Histamine-Induced Excitatory Responses in Mouse Ventromedial Hypothalamic Neurons: Ionic Mechanisms and Estrogenic Regulation

Jin Zhou, Anna W. Lee, Nino Devidze, Qiuyu Zhang, Lee-Ming Kow, and Donald W. Pfaff

Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, New York

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Zhou J, Lee AW, Devidze N, Zhang Q, Kow L-M, Pfaff DW. Histamine-induced excitatory responses in mouse ventromedial hypothalamic neurons: ionic mechanisms and estrogenic regulation. J Neurophysiol 98: 3143–3152, 2007. First published October 17, 2007; doi:10.1152/jn.00337.2007. Histamine is capable of modulating CNS arousal states by regulating neuronal excitability. In the current study, histamine action in the ventromedial hypothalamus (VMH), its related ionic mechanisms, and its possible facilitation by estrogen were investigated using whole cell patch-clamp recording in brain slices from ovariectomized female mice. Under current clamp, a bath application of histamine (20 μM) caused membrane depolarization, associated with an increased membrane resistance. In some cells, the depolarization was accompanied by action potentials. Histamine application also significantly reduced the latency of action potential evoked by current steps. Histamine-induced depolarization was not affected by either tetrodotoxin or Cd2+. However, after blocking K+ channels with tetraethylammonium, 4-aminopyridine, and Cs+, depolarization was significantly decreased. Under voltage clamp, histamine-induced depolarization was associated with an inward current. The current–voltage relationship revealed that this inward current reversed near EK. The histamine effect was mimicked by a histamine receptor 1 (H1) agonist, but not a histamine receptor 2 (H2) agonist. An H1 antagonist, but not H2 antagonist, abolished histamine responses. When ovariectomized mice were treated with estradiol benzoate (E2), histamine-induced depolarization was significantly enhanced with an increased percentage of cells showing action potential firing. These results suggest that histamine depolarized VMH neurons by attenuating a K+ leakage current and this effect was mediated by H1 receptor. E2 facilitated histamine-induced excitation of VMH neurons. This histamine effect may present a potential mechanism by which estrogens modulate the impact of generalized CNS arousal on a sexual arousal–related neuronal group.

INTRODUCTION

The ventromedial nucleus of the hypothalamus (VMH) is a cell group involving a wide range of neuroendocrinological brain functions (Pfaff et al. 2002). In particular, VMH is closely involved in lordosis and sexual arousal. Its roles in governing sexual behavior and related hormone regulation have been extensively studied in our laboratory (Kow and Pfaff 1998; Pfaff 1980, 1999; Zhou et al. 2005). In exploring the molecular mechanisms underlying VMH-mediated arousal, several neurotransmitter systems have emerged in the literature. For example, at least three neurotransmitters signaling generalized CNS arousal affect electrical activity in VMH neurons that are essential for normal lordosis behavior and sexual arousal. They are histamine, norepinephrine, and the opioid peptide enkephalin (Lee et al. 2006). As one of the major arousal-related neurotransmitter systems (Pfaff 2006), histamine is produced by neurons in the tuberomammillary nucleus of the hypothalamus. The efferent fibers of histaminergic neurons project widely throughout the brain (Schwartz et al. 1991; Takada et al. 1987). High densities of histaminergic fibers are found in the hypothalamus, with all nuclei including VMH receiving a strong or moderate innervation (Martinez-Mir et al. 1990; Terao et al. 2004). In CNS, histamine is synthesized from histidine by a specific enzyme, histidine decarboxylase (HDC), and signals through three receptor subtypes: histamine receptor 1, 2, and 3 (H1, H2, and H3). All histamine receptor subtypes present in the CNS were found in the hypothalamus (Brown et al. 2001; Schwartz et al. 1991), with high densities of H1 receptors in the VMH (Bouthenet et al. 1988; Palacios et al. 1981). In fact, histamine acting in the VMH can increase lordosis behavior (Donoso and Broitman 1979).

Histamine functions detected as neurotransmitter-like or neuromodulator-like have been identified in many brain areas. A typical action of histamine is that it excites neurons by producing a depolarization and a subsequent increase in firing frequency (Brown et al. 2001). The mechanisms underlying histamine effects are diverse, depending on brain area and neuronal type (Haas and Panula 2003). The ionic mechanisms include the inhibition of a background potassium conductance (Li and Hatton 1996; McCormick and Williamson 1991), stimulation of a chloride current (Starodub and Wood 2000), stimulation of a nonspecific cation channel or electrogenic Na+/Ca2+ exchanger (Smith and Armstrong 1996), and an increase of intracellular calcium levels (Leopoldt et al. 1997; Leurs et al. 1994). Different histamine receptor subtypes are involved in these diverse mechanisms.

Even though histamine actions have been studied in the VMH (Alvarez and Donoso 1981; Jang et al. 2001; Kow et al. 2005) and the literature indicates its potential in modulating VMH functions, such as sexual behavior (Donoso and Broitman 1979) and feeding behavior (Aou et al. 1995; Magrani et al. 2004; Sakata and Yoshimatsu 1995), electrophysiological characterization of histamine action in the VMH is very limited. In our current study, we used whole cell patch-clamp recording to investigate the effect of histamine on VMH neurons. Our particular interest highlighted its ionic and receptor mechanisms. Because estrogens act as a crucial modulator in VMH-related sexual arousal function, we wondered whether the general arousal function of histamine would interact with the modulator effect of estrogens on sexual arousal in the...
VMH. Thus by comparing histamine effects on VMH neurons from estrogen-versus vehicle-treated animals, the possible action of estrogen treatment on histaminergic function was examined. We found that histamine increased the excitability of VMH neurons as indicated by membrane depolarization and increased firing rate. This effect is produced by the inhibition of potassium leakage currents through the H₁ receptor. Estrogen treatment facilitated histamine-induced excitation, indicating a potential interaction between general arousal and specific sexual arousal functions.

METHODS

Slice preparation

Four- to 5-wk-old, ovariectomized Swiss-Webster female mice (Taconic Farm, Hudson, NY) were used to prepare VMH brain slices. After mice were deeply anesthetized by intraperitoneal injection of urethane (40%, 1–1.5 g/kg), brains were rapidly removed and placed in an ice-cold oxygenated slicing solution consisting of (in mM): 210 sucrose, 3.5 KCl, 1 CaCl₂, 4 MgCl₂, 1.25 NaH₂PO₄, and 10 Na-glucose, pH 7.3. Coronal brain slices (250 µm in thickness) containing VMH were prepared using a vibratome (Leica VT1000, Wetzlar, Germany). Slices were then transferred to a room-temperature oxygenated bath solution and allowed to recover ≥1 h at room temperature before electrophysiology recording.

During the estrogen effect study, ovariectomized mice received daily injections of either estradiol benzoate [E₂, 10 µg/0.1 ml oil, administered subcutaneously (sc)] or sesame oil (0.1 ml) for 2 days before the recording. The dose of estradiol used achieves proestrous levels of estrogen in the blood, 60–90 pg/ml. All animals were cared for in accordance with the Rockefeller University Animal Care and Use Committee protocol.

Electrophysiology

Whole cell patch-clamp recordings were performed at room temperature (22–25°C) from VMH slices using a MultiClamp 700A amplifier (Axon Instruments, Foster City, CA). Patch pipettes were pulled from thin-walled borosilicate glass pipettes (Warner Instrument, Hamden, CT) and had a resistance of between 3 and 5 MΩ. The recording chamber was continuously perfused with an artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 5 KCl, 2 MgCl₂, 2 CaCl₂, 10 glucose, 1.25 NaH₂PO₄, and 26 NaHCO₃. ACSF was aerated with 95% O₂-5% CO₂ to a final pH of 7.3 (osmolarity 300–310 mOsM). The internal pipette solution contained (in mM): 140 K-glucolate, 10 HEPES, 0.6 NaHCO₃, 2 KCl, 1 CaCl₂, 2 MgATP, 2 Na₂ATP, 0.3 Na₂GTP, 8 sucrose, and 5 EGTA (pH 7.3, osmolarity 285–290 mOsM). The series resistance was typically 10–20 MΩ, which was frequently checked during and at the end of the recording. Data were not included if changes were >30% from the starting series resistance. The holding potential for the current clamp was set at −55 mV. Data were acquired by pCLAMP 9.0 software and analyzed by Clampfit software (Axon Instruments).

Drugs and solutions

When the ionic mechanism was examined, tetrodotoxin (TTX, 0.5 µM) or CdCl₂ (100 µM) were added to the ACSF to block Na⁺ or Ca²⁺ channels. To block the K⁺ channel, tetraethylammonium (TEA, 20 mM) and 4-aminopyridine (4-AP, 5 mM) were added to ACSF and the K⁺ in the pipette solution was replaced by Cs⁺ (120 mM). When required, 1,2-bis (2-aminophenoxo) ethane-N,N',N'',N'''-tetracetic acid (BAPTA, 11 mM) was added into the internal pipette solution to block Ca²⁺ release from internal Ca²⁺ storage. When Ca²⁺-free ACSF was required, Ca²⁺ in the ACSF was replaced by the equally molar Mg²⁺.

Histamine, betahistine (H₁ receptor agonist, 100 µM; Tocris), mepyramine (H₁ receptor antagonist, 1 µM; Tocris), dimaprit (H₂ receptor agonist, 50 µM), and cemitidine (H₂ receptor antagonist, 30 µM) were applied by bath perfusion to the slices in respective experiments. All drugs were purchased from Sigma except as indicated. All drugs were diluted in fresh ACSF to final concentration before experiments.

Statistical analysis

Data are represented as means ± SE. Statistical analysis was accessed using different statistical tests in different experiments. Student’s paired t-test was used when drug effects were compared on the same neuron; Mann–Whitney U test was used to compare histamine responses between oil- and E₂-treated neurons; the chi-square test was used to compare action potential firing between oil- and E₂-treated neurons. P < 0.05 was taken to indicate statistical significance.

RESULTS

In our study, the recordings were acquired from cells located in the ventrolateral part of the VMH, where estrogen receptors are highly expressed (Li et al. 1993; Mitra et al. 2003; Pfaff and Keiner 1973). These neurons can be generally classified as midsize, multidendritic neurons. A total of 171 neurons from 55 different mice were collected in this study. The average resting membrane potential of these cells was −59.2 ± 0.6 mV.

Histamine-induced responses in ventromedial hypothalamic neurons

We first examined the effect of histamine on the membrane potential of VMH neurons under current-clamp conditions. Figure 1A1 shows a typical example of membrane potential recorded from VMH neurons under current-clamp conditions. Membrane potentials were usually held at −55 mV. Bath applications of histamine (20 µM, 2 min) reversibly depolarized every VMH neuron recorded. The depolarization started within 10 s after histamine was delivered into the recording chamber and lasted 3 to 6 min, after which the cells repolarized to holding potential during washout. To estimate the membrane resistance, hyperpolarizing current steps (−100 pA, 150-ms duration, 3-s interstep interval) were applied before, during, and after histamine application. Figure 1A2 shows an example in which a reversible increase in membrane resistance was observed with histamine application (baseline: 135 ± 25 MΩ; histamine: 165 ± 30 MΩ; n = 6, P < 0.05). In all 12 neurons recorded, the membrane potential was significantly depolarized by histamine application (5.2 ± 0.6 mV, n = 12, P < 0.001; Fig. 1A3). The depolarization was accompanied by action potential firing in 3 of 12 cells. Under voltage-clamp conditions (with holding potential at −55 mV), histamine application produced an inward current (10.9 ± 1.5 pA, n = 20, P < 0.001; Fig. 1B1 and B2) with the same time course as the depolarization shown in the current clamp. Therefore histamine-induced depolarization is associated with an inward current and increased membrane resistance.

A membrane depolarization accompanied by an increase in membrane resistance can potentially increase the excitability of these neurons by bringing the membrane potential closer to the action potential threshold and increasing the responsiveness of
Histamine-induced membrane depolarization and inward current in ventromedial hypothalamic (VMH) neurons. A1: sample of a continuous whole cell current-clamp recording from a VMH neuron showing reversible membrane depolarization with 20-μM histamine application. Holding potential was −55 mV. A2: sample of a continuous current-clamp recording from another VMH neuron showing membrane depolarization and concomitant action potential firing. Negative deflections were produced by hyperpolarizing rectangular wave current steps (−100 pA, 150-ms duration, 3-s interstep interval) injected before, during, and after histamine application. A reversible increase in membrane resistance was observed during histamine application indicated by the increase in amplitude of electrotonic potentials (negative deflections of the records). A3: summary graph illustrating membrane potential before (baseline) and during histamine application in VMH neurons. Mean value (n = 12) of baseline membrane potential and the peak membrane potential in response to histamine are plotted. Error bars represent SE. B1: sample of a continuous whole cell voltage-clamp recording showing histamine-induced inward current. B2: summary graph illustrating membrane current before and during histamine application. Mean value (n = 20) of baseline membrane current and the peak inward current in response to histamine are shown (means ± SE).

Ionic mechanisms underlying histamine-induced depolarization

We then investigated the ionic basis for histamine-induced depolarization. In this experiment, histamine response was tested when neurons were preperfused with different channel blockers. Shown in Fig. 3A, when VMH neurons were perfused with ACSF containing TTX (0.5 μM), Cd<sup>2+</sup> (100 μM), or combined TTX and Cd<sup>2+</sup>, respectively, histamine still depolarized the neurons. Therefore it is unlikely that Na<sup>+</sup> and Ca<sup>2+</sup> channels were involved in histamine-induced depolarization. However, after blocking K<sup>+</sup> channels with TEA (20 mM) and 4-AP (5 mM) in the ACSF solution and Cs<sup>+</sup> (120 mM) in the pipette solution, histamine failed to induce depolarization. Intracellular Ca<sup>2+</sup> from the endoplasmic reticulum has been shown to play an important role in mediating histamine signaling (Brown et al. 2001). To test Ca<sup>2+</sup> dependence in histamine responses, calcium chelator BAPTA (11 mM) was added to the pipette solution and extracellular Ca<sup>2+</sup> in ACSF was replaced by an equal molarity of Mg<sup>2+</sup>. As shown in Fig. 3A, BAPTA and Ca<sup>2+</sup>-free ACSF did not affect histamine-induced depolarization. Statistical analysis (Fig. 3B) suggests that only K<sup>+</sup> channel blockers significantly reduced histamine-induced membrane responses (0.1 ± 0.1 mV; ACSF control, 4.2 ± 1.1 mV; n = 6; P < 0.01). Neither TTX (3.1 ± 0.6 mV; ACSF control, 3.8 ± 0.5 mV; n = 8; P = 0.342), Cd<sup>2+</sup> (3.1 ± 0.7 mV; ACSF control, 3.6 ± 0.6 mV; n = 7; P = 0.308), nor the combined TTX and Cd<sup>2+</sup> (2.9 ± 0.5 mV; ACSF control, 3.7 ± 0.9 mV; n = 9; P = 0.461) inhibited the histamine-induced depolarization. Moreover, neither BAPTA (3.0 ± 0.6 mV; control, 3.8 ± 0.6 mV; n = 8; P = 0.195) nor BAPTA combined with Ca<sup>2+</sup>-free ACSF (4.3 ± 0.6 mV; control, 3.8 ± 0.5 mV; n = 9; P = 0.436) had significant effect on histamine-induced stimuli compared with the threshold value of 20.0 ± 6.0 pA in the presence of histamine. Therefore histamine significantly decreased the stimulus strength required to evoke an action potential (P < 0.01; Fig. 2B). In addition, at each current step above threshold, action potentials were evoked more rapidly during histamine application compared with those under control conditions (Fig. 2A). The change in excitability can also be seen in a plot of stimulus strength versus latency of action potential under each condition (Fig. 2C). The action potential latency represents the time between the onset of the current stimulus and the first action potential induced. The leftward shift of the latency curve in the presence of 20 μM histamine indicates that histamine accelerates the firing of action potentials given the same stimulation strength. These results suggest an increased sensitivity of VMH neurons in the presence of histamine, so that previously subthreshold stimuli become superthreshold.
induced depolarization. These results suggest that the ionic mechanism underlying histamine responses is likely mediated through K⁺ currents.

To take a closer look at the ionic basis underlying histamine responses, current–voltage relationships (I–V) were recorded before and during histamine application (Fig. 4A). I–V relationships revealed a decrease in the slope of the I–V in the presence of histamine, indicating a decrease in membrane conductance. Subtracting I–V relationships obtained in the presence of histamine from those obtained under baseline conditions revealed that histamine induced an inward current that decreased as membrane potential was hyperpolarized, and had a reversal potential of ~90 mV, which is close to K⁺ equilibrium potential (E_K) (Fig. 4B). The inward current was linear against membrane potential and without voltage dependence, which is similar to the background/leaking potassium current (I_KL) described in many cell preparations (Jafri et al. 1997; Li and Hatton 1996; McCormick and Williamson 1991). Together, these results suggest that histamine may increase VMH neuron excitability by blocking a relatively linear potassium current (I_KL) that contributes substantially to the normal leak membrane conductance.

**Histamine action in VMH neurons is by H₁ receptor activation**

Both H₁ and H₂ receptors have been indicated to involve histamine effect on neuronal activity (McCormick and Williamson 1991). To test which particular histamine receptor mediates histamine-induced response in VMH neurons, agonists of H₁ and H₂ receptors were applied to the VMH neuron. Figure 5A shows that a bath application of H₁ receptor agonist betahistine (100 μM) depolarized VMH neurons in a similar way as did histamine. However, when H₂ receptor agonist dimaprit (50–100 μM) was applied through a bath perfusion, no membrane depolarization was observed. Moreover, when histamine (20 μM) was applied in the presence of H₂ antagonist mepyramine (1 μM), histamine failed to induce depolarization. On the other hand, application of H₂ antagonist cimetidine (50 μM) did not affect histamine-induced depolarization. A histogram (Fig. 5B) shows that betahistine (4.1 ± 0.5 mV; histamine control, 3.1 ± 0.5 mV; n = 12; P = 0.118), but not dimaprit (1.0 ± 0.4 mV; histamine control, 3.8 ± 0.6 mV, n = 13; P < 0.01), has a membrane depolarization effect similar to that of histamine; mepyramine (0.4 ± 0.2 mV; histamine control, 3.2 ± 0.7 mV; n = 8; P < 0.001), but not cimetidine (5.0 ± 1.0 mV; histamine control 3.8 ± 0.7 mV; n = 7; P > 0.3), significantly blocked histamine response. These results indicate that histamine-induced membrane depolarization in VMH neurons was mediated by the H₁ receptor.

**Estrogen treatment enhanced histamine-induced response in VMH neurons**

Estrogen action in the VMH has been intensively studied for its regulatory function in neuroendocrinologic processes. Our previous study (Zhou et al. 2005) showed that estrogen treatment modulated neuronal network activity by increasing spontaneous activity in cultured VMH neurons from female rats. As a generalized arousal neurotransmitter, histamine was shown earlier to trigger membrane depolarization and increased excitability. Previous study in our lab found bath applications of estrogen potentiated histamine-induced spikes in VMH neurons (Kow et al. 2005). We then examined the effect of...
estrogen treatment on the response of VMH neurons to histamine. Ovariectomized (OVX) animals received either estradiol benzoate (E2, 10 μg/0.1 ml, sc) or vehicle (sesame oil) 48 h before experiments. Figure 6A1 shows current-clamp recordings of histamine response in VMH neurons from oil-treated and E2-treated animals. In neurons from E2-treated animals, the amplitude of histamine-induced depolarization is higher.

FIG. 3. Histamine-induced membrane depolarization is blocked by potassium channel blocker. A: samples of continuous whole cell current-clamp recording from VMH neurons showing histamine response in the presence of different channel blockers and Ca\(^{2+}\) chelator. VMH neurons were pretreated with tetrodotoxin (TTX, 0.5 μM), Cd\(^{2+}\) (100 μM), tetraethylammonium (TEA, 20 mM) plus 4-aminopyridine (4-AP, 5 mM), 1,2-bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA, 11 mM), and Ca\(^{2+}\)-free ACSF, respectively, and their membrane potential responses to histamine (20 μM) were shown. Histamine-induced membrane depolarization was abolished by TEA and 4-AP (with Cs\(^{+}\) internal solution), but not affected by TTX, Cd\(^{2+}\), BAPTA, and Ca\(^{2+}\)-free ACSF. B: summary histogram illustrating the pharmacological profile of histamine-induced depolarization in the presence of different channel blockers and Ca\(^{2+}\) chelator. Only potassium channel blocker significantly reduced histamine effect (n = 6, P < 0.01). Data shown as means ± SE.

FIG. 4. Potassium current underlying histamine-induced membrane responses in VMH neurons. A: plot of current–voltage (I–V) relationships before (baseline) and during histamine application. I–V relationships reveal a decrease in the slope of the I–V relationship in the presence of histamine, indicating a decrease in membrane conductance. Note the reversal potential is around −90 mV, close to that for potassium ions under our recording conditions. A reversal potential near −90 mV.
data shown as means ± SE.

Figure 6A shows that the histamine-induced depolarization was significantly increased (7.9 ± 0.8 mV, n = 17; P < 0.01) in neurons from E2-treated animals compared with that of oil-treated animals (4.7 ± 0.5 mV). Out of 17 neurons recorded in each group, only 5 neurons from the oil-treated group showed action potential firing during histamine-induced depolarization. In the E2-treated group, 12 of 17 neurons showed action potential firing. As shown in Fig. 6A3, the percentage of cells showing action potential firing during histamine application was significantly higher in the E2-treated group (70.6%) compared with the oil-treated group (29.4%, n = 17; \( \chi^2 = 5.764, P < 0.05 \)). Under voltage-clamp conditions, the amplitude of inward current produced by histamine application was higher in neurons from E2-treated animals than in those from oil-treated animals (Fig. 6B1). Statistical analysis (Fig. 6B2) showed that histamine-induced inward currents were significantly increased in E2-treated neurons (14.8 ± 1.4 pA) compared with oil-treated neurons (9.2 ± 1.0 pA; n = 18; P < 0.01).

We have shown that histamine application increased the excitability of VMH neurons by decreasing the latency of action potential to depolarizing stimuli (Fig. 2). To compare the effect of histamine application on the action potential latency between neurons from oil- and E2-treated animals, a series of depolarizing current steps were applied to current-clamped neurons before and in the presence of histamine. As shown in Fig. 7A, during histamine application, the action potential latency was decreased in neurons from oil-treated animals (P < 0.001, Fig. 7B). Statistical analysis (Fig. 7B) showed that histamine-induced latency changes were significantly higher in neurons from E2-treated animals than in those from oil-treated animals. Interestingly, in neurons from E2-treated animals (Fig. 7B) there was no difference in terms of the action potential latency at baseline level compared with neurons from oil-treated animals. However, during histamine application, the action potential latency was further decreased in neurons from E2-treated animals than in those from oil-treated animals. After normalization with their baseline action potential latency, histamine-induced latency changes were significantly higher in neurons from E2-treated animals than in those from oil-treated animals (P < 0.001, Fig. 7C). These data suggested that E2 treatment facilitates histamine’s effect of increasing excitability of the VMH neurons.

**DISCUSSION**

Among arousal-related neurotransmitters, histamine appears to be a potential candidate due to its strong ability in regulating neuronal excitability and related behaviors in a broad range of brain areas (Haas and Panula 2003). Although it has long been shown that the VMH has abundant histaminergic innervations and high densities of histamine receptors (Martinez-Miri et al. 1990; Terao et al. 2004), there are few functional studies about how histamine modulates VMH neuronal activity and its possible role in histamine regulation on VMH-related arousal behavior. Here, our study provides evidence of histamine modulation on the electrophysiological properties of VMH neurons. We found that histamine increased the excitability of VMH neurons by producing a membrane depolarization and increased firing frequency. This action was through the inhibition of a potassium leaking current and mediated by H1 receptors.
Importantly, histamine response in VMH neurons is facilitated by estrogen treatment, demonstrating a possible interaction between general arousal and specific sexual arousal systems at the cellular level.

**Histamine enhanced excitability of VMH neurons by membrane depolarization and increased firing frequency**

As one of the neurotransmitter systems related to brain arousal (Pfaff 2006), histaminergic neurons are featured by their abundant efferent fibers to almost all brain areas. In many neuronal networks, histamine showed powerful excitatory action on neuronal activity by depolarizing neurons. Depolarization, a typical response to histamine, has been shown not only in areas of the CNS such as the cortex (Reiner and Kamondi 1994), the thalamus (McCormick and Williamson 1991), and the hypothalamic nucleus (Smith and Armstrong 1996), but also in peripheral nervous systems such as sympathetic preganglionic neurons (Whyment et al. 2006) and intracardiac neurons (Hardwick et al. 2006). Depending on different brain areas and neuronal types, histamine-induced depolarization can mediate important local functions. For example, in the supraoptic nucleus, histamine-induced depolarization might be related to increased vasopressin release (Dogterom et al. 1976; Li and Hatton 1996; Smith and Armstrong 1996).

As one of the neuroendocrine functions managed by VMH neurons, sexual behavior has been studied for decades in our laboratory (Pfaff 1999). Our data here show that histamine application increasing VMH neuron excitability might be the mechanism through which the arousal-related histamine regulates these VMH-related functions, including lordosis.

**Receptor mechanisms of histamine-induced depolarization in VMH neurons**

Even though histamine-induced depolarization is a common phenomenon in many areas, the mechanism behind it is far from simple and unique. Setting aside the recently cloned ionotropic receptors in insect eyes, histamine has three receptor subtypes (H₁, H₂, and H₃) in CNS that all belong to the G-protein–coupled receptor family. These histamine receptors are coupled to different types of G proteins and various second-messenger pathways. This fact could explain histamine’s versatile actions. Among histamine receptors, both H₁ and H₂ receptors have been shown to mediate histamine-induced depolarization (Haas and Panula 2003). Compared with more limited distribution of H₂ receptors (Vizuete et al. 1997), H₁ receptors have a widespread distribution throughout the brain, with especially high densities in areas of the hypothalamus such as the preoptic area, the ventromedial, and most posterior nuclei (Bouthenet et al. 1988). In examining the receptor mechanism of histamine-induced depolarization in VMH neurons, both agonists and antagonists of H₁ and H₂ receptors were tested. The results suggested that histamine
response was well mimicked by the H\textsubscript{1} receptor agonist and abolished by the H\textsubscript{1} antagonist. In addition, the H\textsubscript{2} receptor agonist could not induce a histamine-like response and the H\textsubscript{2} receptor antagonist failed to block histamine response. Therefore, histamine-induced depolarization in VMH neurons is mediated by the H\textsubscript{1} receptor. This matches that fact that compared with the high density of the H\textsubscript{1} receptor, the H\textsubscript{2} receptor showed only weak density in the hypothalamus.

The primary signal transduction event induced by the H\textsubscript{1} receptor action is the activation of phospholipase C (PLC) by a pertussis toxin-insensitive G\textsubscript{q/11} protein (Leopoldt et al. 1997; Leurs et al. 1994). The activation of the H\textsubscript{1}-receptor–coupled G\textsubscript{q/11} protein leads to the stimulation of PLC, which in turn hydrolyzes phosphatidyl-4,5-biphosphate (PIP\textsubscript{2}) to DAG and IP\textsubscript{3}. IP\textsubscript{3} binds to its own receptors located on the endoplasmic reticulum, allowing the release of stored Ca\textsuperscript{2+} into cytoplasm. Among other signaling pathways activated by H\textsubscript{1} receptor activation, many of them appear secondary to changes in intracellular Ca\textsuperscript{2+} concentration. Ca\textsuperscript{2+} dependence in histamine-induced depolarization in VMH neurons was tested by: 1) applying calcium chelator BAPTA within a pipette solution, 2) using Ca\textsuperscript{2+} channel blocker Cd\textsuperscript{2+} in ACSF, and 3) depriving extracellular calcium by Ca\textsuperscript{2+}-free ACSF. None of these attempts affects histamine response, therefore indicating that calcium is not required in histamine-induced depolarization in VMH neurons.

**Ionic mechanisms of histamine-induced excitability in VMH neurons**

Another notable H\textsubscript{1} receptor signaling is that histamine directly blocks a background/leaking potassium current (I\textsubscript{KL}), which leads to depolarization and/or an increase in firing frequency. I\textsubscript{KL} is characterized by a lack of voltage and time dependence, and with a linear current–voltage relationship (Patel and Honore 2001). Background/leaking potassium se-
lective channels play an essential role in setting the resting membrane potential, tuning the action potential duration, and modulating the responsiveness to synaptic inputs. Regulation of background potassium channels by neurotransmitters and second messengers is central for synaptic function (Belardetti and Siegelbaum 1988; Hawkins et al. 1993). The role of $I_{KL}$ in histamine-induced depolarization has been found in many brain areas such as the cortex (Reiner and Kamondi 1994), thalamus (McCormick and Williamson 1991), hypothalamus (Li and Hatton 1996), and striatum (Munakata and Akaike 1994) and might be the mechanism for antihistamine-induced sedation in the human brain (Reiner and Kamondi 1994). In VMH slices, current–voltage relationships revealed that histamine-induced depolarization was associated with an inward current that reversed near $E_K$. This current displayed a linear relationship with voltage, and therefore was not voltage dependent. These characteristics indicated that the depolarization was due to a decrease in potassium current. The possibility that the observed effect was due to a reduction in chloride conductance was ruled out for two reasons: 1) low chloride ACSF did not affect histamine-induced depolarization and 2) low chloride ACSF did not affect $I-V$ curve changes of histamine response. Therefore decreased background/leaking potassium current by histamine application depolarized cell membranes and increased the excitability of VMH neurons. One feature of leaking potassium channel is that it is insensitive to most classical potassium channel blockers including TEA and 4-AP. Our data showed that TEA and 4-AP, together with Cs, actually blocked depolarization. This finding may suggest the involvement of other types of potassium channels.

**Potential caveats**

These recordings were performed at room temperature. The difference between this temperature and normal body temperature would not only affect chemical kinetics but also might influence histamine dose–response curves and other physiological parameters. However, by recording from VMH neurons over the years we have produced a large body of data on norepinephrine effects in which electrophysiological results gathered at room temperature are consistent not only with each other but also with behavioral results (Kow et al. 1992). Further, in an ongoing whole cell recording study with VMH neurons we found no difference in their responses between room temperature and 34°C. In fact, in our experience, hypothalamic slices do not remain healthy as long when maintained at 36°C. We know that higher bath temperatures would be associated with reduced oxygen solubility, but do not know whether that is the only cause of problems with tissue slice health when maintained at higher temperatures.

**Estrogenic regulation of histamine response in VMH neurons**

We propose that neurons in the ventrolateral corner of the VMH are responsible for the integration of nutritional signals with estrogenic signaling because, together, they influence female reproductive behavior. This proposal makes sense in that it would not be biologically adaptive for females to reproduce at times when they do not have an adequate food supply. Estrogens and histamine are both strongly implicated in feeding and energy metabolism, even though the interactions between estrogen and histamine action are less investigated. Hypothalamically caused obese animals produced by VMH and mammillary nuclei lesions showed very low plasma estrogen and estrogen replacement reduced food intake in those animals (Jacoby et al. 1995). During food restriction, estrogen–receptor–containing cell numbers in the VMH decreased (Hileman et al. 1999), which may represent one mechanism whereby undernutrition enhances the ability of estrogens to foster reproduction. Histamine also modulated feeding behavior through its receptors in the VMH. As shown by Sakata (Sakata and Yoshimatsu 1995), food intake was suppressed and drinking was accelerated by either activation of $H_1$ receptors or inhibition of $H_2$ receptors in the VMH. Pharmacological blockage of both $H_1$ and $H_2$ VMH receptors significantly increased overnight food intake and decreased water intake, which may be specifically attributed to the set of histaminergic receptors situated within the VMH (Magrani et al. 2004).

Estrogens also have long been studied in the VMH for their role in facilitating hormone-dependent mating behaviors. LORDosis behavior is a classic model used to examine estrogenic regulation in sexual behavior (Pfaff 1999). The neurons at the ventrolateral quadrant of the VMH express both estrogen receptors, alpha and beta (Ikeda et al. 2003; Li et al. 1993; Pfaff and Keiner 1973), which mediate estrogen-dependent action in governing lordosis behavior. Several neurotransmitter systems have been implicated in estrogenic regulation of lordosis behavior, such as norepinephrine, acetylcholine, and serotonin (Kow and Pfaff 1985). Notably, Donoso and Broitman (1979) have implicated hypothalamic histamine in lordosis behavior performance. At least two ways were found for estrogen engaging its modulation action in VMH neurons: neuronal resting activity and specific neurotransmitter-induced responses. In VMH cultures (Zhou et al. 2005), estrogen treatment increased spontaneous synaptic events in neurons derived from females. In the meantime, the frequency of miniature inhibitory postsynaptic currents was decreased in these neurons with estrogen application, suggesting that estrogen-induced changes in GABAergic inhibition could at least partially explain estrogen effects on neuronal activity. Estrogen treatment also potentiates excitatory responses of VMH neurons to specific neurotransmitters. Extracellular recording showed that estrogen application potentiated N-methyl-D-aspartate (NMDA) or histamine induced an increased spiking rate even though estrogen itself did not have any significant effect on resting activity (Kow et al. 2005). In our study, estrogen treatment did not change membrane potential itself (data not shown); however, it facilitated histamine-induced depolarization. This result indicates that estrogen has multiple cellular mechanisms in controlling VMH neuronal activity.

Supporting specific types of CNS arousal, such as sexual arousal, are the mechanisms of generalized arousal (Pfaff 2006). This fact raises questions regarding exactly how generalized arousal modulators affect VMH neurons managing sexual behavior, a specific form of arousal-dependent motivated behavior. Histamine-induced responses shown in this study indicate a possible mechanism through which the generalized arousal factor (histamine) can affect neurons in a local brain area (VMH), which is crucial for a specific motivated behavior (lordosis). Furthermore, we addressed this question by exam-
ining whether a general arousal modulator, histamine, interacts with a hormonal modulator, estrogen, in VMH neurons. Our results are remarkable in the sense that they show a possible pivotal point where generalized arousal influences and supports a specific (sexual) arousal state. As a generalized arousal neurotransmitter, histamine’s excitatory impact on VMH neurons was further potentiated by estrogen. Thus the interaction between histamine and estrogen might be a key mechanism linking generalized arousal with specific (sexual) arousal.

REFERENCES


