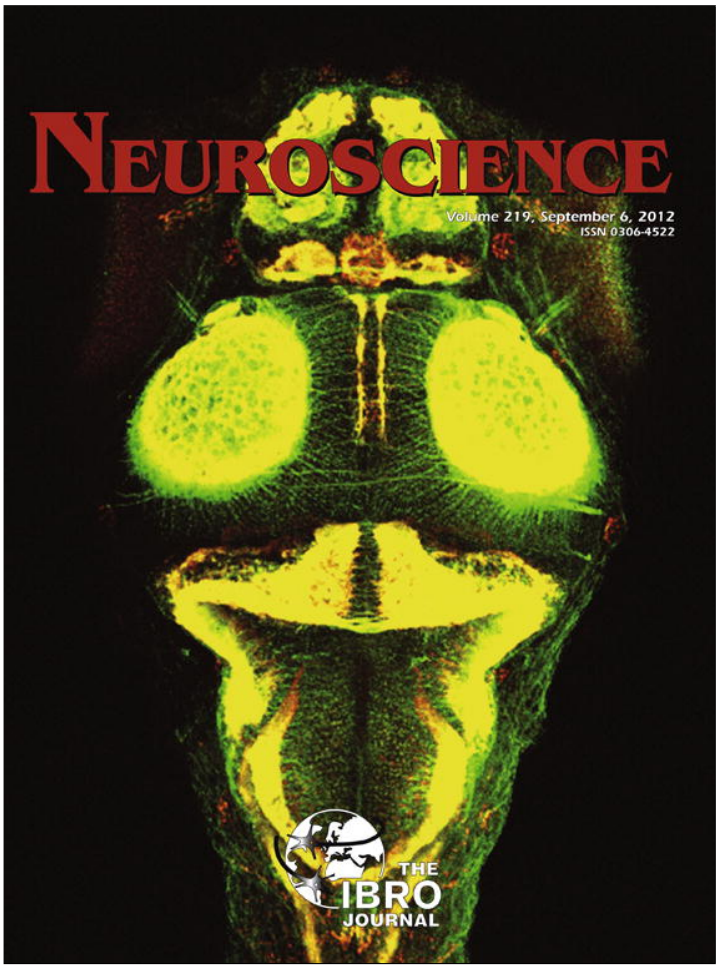

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EFFECTS OF ARGININE VASOPRESSIN ON FIRING ACTIVITY AND THERMOSENSITIVITY OF RAT PO/AH AREA NEURONS

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Abstract—It is well known that the preoptic–anterior hypothalamus (PO/AH), containing temperature-sensitive and -insensitive neurons plays an important role in precise thermoregulatory responses. Previous *in vivo* studies suggest that the arginine vasopressin (AVP) is an important endogenous mediator in thermoregulation, since AVP and V_{1a} vasopressin receptor antagonist can induce hypothermia and hyperthermia, respectively. In the present study, intracellular electrophysiological activity was recorded from temperature-sensitive and -insensitive neurons in rat PO/AH tissue slices, using a whole-cell patch clamp. By monitoring neuron's changes of firing activity and thermosensitivity when perfused with AVP or V_{1a} vasopressin receptor antagonist, we found that AVP increased the spontaneous firing rate in 65% of warm-sensitive neurons and decreased it in nearly 50% of cold-sensitive and temperature-insensitive neurons. These changes are due to the AVP enhancing the rise rate of depolarization prepotential in warm-sensitive neurons and reducing it in the other neurons. Moreover, AVP increased the thermosensitivity of warm-sensitive neurons while it decreased thermosensitivity of cold-sensitive and temperature-insensitive neurons. V_{1a} vasopressin receptor participated in these responses. Since excited warm-sensitive neurons or inhibited cold-sensitive and temperature-insensitive neurons promote heat loss or suppress heat production and retention. These results that AVP excites warm-sensitive neurons and inhibits cold-sensitive and temperature-insensitive neurons suggest a probable mechanism of AVP-induced hypothermia.

Key words: arginine vasopressin, V_{1a} vasopressin receptor, preoptic–anterior hypothalamus area (PO/AH), warm-sensitive neuron, thermosensitivity.

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Abbreviations: aCSF, artificial cerebrospinal fluid; AVP, arginine vasopressin; CSN, cold-sensitive neuron; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; L-TIN, low-slope temperature-insensitive neuron; M-TIN, moderate-slope temperature-insensitive neuron; PO/AH, preoptic–anterior hypothalamus; TRPV, transient receptor potential vanilloid; V_{1a} ant, V_{1a} vasopressin receptor antagonist; WSN, warm-sensitive neuron.

INTRODUCTION

Neurons in the preoptic–anterior hypothalamus (PO/AH) play an important role in thermoregulatory responses. Temperature-sensitive neurons in the PO/AH area can sense local temperature changes and receive synaptic afferent inputs from peripheral skin thermoreceptors (Griffin, 2004; Boulant, 2006). Properly physiological and behavioral thermoregulatory responses are induced following the PO/AH neuron's integration of central and peripheral thermal information. Previous electrophysiological studies have reported the activity of PO/AH neurons during changes in hypothalamic temperature (Kelso et al., 1982; Griffin et al., 1996). According to these reports, the majority of neurons in the PO/AH area are considered to be temperature insensitive. However, at least 20% PO/AH neurons are classified as warm-sensitive neurons whose firing rates significantly increase or decrease during hypothalamic warming or cooling, respectively (Wright et al., 2008). Thermosensitivity is an intrinsic property of warm-sensitive neurons, and some ionic channels may contribute to produce thermosensitive pacemaker potentials (Griffin et al., 1996; Wechselberger et al., 2006). Compared with warm-sensitive neurons, a small proportion of neurons have the opposite firing rate responses to temperature and are classified as cold-sensitive neurons (Boulant, 2006).

Arginine vasopressin (AVP) appears to be an important mediator in the central control of body temperature. Following AVP administration, there was a consistent decrease in body temperature (Steiner et al., 1998; Paro et al., 2003). After administration of V_1 vasopressin receptor antagonist, however, body temperature increased (Steiner et al., 1998). Our previous *in vivo* studies, by extracellular recording, indicated that AVP can excite warm-sensitive neurons and inhibit cold-sensitive neurons in the rabbit PO/AH area and anti-AVP serum can reduce the effect of AVP (Yang and Chen, 1994). Using brain slice extracellular recording, this same effect of AVP on PO/AH temperature-sensitive neurons was verified in rats (Moravec and Pierau, 1994). These results indicate that there exists an association between the AVP-induced hypothermia and the effect of AVP on PO/AH temperature-sensitive neurons. But the central mechanisms of AVP governing the thermoregulatory responses remain unclear.

Therefore, the purpose of the present study was to further investigate the effects of AVP on the firing rate and thermosensitivity of different types of neurons in the PO/AH area. Using whole-cell patch clamp recording, the *in vitro* electrophysiological study was conducted

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using rat hypothalamic tissue slices. In addition, this study pharmacologically examined the physiological role of V_{1a} vasopressin receptor on PO/AH neurons, which are necessary for AVP action.

EXPERIMENTAL PROCEDURES

Preparation of the brain slices

According to procedures reported previously (Dean and Boulant, 1988; Griffin et al., 1996; Wright and Boulant, 2007; Wright et al., 2008), horizontal hypothalamic tissue slices were prepared from male Sprague–Dawley rats (150–200 g). Briefly, each rat was anesthetized with pentobarbital and quickly decapitated in accordance with procedures approved by the NIH and the Chengdu Medical College Laboratory Animal Care and Use Committee. Following removal of the brain, the tissue block containing the hypothalamus was cut and sectioned into 300 μM thick horizontal slices. Tissue slices were allowed to incubate in a chamber that was with humidified 95% O_2–5% CO_2 gas mixtures for 1.5–2 h before any recordings were attempted. Horizontal tissue slices containing the PO/AH were transferred to a recording chamber and were constantly perfused at 1.2 ml/min with a 300 mOsm/kg H_2O artificial cerebrospinal fluid (aCSF) consisting of (in mM) 124 NaCl, 26 $NaHCO_3$, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, and 10 glucose (pH 7.2–7.4). The aCSF was the gas saturated with 95% O_2–5% CO_2 and heated to 35–37 °C using a thermoelectric Peltier assembly (SC-20, Warner Instruments Inc., USA).

Whole-cell patch clamp recordings

The spontaneous firing activity of PO/AH neuron was whole-cell recorded in the current clamp mode. Patch pipettes (5–7 MΩ) were pulled from borosilicate glass and filled with an internal solution consisting of (in mM) 130 potassium gluconate, 10 EGTA, 10 HEPES, 2 Mg–ATP, 2 Na_2GTP, 1 $CaCl_2$. This internal solution was adjusted to 295 mOsm/kg H_2O and pH of 7.2–7.4. Whole-cell recordings were obtained from somata of neurons visualized by infrared differential interference contrast (ID-DIC) videomicroscopy. The ground electrode was maintained at a constant temperature in an outer bath connected to the inner recording chamber (Griffin et al., 1996). The liquid junction potential for this solution has been experimentally determined to be about 12 mV (Griffin and Boulant, 1995), and this value was subtracted from all reported potentials. Recordings were carried out using a Multiclamp 700B amplifier (Axon Instruments, Sunnyvale, CA, USA). When spontaneous activity was recording, no holding current was applied to neurons. The program package pCLAMP 10.1 was used for data acquisition and analysis. Recordings were digitized at 10 kHz and filtered with low-pass filter of 2 kHz.

Experimental protocols

The thermoelectric assembly allowed the tissue slice temperature to be periodically varied 3–5 °C above and below the neutral temperature (35–37 °C) to characterize the thermosensitivity of each recorded neuron. Tissue temperature was monitored continuously by a thermocouple directly in the medium. The spontaneous firing activity of neurons was continuously recorded during cyclic temperature changes. The neuronal thermosensitivity (imp s^{-1} °C^{-1}) was characterized by plotting firing rate as a function of tissue temperature to determine the linear regression coefficient (thermal coefficient) or slope (m) of this plot. According to previous reports (Ranels and Griffin, 2003, 2005; Wright et al., 2008), neurons were classified as warm sensitive if they had a thermal coefficient of at least 0.8 imp s^{-1} °C^{-1} or cold sensitive if they had a thermal coefficient of -0.6 imp s^{-1} °C^{-1} or less. All other neurons were considered temperature insensitive, and these were further divided into two subpopulations: moderate-slope neuron whose thermal coefficient was <0.8 imp s^{-1} °C^{-1} but >0.4 imp s^{-1} °C^{-1} and low-slope neuron whose thermal coefficient was $\leqslant 0.4$ imp s^{-1} °C^{-1}. When control firing activity and thermosensitivity were determined, the perfusion aCSF was switched to an experimental aCSF containing 1 μM AVP ([Arg^8]-Vasopressin acetate salt, Sigma Chemical Co., St. Louis, MO, USA) or V_{1a} vasopressin receptor antagonist ([β-Mercapto-β, β-cyclopentamethylenepropionyl^1, O-me-Tyr^2, Arg^8]-Vasopressin, Sigma Chemical Co., St. Louis, MO, USA). This concentration of AVP or V_{1a} vasopressin receptor antagonist could affect activities of V_{1a} vasopressin receptor (Allaman-Exertier et al., 2007). Then, the experimental aCSF was switched back to the control aCSF to display that the effects of the experimental aCSF could be reversed.

Data analysis

All data are expressed as means ± SE. Mean firing rates (impulse/s) were determined at neutral (36–38 °C) and warm temperature (39–41 °C). The firing rate continuously recorded from 39 to 41 °C was averaged. Then, this mean firing rate was defined as neuron's mean firing rate at the warm temperature. The thermal coefficients (imp s^{-1} °C^{-1}) were recalculated during AVP or V_{1a} vasopressin receptor antagonist treatment. The rise rate of depolarizing prepotential was calculated from the slope of the membrane potential during the 4–20-ms period immediately preceding the action potential (Burgoon and Boulant, 2001). Control firing rates were compared during AVP or V_{1a} vasopressin receptor antagonist treatment. The criteria for a change in firing rate were a 10% change that was $\geqslant 1$ impulse/s and a firing rate response displaying at least partial recovery to control conditions (Wright et al., 2008). In all reported comparisons of firing rate, thermal coefficient, and rise rate of depolarizing prepotential changes, statistical differences were determined by Student's t-tests or ANOVA (one-way). Differences were considered significant at $P < 0.05$. All statistical tests were performed using IBM SPSS Statistics 19 software (SPSS Inc., an IBM Company, Chicago, IL, USA).

RESULTS

Intracellular activity was recorded in 89 neurons during at least two cyclic temperature changes. These neurons were classified according to their thermosensitivity as well as their firing rate responses recorded at two different temperatures before and during exposure to AVP or V_{1a} vasopressin receptor antagonist. These neurons included 19 warm-sensitive neurons (21%), 10 cold-sensitive neurons (11%), 12 moderate-slope temperature-insensitive neurons (14%), and 48 low-slope temperature-insensitive neurons (54%). These proportions are similar to those reported previously (Dean and Boulant, 1989; Griffin et al., 2001; Wright and Boulant, 2007; Wright et al., 2008).

Effects of AVP and V_{1a} vasopressin receptor antagonist on firing rate in temperature-sensitive neurons

In the present study, 65% (11/17) of the recorded warm-sensitive neurons exhibited firing rate increases (AVP-excited) during AVP perfusion (Table 1 and Fig. 1A). Fifty percent (5/10) of cold-sensitive neurons, however, decreased firing rate (AVP-inhibited) in the present AVP (Table 1 and Fig. 1B). At warm temperature (Table 2), the proportions of AVP-excited warm-sensitive and AVP-inhibited cold-sensitive neuron are 41% (7/17)

and 44% (4/9), respectively (Fig. 1A, B). In Fig. 2A, every neuron's firing rate during AVP is plotted as a function of its control firing rate before AVP exposure. If there was no observed net change in firing rate of the neuronal population, linear regression analysis would show a slope (m) of 1.0. Actually, Fig. 2A shows that the slope increased in warm-sensitive neurons (m = 1.1; m = 1.09) and decreased in cold-sensitive neurons (m = 0.44; m = 0.81) at two different recorded temperatures. This is confirmed in Fig. 2B, where the firing rate change differed significantly between warm-sensitive neurons (89 ± 24%, n = 11; 99 ± 26%, n = 7) and cold-sensitive neurons (−72 ± 14%, n = 5; −84 ± 10%, n = 4) in the presence of AVP, at two recorded temperatures respectively (P < 0.01).

At the neutral temperature, 46% (6/13) of warm-sensitive neurons decreased firing rate (V_{1a} ant-inhibited) and 43% (3/7) of cold-sensitive neurons increased firing rate (V_{1a} ant-excited) during V_{1a} vasopressin receptor antagonist perfusion (Table 1). However, at the warm temperature, the proportion of V_{1a} ant-inhibited warm-sensitive neurons or V_{1a} ant-excited cold-sensitive neuron is enhanced to 77% (10/13) or 71% (5/7), respectively (Table 2). The slope of linear regression decreased in warm-sensitive neurons (m = 0.63; m = 0.59) and increased in cold-sensitive neurons (m = 1.18; m = 1.59) at two different recorded temperatures (Fig. 3A). There were significant differences in the firing rate changes between the warm-sensitive neurons (−67 ± 8%, n = 6; −50 ± 9%, n = 10) and cold-sensitive neurons (68 ± 36%, n = 3; 201 ± 81%, n = 5) when treatment with V_{1a} vasopressin receptor antagonist was recorded at two temperatures (P < 0.01; P < 0.05; Fig. 3B).

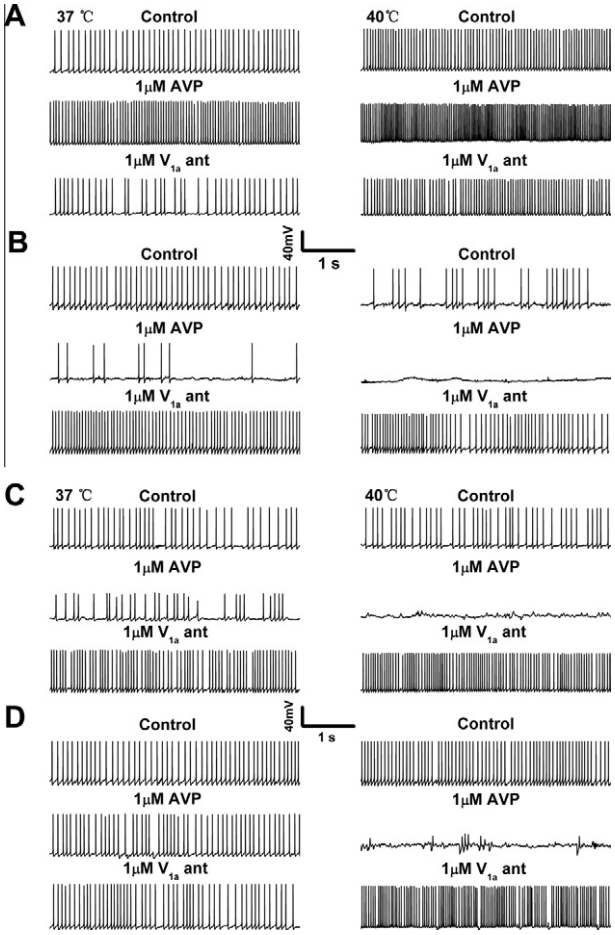

Fig. 1. Effects of AVP and V_{1a} vasopressin receptor antagonist on spontaneous firing of different temperature-sensitive type neurons in the PO/AH at two different temperatures. Left: recording temperature was 37 °C. Right: recording temperature was 40 °C. The potential traces, obtained in current-clamp mode, show recorded spontaneous firing rate of a warm-sensitive neuron (A, m = 1.21 imp s^{-1} °C^{-1}), cold-sensitive neuron (B, m = −1.24 imp s^{-1} °C^{-1}), low-slope (C, m = −0.11 imp s^{-1} °C^{-1}) and moderate-slope temperature-insensitive neuron (D, m = 0.65 imp s^{-1} °C^{-1}) in control conditions, in the presence of AVP (1 μM) and in the presence of V_{1a} ant (1 μM) at two recorded temperatures. Note that AVP excited the spontaneous firing rate of warm-sensitive neuron and suppressed the spontaneous firing rate of cold-sensitive neuron, low-slope and moderate-slope temperature-insensitive neuron. The effect of V_{1a} ant was on the contrary. V_{1a} ant, V_{1a} vasopressin receptor antagonist.

Effects of AVP and V_{1a} vasopressin receptor antagonist on firing rate in temperature-insensitive neurons

Fig. 4A shows that the firing rate change of moderate-slope temperature-insensitive neurons has significant difference for AVP-excited neurons (M-TIN 1, 44 ± 16%, n = 4; 40 ± 13%, n = 3) and AVP-inhibited neurons (M-TIN 2, −29 ± 8%, n = 5; −54 ± 10%, n = 7), respectively, at two recorded temperatures (P < 0.01). When perfused with V_{1a} vasopressin receptor antagonist, the mean firing rate change between V_{1a} ant-excited neurons (M-TIN 1, 150 ± 85%, n = 3; 62 ± 19%, n = 3) and V_{1a} ant-inhibited neurons (M-TIN 2, −75 ± 12%, n = 3; −72 ± 15%, n = 4) differed, but there was statistical significance only at the warm temperature (Fig. 4C, P < 0.01). For low-slope temperature-insensitive

Table 1. Effects of AVP and V_{1a} ant on firing rate in PO/AH neurons at neutral temperature

Firing rate	WSN		CSN		M-TIN		L-TIN	
	AVP	V_{1a} ant	AVP	V_{1a} ant	AVP	V_{1a} ant	AVP	V_{1a} ant
Inc	11	5	2	3	4	3	16	17
Dec	5	6	5	2	5	3	12	11
NC	1	2	3	2	2	2	9	5
n	17	13	10	7	11	8	37	33

n = no. of cells recorded. AVP, arginine vasopressin; V_{1a} ant, V_{1a} receptor antagonist; WSN, warm-sensitive neuron; CSN, cold-sensitive neuron; M-TIN, moderate-slope temperature-insensitive neuron; L-TIN, low-slope temperature-insensitive neuron; Inc, increased; Dec, decreased; NC, no change. The criterion for a change in firing rate was a 10% change that was ⩾1 impulse/s. The percent change in firing rate = (firing rate during perfusion with AVP or V_{1a} vasopressin receptor antagonist − spontaneous firing rate)/spontaneous firing rate. Neutral temperature (36–38 °C).

Table 2. Effects of AVP and V_{1a} ant on firing rate in PO/AH neurons at warm temperature

Firing rate	WSN		CSN		M-TIN		L-TIN	
	AVP	V_{1a} ant	AVP	V_{1a} ant	AVP	V_{1a} ant	AVP	V_{1a} ant
Inc	7	2	2	5	3	3	17	19
Dec	7	10	4	0	7	4	13	6
NC	3	1	3	2	1	1	7	8
n	17	13	9	7	11	8	37	33

Warm temperature (39–41 °C).

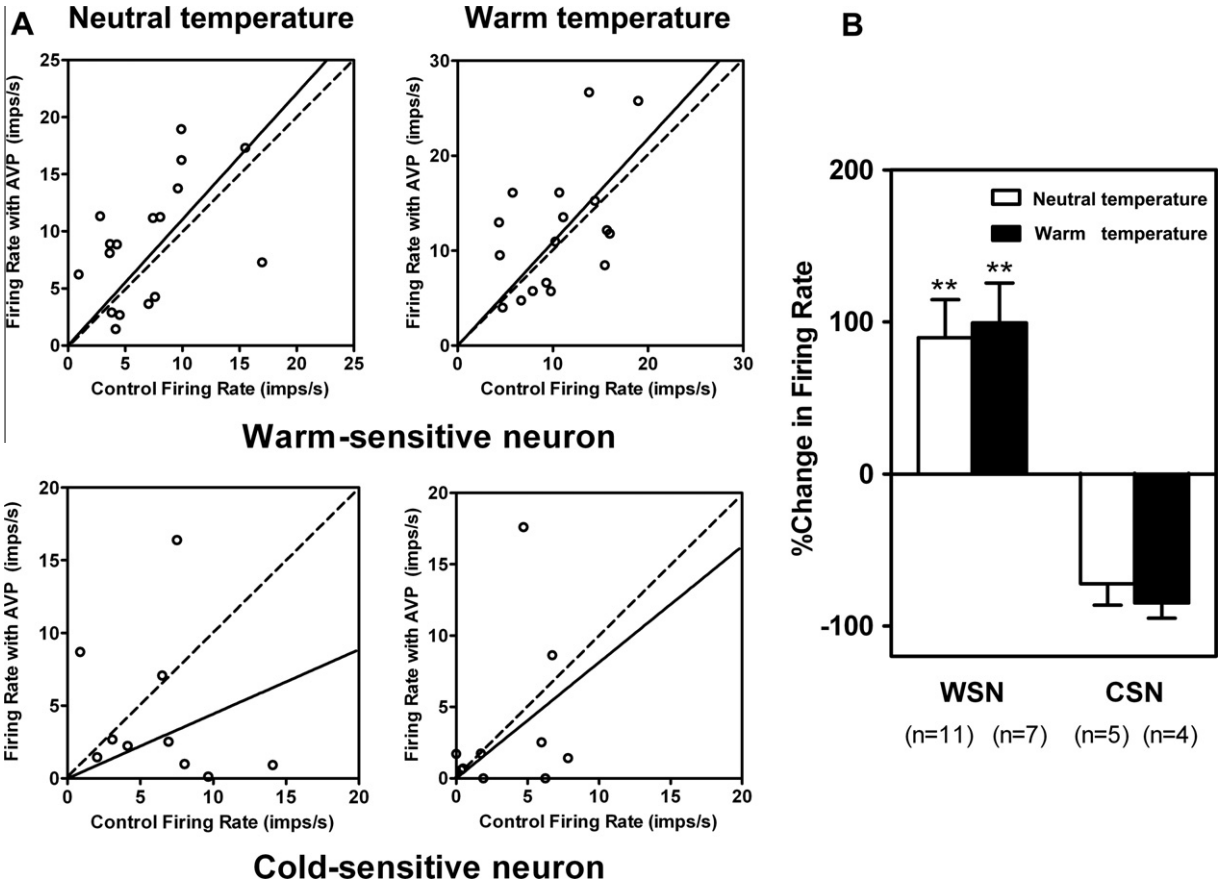

Fig. 2. Effect of AVP on firing rate of temperature-sensitive neurons. (A) Left: neutral temperature (36–38 °C). Right: warm temperature (39–41 °C). Firing rate during perfusion with 1 μM AVP is plotted as a function of firing rate before perfusion. Data points that lie along the dashed line ($m = 1.0$) represent neurons that did not alter the firing rate. Data points lying above or below this dashed line represent neurons that increased or decreased their firing rate, respectively. Linear regression analysis showed that the slope increased in warm-sensitive neurons ($m = 1.1$; $m = 1.09$) and decreased in cold-sensitive neurons ($m = 0.44$; $m = 0.81$) at two different recorded temperatures. (B) Change in firing rate during perfusion with AVP for temperature-sensitive neurons at two different recorded temperatures. The percent change in firing rate = (firing rate during perfusion AVP − spontaneous firing rate)/spontaneous firing rate. Bars represent the mean change in firing rate, and the error bars represent SE. $^{**}P < 0.01$. SE, standard error.

neurons, the observed firing rate change of AVP-excited (L-TIN 1) was 171 ± 53% ($n = 16$) or 117 ± 28% ($n = 17$) and for AVP-inhibited (L-TIN 2) was −42 ± 6% ($n = 12$) or −41 ± 7% ($n = 13$), respectively, at two recorded temperatures. There is statistical significance between the firing rate change of AVP-excited neurons and AVP-inhibited neurons (Fig. 4B, $P < 0.01$). There was also a significant difference between the firing rate change for V_{1a} ant-excited neurons (L-TIN 1, 179 ± 36%, $n = 17$; 175 ± 47%, $n = 19$) and V_{1a} ant-inhibited neurons (L-TIN 2, −58 ± 6%, $n = 11$; −82 ± 6%, $n = 6$) at two different temperatures (Fig. 4D, $P < 0.01$). These data indicate that AVP and V_{1a} vasopressin receptor antagonist increase firing rates of some temperature-insensitive neurons and decrease it for others at the two different recorded temperatures.

Effects of AVP and V_{1a} vasopressin receptor antagonist on thermosensitivity in temperature-sensitive neurons

To determine whether AVP affected the thermosensitivity of temperature-sensitive neurons, we studied the effect of the peptide and V_{1a} vasopressin receptor antagonist

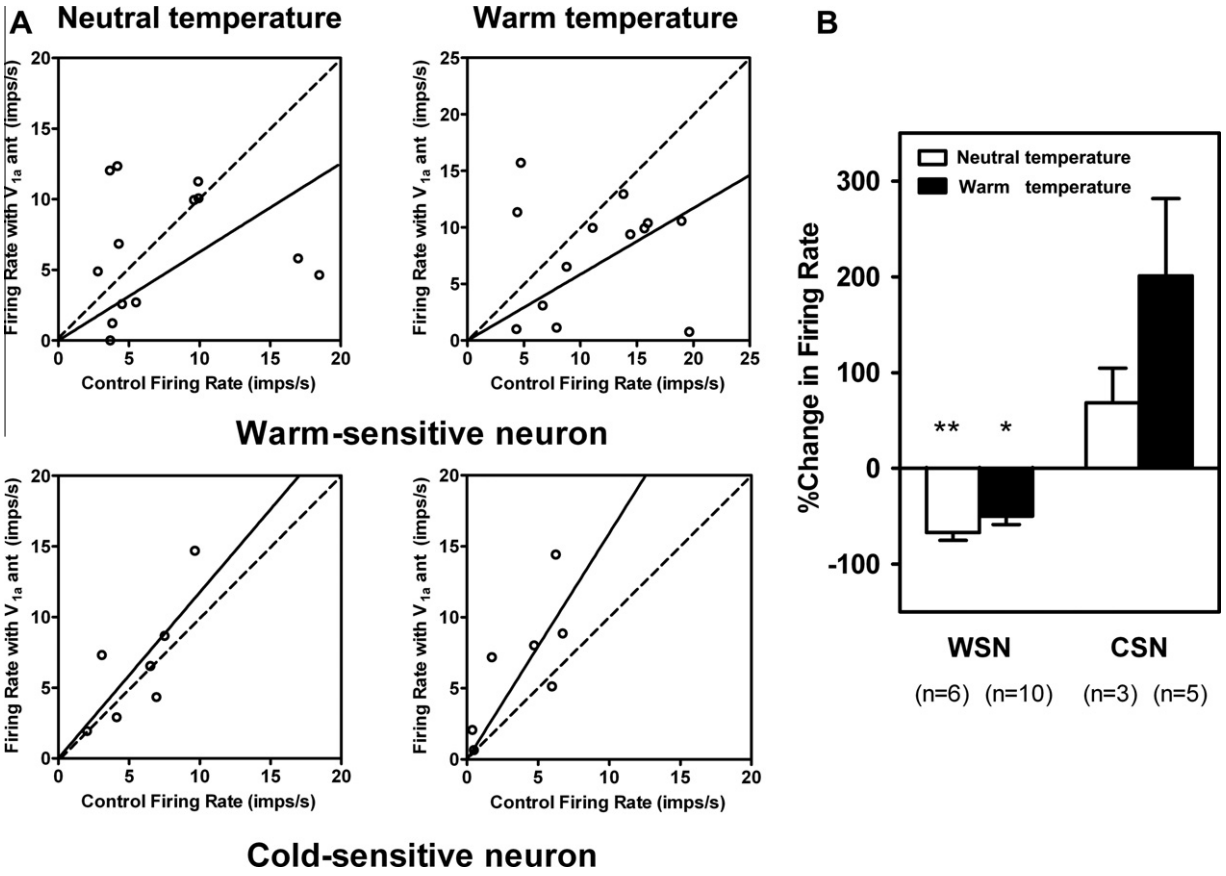

Fig. 3. Effect of V_{1a} vasopressin receptor antagonist on firing rate of temperature-sensitive neurons. (A) Left: neutral temperature (36–38 °C). Right: warm temperature (39–41 °C). Firing rate during perfusion with 1 μM V_{1a} ant is plotted as a function of firing rate before perfusion. Linear regression analysis showed that the slope decreased in warm-sensitive neurons (m = 0.63; m = 0.59) and increased in cold-sensitive neurons (m = 1.18; m = 1.59) at two different recorded temperatures. (B) Change in firing rate during perfusion with V_{1a} ant for temperature-sensitive neurons at two different recorded temperatures. The percent change in firing rate = (firing rate during V_{1a} ant − spontaneous firing rate)/spontaneous firing rate. Bars indicate the mean change in firing rate, and the error bars represent SE. $^{**}P < 0.01$; $^{*}P < 0.05$.

on thermal coefficients of temperature-sensitive neurons. During the control aCSF perfusion, a warm-sensitive neuron's thermal coefficient was 1.21 imp s^{-1} °C^{-1} (Fig. 5A, bottom, 1); then, this increased to 2.75 imp s^{-1} °C^{-1} during AVP exposure (Fig. 5A, bottom, 2). After washout, when the aCSF perfusion contained 1 μM V_{1a} vasopressin receptor antagonist, the thermal coefficient declined to 0.92 imp s^{-1} °C^{-1} (Fig. 5A, bottom, 3). In the present study, the thermosensitivity of 10/15 warm-sensitive neurons was increased during AVP perfusion. AVP applications, however, induced a decrease in thermosensitivity of 9/10 cold-sensitive neurons (Fig. 6A). There was a significant difference in the thermosensitivity changes between thermosensitivity-increased warm-sensitive neurons (0.86 ± 0.28, n = 10) and thermosensitivity-decreased cold-sensitive neurons (−0.80 ± 0.16 n = 9) during AVP perfusion (Fig. 6B, $P < 0.01$). As a population, warm-sensitive neurons (−0.97 ± 0.24, n = 12) and cold-sensitive neurons (−1.08 ± 0.19, n = 7) all exhibited the dramatic decrease in thermosensitivity during treatment with the V_{1a} vasopressin receptor antagonist. There was not a significant difference in the thermosensitivity changes between them ($P > 0.05$).

Effects of AVP and V_{1a} vasopressin receptor antagonist on thermosensitivity in temperature-insensitive neurons

To determine whether AVP affected the thermosensitivity of temperature-insensitive neurons, we studied the effect of the peptide or V_{1a} vasopressin receptor antagonist on thermal coefficients of temperature-insensitive neurons. Fig. 5B is an example of low-slope temperature-insensitive neurons (m = −0.11 imp s^{-1} °C^{-1}, Fig. 5B, bottom, 1) recorded in the PO/AH region. V_{1a} vasopressin receptor antagonist (1 μM) induced a dramatic increase in thermosensitivity (m = 0.52 imp s^{-1} °C^{-1}, Fig. 5B, bottom, 2) but there was a decrease in its thermosensitivity (m = −1.07 imp s^{-1} °C^{-1}, Fig. 5B, bottom, 3) when perfused with 1 μM AVP. However, the thermosensitivity changes of some low-slope temperature-insensitive neurons were inverted with Fig. 5B during experimental perfusion. Furthermore, this is confirmed in Fig. 7B. There was a significant difference in the thermosensitivity changes between thermosensitivity-increased neurons (0.60 ± 0.12, n = 20) and thermosensitivity-decreased neurons (−0.51 ± 0.14, n = 16) during AVP exposure ($P <$

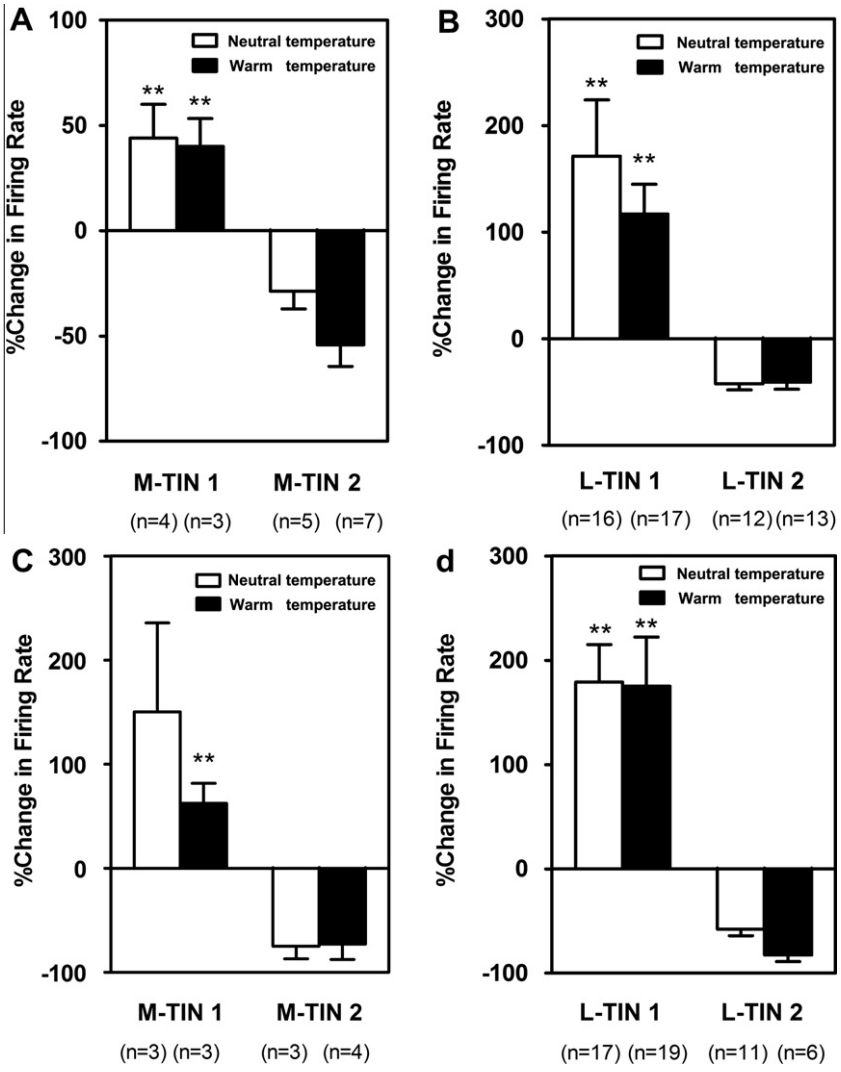

Fig. 4. The changes in firing rate of moderate-slope (A, C) or low-slope temperature-insensitive neurons (B, D) during slice were perfused with 1 μM AVP (top) or V_{1a} vasopressin receptor antagonist (bottom). The percent change in firing rate = (firing rate during AVP or V_{1a} ant − spontaneous firing rate)/spontaneous firing rate. TIN 1 represents AVP or V_{1a} ant-excited neurons and TIN 2 represents AVP or V_{1a} ant-inhibited neurons. Bars indicate the mean change in firing rate, and the error bars represent SE. M-TIN, moderate-slope temperature-insensitive neuron; L-TIN, low-slope temperature-insensitive neuron. $^{**}P < 0.01$.

0.01). Similarly, V_{1a} vasopressin receptor antagonist increased the thermosensitivity of 19 low-slope temperature-insensitive neurons (0.71 ± 0.13, $n = 19$) and decreased thermosensitivity for 13 neurons (−0.43 ± 0.10, $n = 13$). This difference has statistical significance (Fig. 7D, $P < 0.01$).

For moderate-slope temperature-insensitive neurons, there was a significant difference in the thermosensitivity changes between thermosensitivity-increased neurons (0.51 ± 0.15, $n = 5$) and thermosensitivity-decreased neurons (−0.71 ± 0.19, $n = 6$) during AVP perfusion (Fig. 7A, $P < 0.01$). In the same way, V_{1a} vasopressin receptor antagonist increased the thermosensitivity of three moderate-slope temperature-insensitive neurons (0.66 ± 0.03) and decreased it for five neurons (−0.53 ± 0.11). There was significant difference in thermosensitivity changes between them (Fig. 7C, $P < 0.01$).

Effects of AVP or V_{1a} vasopressin receptor antagonist on depolarizing prepotential

Most PO/AH neurons displayed depolarizing prepotentials that induced the membrane potential to depolarize to the threshold, thereby producing action potentials. To determine whether AVP affecting depolarizing prepotential contributed to changes of firing rate, we studied the effects of the peptide or antagonist on prepotential's rate of depolarization at 37 °C. In control conditions, the depolarizing prepotential's rate of warm- and cold-sensitive neurons was 0.24 ± 0.01 mV ms^{-1} ($n = 15$) and 0.24 ± 0.01 mV ms^{-1} ($n = 10$), whereas in the presence of AVP this was 0.29 ± 0.01 mV ms^{-1} ($n = 15$) and 0.19 ± 0.02 mV ms^{-1} ($n = 10$), respectively (Fig. 8A, B). Hence, AVP caused an increase in the depolarizing prepotential's rate of warm-sensitive neurons ($P < 0.01$) and a decrease of cold-sensitive neurons ($P < 0.01$).

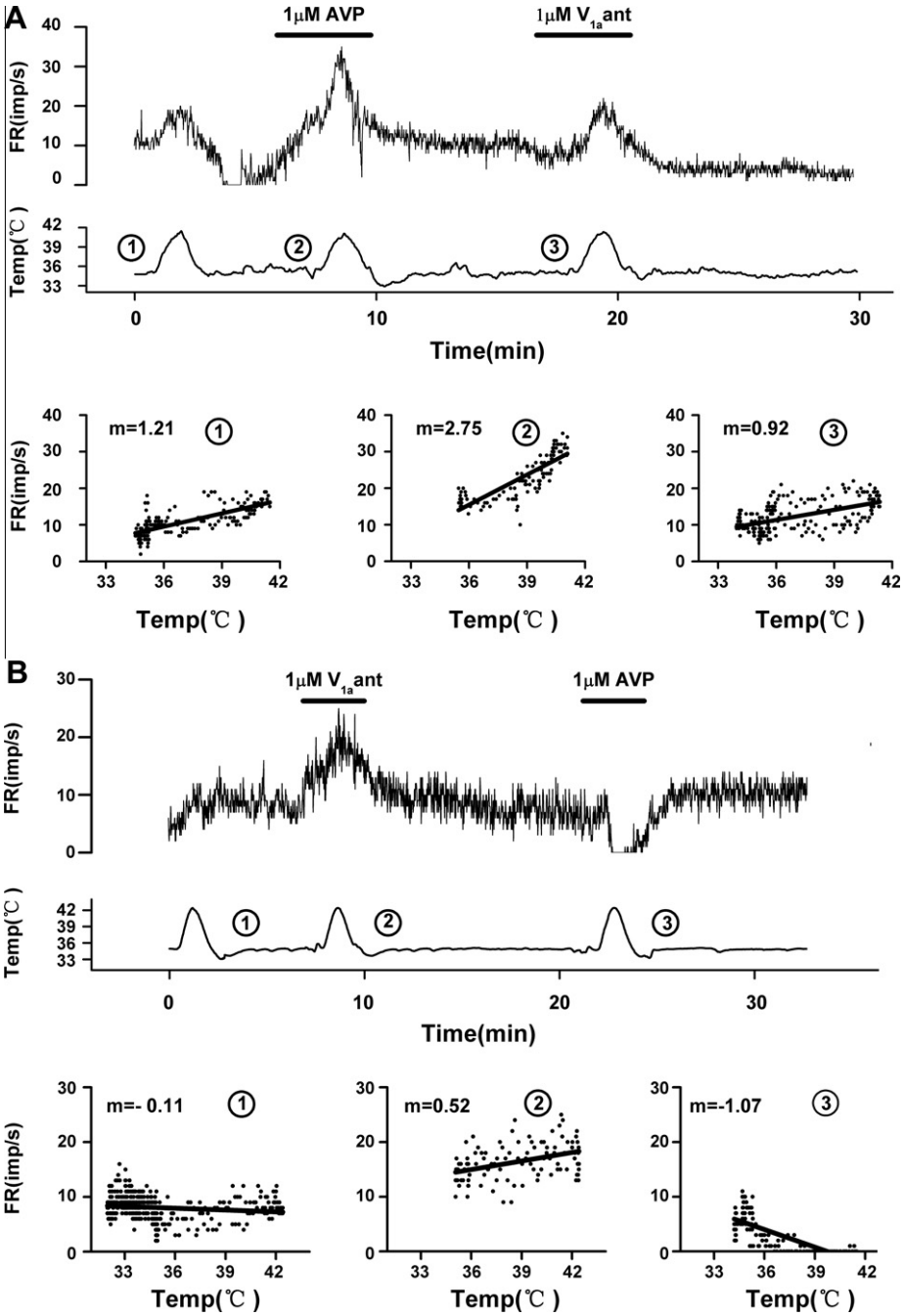

Fig. 5. Effects of AVP and V_{1a} vasopressin receptor antagonist on the thermosensitivity of a warm-sensitive neuron (A) and temperature-insensitive neuron (B) recorded in PO/AH area. (A, B) Top: experimental record of spontaneous firing rate and tissue slice temperature. (A, B) Bottom: thermoresponse plots of spontaneous firing rate as a function of tissue slice temperature and its corresponding thermosensitivity at different times (1, 2, and 3) indicated at TOP. FR, firing rate; Temp, temperature.

Conversely, V_{1a} vasopressin receptor antagonist caused a decrease in the depolarizing prepotential's rate for warm-sensitive neurons (0.20 ± 0.01 mV ms^{-1}, $n = 15$, $P < 0.01$) and an increase for cold-sensitive neurons (0.32 ± 0.01 mV ms^{-1}, $n = 10$, $P < 0.01$).

For temperature-insensitive neurons, although AVP inhibited spontaneous firing rate, the effect of AVP on prepotential's rate of depolarization was different. In control conditions, the depolarizing prepotential's rate of low- and moderate-slope temperature-insensitive neurons was 0.21 ± 0.01 mV ms^{-1} ($n = 15$) and 0.28 ± 0.01 mV ms^{-1} ($n = 15$), whereas in the presence of AVP rate was 0.32 ± 0.02 mV ms^{-1} ($n = 10$) and 0.23 ± 0.01 mV ms^{-1} ($n = 15$), respectively. Hence, AVP increased the depolarizing prepotential's rate of low-slope temperature-insensitive neurons ($P < 0.01$) and decreased rate of moderate-slope neurons (Fig. 8C, $P < 0.01$). V_{1a} vasopressin receptor antagonist, however, did not affect

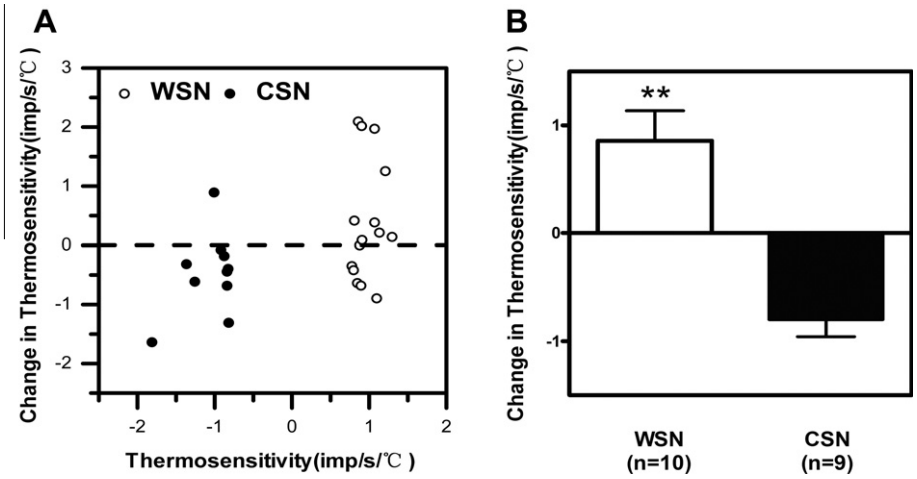

Fig. 6. The effects of AVP on the thermosensitivity of PO/AH temperature-sensitive neurons. (A) The change in thermosensitivity (thermosensitivity during AVP − thermosensitivity) is plotted for each warm- and cold-sensitive neuron, with respect to thermosensitivity. Dashed line indicates a 0 change. (B) Change in thermosensitivity of temperature-sensitive neurons during perfusion with AVP. Bars indicate the mean change in thermosensitivity, and error bars represent SE. $^{**}P < 0.01$. WSN, warm-sensitive neuron; CSN, cold-sensitive neuron.

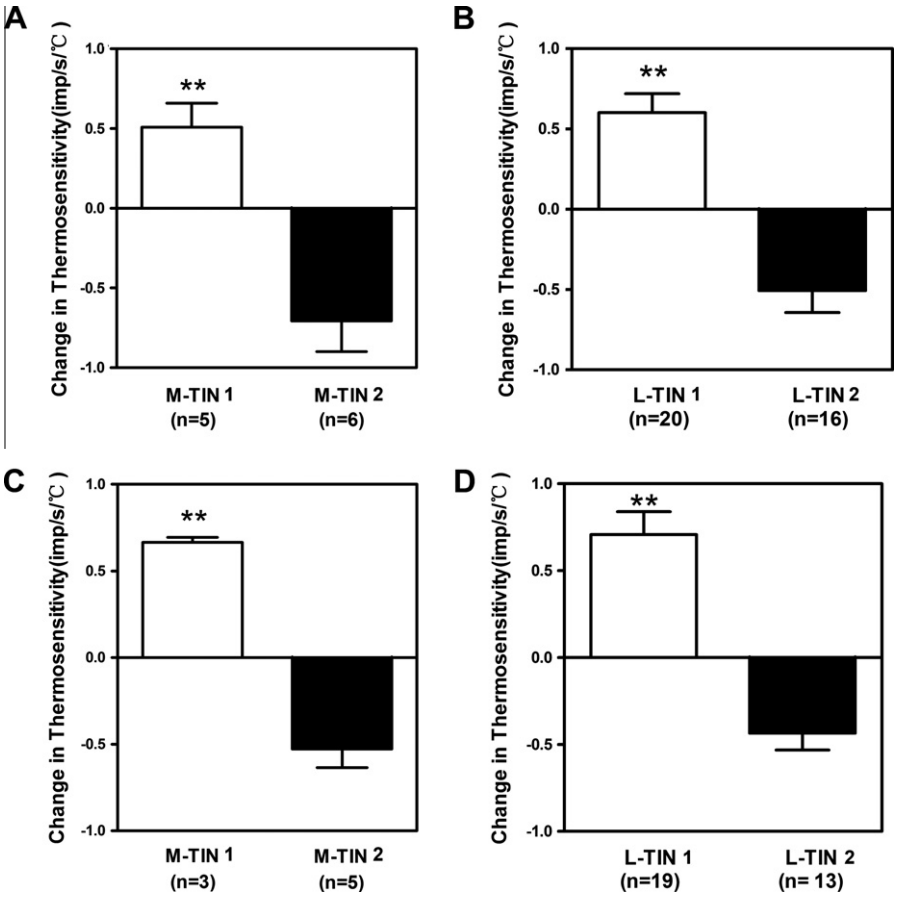

Fig. 7. The changes in thermosensitivity of moderate-slope (A, C) and low-slope temperature-insensitive neurons (B, D) during slice were perfused with 1 μM AVP (top) and V_{1a} vasopressin receptor antagonist (bottom). Change in thermosensitivity = thermosensitivity during AVP or V_{1a} ant − control thermosensitivity. TIN 1 represents thermosensitivity-increased neurons, and TIN 2 represents thermosensitivity-decreased neurons. Bars represent change in thermosensitivity, and the error bars represent SE. $^{**}P < 0.01$.

the depolarizing prepotential's rate of temperature-insensitive neurons (Fig. 8C), even though it excited those neurons. Thus, there is evidence that the AVP or V_{1a} vasopressin receptor antagonist can affect ionic currents

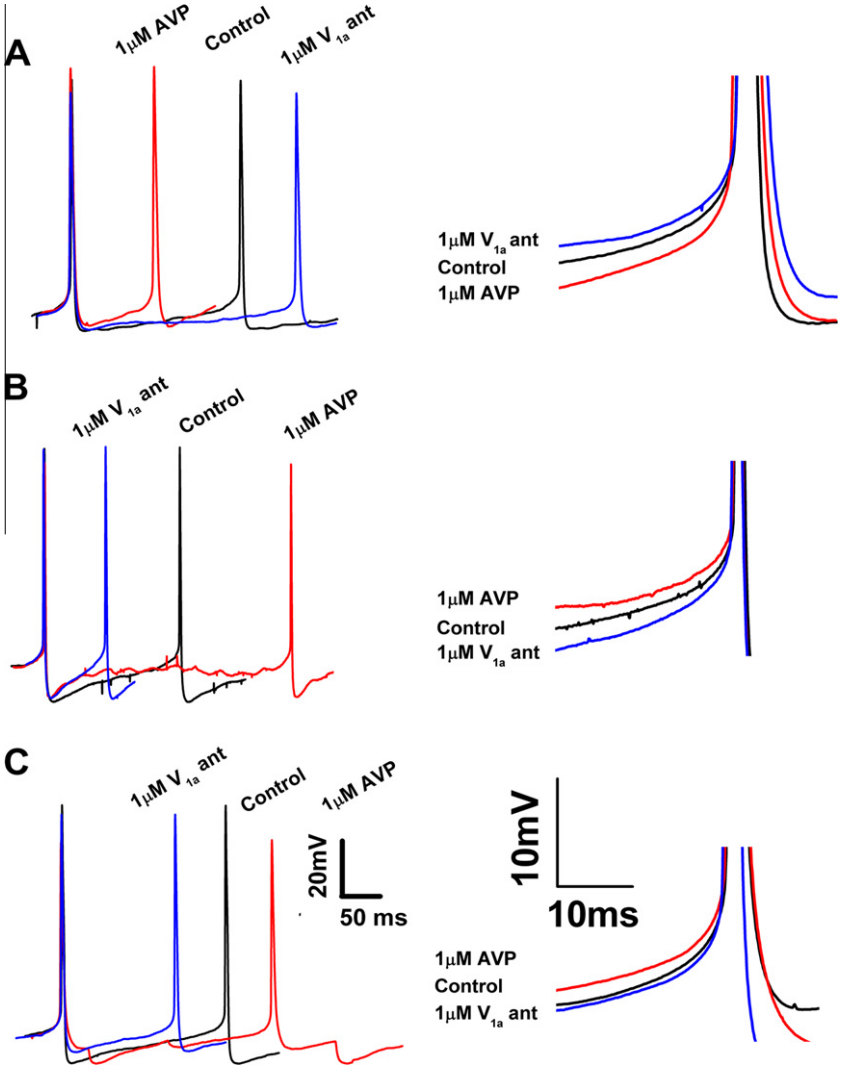

Fig. 8. Effects of AVP and V_{1a} vasopressin receptor antagonist on the depolarizing prepotential in three different types of PO/AH neurons. Left: superimposed single records of action potentials before and during perfusion with AVP and V_{1a} ant. Right: superimposed averaged prepotentials. AVP caused an increase in the depolarizing prepotential's rate of warm-sensitive neurons (A) and a decrease of cold-sensitive neurons (B). The effect of V_{1a} ant was opposite with AVP for temperature-sensitive neurons. AVP decreased the depolarizing prepotential's rate of temperature-insensitive neurons (C). However, V_{1a} ant did not affect the depolarizing prepotential's rate of temperature-insensitive neurons. The neuron (A, B, C) in this figure is the same one in Fig. 1A, B, D. Recording temperature was 37 °C.

determining the prepotential, which is important for a determinant of neuronal firing rate, especially in temperature-sensitive neuron.

DISCUSSION

In this study, we investigated the effect of AVP on firing activity and thermosensitivity of rat PO/AH area neurons by whole-cell patch clamp. Our data demonstrate that AVP increased the firing rate and thermosensitivity of warm-sensitive neurons while decreasing those of cold-sensitive neurons. These changes are partly due to the fact that AVP enhances the rise rate of depolarization prepotential in warm-sensitive neurons and reduces it in cold-sensitive neurons. V_{1a} vasopressin receptor antagonist could decrease or invert these effects. The percentage of increase and decrease for the temperature-insensitive neurons' proportion of firing rate and changes in thermosensitivity are almost equivalent during AVP or V_{1a} vasopressin receptor antagonist perfusion. These results suggest that the mechanisms that AVP moderates, the firing rate and thermosensitivity of temperature-sensitive and -insensitive neurons may be different, and V_{1a} receptor is involved in regulating firing activity and thermosensitivity of neurons.

The AVP acts both as a hormone and as a neurotransmitter/neuromodulator. As a hormone, it targets organs including kidney and blood vessels; as a neurotransmitter/neuromodulator, AVP plays a role in autonomic functions, such as temperature regulation and cardiovascular regulation (Raggenbass, 2008). AVP could induce the hypothermia response (Steiner et al., 1998; Paro et al., 2003). Whereas with administration of V_1 vasopressin receptor antagonist, body temperature increases (Steiner

et al., 1998). It is well known that the PO/AH is an important neural region regulating body temperature by heat production and heat loss responses. The firing activity of PO/AH neuron in the present study was recorded by whole-cell patch clamp. Compared with extracellular recording, whole-cell recording can obtain much more electrophysiological parameter of action potential, such as afterhyperpolarization potential, depolarizing prepotential.

Our result that AVP modifies the firing rate and thermosensitivity of the temperature-sensitive neurons in rat PO/AH area is consistent with that reported by Moravec and Pierau (1994). These authors performed extracellular recording and found that in rat, AVP induced a significant decrease of cold sensitivity in 80% of the cold-sensitive neurons, and a significant increase of the temperature sensitivity in 50% of the warm-sensitive neurons in the medial preoptic area. In this study, our data indicate that AVP increases the spontaneous activity and temperature sensitivity in the majority of warm-sensitive neurons and decreases it in most of the cold-sensitive neurons. However, for temperature insensitive neuron, whatever is moderate-slope or low-slope, AVP excites some neurons and inhibits some others. The proportion of AVP-excited and -inhibited neuron is almost equal. These are some differences with previous report that AVP increased the firing rate in 65% of temperature-insensitive neurons (Moravec and Pierau, 1994). The difference may result from the difference of criteria defining the temperature-sensitive type. In Moravec's study, neurons with a positive or negative value of $m \geqslant 0.6$ imp s^{-1} $°C^{-1}$ were classified as warm sensitive or cold sensitive, respectively; all other neurons were regarded as temperature insensitive. But we used a functional criterion of $m \geqslant 0.8$ imp s^{-1} $°C^{-1}$ in this paper. Because according to previous reports *in vivo* recordings, neurons meeting this functional criterion not only responded to local changes in temperature, but also many were directly influenced by afferent thermal input or displayed a correlation between firing rate and the activation of specific thermoregulatory responses (Boulant and Bignall, 1973; Boulant and Hardy, 1974; Ranels and Griffin, 2003). Furthermore, compared with previous reports (Moravec and Pierau, 1994; Wright et al., 2008), our data not only include the effect of AVP on neurons at the neutral temperature (36–38 °C), but also include that at the warm temperature (39–41 °C). These results suggest that the effect of AVP on firing rate of PO/AH neuron is almost the same at the neutral and warm temperatures.

Then, we investigated the physiological role of V_{1a} receptor in regulating firing activity and thermosensitivity of PO/AH area neuron. The V_{1a} receptor is present in liver, platelets, smooth muscle vascular cells, and the central nervous system, including the basal ganglia, the limbic system, the hypothalamus, and the thalamus, but its physiological role in these diverse tissues remains unknown (Birnbaumer, 2000; Raggenbass, 2001). In lateral septal area, AVP appears to act in a dual mode via V_{1a} but not V_{1b} receptors: by causing a direct excitation of a subpopulation of neurons, and by causing an indirect inhibition of virtually all lateral septal neurons (Allaman-Exertier et al., 2007). In the study of Dong et al., ventral septal area regulates the activities of temperature-sensitive neurons in PO/AH through AVP V_1 but not V_2 receptor (Dong et al., 2007). Based on neuron's responses to V_{1a} vasopressin receptor antagonist, the results of our study suggest that temperature-insensitive neurons were functionally classified as two distinct groups. One is V_{1a} vasopressin receptor antagonist-excited and the other is V_{1a} vasopressin receptor antagonist-inhibited. For temperature-sensitive neuron, however, V_{1a} vasopressin receptor antagonist decreased the firing rate of the warm-sensitive neurons and increased the firing rate of the cold-sensitive neurons. These data indicate that the activity of the majority of temperature-sensitive and temperature-insensitive PO/AH neurons appears to be tonic regulated by AVP through activation of V_{1a} vasopressin receptor, since the V_{1a} vasopressin receptor antagonist changes the spontaneous activity and thermosensitivity of temperature-sensitive as well as temperature-insensitive neurons. Treatment with the V_{1a} vasopressin receptor antagonists, the activation of V_{1a} vasopressin receptor in the PO/AH neuron was attenuated while the activation was potentiation by perfusion with AVP. The changes of V_{1a} vasopressin receptor activation in the PO/AH neurons might lead to the changes of firing rate and thermosensitivity of PO/AH neurons. Although these results suggest that V_{1a} receptor maybe participate in the regulation of their firing activity and thermosensitivity, we cannot exclude the possible role of V_{1b} and V_2 receptors. To determine whether the action of AVP is mediated by V_{1a} but not V_{1b} or V_2 receptor, future patch clamp recording studies should compare the effects of AVP and V_{1b}, V_2 receptor agonists or AVP perfused together with V_{1a} vasopressin receptor antagonist on the spontaneous firing activity of PO/AH neurons.

In the present study, horizontal hypothalamic tissue slices contained the PO/AH, the hypothalamic paraventricular nucleus and the choroidal epithelium of the third cerebral ventricle (Dean and Boulant, 1988). AVP is synthesized by neurosecretory cells located predominantly in the hypothalamic supraoptic and paraventricular nuclei. The epithelium of the choroid plexus located in all four cerebral ventricles can produce AVP and during chronic hyperosmotic stress, the choroidal synthesis of AVP is upregulated in a manner similar to that observed in the hypothalamic paraventricular and supraoptic nuclei (Chodobski et al., 1997; Szmydynger-Chodobska et al., 2011). These previous reports suggest that the brain slice may contain vasopressin neurons that can synthesise and release the AVP at least in the choroidal epithelium located in the third cerebral ventricle or in the paraventricular nucleus. Neuropeptide that is released from dendrites, such as AVP, functions as autocrine or paracrine signals at their site of origin (Ludwig and Leng, 1997). On the one hand the paracrine signals may diffuse locally from the hypothalamic paraventricular nucleus to PO/AH. On the other hand, warm-sensitive neurons in PO/AH oriented their dendrites perpendicular to the third ventricle, with medial dendrites directed toward the periventricular region (Griffin et al., 2001). So the medial dendrites of warm-sensitive neurons directed toward the

third ventricle or periventricular region maybe could receive the signal of AVP released by choroidal epithelium located in the third cerebral or neurosecretory cells located in the paraventricular nucleus. To further determine whether vasopressin neurons locate in this brain slice, future studies of immunohistochemistry and *in situ* hybridization histochemistry are required.

According to Hammel's model, the homeothermia is achieved through integration of a baseline level of nonthermal activation, with both afferent and central thermal information by neurons within the hypothalamus (Ranels and Griffin, 2003). The warm-sensitive and cold-sensitive neurons form with effector neurons controlling specific thermoregulatory responses. Excited warm-sensitive neurons increase the heat loss, whereas excited cold-sensitive neurons increase heat production. It can then be hypothesized that either an increase in the activity of warm-sensitive neurons or a decrease in the activity of cold-sensitive neurons could result in regulation of the body temperature into the hypothermic range. Our electrophysiology studies, in the present paper, present support for this hypothesis. AVP excited the warm-sensitive neurons and inhibited cold-sensitive neurons, thereby increasing heat loss and reducing heat production. These results can explain, at least in part, that AVP induces the hypothermia response *in vivo* (Steiner et al., 1998; Boulant, 2006). Instead, V_{1a} vasopressin receptor antagonist decreased the activity of the warm-sensitive neurons and increased it of cold-sensitive neurons, thereby suppressing heat loss and increasing heat production. These results could indicate a possible mechanism for V_{1a} vasopressin receptor antagonist increasing the body temperature *in vivo* (Steiner et al., 1998).

The importance of temperature-insensitive neurons is to provide a constant, unchanging reference signal to the heat loss and heat production effector neurons. The excitatory synaptic inputs coming from the warm-sensitive neurons are compared with the inhibitory synaptic inputs coming from the temperature-insensitive neurons. This synaptic comparison determines the thermoregulatory responses (Boulant, 2006). Our results that AVP excited warm-sensitive neurons and suppressed partly temperature-insensitive neurons suggest that in *in vivo* studies, AVP maybe increased the excitatory inputs and decreased the inhibitory inputs to effector neurons, in turn, increased heat loss and suppressed heat production. But for some temperature-insensitive neurons being excited by AVP, the results indicate that these neurons probably participate in the thermoregulation by other mechanisms.

Although the mechanisms of neuronal thermosensitivity remain unknown, the depolarizing prepotential is deemed as an important determinant of neuronal warm sensitivity (Curras et al., 1991; Griffin et al., 1996; Burgoon and Boulant, 2001). In the present study, our data suggest that AVP increases the depolarization prepotential's rate of the warm-sensitive neurons and decreases that of the cold-sensitive neurons. But the effect of V_{1a} vasopressin receptor antagonist on rise rate of depolarization prepotential is opposite. These results indicate a possible mechanism, at least partly, explaining the influence of AVP on the firing rate of PO/AH neurons. AVP increases the depolarizing prepotential's rate of warm-sensitive neurons, in turn, shortens the interspike interval and increases firing rate. For cold-sensitive neurons, AVP prolongs the interspike interval and reduces firing rate by decreasing the rate. In the meantime, these data suggest that V_{1a} receptor participates in regulating the firing activity of PO/AH neuron by changing the prepotential's rate of depolarization. The study of Griffin et al. has noted that the A-current exists in all types of PO/AH neurons, including warm-sensitive, temperature-insensitive neurons (Griffin et al., 1996). The A current is a transient outward potassium current that hyperpolarizes the membrane after each action potential. Warming will increase inactivation of A-current and result in the increase of prepotential's rate of depolarization, thus increasing the firing rate of warm-sensitive neurons (Zhao and Boulant, 2005). Hence, we conjecture that AVP could suppress A-current to increase depolarization rate of prepotential of warm-sensitive neurons.

The topic of a mechanism underlying neuronal thermosensitivity is still controversial. Recent studies propose that thermosensitivity of the neuron is due to thermally induced changes in persistent, inward, cationic currents that determine the resting membrane potential, especially by transient receptor potential vanilloid (TRPV) channels (Voets et al., 2004; Zhang et al., 2006). It has been verified that the TRPV1 gene is required for thermosensory transduction and anticipatory secretion from vasopressin neurons during hyperthermia (Sharif-Naeini et al., 2008). Some previous reports indicate that PO/AH neuronal thermosensitivity is due to a warm-induced membrane depolarization caused by the non-inactivating, inward sodium current (Kiyohara et al., 1990; Kobayashi and Takahashi, 1993). Immunohistochemical results suggest that PO/AH neurons exist in hyperpolarization-activated cyclic nucleotide-gated channels (HCN), background potassium leak channels (TASK-1 and TRAAK), and transient receptor potential channel (TRP) TRPV4. Based on these studies, Hodgkin-Huxley-like models were constructed by Wechselberger et al. (2006). These models suggest that most PO/AH neurons have the same types of ionic channels, but different levels of channel expression can explain the inherent properties of the various types of temperature-sensitive and insensitive neurons. The study of Zhang et al. reported that AVP not only decreased an inwardly rectifying potassium conductance and hyperpolarization-activated current (I_h), but also increased the low-threshold spikes (LTSs) in the rat thalamic paraventricular nucleus neurons (Zhang et al., 2006). Hence, further investigations are required in order to demonstrate whether AVP can affect A-current or other ion channel currents that underlie the thermosensitivity of PO/AH neurons.

The present study did not block synaptic activity, so it could contribute to the measured action potentials and firing rate. In PO/AH neurons, postsynaptic potentials have short durations and frequencies of less than 4 events s^{-1}, the contribution of postsynaptic activity to the measurements would be minimal in control (Griffin et al., 2001). AVP, however, greatly increased GABAergic inhibitory

input in lateral septal, hypothalamic paraventricular nucleus neurons and developing hypoglossal motoneurons, act via V_{1a} receptor (Hermes et al., 2000; Reymond-Marron et al., 2005; Allaman-Exertier et al., 2007). Meantime, AVP inhibited the glutamate release via two distinct modes in the brainstem (Bailey et al., 2006) and ionotropic receptors of L-glutamate in the central nervous system participated in peripheral AVP-induced hypothermia (Paro et al., 2003). Therefore, it is likely that synaptic activity that is modulated by AVP contributed to the results reported here. We conjecture that AVP could increase GABAergic inhibitory input in cold-sensitive neurons of the PO/AH area, thereby suppressing neural firing activity. Hence, further investigations are required in order to confirm whether AVP, acting via V_{1a} but not V_{1b} receptors, modulates the excited or inhibited synaptic activity in PO/AH neurons.

CONCLUSION

In response to AVP perfusion, the local AVP within brain slices results in a thermosensitivity-dependent change in the firing rates of PO/AH neurons. Through a direct effect on the rise rate of depolarizing prepotential, which is a determinant of neural firing rates and thermosensitivity, AVP excites warm-sensitive neurons and inhibits cold-sensitive neurons in the PO/AH area by acting on V_{1a} receptors. The results of the present study provide a direction for further investigation on the conductance and synapse transmission mechanisms of AVP effect. Meanwhile, it supports a novel mechanism to explaining AVP-induced hypothermia.

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