Phosphatidylinositol 4,5-bisphosphate hydrolysis mediates histamine-induced KCNQ/M current inhibition

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Liu B, Liang H, Liu L, Zhang H. Phosphatidylinositol 4,5-bisphosphate hydrolysis mediates histamine-induced KCNQ/M current inhibition. Am J Physiol Cell Physiol 295: C81–C91, 2008. First published April 30, 2008; doi:10.1152/ajpcell.00028.2008.—The M-type potassium channel, of which its molecular basis is constituted by KCNQ2-5 homo- or heteromultimers, plays a key role in regulating neuronal excitability and is modulated by many G protein-coupled receptors. In this study, we demonstrate that histamine inhibits KCNQ2/Q3 currents in human embryonic kidney (HEK)293 cells via phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis mediated by stimulation of H1 receptor and phospholipase C (PLC). Histamine inhibited KCNQ2/Q3 currents in HEK293 cells coexpressing H1 receptor, and this effect was totally abolished by H1 receptor antagonist mepyramine but not altered by H2 receptor antagonist cimetidine. The inhibition of KCNQ currents was significantly attenuated by a PLC inhibitor U-73122 but not affected by depletion of internal Ca2+ stores or intracellular Ca2+ concentration ([Ca2+]i) buffering via pipette dialyzing BAPTA. Moreover, histamine also concentration dependently inhibited M current in rat superior cervical ganglion (SCG) neurons by a similar mechanism. The inhibitory effect of histamine on KCNQ2/Q3 currents was entirely reversible but became irreversible when the resynthesis of PIP2 was impaired with phosphatidylinositol-4-kinase inhibitors. Histamine was capable of producing a reversible translocation of the PIP2 fluorescence probe PLCalpH-GFP from membrane to cytosol in HEK293 cells by activation of H1 receptor and PLC. We concluded that the inhibition of KCNQ/M currents by histamine in HEK293 cells and SCG neurons is due to the consumption of membrane PIP2 by PLC.

M CURRENT IS A VOLTAGE-GATED, slowly activating, slowly deactivating, and noninactivating K+ current, which presents in many types of neurons and plays a key role in modulation of cell excitability (8). Blocking of M channel results in membrane depolarization, which makes the cell more likely to fire action potentials (2). At present, the molecular correlates of M current have been identified as the KCNQ2-5 homo- or heteromultimers (9). It has now become widely known that M current is inhibited not only by muscarinic M1 receptor activation but also by the activation of many other G protein-coupled receptors (GPCR), agonists for such receptors including bradykinin, angiotensin II, UTP, etc. (12, 26, 34). Until now, GPCRs that mediate M current inhibition all share a common feature, which is, they are coupled to the Gαq/11 signaling pathway, which leads to activation of phospholipase C (PLC), and then the hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP2). In general, many effectors involved in the Gαq/11 pathway have been reported to participate in the receptor-mediated M current modulation, such as PIP2, Ca2+, calmodulin (CaM), phosphokinase C (PKC), and AKAP150, etc. (16, 24, 32, 45).

Muscarinic M1 and bradykinin B2 receptors are among the receptors that have received the most extensive studies. In rat superior cervical ganglion (SCG) neurons, activation of these two receptors can both produce prominent M current inhibition. Although both receptors are coupled to Gαq/11 protein, they take distinct downstream signaling pathways to fulfill their role in M current modulation in the SCG. Bradykinin, a B2 receptor agonist, evokes intracellular Ca2+ concentration ([Ca2+]i) signals through PLC signaling pathway. Its modulation of M current is largely dependent on Ca2+-CaM pathway (16), whereas oxo-M, an M1 receptor agonist that hardly elicits [Ca2+]i signals, suppresses M current by PIP2 depletion. Such a distinction in these two Gαq/11-mediated M current modulations may originate from the specificity of the “signaling microdomains,” formed by clustering of some Gαq/11-coupled receptors together with inositol 1,4,5-trisphosphate (IP3) receptors, which allows some certain agonists like bradykinin, but not others, like oxo-M, to raise [Ca2+]i (10, 11).

Histamine is an endogenous and widely distributed amine transmitter that mediates numerous physiological processes. Among the four different types of histamine receptors that have been identified (H1, H2, H3, and H4), H1 receptor is known to proceed via Gαq/11 to activate PLC and hydrolyze membrane PIP2 (4). Evidence has been accumulating which suggests that histamine produces depolarization and promotes neuron excitability by inhibiting K+ currents via H1 receptor stimulation (27, 41, 46). As far as KCNQ/M channels are concerned, histamine inhibits heteromeric KCNQ2/Q3 channels translocated in human embryonic kidney (HEK)293 and Hela cells via recombinant H1 receptor (20, 21). In addition, it is also demonstrated that histamine inhibits M current in bovine adrenal chromaffin cells via H1 receptor stimulation, leading to enhanced membrane excitability (40). It is suggested that the activation of a pertussis toxin-insensitive G protein is involved in histamine H1 receptor modulation of KCNQ2/Q3 currents (20), yet the downstream signals are still not clearly revealed. It is still not known whether histamine modulates M current in native neurons.

In the present study, we use pharmacological and electrophysiological approaches to show that histamine-induced inhibition of KCNQ2/Q3 currents in HEK293 cells is due to membrane PIP2 hydrolysis via PLC stimulation. We further demonstrate that histamine also inhibits M current in rat SCG...
neurons via stimulation of $H_1$ receptor and PLC. By comparing the effects of histamine on SCG neurons with those of the well-characterized $M_1$ and $B_2$ receptor agonist, oxo-M, and bradykinin, we suggest that histamine inhibits M current in a similar mechanism with oxo-M.

MATERIALS AND METHODS

cDNA constructs. Plasmids encoding human KCNQ2 and rat KCNQ3 (GenBank accession numbers: AF110020 and AF091247) and PLC$_{C51}$-PH-GFP were kindly provided by Diomedes E. Logothetis (Mount Sinai School of Medicine, New York, NY). Human histamine $H_1$ receptor was obtained from Missouri S&T cDNA Resource Center (Rolla, MO).

HEK293 cell culture and transfection. HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (Maveric) and antibiotics in a humidified incubator at 37°C (5% CO$_2$). Cells were seeded on poly-d-lysine-coated glass coverslips in a 24-multwell plate and transfected when 60–70% confluence was reached. For transfection of six wells of cells, a mixture of 3$\mu$g KCNQ, pEGFP-N1 cDNAs, $H_1$ receptor, and 3$\mu$l Lipofectamine 2000 reagent (Invitrogen) was prepared in 1.2 ml of DMEM and incubated for 20 min according to manufacturer’s instruction. The mixture was then applied to the cell culture wells and incubated for 4–6 h. Recordings were made 24 h after cell transfection and cells were used within 48 h.

Rat SCG neuron culture. SCGs were isolated from 10- to 17-day-old Sprague-Dawley rats, cut into pieces, and then transferred into collagenase solution (1 mg/ml) and incubated for 30 min at 37°C. The ganglia were then placed into trypsin solution (2.5 mg/ml) for 30 min at 37°C. The digested fragments were then rinsed three times with 2 ml DMEM plus 10% fetal bovine serum, centrifuged, and dissociated by trituration. The ganglia were plated onto glass coverslips precoated with poly-d-lysine and incubated at 37°C. After the neurons had attached to the coverslips, cell culture medium was changed to Neurobasal plus B27 supplement (Invitrogen). Neurons were cultured for 1 day and used within 24 h.

Electrophysiology. For current measurements in SCG neurons and HEK293 cells, recordings were performed using whole cell configuration of patch-clamp technique. Signals were amplified using an Axopatch 700B patch-clamp amplifier (Axon Instruments) and filtered at 2 kHz. Patch electrodes were pulled with a Flaming/Brown micropipette puller (Sutter Instruments) and fire-polished to a final resistance of 4–6 M$\Omega$ when filled with internal solution. Data acquisition was achieved using pClamp 9.1 software. Current recording was repeated every 4 s. The normal internal solution (low BAPTA) for HEK293 cell and rat SCG neuron recording was as follows (in mM): 175 KCl, 5 MgCl$_2$, 5 HEPES, 0.1 BAPTA, 3 K$_2$ATP, and 0.1 NaGTP,
Histamine inhibits KCNQ/M current via PI(4,5)P2 hydrolysis

Histamine suppresses KCNQ/M current in HEK293 cells and rat SCG neurons via activation of H1 receptor. We first evaluated the effects of histamine H1 receptor stimulation on KCNQ2/Q3 channel currents heterologously expressed in HEK293 cells. Upon transfection with KCNQ2, KCNQ3, and H1 receptor cDNAs, HEK293 cells yielded a slowly activating, slowly deactivating, and nonactivating outward current with neuronal M current-like properties (Fig. 1A). The KCNQ currents were measured from the deactivation tail current at −60 mV. Bath application of 10 μM histamine caused a significant inhibition of KCNQ2/Q3 currents (Fig. 1A). To confirm the inhibitory effect of histamine on KCNQ2/Q3 currents was due to H1 receptor activation but not some unspecific actions, mepyramine and cimetidine, the specific H1 and H2 receptor antagonists, respectively, were applied before histamine application. H1 receptors mediated the inhibitory effect of histamine on KCNQ2/Q3 currents since mepyramine (1 μM) totally abolished the inhibitory effect (Fig. 1B), whereas cimetidine (10 μM) did not (Fig. 1C). The KCNQ2/Q3 currents usually fully recovered after the removal of histamine under whole-cell patch configurations (Fig. 1A), and no obvious desensitization of H1 receptor effect was observed even after successive applications of histamine (data not shown). Histamine suppression applied the KCNQ2/Q3 currents by 77.9 ± 3.5% (n = 28) and 76.8 ± 6.1% (n = 6) in control cells and in cells treated with cimetidine, respectively (Fig. 1G). But the suppression was only 1.8 ± 0.4% (P < 0.01 vs. control, n = 7, Fig. 1G) when cells were treated with mepyramine. Thus these data indicate that H1 receptor stimulation can induce KCNQ2/Q3 current inhibition in HEK293 cells, which is consistent with a previous report (21). It is also noteworthy that in the present study (data not shown) and in our previous report (29), we found that mepyramine would have its own cause a remarkable inhibition of

**RESULTS**

**Histamine suppresses KCNQ/M current in HEK293 cells and rat SCG neurons via activation of H1 receptor.** We first evaluated the effects of histamine H1 receptor stimulation on KCNQ2/Q3 channel currents heterologously expressed in HEK293 cells. Upon transfection with KCNQ2, KCNQ3, and H1 receptor cDNAs, HEK293 cells yielded a slowly activating, slowly deactivating, and nonactivating outward current with neuronal M current-like properties (Fig. 1A). The KCNQ currents were measured from the deactivation tail current at −60 mV. Bath application of 10 μM histamine caused a significant inhibition of KCNQ2/Q3 currents (Fig. 1A). To confirm the inhibitory effect of histamine on KCNQ2/Q3 currents was due to H1 receptor activation but not some unspecific actions, mepyramine and cimetidine, the specific H1 and H2 receptor antagonists, respectively, were applied before histamine application. H1 receptors mediated the inhibitory effect of histamine on KCNQ2/Q3 currents since mepyramine (1 μM) totally abolished the inhibitory effect (Fig. 1B), whereas cimetidine (10 μM) did not (Fig. 1C). The KCNQ2/Q3 currents usually fully recovered after the removal of histamine under whole-cell patch configurations (Fig. 1A), and no obvious desensitization of H1 receptor effect was observed even after successive applications of histamine (data not shown). Histamine suppression applied the KCNQ2/Q3 currents by 77.9 ± 3.5% (n = 28) and 76.8 ± 6.1% (n = 6) in control cells and in cells treated with cimetidine, respectively (Fig. 1G). But the suppression was only 1.8 ± 0.4% (P < 0.01 vs. control, n = 7, Fig. 1G) when cells were treated with mepyramine. Thus these data indicate that H1 receptor stimulation can induce KCNQ2/Q3 current inhibition in HEK293 cells, which is consistent with a previous report (21). It is also noteworthy that in the present study (data not shown) and in our previous report (29), we found that mepyramine would have its own cause a remarkable inhibition of

**METHODS**

Western blot. Isolated rat SCGs were cut into small pieces and sonicated in 200 μl of radioimmunoprecipitation assay lysis buffer containing protease inhibitors (0.01 μg/μl pepstatin A, 0.025 μg/μl leupeptin, 0.01 μg/μl aprotonin, 0.03 μg/μl PMSF) for 20 min at 0°C. SCG homogenates were centrifuged at 18,000 rpm at 4°C for 20 min, and the pellets were discarded. The concentration of protein in the supernatant was assayed by BCA assay (Pierce, Rockford, IL), a loading buffer (10% SDS, 0.5 M Tris-HCl, 50% glycerol, 0.24% bromophenyl blue, and 20% ME) was added, and it was incubated at 70°C for 10 min. Proteins were fractionated on SDS-PAGE, transferred to polyvinyl difluoride membranes, and blotted with a rabbit anti-histamine receptor antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized with chemiluminescence from goat anti-rabbit horseradish peroxidase coupled secondary antibodies (Zhongshan, Beijing, China).

**Chemicals.** Histamine, mepyramine, cimetidine, o xo-M, bradykinin, phenylarsine oxide, wortmannin, U-73122, and fura-2 AM were purchased from Sigma (St. Louis, MO). The solutions containing antihistamines, U-73122, and wortmannin were all freshly prepared from stock solutions before each experiment and kept away from light exposure.

**Data analysis and statistics.** Currents were analyzed and fitted using Clampfit 9.1 (Axon Instrument) and Origin 7.0 (Originlab) software. The concentration-response curve was fitted by logistic equation: \( y = A_2 + \frac{A_1 - A_2}{1 + (x/x_0)^p} \), where y is the response, \( A_1 \) and \( A_2 \) are the maximum and minimum response, respectively, x is the drug concentration, and \( p \) is the Hill coefficient.

Results were expressed as means ± SE. Statistical analysis of differences between groups was carried out using Student’s t-test, paired t-test or one-way ANOVA. The differences were considered significant if \( P < 0.05 \).

**RESULTS**

Histamine inhibits KCNQ2/Q3 current in HEK293 cells and rat SCG neurons via activation of H1 receptor. We first evaluated the effects of histamine H1 receptor stimulation on KCNQ2/Q3 channel currents heterologously expressed in HEK293 cells. Upon transfection with KCNQ2, KCNQ3, and H1 receptor cDNAs, HEK293 cells yielded a slowly activating, slowly deactivating, and nonactivating outward current with neuronal M current-like properties (Fig. 1A). The KCNQ currents were measured from the deactivation tail current at −60 mV. Bath application of 10 μM histamine caused a significant inhibition of KCNQ2/Q3 currents (Fig. 1A). To confirm the inhibitory effect of histamine on KCNQ2/Q3 currents was due to H1 receptor activation but not some unspecific actions, mepyramine and cimetidine, the specific H1 and H2 receptor antagonists, respectively, were applied before histamine application. H1 receptors mediated the inhibitory effect of histamine on KCNQ2/Q3 currents since mepyramine (1 μM) totally abolished the inhibitory effect (Fig. 1B), whereas cimetidine (10 μM) did not (Fig. 1C). The KCNQ2/Q3 currents usually fully recovered after the removal of histamine under whole-cell patch configurations (Fig. 1A), and no obvious desensitization of H1 receptor effect was observed even after successive applications of histamine (data not shown). Histamine suppression applied the KCNQ2/Q3 currents by 77.9 ± 3.5% (n = 28) and 76.8 ± 6.1% (n = 6) in control cells and in cells treated with cimetidine, respectively (Fig. 1G). But the suppression was only 1.8 ± 0.4% (P < 0.01 vs. control, n = 7, Fig. 1G) when cells were treated with mepyramine. Thus these data indicate that H1 receptor stimulation can induce KCNQ2/Q3 current inhibition in HEK293 cells, which is consistent with a previous report (21). It is also noteworthy that in the present study (data not shown) and in our previous report (29), we found that mepyramine would have its own cause a remarkable inhibition of
KCNQ/M currents at higher concentrations (>10 μM), which in turn would mask its effect on antagonizing H1 receptor.

We then investigated whether histamine also inhibited M current in native rat SCG neurons. M currents were recorded using whole cell patch-clamp configuration and measured from the deactivation tail current at −60 mV (for details, see MATERIALS AND METHODS). For this part of the experiments, we used a slightly different voltage-clamp protocol from that used for HEK293 cells; membrane potential was held continuously at −20 mV before stepping to −60 mV. This would inactivate many voltage-dependent channels (Na+, Ca2+) present in SCG neurons but was negligible in HEK293 cells. Application of histamine (10 μM) induced an obvious inhibition of M current. The mean inhibition of M current by 10 μM histamine was 33.1 ± 2.9% (n = 11, Fig. 1, D and G). Histamine receptors have been demonstrated in SCG by electrophysiological studies (6, 36). To determine which receptor subtype was responsible for histamine-induced M current inhibition, histamine was applied to neurons in either the presence of mepyramine (1 μM) or cimetidine (10 μM). In the presence of mepyramine, histamine-induced M current inhibition was almost completely abolished (1.1 ± 0.5%, n = 7, P < 0.01 vs. control, Fig. 1, E and G), whereas in the presence of cimetidine, the inhibitory response was 30.2 ± 3.1% (n = 5), which was not significantly different from that of the control (P > 0.05, Fig. 1, F and G). Therefore, the above results suggest that histamine inhibits M current in rat SCG neurons via H1 receptor stimulation. We noticed that the baseline of KCNQ/M current (recorded at −60 mV) in SCC neurons also decreased in the presence of histamine. This could be due to inhibition of a nondeactivating component of KCNQ/M current or/and induction of an inward potassium current (5).

The concentration-dependent effects of histamine on rat SCG neurons were then tested, and the concentration response relationship was established. Histamine began to inhibit M current at a concentration of 1 μM and reached the maximal inhibition (~40%) at a concentration of 100 μM. The concentration of histamine needed to produce half-maximal inhibitory effect (EC50) of M currents was estimated to be 3.3 ± 1.1 μM (Fig. 2). To provide additional evidence that the observed inhibitory effects of histamine were mediated through activation of H1 receptor, a Western blot study was performed to confirm the presence of H1 receptor in rat SCG neurons. A band with the expected size of H1 receptor was recognized by anti-H1 receptor antibody (Fig. 2, inset) from SCG preparations.
Histamine suppresses KCNQ/M current via activation of PLC. It is well established that H<sub>1</sub> receptor couples to G<sub>q/11</sub>, which commonly activates PLC. Our next step was to verify the involvement of PLC activity in histamine action on KCNQ/M channels. First, we tested the role of PLC in H<sub>1</sub> receptor-mediated KCNQ2/Q3 current suppression in HEK293 cells. To address this issue, we used U-73122, which is a PLC inhibitor and is widely used as a quick test for the involvement of PLC in the signaling pathway (23). HEK293 cells, which were transfected with KCNQ2, KCNQ3, and H<sub>1</sub> receptors, were challenged with histamine (10 μM) either in the presence of normal extracellular solution or 5 μM U-73122. Such a typical experiment is illustrated in Fig. 3, A and B. Histamine produced 74.5 ± 0.6% (n = 5) inhibition of KCNQ2/Q3 currents, and this was significantly reduced to 7.1 ± 0.6% (n = 5, P < 0.01, Fig. 3E, Student’s paired t-test) when cells were treated with 5 μM U-73122 for 3 min. Interestingly, U-73122 on its own produced a transient augmentation of KCNQ2/Q3 currents, consistent with some previous reports (13, 23).

Next, the role of PLC in native SCG neurons was tested. For this study, the effect of histamine was compared with that of muscarinic M<sub>1</sub> receptor activation, a well-established system for modulation of M currents by PLC activity (23). In non-treated neurons, 10 μM histamine and 10 μM oxo-M, a specific M<sub>1</sub> receptor agonist, could both suppress M currents by 36.6 ± 4.3% (n = 5) and 94.2 ± 3.0% (n = 5), respectively (Fig. 3, C and E). As expected, the inhibitory effect of oxo-M was significantly reduced to 10.7 ± 2.0% (Fig. 3, D and E; n = 5, P < 0.01 vs. inhibition in the absence of U-73122) when treated with U-73122 (5 μM), in accordance with some previous reports (23, 25). Furthermore, the inhibitory effect of histamine was also markedly reduced to 4.2 ± 0.8%, significantly different from the inhibition in the absence of U-73122 (Fig. 3, D and E; n = 5, P < 0.01). Taken together, the above data suggest that PLC activity is involved in histamine-induced KCNQ/M current inhibition.

Histamine-induced inhibition of KCNQ2/Q3 currents is the result of membrane PIP<sub>2</sub> hydrolysis. PIP<sub>2</sub> depletion, as a result of its hydrolysis triggered by PLC stimulation, was proposed to underlie KCNQ/M current inhibition by stimulation of certain G<sub>q/11</sub> protein-coupled receptors (17). Since PLC activity is involved in the histamine-induced inhibitory effect on KCNQ/M current, we thus hypothesize that histamine-induced KCNQ/M current inhibition may be due to membrane PIP<sub>2</sub> hydrolysis. To address this issue, we start with confocal experiments to monitor the effect of histamine H<sub>1</sub> receptor activation on the hydrolysis of membrane PIP<sub>2</sub> in HEK293 cells. For this study, PLC<sub>51</sub>-PH-GFP, H<sub>1</sub> receptor, and
KCNQ2/Q3 channels were coexpressed in HEK293 cells. PLC_{\alpha1}-PH-GFP is a green fluorescence probe known to bind PIP2, and its translocation between the membrane and the cytosol has been successfully used as a reporter of PIP2 breakdown and its resynthesis (37). Figure 4A (a–c) shows a typical HEK293 cell expressed with PLC_{\alpha1}-PH-GFP probe before, during, and after application of 10 \( \mu \)M histamine. Figure 4B examines the time course of histamine action on the fluorescence intensity of the membrane and cytoplasmic region of interest. The fluorescence intensity was normalized based on its initial level before application of histamine. Histamine induced a remarkable and reversible translocation of green fluorescence signal, suggesting translocation of the probe from the membrane to the cytosol and vice versa upon its application and washout (Fig. 4B). The translocation of the fluorescence probe was completely blocked when cells were treated with mepyramine (Fig. 4C) or U-73122 (Fig. 4D). These data are summarized in Fig. 4E. In these experiments, the rise of fluorescence signals in the cytosol evoked by application of histamine is 80.4 ± 6.0% \((n = 13)\) but decreased to 1.5 ± 0.5% \((n = 10, P < 0.01)\) and 2.5 ± 0.7% \((n = 12, P < 0.01)\) in cells treated with mepyramine and U-73122, respectively. Thus our results indicate that histamine induces membrane PIP2 hydrolysis via stimulation of H1 receptor and PLC.

One strong piece of evidence suggesting PIP2 as the molecular determinant responsible for some GPCRs (such as M1 receptor)-mediated inhibition of KCNQ/M current came from experiments showing that a block of PIP2 resynthesis after its hydrolysis will lead to a block in the recovery of the inhibited KCNQ/M currents (38, 45). If PIP2 depletion is involved in histamine-induced KCNQ/M current inhibition, then its resynthesis would be required for the recovery of the current. To test this hypothesis, wortmannin, a fungal metabolite that could block phosphatidylinositol-4-kinase (PI4 kinase) at micromolar concentrations and lead to the block of PIP2 resynthesis (38), was used in this study. HEK293 cells were cotransfected with KCNQ2/Q3 channels and H1 receptor. Figure 5A shows the different recovery rates of KCNQ2/Q3 currents in the absence and presence of 30 \( \mu \)M wortmannin. As before, application of histamine (10 \( \mu \)M) induced a strong inhibition of KCNQ2/Q3 current, which could recover almost back to its initial level after histamine removal in <2 min. However, the recovery phase of the histamine response was greatly slowed when the cell was treated with 30 \( \mu \)M wortmannin (Fig. 5A). In control conditions, the KCNQ2/Q3 currents recovered to 88.4 ± 6.2% \((n = 7)\) of its initial level after 2 min, but in cells treated with wortmannin, the recovery was only 10.5 ± 3.4% \((n = 8)\), which was significantly attenuated \((P < 0.01)\). Notably, treating cells with 30 \( \mu \)M wortmannin for 3 min would on its own cause a gradual reduction (~50%) of the current, a phenomenon that may suggest a critical role for the tonic PI4 kinase activity in maintaining KCNQ2/Q3 channel activity.

We next proceeded to use phenylarsine oxide (PAO) to block the resynthesis of PIP2. PAO, an arsenic-based compound that inhibits PI4 kinase at a concentration of 10 to 30 \( \mu \)M, has been used successfully to prevent the recovery of KCNQ2/Q3 current from M1 receptor-induced inhibition (38). HEK293 cells were pretreated with 30 \( \mu \)M PAO for 4 min before histamine (10 \( \mu \)M) application. Unexpectedly, application of PAO by itself produced a transient but pronounced argumentation of the current, which in turn returned to normal in the continued presence of PAO. As shown in Fig. 5B, 30 \( \mu \)M PAO almost completely blocked the current recovery after histamine application. In nontreated cells, KCNQ2/Q3 current recovered to 95.4 ± 4.6% \((n = 5)\); however, in PAO-treated cells, the recovery was dramatically reduced to only 6.3 ± 0.9% \((n = 5, P < 0.01)\). These data are summarized in Fig. 5C. In addition, wortmannin and PAO also strongly slowed the rate of membrane PIP2 recovery indicated by PLC_{\alpha1}-PH-GFP translocation after histamine application (data not shown). All these results support the notion that delaying KCNQ2/Q3 channel recovery following histamine-induced inhibition by wortmannin or PAO is due to the attenuation of membrane PIP2 resynthesis. Thus the above results provide evidence that activation of histamine H1 receptor inhibits KCNQ2/Q3 currents in a mechanism that may involve PIP2 depletion.

Histamine inhibits KCNQ2/Q3 currents in HEK293 cells deprived of [Ca\(^{2+}\)]\(_i\) rises. In mammalian cell lines, the inhibition of KCNQ2/Q3 currents produced by activation of heterologously expressed G_{\alphaq11}-coupled M1 or AT1 receptors was...
accompanied by robust $[Ca^{2+}]$, rises (33, 43). To check if stimulation of $H_1$ receptor expressed in HEK293 cells would also result in a similar effect, HEK293 cells were heterologously expressed with $H_1$ receptor and loaded with a cell-permeant calcium indicator fura-2 AM for calcium imaging studies. $[Ca^{2+}]$, was monitored by the fluorescence ratio at 340 and 380 nm (F340/F380). As shown in Fig. 6A, the application of 10 $\mu$M histamine evoked a transient rise of $[Ca^{2+}]$, which then gradually declined to the baseline level within 100 s. This response involves PLC-mediated intracellular $Ca^{2+}$ mobilization since the $[Ca^{2+}]$, rise was not only partially reduced in $Ca^{2+}$-free bath solution but almost completely abolished by 5 $\mu$M U-73122 (Fig. 6A).

We proceeded to test whether the histamine-evoked $[Ca^{2+}]$, signal was involved in KCNQ2/Q3 modulation. To address this issue, we perform two types of experiments to study the extent of KCNQ2/Q3 current inhibition when such $[Ca^{2+}]$, rises are suppressed. First, we used different internal solutions containing either 0.1 mM BAPTA (low BAPTA) or 20 mM BAPTA plus 10 mM CaCl$_2$ (high BAPTA) to dialyze cells by whole cell patch recording. The dialysis with low BAPTA internal solution did not disturb $[Ca^{2+}]$, rises (Fig. 6B, bottom), whereas the dialysis with high BAPTA solution was capable of eliminating $[Ca^{2+}]$, signals evoked by agonist stimulation (Fig. 6C, bottom) and clamping $[Ca^{2+}]$, at a physiological level (~170 nM) (1, 7, 33). With a pipette containing low BAPTA solution, the KCNQ2/Q3 currents inhibition induced by 10 $\mu$M histamine is 89.1 $\pm$ 5.8% ($n = 6$) after 12 min dialysis (Fig. 6, B and E). For cells dialyzed with high BAPTA solution for the same period, the KCNQ2/Q3 currents were inhibited with a slower time course than that seen in low BAPTA-treated cells. Nevertheless, the current inhibition was 81.3 $\pm$ 4.0% (Fig. 6, C and E; $n = 11$), which was only slightly but not significantly reduced ($P > 0.01$).

We then tried another approach to prevent the release of $Ca^{2+}$ from internal $Ca^{2+}$ stores. We used thapsigargin, a sarcoplasmic-endoplasmic reticulum $Ca^{2+}$ pump inhibitor, to empty the $Ca^{2+}$ stores, thus suppressing the $[Ca^{2+}]$, signals (33). Figure 6D shows such a typical experiment on a HEK293 cell cotransfected with KCNQ2/Q3 channels and $H_1$ receptor. As before, application of 10 $\mu$M histamine inhibited KCNQ2/Q3 currents by 90.0 $\pm$ 1.6% (Fig. 6, D and E; $n = 6$). Then the same cell was bath perfused with thapsigargin (2.5 $\mu$M) for 5 min to deplete the internal $Ca^{2+}$ stores. Thapsigargin on its own had little effect on the basal KCNQ2/Q3 currents amplitude during application. A subsequent applica-

![Fig. 6. Suppression of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]$) rises does not abolish histamine-induced inhibition of KCNQ2/Q3 currents in HEK293 cells. HEK293 cells were transiently transfected with KCNQ2/Q3 subunits and $H_1$ receptors. A: superimposed time courses for changes in F340/F380 ratio of HEK293 cells upon application of histamine (10 $\mu$M) in extracellular solutions containing 2 mM Ca$^{2+}$, Ca$^{2+}$-free, or 5 $\mu$M U-73122. HEK293 cells were bath loaded with fura-2 AM before calcium photometry study. Whole cell recordings of HEK293 cells dialyzed with pipettes containing either the low BAPTA (B) or high BAPTA intracellular solution (C) are shown. Each cell was allowed for dialysis at least for 12 min before histamine application. Bottom panels indicate histamine-induced $[Ca^{2+}]$, changes in HEK293 cells dialyzed with either low or high BAPTA solutions. D: effect of depleting internal $Ca^{2+}$ stores by thapsigargin (2.5 $\mu$M) on histamine-induced current inhibition. Drugs were bath applied to the cell as indicated by the bars. Insets denote the corresponding current traces recorded. E: summary of KCNQ2/Q3 current inhibitions under the conditions described above. Three, four, and three separate experiments were done to get the statistic data illustrated.](http://ajpcell.physiology.org/10.2203.34.4)
tion of histamine produced a slower but remarkable inhibition of KCNQ2/Q3 currents (Fig. 6, D and E, 88.6 ± 3.9%, n = 6, P > 0.05 vs. control, paired t-test). Thus, although a fast onset of histamine action requires the presence of [Ca\textsuperscript{2+}], signals, which presumably can promote the PLC activity (23), it is clear that calcium changes are not required for the block of KCNQ2/Q3 currents by histamine.

H\textsubscript{1} receptor stimulation does not evoke [Ca\textsuperscript{2+}], signal in SCG neurons. In rat SCG neurons, PLC-mediated Ca\textsuperscript{2+} signals diverge among G\textsubscript{q/11}-coupled receptors such that activation of bradykinin B\textsubscript{2} and purinergic P\textsubscript{2Y} receptors can induce [Ca\textsuperscript{2+}], rises from IP\textsubscript{3}-gated internal Ca\textsuperscript{2+} stores, whereas muscarinic M\textsubscript{1} and angiotensin AT\textsubscript{1} receptors cannot. This may be explained by the hypothesis that B\textsubscript{2}, but not M\textsubscript{1}, receptor colocalizes with endoplasmic reticulum membrane IP\textsubscript{3} receptor to form the “signaling microdomains” that provide easier accesses for released IP\textsubscript{3} to bind with IP\textsubscript{3} receptor and evoke [Ca\textsuperscript{2+}], signals (11). Recently, it has been postulated that the mechanisms involved in M current modulation by G\textsubscript{q/11}-coupled receptors in SCG neurons may be different based on the ability of receptors to evoke [Ca\textsuperscript{2+}], signals (44). Thus we began our study by examining whether histamine could evoke [Ca\textsuperscript{2+}], signals in rat SCG neurons. SCG neurons were bath loaded with fura-2 AM for calcium imaging. We tested histamine and compared it with oxo-M and bradykinin, of which the effects on [Ca\textsuperscript{2+}], have been well characterized (10). As shown in Fig. 7, A and B, bath application of oxo-M (10 \mu M) and histamine (10 \mu M) did not result in a measurable [Ca\textsuperscript{2+}], signal. However, a subsequent application of high K\textsuperscript{+} (containing 50 mM K\textsuperscript{+}) solution caused a significant rise in [Ca\textsuperscript{2+}],. Application of bradykinin (100 nM) evoked an obvious and reversible [Ca\textsuperscript{2+}], signal (Fig. 7C), which is consistent with the previous report (7). The rises in the F\textsubscript{340}/F\textsubscript{380} ratio produced by histamine, oxo-M, and bradykinin were 0.04 ± 0.008 (n = 13), 0.03 ± 0.004 (n = 12), and 0.32 ± 0.05 (n = 7, P < 0.01 vs. oxo-M and histamine, one-way ANOVA), respectively (Fig. 7D). Thus these results suggest that like oxo-M, but unlike bradykinin, histamine does not induce [Ca\textsuperscript{2+}], rises in rat SCG neurons.

Histamine does not require [Ca\textsuperscript{2+}], signal for M current inhibition in SCG neurons. The inability of histamine to provoke [Ca\textsuperscript{2+}], signals in SCG neurons lets us hypothesize that histamine and muscarinic modulations of M currents are mediated by a congruent signaling mechanism that does not require a [Ca\textsuperscript{2+}], signal. Previous work on the actions of [Ca\textsuperscript{2+}], on M current modulation by GPCR has revealed that Ca\textsuperscript{2+} is not the messenger for the muscarinic M\textsubscript{1} receptor-induced inhibition in rat SCG neurons. On the contrary, Ca\textsuperscript{2+} is indispensable for bradykinin to achieve its inhibitory effect on M current via B\textsubscript{2} receptor (9). Thus we test our hypothesis by [Ca\textsuperscript{2+}], clamping study and compare its impact among histamine, oxo-M, and bradykinin-induced M current inhibitions in SCG neurons. We probe the effects of these agonists on M currents using pipettes containing either the low BAPTA or high BAPTA internal solutions described above. After several minutes to allow for dialysis by the pipette, neurons were successively challenged with these agonists as indicated. For neurons dialyzed with the low BAPTA internal solutions (Fig. 8A), application of histamine (10 \mu M), oxo-M (10 \mu M), and bradykinin (100 nM) suppressed M currents by 38.3 ± 7.9% (n = 6), 98.8 ± 0.4% (n = 6), and 85.3 ± 4.4% (n = 6), respectively. For neurons dialyzed with high BAPTA internal solutions (Fig. 8B), the suppression of M currents by histamine and oxo-M was 32.1 ± 3.1% (n = 10) and 87.3 ± 7.0% (n = 6), which was not significantly altered. In contrast, the inhibitory effect of bradykinin was significantly reduced to 20.5 ± 2.6% (n = 6) (P < 0.01). The results from the above experiments are summarized in Fig. 8C. Thus we suggest that, like oxo-M, but unlike bradykinin, histamine modulation of M

Fig. 7. Histamine does not induce [Ca\textsuperscript{2+}], signal in SCG neurons. A–C: time courses for changes in F\textsubscript{340}/F\textsubscript{380} ratio of SCG neurons upon application of histamine (10 \mu M), oxo-M (10 \mu M), and bradykinin (BK, 100 nM). Extracellular solution containing 50 mM K\textsuperscript{+} was applied at the end of oxo-M and histamine application to be a positive control. D: summary of the changes in F\textsubscript{340}/F\textsubscript{380} ratio of these studies. Error bars indicate SE. **P < 0.01.
current in rat SCG neurons is not mediated by a [Ca\(^{2+}\)]\(_i\) signal, in accordance with its inability to raise [Ca\(^{2+}\)]\(_i\).

**DISCUSSION**

In the present work, we demonstrated that histamine, via stimulation of H\(_1\) receptor, inhibits KCNQ2/Q3 and M currents in HEK293 cells and rat SCG neurons. The inhibition involves PLC activation and subsequent hydrolysis of membrane PIP\(_2\). Histamine also inhibited KCNQ/M currents when intracellular calcium was clamped or Ca\(^{2+}\) stores were depleted. Activation of H\(_1\) receptor inhibits M currents in SCG neurons through a similar mechanism described for activation of M\(_1\) receptor.

Several lines of evidence indicate that histamine-induced KCNQ/M current inhibition results from PIP\(_2\) hydrolysis: 1) The inhibition was mediated via H\(_1\) receptor stimulation. H\(_1\) receptor is generally believed to couple with G\(_{q/11}\) to stimulate PLC\(_\beta\), which catalyzes the hydrolysis of membrane PIP\(_2\) (4). 2) U-73122, a PLC inhibitor, significantly antagonized histamine-induced KCNQ/M current inhibition. 3) Treatment with PI4 kinase inhibitor wortmannin or PAO, which blocks the synthesis of PIP\(_2\), makes histamine-induced KCNQ2/Q3 currents inhibition irreversible. 4) The reversible translocation of PIP\(_2\) fluorescence reporter PLC\(_{61}\)-PH-GFP induced by histamine indicates the hydrolysis of membrane PIP\(_2\) in the presence of histamine and its resynthesis upon washout. The time course is also consistent with that of the current inhibition.

Recently, it is proposed that G\(_{q/11}\) protein-coupled receptors in rat SCG neurons can be divided into two fundamental modes based on their capabilities of depleting membrane PIP\(_2\) and evoking [Ca\(^{2+}\)]\(_i\) signals (22, 44). For receptors such as M\(_1\), and probably AT1 receptors as well, stimulation of these receptors inhibits M currents via membrane PIP\(_2\) depletion (39), but this process is not accompanied with an obvious [Ca\(^{2+}\)]\(_i\) signal. The second mode is well characterized with bradykinin B2 and purinergic P2Y receptors. The stimulation of these receptors will commonly evoke [Ca\(^{2+}\)]\(_i\) signals mediated by IP3. The evoked [Ca\(^{2+}\)]\(_i\) can act as a feedback signal to stimulate the synthesis of PIP\(_2\) by PI4 kinase through neuronal calcium sensor-1, concurrently with the activation of PLC (15, 42). Hence, in all, membrane PIP\(_2\) level will not fall considerably upon stimulation of these receptors. In this case, M current is inhibited by an alternative pathway that involves Ca\(^{2+}\)-mediated CaM modulation of channel (3, 14, 16). In the present study, our results indicate that activation of H\(_1\) receptor inhibits M currents in rat SCG neurons through a similar mechanism with that of M\(_1\) receptor, since both receptors failed to evoke [Ca\(^{2+}\)]\(_i\) signals.
We noticed that the KCNQ2/Q3 currents were inhibited to a less degree or more slowly in cells either dialyzed with high BAPTA solutions or treated with thapsigargin (Fig. 6, C and D). This is likely due to a reduced rate of PLC activation under the condition of intracellular Ca\(^{2+}\) clamping or reduction. Indeed, it was reported that M\(_1\) receptor-induced PiP\(_2\) hydrolysis, which was monitored by the translocation of PLC\(_{5\alpha}\)-PH-GFP, was slowed and reduced when [Ca\(^{2+}\)] was clamped at resting levels with BAPTA or stores were depleted with thapsigargin (23). Thus, although clamping [Ca\(^{2+}\)] or depletion of Ca\(^{2+}\) stores with the two maneuvers in the present study did not eliminate the large action of histamine, calcium did play a role in promoting a fast onset of histamine action.

The inhibitory effect of histamine on M currents in SCG neurons is slower compared with the fast response in HEK293 cells (Fig. 1, A and D) and to the fast response of M\(_1\) receptor agonist Oxo-M in SCC neurons (Fig. 3C). These differences may reflect a lower expression level of histamine H\(_1\) receptor and/or lower coupling efficiency between H\(_1\) receptors and downstream signaling pathway in native neurons.

In our Ca\(^{2+}\) imaging studies on HEK293 cells and SCG neurons, we saw a marked difference in histamine-evoked [Ca\(^{2+}\)]\(_i\) signals. Histamine induces robust Ca\(^{2+}\) rising in HEK293 cells but failed to provoke [Ca\(^{2+}\)]\(_i\) rising in SCG neurons. Such difference could arise from the existence of a signaling microdomain, formed by specific G\(_{q/11}\)-coupled receptors (e.g., B\(_2\) and probably P\(_2Y\) receptor) with IP\(_3\) receptors in native neurons (10) but not in HEK293 cells. In HEK293 cells, such delicate signaling specificity may be greatly disturbed with overexpression of receptors. For this reason, while only activation of B\(_2\) and P\(_2Y\) evokes rising of [Ca\(^{2+}\)]\(_i\) signals in SCG neurons, activation of H\(_1\), P\(_2Y\), M\(_1\), and B\(_2\) receptors all induces robust [Ca\(^{2+}\)]\(_i\) rising in HEK293 cells (unpublished data). Thus signaling microdomains such as local PiP\(_2\) sequestration by lipid raft (18) or spatial clustering of GPCR and IP\(_3\) receptors (11) may confer specificity to receptor-mediated M current modulation in native neurons.

It is worthwhile putting a note on the effects of two different PI4 kinase inhibitors, wortmannin and PAO. Both drugs have been used to block the synthesis of PiP\(_2\) by inhibiting PI4 kinase activity (38). In the present study, the KCNQ2/Q3 currents gradually decline upon application of 30 \(\mu M\) wortmannin (Fig. 5A). This likely reflects ongoing endogenous hydrolysis of PiP\(_2\) under inhibition of PI4-kinase by wortmannin. On the other hand, PAO transiently but remarkably increases basal KCNQ2/Q3 currents (Fig. 5B). PAO is an arsenic-based compound that affects a variety of cell functions. In addition to its PI4 kinase blocking activity, it is also a potent sulfhydryl oxidant that is capable of altering ion channel functions (35). It is recently reported that oxidative modification can enhance KCNQ/M current by modifying the cysteines in sulfhydryl groups located in channel proteins (19). Therefore, we hypothesize that the transient augmentation of KCNQ2/Q3 currents upon PAO application may be the combined results of oxidative modification of channels and PI4 kinase inhibition.

It is established that functional histamine receptors (H\(_1\)–H\(_3\)) are expressed in SCG neurons of many animals, based on electrophysiology studies (5, 6, 28, 31, 36). In our current study, histamine inhibits M current at a concentration of 1 \(\mu M\) and the EC\(_{50}\) is 3.3 \(\mu M\), which falls in the proposed physiological concentration range (4). There is growing evidence showing that histamine can affect neuronal excitabilities (30, 41, 46). Furthermore, exogenous applied (10 \(\mu M\)) or endogenous histamine released by immunological responses both induced a remarkable membrane depolarization in SCG neurons (5). Since M channels open at resting membrane potential and is considered to be the major contributor to resting membrane potential in SCG neurons (9), thus M current inhibition by physiological concentration of histamine will generally induce membrane depolarization and increase excitability. The inhibitory effect on M current mediated by histamine may provide new insights into the understanding of the excitatory effects of histamine on neurons.

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REFERENCES


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