Acetylcholine enhances excitability by lowering the threshold of spike generation in olfactory receptor cells

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Ohkuma M, Kawai F, Miyachi E. Acetylcholine enhances excitability by lowering the threshold of spike generation in olfactory receptor cells. J Neurophysiol 110: 2082–2089, 2013. first published August 7, 2013; doi:10.1152/jn.01077.2012.—Olfactory perception is influenced by behavioral states, presumably via efferent regulation. Using the whole cell version of patch-clamp recording technique, we discovered that acetylcholine, which is released from efferent fibers in the olfactory mucosa, can directly affect the signal encoding in newt olfactory receptor cells (ORCs). Under current-clamp conditions, application of carbachol, an acetylcholine receptor agonist, increased the spike frequency of ORCs and lowered their spike threshold. When a 3-pA current to induce near-threshold depolarization was injected into ORCs, 0.0 spikes/s were generated in control solution and 0.5 spikes/s in the presence of carbachol. By strong stimuli of injection of a 13-pA current into ORCs, 9.1 and 11.0 spikes/s were generated in control and carbachol solutions, respectively. A similar result was observed by bath application of 50 μM acetylcholine. Under voltage-clamp conditions, carbachol increased the peak amplitude of a voltage-gated sodium current by 32% and T-type calcium current by 39%. Atropine, the specific muscarinic receptor antagonist, blocked the enhancement by carbachol of the voltage-gated sodium current and T-type calcium current, suggesting that carbachol increases those currents via the muscarinic receptor rather than via the nicotinic receptor. In contrast, carbachol did not significantly change the amplitude of the L-type calcium current or the delayed rectifier potassium current in the ORCs. Because T-type calcium current is known to lower the threshold in ORCs, we suggest that acetylcholine enhances excitability by lowering the threshold of spike generation in ORCs via the muscarinic receptor.

action potential; muscarinic receptor; patch clamp; voltage-gated current

Sensory organs are generally modulated by efferent nerves (Hall 2011). In the visual system, efferent inputs to the retina are known to regulate visual sensitivity of rod photoreceptors (Barlow 2001). The efferents also regulate a selective visual attention by modulating the light response of retinal ganglion cells (Uchiyama et al. 1998). In the auditory system, it is proposed that activation of the efferents can alter micromechanical event within the cochlear partition and provide a gain control of the cochlear amplifier (Guinan 1996). Olfactory perception, which starts in the olfactory receptor cells (ORCs) of the olfactory epithelium (Gold and Nakamura 1987; Bakalyar and Reed 1991; Breer and Boekhoff 1992; Ronnett and Snyder 1992; Firestein 1992; Kurahashi and Yau 1994; Restrepo et al. 1996; Hall 2011), is influenced by behavioral states (Takagi 1989). Because the olfactory epithelium is innervated by efferent neurites (Zielinski et al. 1989), olfactory perception may be modulated efferently by neurotransmitters. We have shown that epinephrine or norepinephrine, which is released from sympathetic nerves, enhances odorant contrast in ORCs (Kawai et al. 1999).

The olfactory mucosa is also innervated by cholinergic fibers (Zielinski et al. 1989; Hall 2011), and muscarinic receptors are present in the olfactory epithelium (Hedlund and Shepherd 1983; Hall 2011; Li and Matsunami 2011). Parasympathetic nerve stimulation releases acetylcholine and modulates discharge rates of ORCs (Bouvet et al. 1987). Direct application of acetylcholine to the intact olfactory epithelium also affects spiking activity in the olfactory nerve (Bouvet et al. 1988). These observations raise the possibility that acetylcholine may affect odor responses of ORCs. Recently, it is found that the type 3 muscarinic acetylcholine receptor, which is found in the cilium membrane of ORCs, modulates olfactory responses by calcium imaging (Hall 2011; Li and Matsunami 2011). In the present experiment, we investigated how acetylcholine modulates ORC responses by using the electrophysiological methods, focusing on electrical activity in the somatic membrane of ORCs, because acetylcholine modulates the ciliary membrane of ORCs (Hall 2011; Li and Matsunami 2011) and could also affect the somatic membrane of ORCs. Single ORCs were dissociated from the newt olfactory epithelium, and their spiking activity was investigated by using whole cell patch-clamp recording. We discovered that acetylcholine, which may be released from efferent fibers, enhances excitability by lowering the threshold of spike generation in ORCs.

Materials and methods

Preparation. ORCs were dissociated enzymatically from the olfactory epithelium of the newt, Cynops pyrrhogaster. Dissociation protocols were similar to those reported previously (Kurahashi 1989; Kawai et al. 1996). In short, the animal was chilled on ice and pithed. The mucosae excised from the olfactory cavity were incubated for 10 min at 30°C in a Ringer solution containing 0.1% collagenase (Sigma, St Louis, MO) with no added calcium. The tissue was then rinsed twice and triturated with a normal Ringer solution (in mM): 110 NaCl, 2.6 KCl, 3 CaCl2, 10 HEPES, 10 glucose, and 10 ppm phenol red (pH adjusted to 7.4 with KOH). Isolated cells were plated on the concavalin A-coated glass cover slips. All animal experiments are approved by the Ethics Committee of Fujita Health University.

Recording procedures and statistical analysis. Membrane voltages and currents were recorded in the whole cell-recording configuration (Hamill et al. 1981). The recording pipette was filled with pseudointracellular (potassium) solution (in mM): 116 CsCl, 1 Mg-ATP, 0.5 Na-GTP, 5 Ba Cl2, and 0.5 Na-GTP, 5 Ba Cl2, and 0.5 HEPES (pH adjusted to 7.4 with CsOH). The resistance of the pipette was about 8–10 MΩ. For recording, the

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culture dish was mounted on the stage of an inverted microscope with phase contrast optics (TMD300; Nikon, Tokyo). The indifferent electrode was an Ag-AgCl wire connected to the culture dish via an agarose bridge. A patch-clamp amplifier (Axopatch-200B; Axon Instruments, Burlingame, CA), linked to a computer, was used to measure membrane current and voltage. The voltage- and current-clamp procedures were controlled by pCLAMP software (Axon Instruments, Burlingame, CA). Data were low-pass filtered with a cut-off frequency of 5 kHz and then digitized at 10 kHz by an analog-to-digital interface (Digidata 1320A; Axon Instruments). For statistical analysis, paired t-tests were used to examine the effects of chemicals on ORC responses. For all tests, the significance level was set at \( P < 0.05 \). Data are given as means ± SE.

**Solutions and drugs.** For the current-clamp experiment, a normal Ringer’s solution was the same as the dissociation Ringer’s solution (in mM): 110 NaCl, 2.6 KCl, 3 CaCl\(_2\), 10 HEPES, 10 glucose, and 10 ppm phenol red. For the voltage-clamp experiments, several pharmacological agents were used for isolating each component of ionic currents. Choline chloride was substituted for NaCl to suppress sodium currents. CoCl\(_2\) (1 mM), CdCl\(_2\) (0.1 mM), and NiCl\(_2\) (0.1 mM) were used to block all voltage-gated calcium currents, an L-type calcium current, and a T-type calcium current, respectively. The control extracellular solution used to record a voltage-gated sodium current contained (in mM): 80 NaCl, 2.6 KCl, 1 CoCl\(_2\), 35 TEA, 10 HEPES, and 10 glucose, and that used to record T-type calcium current contained 65 choline-Cl, 2.6 KCl, 10 CaCl\(_2\), 0.1 CdCl\(_2\), 35 TEA, 10 HEPES, and 10 glucose. The bath solution for an L-type calcium current contained (in mM): 110 NaCl, 2.6 KCl, 3 CaCl\(_2\), 10 HEPES, 10 glucose, and 10 CoCl\(_2\), 10 mannitol, 10 TEA, 10 HEPES, and 10 glucose. The acetylcholine receptor agonist carbachol (100 \( \mu \)M), the muscarinic acetylcholine receptor antagonist atropine (50 \( \mu \)M), or the selective type 3 muscarinic acetylcholine receptor (M3-R) antagonist DAU5884 (10 \( \mu \)M) was applied from the bath. All experiments were performed at room temperature (23–25°C). DAU5884 was purchased from Tocris, and other chemicals were from Sigma.

**RESULTS**

Acetylcholine facilitates spike generation of ORCs. Isolated ORCs can be clearly distinguished from supporting cells in the olfactory epithelium by their shape (Kurahashi 1989; Schild and Restrepo 1998). The somatic diameter of newt ORCs was much smaller than that of isolated supporting cells. After dissociating, an olfactory cilia, dendrite and soma were visible (Fig. 1A). However, an axon was lost in most of the isolated ORCs. Figure 1B shows an ORC soma without the cilia, dendrite, and axon. The lack of cilia, consequently the smaller cell capacitance and relatively simple geometry, allowed for excellent space- and voltage-clamp conditions with tight-seal electrodes in the whole cell configuration. Thus we recorded mainly from ORCs that lacked cilia to investigate effects of acetylcholine on the somatic membrane.

We examined the effect of the acetylcholine receptor agonist carbachol on spike generation in isolated ORCs. In the present experiment, carbachol was mainly used, because carbachol is not hydrolyzed by acetylcholinesterase. Under current-clamp condition, action potentials were recorded from the soma of ORCs that had lost their cilia during the dissociation procedure (Fig. 1B).

Bath application of carbachol did not change significantly the membrane potentials of ORCs but markedly modulated spike generation. The resting potentials of the newt ORC in control and carbachol solution were –71 mV, which are similar values to those reported previously (–70 mV; Kawai et al. 1996). In all current-clamp recordings of the present experiment, no holding current was applied for the resting condition. When a 3-pA current was injected into an ORC, carbachol facilitated spike generation (Fig. 2A). In control Ringer’s solution no spike was observed, whereas in the presence of 100 \( \mu \)M carbachol four spikes were generated by near-threshold depolarization. For strong stimuli, carbachol also increased spike frequency (Fig. 2B). When a 3-pA current was injected into ORCs, 0.0 ± 0.0 spikes/s were generated in control solution and 0.5 ± 0.5 spikes/s in the presence of carbachol. By injection of a 13-pA current into ORCs, 9.1 ± 1.4 and 11.0 ± 1.3 spikes/s were generated in control and carbachol solutions, respectively (\( n = 7 \)).

With the increase of current injection from 0 to 13 pA, the spike frequency both in control solution (Fig. 2C, filled squares) and after addition of carbachol (Fig. 2C, filled circles) increased monotonously. In the stimulus-response relationship, carbachol significantly lowered the current threshold of spike generation from 5.3 ± 0.6 to 3.8 ± 0.5 pA (28% decrease; \( n = 7 \); \( P < 0.05 \), paired t-test) and increased the spike frequency (Fig. 2C). A similar result was observed by bath application of 50 \( \mu \)M acetylcholine. Acetylcholine significantly lowered the current threshold of spike generation from 6.3 ± 0.6 to 4.5 ± 0.5 pA (29% decrease; \( n = 4 \); \( P < 0.01 \), paired t-test). These results suggest carbachol directly modulates spike generation in ORCs.

Carbachol increases a transient inward current of ORCs. To understand the mechanism underlying modulation of spikes by acetylcholine, we examined the effects of carbachol on voltage-gated currents under voltage clamp (Fig. 3). In most of the voltage-clamp experiments, we used the ORCs without cilia to test selectively the effects of carbachol and acetylcholine on voltage-gated currents on the somatic membrane of ORCs. Depolarizing voltage steps between –90 and +30 mV from a holding voltage (\( V_h \)) of –100 mV induced a rapidly decaying initial inward current and a late outward current (Fig. 3A). The transient inward current was activated at potentials more positive than –60 mV and was maximal at approximately –20 mV.
mV (Fig. 3C, filled squares). The outward current was activated at potentials more positive than -30 mV (Fig. 3D, filled squares). Bath application of 100 μM carbachol markedly increased the transient inward current (Fig. 3, B and C, filled circles) but did not change the outward current significantly (Fig. 3, B and D, filled circles). At the voltage step of -20 mV, the peak amplitude of the transient inward current was -352 ± 109 pA in control solution and -430 ± 117 pA in the presence of carbachol. Carbachol significantly increased the peak amplitude of the transient inward current by 30.3 ± 6.0% (n = 6; P < 0.001, paired t-test). At the voltage step of +40 mV, the amplitude of the outward current was 636 ± 122 pA in control solution and 629 ± 128 pA in the presence of carbachol (n = 6). Similar increase of the transient inward current was also obtained from ORCs with their cilia attached. Carbachol increased the peak amplitude of the transient inward current of those ORCs by 29.7 ± 7.8% (n = 3), suggesting that the enhancement of the transient inward current is mainly due to acetylcholine receptors on the soma rather than on the cilia.

Carbachol increases voltage-gated sodium and T-type calcium currents. In the somatic membrane of the newt ORCs, four major voltage-gated currents have been identified: volt-
age-gated sodium ($I_{Na}$), T-type calcium ($I_{CaT}$), an L-type calcium current, and a delayed rectifier potassium current (Kawai et al. 1996). Spikes in ORCs are triggered by activation of $I_{Na}$ and $I_{CaT}$ currents (Kawai et al. 1996, 1997). To identify the voltage-gated current that is modulated by acetylcholine, we first examined the effects of carbachol on $I_{Na}$. Carbachol increased the peak amplitude of $I_{Na}$ by 32% without changing its kinetics (Fig. 4A). This effect was reversible and evident between −50 and +20 mV (Fig. 4B). At the voltage step of −30 mV, carbachol significantly increased the peak amplitude of $I_{Na}$ by 29.0 ± 5.3% ($n = 7; P < 0.01$, paired t-test).

In control solution, the activation and inactivation curves were fitted by a single Boltzmann function with a half-activation voltage of −33 mV and a half-inactivation voltage of −62 mV respectively (Fig. 4C), as previously reported (Kawai et al. 1996). A slope factor of the Boltzmann function was 6.5 mV for the activation curve and 11 mV for the inactivation curve. In the presence of carbachol, the half-activation voltage was −33 mV and the half-inactivation voltage −63 mV, and the slope factors of the activation and inactivation curves were 6.3 and 11 mV, respectively. Carbachol did not significantly change the activation or inactivation curves. Thus enhancement of $I_{Na}$ by carbachol results from an increase in the total sodium conductance rather than modulation of its voltage dependence. Noise analysis is needed to demonstrate which parameters (total number of sodium channels, open probability, or single sodium channel conductance) were modified by carbachol.

Acetylcholine receptors consist of nicotinic and muscarinic receptors. Because carbachol activates both nicotinic and muscarinic receptors, to identify which type modulates $I_{Na}$, we tested the effects of atropine, the specific muscarinic receptor antagonist, on $I_{Na}$. In the presence of 50 μM atropine, carbachol did not increase the amplitude of $I_{Na}$ (Fig. 5, A and B; $n = 7$). This result suggests that carbachol increases $I_{Na}$ via the muscarinic receptor rather than via the nicotinic receptor.

The peak amplitude of $I_{Na}$ in the presence of atropine was markedly smaller than that of $I_{Na}$ (see Figs. 4A and 5A). At the voltage step of −10 mV, 50 μM atropine decreased the amplitude of $I_{Na}$ by 57.8 ± 2.9% ($n = 4$). The reason why atropine decreased $I_{Na}$ is still unclear. However, because carbachol, the muscarinic and nicotinic receptor agonist, increased the amplitude of $I_{Na}$ (Fig. 4A), atropine, the specific muscarinic receptor antagonist, might decrease $I_{Na}$ via the muscarinic receptors in the newt ORCs.

Recently, in mouse ORCs Li and Matsunami (2011) have shown that the type 3 muscarinic acetylcholine receptor (M3-R) can form complexes with odorant receptors in the olfactory cilia to directly control odorant activity. To identify the subtype of muscarinic acetylcholine receptors in newt ORCs, we also examined the effects of DAU5884, a selective M3-R antagonist, on $I_{Na}$

In the presence of 10 μM DAU5884,
carbachol did not increase the amplitude of $I_{Na}$ (Fig. 6, A and B; $n = 5$). Thus it is likely that carbachol increased $I_{Na}$ through the M3-R. It is interesting to investigate whether M3-R is expressed on ORC somata by using immunohistochemistry. However, since there is no good mAChR antibody for newt ORCs, we did not perform immunohistochemical experiments in the present study.

Because $I_{Ca,T}$ is also crucial for spike generation in ORCs (Kawai et al. 1996, 1997; Kawai and Miyachi 2001; Gautam et al. 2007), next we examined the effects of carbachol on $I_{Ca,T}$. The depolarizing voltage step from $-100$ to $-40$ mV induced $I_{Ca,T}$ of $23$ pA in control solution in the bath solution containing $10 \mu M$ DAU5884 (thin line) or in the solution containing $10 \mu M$ DAU5884 and $100 \mu M$ carbachol (thick line). To enhance the peak amplitude of $I_{Ca,T}$, we used $10 \mu M [Ca^{2+}]_o$ instead of $3 \mu M [Ca^{2+}]_o$, which was used for a normal Ringer’s solution. In the presence of $100 \mu M$ carbachol, the same voltage step induced $I_{Ca,T}$ of $32$ pA. Thus carbachol increased $I_{Ca,T}$ by $39\%$. This effect was reversible and evident between $-60$ mV and $0$ mV (Fig. 7B). A similar significant increase of $I_{Ca,T}$ ($32 \pm 4\%$) was obtained in five cells ($P < 0.01$, paired t-test). Thus carbachol enhanced the transient inward currents of ORCs as shown in Fig. 3 by increasing both $I_{Na}$ and $I_{Ca,T}$.

**Effects of carbachol on other voltage-gated currents in ORCs.** We also tested the effect of carbachol on the L-type calcium current and the delayed rectifier potassium current. However, carbachol did not significantly change the amplitude of the L-type calcium current (Fig. 8, A and B) or the delayed rectifier potassium current (Fig. 8, C and D). Therefore, we suggest that carbachol facilitates spike generation of ORCs not by modulating the L-type calcium current or the delayed rectifier potassium current, but by increasing the amplitude of $I_{Ca,T}$ and $I_{Na}$.

**DISCUSSION**

**Comparison with other sensory systems.** We have shown that carbachol enhances excitability in ORCs. A similar modulation by efferent nerves of cellular sensitivities is reported in other sensory systems. In zebrafish efferent inputs regulate visual sensitivity of rod photoreceptors via dopamine (Li and Dowling 2000; Barlow 2001). Birds have a well-developed efferent pathway, and efferent increases the light response of retinal ganglion cells (Uchiyama et al. 1998). In the auditory system, it is proposed that cochlear outer hair cells are dominantly innervated by efferents, with acetylcholine being their principal neurotransmitter (He and Dallos 1999). Acetylcholine activation of the cholinergic receptors induced calcium influx through the ionotropic receptors, followed by a large outward potassium current through nearby calcium-activated potassium channels (Fuchs and Murrow 1992a,b). Activation of the efferent nerve may alter micromechanical event within the cochlear partition and provide a gain control of the cochlear amplifier (Guinan 1996).

Because activation voltage of $I_{Ca,T}$ in ORCs is $-10$ mV more negative than that of $I_{Na}$, $I_{Ca,T}$ contributes to lower the threshold of spike generation in ORCs (Kawai et al. 1996). Previously, we have reported that $I_{Ca,T}$ indeed enhances odorant sensitivity in ORCs (Kawai and Miyachi 2001). In the present study, we have shown that carbachol enhances the amplitude of $I_{Ca,T}$ by $32 \pm 4\%$ and $I_{Na}$ by $29 \pm 5\%$. Thus it is likely that acetylcholine lowers the current threshold of spike generation in ORCs.

![Fig. 6. DAU5884 blocks the enhancement by carbachol of $I_{Na}$. A: $I_{Na}$ induced by depolarization to $-10$ mV ($V_h = -100$ mV) in control solution containing $10 \mu M$ DAU5884 (thin line) or in the solution containing $10 \mu M$ DAU5884 and $100 \mu M$ carbachol (thick line). B: effects of $100 \mu M$ carbachol on the $I-V$ relationship of the cell shown in A in the presence of $10 \mu M$ DAU5884.](Image)

![Fig. 7. Carbachol augments T-type calcium ($I_{Ca,T}$) in ORCs. A: $I_{Ca,T}$ induced by depolarization to $-40$ mV ($V_h = -100$ mV) in control solution (thin line) or in $100 \mu M$ carbachol (thick line). Recording pipette was filled with cesium solution. B: effects of $100 \mu M$ carbachol on the $I-V$ relationship of the cell shown in A.](Image)

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generation in ORCs by enhancing the amplitude of $I_{Ca,T}$ and $I_{Na}$. In cochlear hair cells, acetylcholine regulates auditory sensitivity by modulating calcium-activated potassium currents via an influx of calcium, whereas in ORCs acetylcholine may regulate odorant sensitivity by modulating $I_{Ca,T}$. Thus, to control cellular sensitivity by efferent nerves, different mechanisms are employed for the individual sensory system.

**Effects of acetylcholine on voltage-gated currents in other preparations.** We have shown that carbachol and acetylcholine enhanced the peak amplitude $I_{Ca,T}$ and $I_{Na}$ in ORCs but did not significantly change the $L$-type calcium current and potassium current. Acetylcholine exhibits various effects on voltage-gated currents in other preparations. In NIH 3T3 cells, activation of muscarinic receptors increases the peak amplitude of $I_{Ca,T}$ by $\sim 20\%$ via protein kinase A (Pemberton et al. 2000). In rat striatal neurons, muscarinic receptors reduce $L$-type calcium currents by $\sim 50\%$ via protein kinase A (Howe and Surmeier 1995). This effect of muscarinic receptors on $I_{Ca,T}$ is similar to our result, but the effect on the $L$-type calcium currents is different from ours.

Acetylcholine also modulates $I_{Na}$ in other preparations. In rat hyppocampal neuron, 100 $\mu M$ carbachol reduced $I_{Na}$ by $30\%$ by phosphorylating sodium channel subunits via protein kinase C (Cantrell et al. 1996). This effect is opposite to our data, indicating that acetylcholine may increase $I_{Na}$ in ORCs via another pathway besides protein kinase C. The peak amplitude of $I_{Na}$ in the presence of atropine was markedly smaller than that of $I_{Na}$ in the control solution (see Figs. 4 and 5). It is still unclear the reason why atropine decreased the peak amplitude of $I_{Na}$ via the muscarinic receptors in the ORCs. Because atropine inhibits muscarinic receptors, atropine may lower the resting activity of a second messenger of the muscarinic receptors and may decrease the peak amplitude of $I_{Na}$. How muscarinic receptors in ORCs modulate $I_{Na}$ and $I_{Ca,T}$ remains to be established.

**Functional significance of modulation by acetylcholine of spiking responses in ORCs.** Several findings in vivo suggest that modulation by acetylcholine of spiking responses in ORCs may occur under physiological conditions. Muscarinic receptors have been shown in the olfactory epithelium (Hedlund and Shepherd 1983), and acetylcholine is contained in vesicles of nerve endings and varicosities of the ophthalmic branch of the trigeminal nerve (Zielinski et al. 1989). Binding studies have shown that salamander ORCs indeed bind carbachol and other muscarinic agonists (Hedlund and Shepherd 1983). Antidromic stimulation of the ophthalmic branch releases acetylcholine from the nerve endings and modulates discharge rates of ORCs (Bouvet et al. 1987). In addition, direct application of acetylcholine to the intact epithelium, not isolated ORCs, increased spiking activity in the frog olfactory nerve (Bouvet et al. 1988). This enhancement by acetylcholine of spiking activity is consistent with our data recorded from isolated ORCs. Using in situ spike recording, Frings (1993) has also reported that carbachol increases the spiking rate of ORCs by modulating the ciliary transduction pathway. In the present study, however, enhancement by acetylcholine of spiking activity was observed not only in intact ORCs but also in the ORCs that had lost their cilia during the dissociation procedure. Thus this finding suggests acetylcholine enhances spiking activity of ORCs at least via their somatic membrane.

Recently, in mouse ORCs Li and Matsunami (2011) have shown that the type 3 muscarinic acetylcholine receptor can form complexes with odorant receptors in the olfactory cilia to directly control odorant activity. Using the calcium imaging
technique, they have found that acetylcholine broadly increases response to odorants by modulating the ciliary membrane (Hall 2011; Li and Matsunami 2011). In the present experiment, by analyzing the somatic membrane of ORCs, we have also discovered that acetylcholine enhances excitability by lowering the threshold of spike generation in ORCs. Therefore, it is highly likely that acetylcholine broadly enhances responses to odorants by modulating both of the ciliary and somatic membranes of ORCs through the different mechanisms.

Acetylcholine may also affect ORC responses by regulating vasomotor tone and secretion from Bowman’s glands, which modulate odorant access to and clearance from the olfactory epithelium. Muscarinic receptors are expressed in nasal glands as well as ORCs (Hedlund and Shepherd 1983; Hildebrandt and Shuttleworth 1994). Acetylcholine induces electrolyte secretion from nasal glands in the guinea pig (Wu et al. 1994; Ikeda et al. 1995). Our data do not exclude that possibility, but acetylcholine-induced modulation of spiking activity at the soma of ORCs should be regarded as an important effect of acetylcholine, as a 28% decrease in the current threshold (5.3 ± 0.6 to 3.8 ± 0.5 pA; P < 0.05, paired t-test) seems likely to be functionally significant.

The autonomic nervous system is well known to regulate the activity of not only the sensory organs but also other organs such as cardiac, smooth muscle, and glands. When sympathetic stimulation excites a particular organ, parasympathetic stimulation sometimes inhibits it, demonstrating that the two systems occasionally act reciprocally to each other. We have reported that epinephrine or norepinephrine, which is released from the sympathetic nerves, activates β-adrenergic receptors on the soma of ORCs, which shifts the current threshold of spike generation to a higher intensity by suppressing $I_{Ca,T}$ (Kawai et al. 1999). In contrast, in the present study we found that acetylcholine shifts the current threshold to a lower intensity by enhancing $I_{Ca,T}$. Thus we suggest that the sympathetic and parasympathetic nerve systems regulate competitively excitability of ORCs by modulating the amplitude of $I_{Ca,T}$. Carbachol not only lowered the current threshold of spike generation but also increased the maximum spike frequency of ORCs (see Fig. 2C). Because $I_{Na}$ is the major component of spike generation, it is likely that the enhancement by carbachol of $I_{Na}$ is involved in the increase of the maximum spike frequency of ORCs.

Animals are quite sensitive to odorous airborne chemicals. Psychophysical measurements indicate that a minimum of 50 odorant molecules are necessary for human olfactory detection (Takagi 1989), suggesting that an individual ORC may be activated by a few odorant molecules. Thus detection of odorant molecules is mainly limited by the nature of odorant receptor proteins. However, detection of odorants is also regulated by the nature of the somatic membrane of ORCs. Because the input resistance of ORCs in various species is quite high (1–50 GΩ; Schild and Restrepo 1998), even a small amplitude of transduction current can generate the receptor potentials in ORCs, which also generate action potentials by activating the voltage-gated currents in the somatic membrane. Because carbachol increases the amplitude of $I_{Ca,T}$, we suggest that the activation of acetylcholine receptors act to enhance excitability by lowering the current threshold of spike generation in ORCs. This finding may explain the reason why olfactory perception is quite sensitive.

**REFERENCES**


