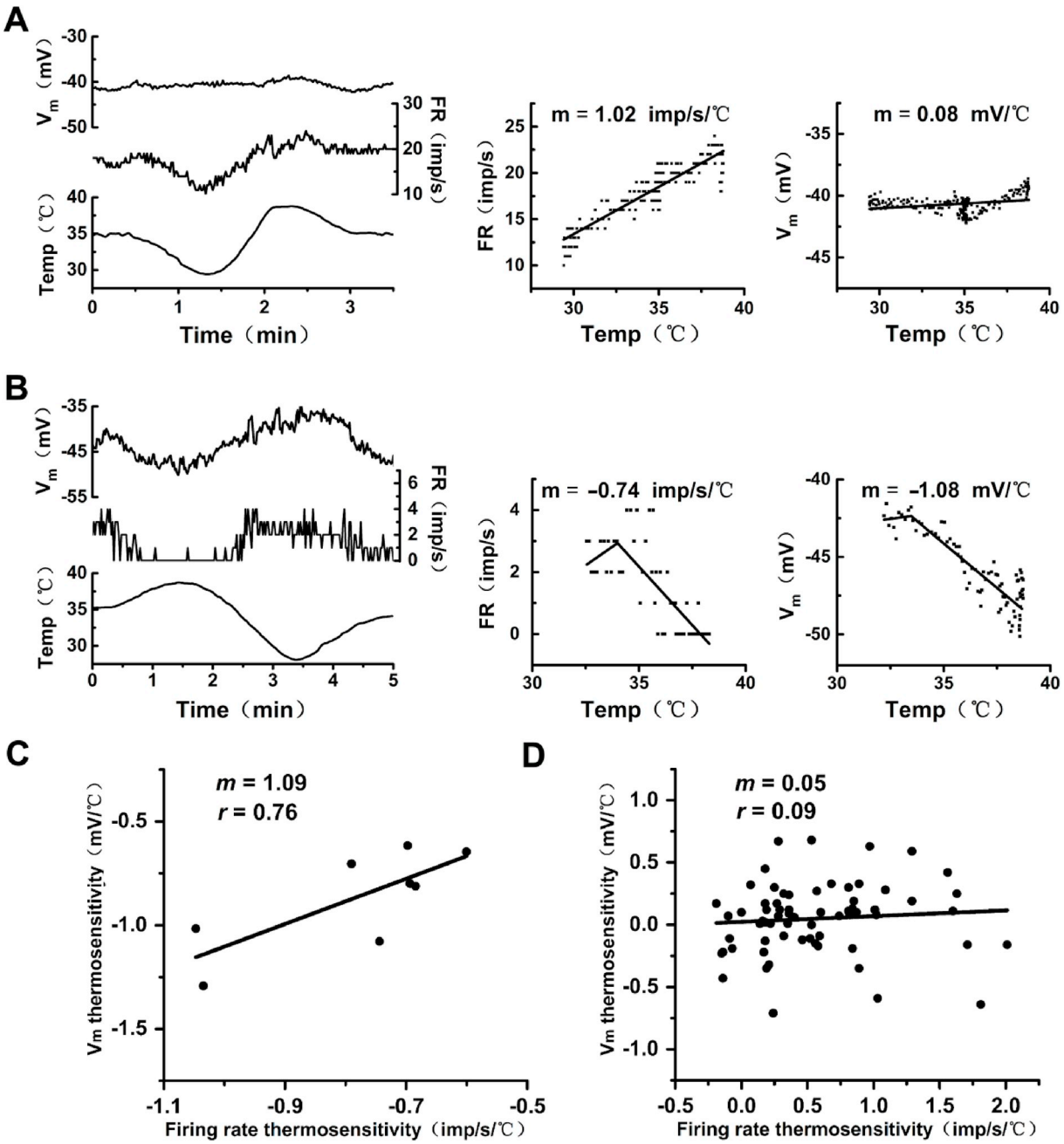

Fig. 2. Effects of temperature on the membrane potential and firing rate of thermosensitive neurons in the LPB. (A) Warm-sensitive neuron with a firing rate thermosensitivity of 1.02 imp/s/°C and a membrane potential thermosensitivity of 0.08 mV/°C. Left panels: time records of the membrane potential (V_m) and firing rate (FR) during tissue temperature (Temp) changes. Center panels: firing rate plotted as a function of tissue temperature. Right panels: plots of the membrane potential as a function of tissue temperature. m is the regression coefficient of firing rate or V_m plots. (B) Cold-sensitive neuron with a firing rate thermosensitivity of –0.74 imp/s/°C and a membrane potential thermosensitivity of –1.08 mV/°C. Temperature had little effect on the membrane potential of the warm-sensitive neuron, but it led to noticeable differences in the membrane potential of the cold-sensitive neuron, in which warming produced membrane hyperpolarization and cooling produced membrane depolarization. (C) The membrane potential (V_m) thermosensitivity of each cold-sensitive neuron as a function of the neuron's firing rate thermosensitivity (imp/s/°C). The high regression coefficient (*m*) and correlation coefficient (*r*) indicated that there was a strong correlation between the firing rate and membrane potential thermosensitivity of cold-sensitive neurons. (D) For warm-sensitive, moderate-slope temperature-insensitive, and low-slope temperature-insensitive neurons, the low regression coefficient and correlation coefficient indicated that there was no correlation between membrane potential thermosensitivity and firing rate thermosensitivity.

3.4. Effects of temperature on action potential amplitude and duration

The amplitudes of action potential were reduced during warming (cool = 73.73 ± 1.35 mV, neutral = 71.41 ± 1.34 mV, warm = 66.80 ± 1.37 mV; $P < 0.01$). In addition, warming shortened the duration at half-amplitude (cool = 1.36 ± 0.06 ms, neutral = 1.24 ± 0.05 ms, warm = 1.17 ± 0.05 ms; $P < 0.01$). Despite the fact that these variables were correlated with temperature, no statistically significant differences were found among the four types of neurons.

3.5. Effects of temperature on threshold potential and AHP

Fig. 5A demonstrates the impacts of temperature on the threshold potential of a warm-sensitive neuron and a cold-sensitive neuron,

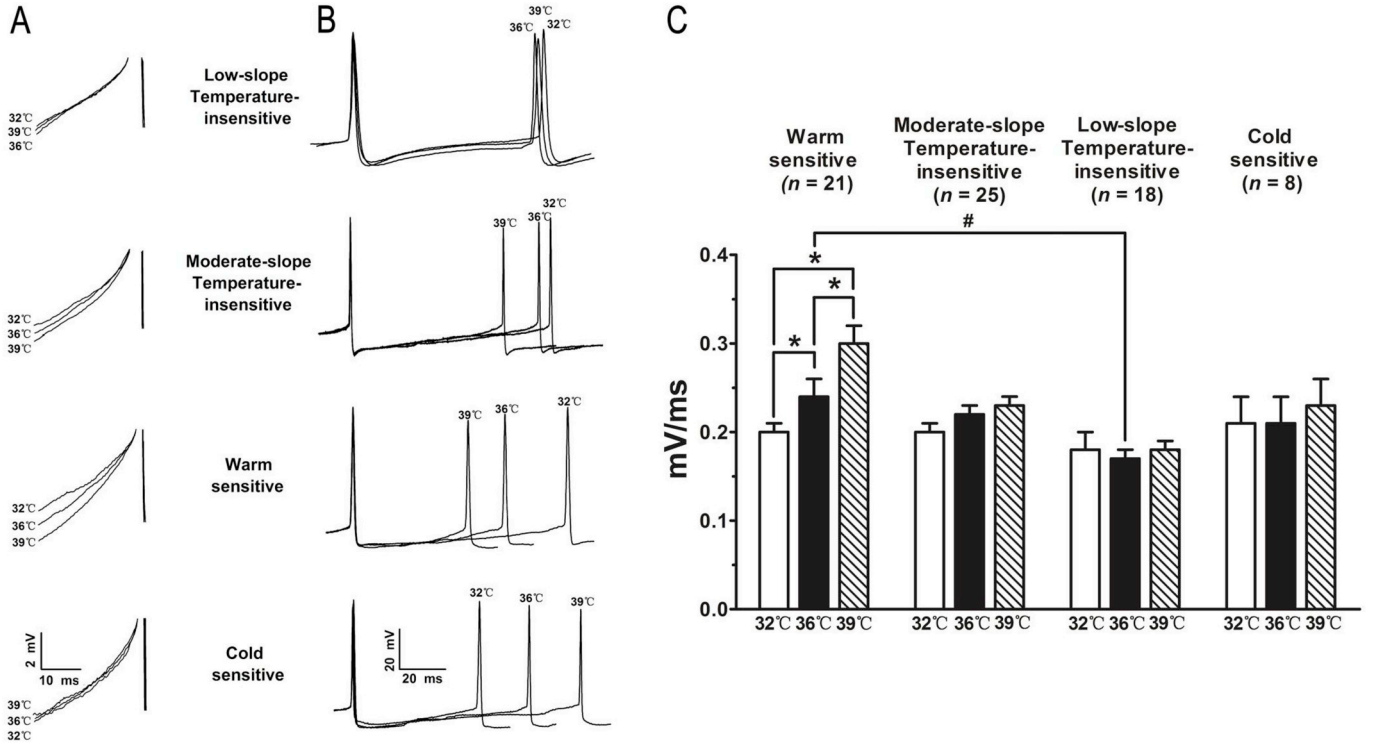

Fig. 3. Effects of temperature on the depolarizing prepotential of LPB neurons. (A) Superimposition of averaged prepotentials. (B) Superimposition of single action potential records at three temperature ranges. Temperature did not affect the prepotential in the low-slope temperature-insensitive neuron. Warming increased the rate of rise of the prepotential in warm-sensitive and moderate-slope temperature-insensitive neurons, and shortened their interspike interval. On the contrary, cooling reduced the rate of rise of the prepotential in the warm-sensitive neuron, and lengthened its interspike interval. Besides, temperature exerted little effect on the prepotential in the cold-sensitive neuron. (C)*For warm-sensitive neurons, the mean prepotential rate of rise was significantly greater in both normothermic and hyperthermic ranges compared to hypothermic range, as well as in hyperthermic range compared to normothermic range ($P < 0.01$). #In normothermic range, the mean prepotential rate of rise was significantly higher in warm-sensitive neurons than in low-slope temperature-insensitive neurons ($P < 0.01$).

respectively. The threshold potential of these two neurons did not markedly change at different temperatures. As summarized in Table 2, the threshold potential of warm- and cold-sensitive, as well as temperature-insensitive neurons remained stable during temperature changes. Therefore, there were no consistent changes in the threshold that may explain the heat-induced changes in firing rate.

Fig. 5B reveals the signal-averaged traces of a warm-sensitive neuron and a cold-sensitive neuron at three different temperatures. Warming exerted variable effects on their AHP amplitudes. As presented in Fig. 5C, warming decreased the AHP amplitudes of warm-

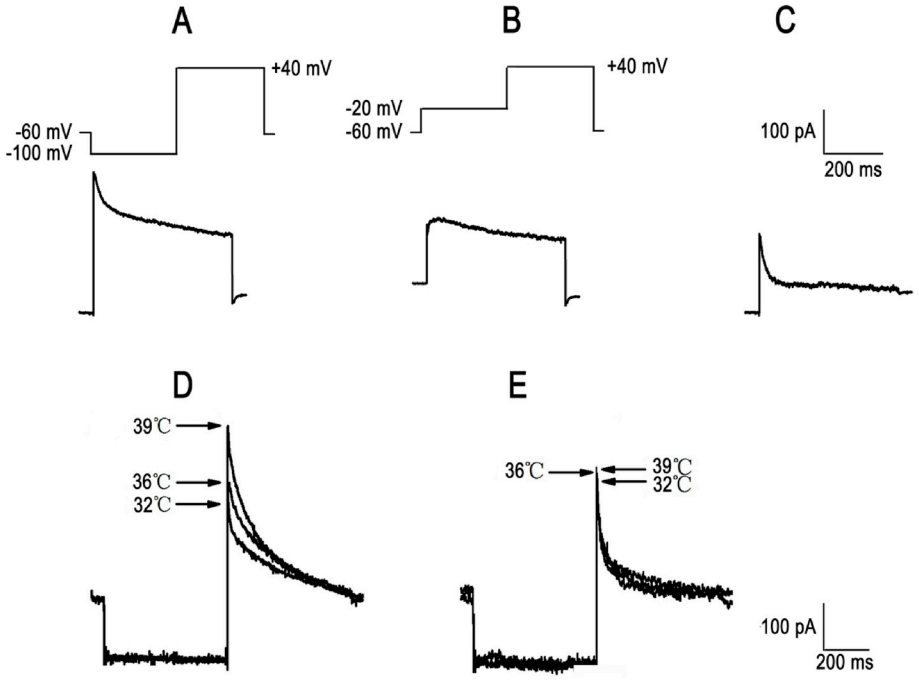

Fig. 4. Effects of temperature on the I_A currents of LPB neurons. (A) Total outward K^+ current triggered with a 500 ms depolarizing pulse to +40 mV following a hyperpolarizing prepulse of 500 ms to −100 mV. (B) Non-inactivating outward K^+ current triggered with a 500 ms depolarizing pulse to +40mV following a depolarizing prepulse of 500 ms to −20 mV. (C) Subtraction of current trace in (B) from that in (A) for isolating I_A. The responses were obtained from a warm-sensitive neuron (m = 1.36 imp/s/°C; D) and a temperature-insensitive neuron (m = 0.12 imp/s/°C; E). Warming increased the amplitude and inactivation rate of I_A currents in the warm-sensitive neuron, but exerted no significant effect on those of the temperature-insensitive neuron.

Table 1
Effects of temperature on the amplitude and inactivation rate of I_A currents at +40 mV in different types of LPB neurons.

Temperature	Amplitude (pA)		Rate of inactivation (pA/ms)	
	Warm-sensitive (n = 5)	Temperature-insensitive (n = 7)	Warm-sensitive (n = 5)	Temperature-insensitive (n = 7)
32–33 °C	296.72 ± 43.44[b]	150.47 ± 18.99	–0.62 ± 0.17[b]	–0.22 ± 0.05
36–37 °C	358.69 ± 50.74[b]	159.35 ± 10.84	–0.81 ± 0.24[b]	–0.21 ± 0.05
39–40 °C	395.25 ± 48.06[a b]	144.15 ± 12.03	–1.02 ± 0.25[a b]	–0.23 ± 0.05

[a] For warm-sensitive neurons, the mean amplitude and inactivation rate of I_A were significantly higher in hyperthermic range compared to hypothermic range ($P < 0.05$).
[b] For the same temperature range, the mean amplitude and inactivation rate of I_A were significantly greater in warm-sensitive neurons than those in temperature-insensitive neurons ($P < 0.05$).

sensitive neurons and elevated those of cold-sensitive neurons, but did not reach statistical significance. Besides, the AHP amplitudes of low-slope and moderate-slope temperature-insensitive neurons remained stable during temperature changes.

4. Discussion

Our previous work (Xue et al., 2016) has identified that both warm- and cold-sensitive neurons are presented in the LPB via a common method used to characterize preoptic neuronal thermosensitivity. Considering the anatomical and functional correlation of the spinal cord, LPB and POA in the spinal-LPB-POA afferent pathway, it can be hypothesized that skin temperature information from the spinal cord is presumably integrated at synapses in the LPB, where cool and warm afferent signals are processed within different subnuclei and combined with local temperature signals and subsequently transmitted to the POA to promote defensive thermoregulatory responses (Morrison and Nakamura, 2011; Xue et al., 2016). To the best of our knowledge, this study is the first to investigate the electrophysiological properties of LPB neurons to explore the basic mechanisms underlying thermosensitivity.

The thermosensitive changes in neuronal firing rate can be attributed to the temperature-dependent changes in RMP, threshold potential, AHP, and/or prepotential. For warm-sensitive neurons, warming may depolarize the membrane potential, elevate the prepotential rate of depolarization, and reduce the threshold potential or AHP value, leading to increased firing rates. The findings of this study reveal that the prepotential may serve as a primary mechanism underlying LPB neuronal warm sensitivity, as supported by several other studies (Griffin et al., 1996; Burgoon and Boulant, 2001). First, LPB neuronal warm sensitivity is mainly attributed to the impacts of temperature on depolarizing prepotentials or pacemaker potentials that regulate the intervals among successive action potentials. As shown in Fig. 3, warming increased the prepotential rates of depolarization, reduced the interspike intervals and elevated the firing rates in warm-sensitive neurons. In contrast, temperature exerted a non-significant effect on the depolarizing prepotentials of low-slope temperature-insensitive, moderate-slope temperature-insensitive and cold-sensitive neurons. Moreover, warm-sensitive neurons exhibited stronger thermal effects on the average prepotential and a higher Q_{10} value for the rate of rise compared to the other three types of neurons. Second, no significant correlation was found between firing rate thermosensitivity and RMP thermosensitivity in warm-sensitive neurons, thus excluding RMP as a possible determinant of warm-sensitive neuronal firing rate. Third, temperature did not affect the threshold potential in both temperature-sensitive and -insensitive LPB neurons despite affecting the amplitude and duration of action potentials. This notion negates the idea that warming triggers lower threshold potential, thereby producing an increase in firing rates. Fourth, although AHP amplitudes were reduced in warm-sensitive neurons during warming, this trend did not reach statistical significance, indicating that AHP is not an important factor for LPB neuronal warm sensitivity. Similar thermosensitive changes in the

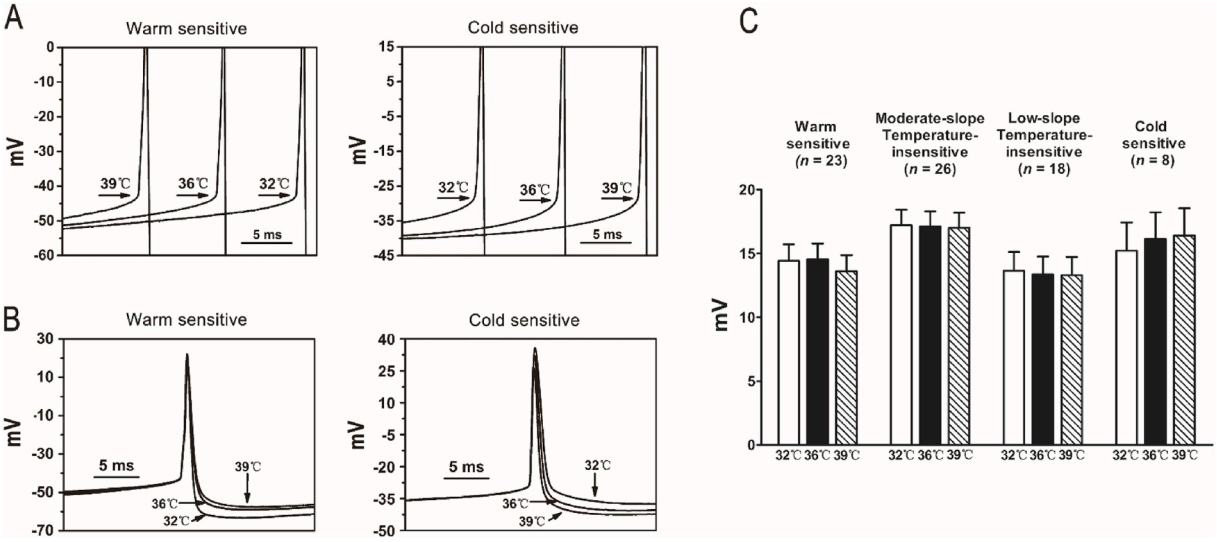

Fig. 5. Effects of temperature on the thresholds and AHPs of warm-sensitive and cold-sensitive neurons in the LPB. (A) Effects of temperature on the thresholds of a warm-sensitive neuron (m = 0.97 imp/s/°C), and a cold-sensitive neuron (m = –1.05 imp/s/°C). Temperature did not affect the threshold potential of these neurons. (B) Effect of temperature on the AHPs of the warm-sensitive and cold-sensitive neurons. Warming exerted variable effects on their AHP amplitudes. Warming decreased the AHP amplitudes of the warm-sensitive neuron, while increased those of the cold-sensitive neuron. (C) Effects of temperature on the AHPs of the warm-sensitive and cold-sensitive neurons did not reach statistically significance.

Table 2
Action potential threshold of LPB neurons classified by firing rate thermosensitivity.

Classification	*n*	Threshold (mV)		
		32–33 °C	36–37 °C	39–40 °C
Warm-sensitive	23	–37.14 ± 1.57	–36.53 ± 1.70	–36.55 ± 1.73
Moderate-slope temperature-insensitive	26	–39.76 ± 1.71	–39.37 ± 1.72	–39.38 ± 1.81
Low-slope temperature-insensitive	18	–39.59 ± 2.88	–39.73 ± 2.81	–39.25 ± 2.77
Cold-sensitive	8	–36.39 ± 3.25	–37.50 ± 3.09	–37.87 ± 3.21
All neurons	75	–38.56 ± 1.08	–38.38 ± 1.08	–38.32 ± 1.10

prepotential were observed in warm-sensitive neurons located at the hypothalamic preoptic region (Griffin et al., 1996) and suprachiasmatic nucleus (Burgoon and Boulant, 2001), thus explaining their similar thermoresponsiveness as previously reported by others (Griffin et al., 1996; Burgoon and Boulant, 2001) and our group (Xue et al., 2016).

In preoptic/anterior hypothalamic neurons, the inactivation rate of I_A currents is considered to be an important contributor to neuronal warm sensitivity (Griffin et al., 1996; Radicke et al., 2013). In the present experiments, the inactivation rates of I_A currents were elevated in warm-sensitive LPB neurons during warming, with a Q_{10} value similar to that of depolarizing prepotential. It is possible that warming triggers a more rapid inactivation of outward I_A currents, resulting in a faster depolarizing rate of membrane potential toward the threshold, i.e., the steeper prepotential observed in warm-sensitive LPB neurons. Hence, the effects of temperature on I_A inactivation may offer an explanation for the firing rate changes observed in warm-sensitive LPB neurons.

We have previously reported that many cold-sensitive LPB neurons exhibited different responses to temperature changes as compared to POA neurons (Xue et al., 2016). Cold-sensitive POA neurons have been found to display thermoresponse curves that appear to “peak” close to thermoneutrality, with an optimum firing rate at 36-38 °C (Kelso et al., 1982). In the LPB, some cold-sensitive neurons appeared silence at 36-38 °C, and the firing rates reduced rapidly to 0 imp/s at approximately 37 °C. In addition, the cold sensitivity of LPB neurons is within a specific temperature range of less than 37 °C (Xue et al., 2016). These different thermoresponsiveness may result from a distinct neuronal mechanism underlying cold sensitivity.

In the POA, cold sensitivity is determined by inhibitory synaptic inputs from surrounding warm-sensitive neurons based on their similar changes in thermosensitivity to skin or spinal cord temperature (Hammel, 1968). Meanwhile, the neuronal mechanism of cold sensitivity in the LPB appears to be determined by their temperature dependence of RMP, as cooling and warming produced membrane depolarization and hyperpolarization, respectively. Moreover, the membrane potential thermosensitivity is almost identical to firing rate thermosensitivity. A similar effect of temperature on RMP has been reported in cat spinal motoneurons (Pierau et al., 1969), rat visual cortical cells (Volgushev et al., 2000), rat dorsal root ganglion cells (Reid and Flonta, 2001), and mouse trigeminal ganglion cells (Viana et al., 2002). Besides, the prepotential rate of depolarization remained stable during temperature changes in cold-sensitive LPB neurons, indicating that neuronal cold sensitivity is not associated with depolarizing prepotential. The present study reveals an inherent mechanism of cold sensitivity in the LPB, but it does not exclude the possibility that synaptic inputs from nearby neurons may be partly responsible for the thermosensitivity of cold-sensitive neurons. Therefore, further research is needed to determine whether local synaptic networks are involved in the thermosensitivity of LPB neurons.

In contrast to the advanced understanding of the ionic mechanisms underlying central warm sensitivity, the ionic mechanism that mediates central cold sensitivity remains largely unknown, probably due to a lower number of cold-sensitive neurons in the central nervous system. Furthermore, I_A current was not apparent in 5 (of 5) cold-sensitive LPB neurons, and thus was excluded as a potential contributor of firing rate cold sensitivity. The two-pore domain leak potassium channels, such as TREK1, are essential for maintaining hyperpolarized RMPs (Wechselberger et al., 2006; Renigunta et al., 2015). In addition, TREK1 has been proposed as a promising candidate of physiological thermoreceptor (Maingret et al., 2000; Stebe et al., 2014). Hence, the thermosensitive TREK1 may act as an ionic basis for cold sensitivity in the LPB. Typically, the greater the conductance of K^+ leak current, the lower the firing rate and thermal coefficient (Griffin and Boulant, 1995). This may be the reason why the firing rates of cold-sensitive LPB neurons are lower than those of warm-sensitive neurons. The molecular identity of neuronal background K^+ channel suppressed during cooling in cold-sensitive LPB neurons will be investigated in future studies.

In conclusion, our study reveals that warm- and cold-sensitive LPB neurons exhibit thermal-dependent differences in resting membrane potentials and depolarizing prepotential rate. Similar to POA, the primary mechanism of LPB neuronal warm sensitivity may reside in the depolarizing prepotential, which may result from the temperature-dependent inactivation of I_A currents. Additionally, LPB neuronal cold sensitivity is determined not by the depolarizing prepotential, but instead the temperature dependence of RMP. The findings also reveals why warm-sensitive neurons in the LPB are similar in thermoresponsiveness to those in the POA, while cold sensitivity in the LPB is distinct from that in the POA.

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