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Identification of Histamine Receptors and Effects of Histamine on Murine and Simian Colonic Excitability

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Abstract

Background—Inflammatory responses can include recruitment of cells of hematopoietic origin to the *tunica muscularis*. These cells can secrete a variety of factors which can reset the gain of smooth muscle cells (SMC) and influence motor patterns. Histamine, a major mediator in inflammation, is released by mast cells and exerts diverse effects in SMC by binding to histamine (H) receptors. The profiles of H receptor expression in animal models used to study inflammatory diseases are unknown.

Methods—H receptor expression and electro-mechanical responses to histamine were tested in simian and murine colonic smooth muscle using qualitative and quantitative PCR, isometric force measurements, microelectrode recordings and patch clamp techniques.

Key results—H1, H2 and H4 receptor transcripts were expressed at similar levels in simian colonic tissue whereas only the H2 receptor transcript was detected in murine colonic tissue. Stimulation of simian colonic muscles with histamine caused depolarization and contraction in the presence of TTX. Histamine activated non-selective cation channels in simian SMC. In contrast, histamine caused hyperpolarization and inhibited contractions of murine colon. The hyperpolarization was inhibited by the K_{ATP} channel blocker, glibenclamide. Histamine-activated K^+ currents were inhibited by glibenclamide in murine colonic SMC.

Conclusions & Inferences—H receptor expression in simian SMC was similar to that reported in humans. However, H receptor profile and responses to histamine were considerably different in mice. Thus, monkey colon may be a more suitable model to study how inflammatory mediators affect the gain of smooth muscle excitability.

Keywords

histamine receptors; colonic excitability; contractility

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Introduction

Colonic motility results from contractions of smooth muscle cells (SMC) that are regulated by intrinsic excitability and Ca^{2+} sensitivity mechanisms and inputs from a variety of higher-order control systems (e.g. interstitial cells of Cajal, enteric motor neurons, hormones, and paracrine substances). The responses of colonic muscles to regulatory signals depends upon the gain on excitability that is set in individual SMC. In inflammatory diseases new regulatory influences are imposed due to the release of bioactive substances from activated, resident immune cells and cells of hematopoietic origin that are recruited to the muscularis as part of the inflammatory response. Among these cells are mast cells that can be found in abundance in the *tunica muscularis* in response to inflammation.[1] Mast cells release a variety of bioactive substances, including histamine, cytokines and interleukins.[2]

Histamine, a major mediator in inflammatory and allergic reactions,[3] is a biogenic amine synthesized from the basic amino acid histidine. Many stimuli, including allergens, neuropeptides, and stress can activate mast cells and cause release of histamine.[4] Histamine, acting as a paracrine, may be able to significantly reset the excitability of colonic SMC. In the guinea pig enteric nervous system, histamine suppresses synaptic transmission presynaptically, whereas in human submucous plexus, histamine excites enteric neurons suggesting histamine effects can differ depending on species.[5,6] There are four histamine (H) receptors, H1, H2, H3 and H4, through which histamine can exert it effects. H1 receptors are widely expressed in enterocytes, muscle layer, blood vessels, immune cells and ganglion cells of the myenteric plexus in the human GI tract.[7] H2 receptors are located on parietal cells in the fundic mucosa, intestinal epithelium, immune cells and myenteric ganglia.[7,8] Although H3 agonists had pronounced excitatory effects on submucosal neurons from human small and large intestine, which suggests expression of the receptor in human bowel,[6] minimal expression levels of H3 receptor mRNA were detected in the human GI tract (5 out of 66 samples).[7] Héron *et al.* found that H3 receptor was expressed in the mucosa of the rat GI tract.[9] Transcriptional expression of the H4 receptor is lower than H1 and H2 in human stomach, small intestine and colon and is mainly expressed in leucocytes in mucosa and submucosal blood vessels in the colon.[7,10,11]

Surprisingly, few studies have examined the effect of histamine on GI smooth muscle excitability. One study reported that histamine induced a dominant contractile response in human colon tissue strips, and these effects were not affected by hyoscine (a ganglion blocker) or anti-adrenergic drugs, suggesting that the effects of histamine were not dependent upon nervous activity.[12] Bolton *et al* showed that histamine depolarized longitudinal SMC and increased action potential discharge in guinea-pig ileum.[13] In the submucosal plexus of the human colon, local microinjection of H receptor-specific agonists resulted in excitation of enteric neurons.[6] Many inflammatory studies are performed on rodents without an appreciation for the cellular responses of inflammatory mediators. Here we have evaluated the profile of histamine receptors in mice and in a non-human primate model (*Macaca fascicularis*) and characterized the electromechanical responses to histamine and a variety of H receptor-specific agonists.

Materials and methods

Animals

Male BALB/c mice (10-12 weeks old) were used for electro-mechanical experiments in this study. These mice were killed by inhalation of isoflurane (Baxter Healthcare, Deerfield, IL, USA) followed by cervical dislocation. Colons were removed following abdominal incision and rinsed with Krebs–Ringer bicarbonate buffer (KRB, see solutions and drugs section). *Cynomolgus simians* of either sex (13 simians, 2.5–7 years of age) were donated by Charles

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River Laboratories (Preclinical Services, Sparks, NV, USA). The animals used were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All experiments performed and protocols were approved by the Institutional Animal Use and Care Committee at the University of Nevada, Reno.

Expression Studies

Following dispersion of simian SMC from the longitudinal muscle layer (see *Isolation of SMC* section), SMC were collected by applying suction to the pipette resulting in aspiration of the cells into the pipette. Total RNA isolation, cDNA preparation and amplification of simian and murine colonic longitudinal muscle strips (mucosa and submucosa removed) and simian colonic longitudinal SMC were performed as previously reported.[14] Briefly, RNA was prepared using a SNAP Total RNA isolation kit (Invitrogen, San Diego, CA) as per the manufacturer's instructions. RNA was treated with Rnase-free Dnase I (2 units) at 37°C (New England Biolabs) prior to cDNA preparation. First strand cDNA was synthesized from each RNA using Superscript II Reverse Transcriptase with 500 μg μl^{-1} of oligo dT primers cDNA. To investigate the expression of H receptors, the following PCR primers designed against murine sequences were used (genebank accession number is given in parenthesis for the reference nucleotide sequence used): H1 (NM_008285), H2 (NM_008286), H3 (NM_133849), H4 (NM_153087). Similarly, primers were designed against the following simian sequences: H1 (XM_001088286), H2 (XM_001082927), H3 (XM_001032898), H4 (XM_001096657). The relative expression levels of H receptors in simian colons was determined by real-time quantitative PCR performed on a ABI PrismM 7000 sequence detector using SYBR® Green chemistry (Applied Biosystems, CA). Standard curves were generated for each receptor and the constitutively expressed GAPDH from regression analysis of the mean values of RT-PCRs for the $\log_{10}$ diluted cDNA. Unknown quantities relative to the standard curve for the H receptor primers were calculated, yielding transcriptional quantization of H receptor cDNA relative to the endogenous standard GAPDH. Each cDNA sample was tested in triplicate and cDNA was obtained from 4 different simian colons. The reproducibility of the assay was tested by analysis of variance comparing repeat runs of samples, and the mean values generated at individual time points were compared by Student's *t* test.

Isometric Force Measurements

Standard organ bath techniques were employed to measure the changes in force generated by murine and simian longitudinal smooth muscle strips. One end of a smooth muscle strip was attached to a fixed mount and the opposite end to an isometric strain gauge (Fort 10, WPI, Sarasota, FL, USA) in oxygenated KRB solution maintained at 37.5±0.5°C. A resting force of 500 mg (murine colon) and 1g (simian colon) was applied to set the muscles at optimum length, and the muscles were allowed to equilibrate for 1-2 h with constant perfusion with KRB solution. Mechanical responses were recorded on a computer running Axoscope (Axon instrument, Foster City, CA, USA) and measurements of the area under the curve (AUC) obtained. The AUC was determined as the integral values above the baseline of selected area for 5 min recordings (mNxmin). The AUC for the tissues exposed to tested drugs were compared to the AUC for tissues under control conditions, during an equivalent period of time. Bathing solutions were exchanged by switching the perfusion to the drug-containing solution.

Intracellular Microelectrode Recordings

After preparing the longitudinal smooth muscle tissue, impalements of cells were made with glass microelectrodes having resistances of 80–120 MΩ. Transmembrane potentials were recorded with a standard electrometer (Duo 773; WPI, Sarasota, FL, USA). Data were recorded using Axoscope (Axon instrument, Foster City, CA, USA).

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Isolation of SMC

Simian colon segments were placed in KRB solution. Each segment was opened and pinned to the base of a dissecting dish coated with Sylgard elastomer (Dow Corning Corp., Miland, MI, USA) and the adhering mucosa, submucosa and circular muscle layer were removed. Freshly dispersed colonic SMC were prepared from colonic longitudinal muscle strips using Ca^{2+}-free Hank's solution containing (mmol L^{-1}): 125 NaCl, 5.36 KCl, 15.5 NaOH, 0.336 Na_2HPO_4, 0.44 KH_2PO_4, 10 glucose, 2.9 sucrose and 11 Hepes, adjusted to pH 7.4 with Tris. Pieces of muscle were incubated for 50-55 minutes at 37°C in a Ca^{2+}-free solution (2 ml) containing collagenase (4 mg mL^{-1}, Worthington Biochemical, Lakewood, NJ), trypsin inhibitor (8 mg mL^{-1}), fatty acid-free bovine serum albumin (8 mg mL^{-1}), papain (2 mg mL^{-1}), and L-dithiothreitol (LDTT, 0.3 mg mL^{-1}, Sigma-Aldrich, MO, USA). Tissue pieces were washed with Ca^{2+}-free solution and then gently agitated to create a cell suspension. Dispersed SMC were stored at 4°C in Ca^{2+}-free solution. Drops of the cell suspensions were placed on the bottom of a 300 μl chamber mounted on an inverted microscope and allowed to adhere to the bottom of the chamber for 5 minutes before recording.

Colons from BALB/c mice were dissected in the same fashion as simian colons. Pieces of longitudinal muscle were incubated for 35-40 minutes at 37°C in a Ca^{2+}-free solution (2 ml) containing collagenase (2 mg mL^{-1}, Worthington Biochemical, Lakewood, NJ), trypsin inhibitor (4 mg mL^{-1}), fatty acid-free bovine serum albumin (4 mg mL^{-1}), papain (1 mg mL^{-1}) and L-DTT (0.3 mg mL^{-1}).

Patch Clamp Experiments

The whole-cell voltage clamp technique was used to record membrane currents from dissociated murine colonic SMC. Currents were amplified with an Axopatch 200B (Axon Instruments). Data were digitized with 16-bit analogue to digital converter (Digidata 1322A, Axon instruments, Foster City, CA, USA). Data were stored directly and digitized online using pClamp software (version 9.0, Axon instrument, Foster City, CA, USA). The data were sampled at 5 KHz with low pass filtered at 2 KHz using an eight-pole Bessel filter. Conventional and perforated whole cell patch-clamp techniques were used for recording ionic currents under voltage clamp. For perforated patches, amphotericin B (60 mg mL^{-1}) was dissolved in DMSO, sonicated, and diluted in the pipette solution to give a final concentration of 270 μg mL^{-1}. Experiments were performed at room temperature (between 22 and 25°C).

Solutions and Drugs

In intracellular microelectrode recordings and mechanical experiments, the tissue chamber housing longitudinal muscles was constantly perfused with oxygenated KRB solution of the following composition (in mmol L^{-1}): NaCl 118.5; KCl 4.5; $MgCl_2$ 1.2; $NaHCO_3$ 23.8; KH_2PO_4 1.2; dextrose 11.0; $CaCl_2$ 2.4. The pH of the KRB was 7.3–7.4 when bubbled with 97% O2–3% CO_2 at 37.0 ± 0.5°C. In order to measure inward currents, colonic SMC were bathed in a Ca^{2+}-containing physiological salt solution (CaPSS) containing (in mmol L^{-1}): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose, 10 HEPES adjusted to pH 7.4 with Tris. The pipette solution for the study of inward currents in simian colonic SMC contained (in mmol L^{-1}): 30 CsCl, 110 Cs-aspartate, 0.1 EGTA, 0.1 Na_2GTP, 3 MgATP, 10 glucose, 2.5 creatine phosphate disodium and 10 HEPES. This solution was adjusted to pH 7.2 with Tris. In order to increase the driving force for K^+ influx in murine colonic SMC, cells were perfused in High K^+-containing solution (135 mmol L^{-1} Na^+ was replaced with equimolar K^+) and the pipette solution contained (in mmol L^{-1}): 135 KCl, 10 BAPTA, 0.1 Na_2GTP, 3 MgATP, 10 glucose, 2.5 creatine phosphate disodium and 10 HEPES and was adjusted to pH 7.2 with Tris. H1 agonist (histamine-trifluoromethyl-toluidine, HTMT), H2 agonist (Dimaprit), H3 agonist ((R)-α-methylhistamine dihydrobromide), H4 agonist (4-

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methylhistamine dihydrochloride, MHDC) and H2 antagonist (Zolantidine dimaleate) were purchased from Tocris (Park Ellisville, MO, USA). Histamine, glibenclamide (GBC) and tetrodotoxin (TTX) were obtained from Sigma Chemical Co (St Louis, MO, USA).

Statistical Analysis

Data were expressed as means ± S.E.M. The Student's t-test was used where appropriate to evaluate differences in the data. *P*-values less than 0.05 were taken as statistically significant differences. n values refer to the number of recordings from muscle strips in electro-mechanical experiments or from cells in patch clamp experiments.

Results

Transcriptional expression of histamine receptors in murine and simian colonic muscles

We investigated gene expression of H receptor isoforms by performing RT-PCR on colonic longitudinal muscles from monkey and mouse. Detectable amplicons for H1, H2 and H4 were revealed in simian colonic muscles (Fig. 1A). Only H2 receptor expression was detected in murine colonic longitudinal muscles (Fig. 1B). Quantitative analysis of H1, H2 and H4 receptors in simian colonic muscle revealed no significant difference in expression levels of these receptors (Fig. 1C). Since transcripts at the tissue level could include genes expressed in a variety of cell types (e.g. neurons, fibroblasts, etc), we also examined transcript expression in freshly dispersed SMC collected by suction into the pipette (~100 cells per sample). H1, H2 and H4 receptors were expressed in SMC samples collected from 6 monkeys. These SMC samples were negative to c-Kit (interstitial cell of Cajal marker) and PGP9.5 (neuronal cell marker).

Histamine had different effects on contractility in simian and murine colonic smooth muscles

We compared the effects of histamine on contractions in simian and murine colons because the expression of H receptors was markedly different in the 2 species. Contractions were quantified by calculating the averaged areas under the contraction curves (AUC) for 5-min recording periods (see materials and methods section). Histamine (10 μmol L^{-1}) increased AUC from 14.0±3.4 to 157.4±54.8 mNxmin in simian muscles (Fig 2A & C, n=4, $P<0.05$). In contrast, histamine (10 μmol L^{-1}) had inconsistent effects in murine colonic muscles. In 8 of 15 tissues, histamine (10 μmol L^{-1}) transiently decreased AUC from 27.7±2.3 to 21±2.2 mNxmin ($P<0.01$) (Fig. 2B). However, in the other 7 murine tissues tested, there was no significant change in AUC. Analysis of all 15 tissue experiments found that the AUC for the initial 5min period after histamine application was not significantly different (31.0±2.1 to 30.3±3.4 mNxmin) (Fig. 2C; n=15).

Neuronal cells also express H receptors, so the effects of histamine could also be due to stimulation or inhibition of enteric motor neurons. Therefore, we repeated contractile responses in the presence of tetrodotoxin (TTX, 1 μmol L^{-1}). As previously reported, TTX application resulted in a significant increase in contractile force in murine colonic muscles reflecting a dominant inhibitory influence of neurons on spontaneous contractile activity (Fig. 3C & 3D).[15] In comparison, TTX had a minor effect on contractile force in simian colonic muscles suggesting a minor neuronal influence on basal spontaneous contractility. Histamine (10 μmol L^{-1}) caused a significant increase in contractile force in simian muscles (AUC from 45.2±21.3 to 260.9±56 mNxmin) in the presence of TTX (Fig. 3A & 3B; n=9, $P<0.005$). In murine colonic muscles, a sustained decrease in contractile force and frequency of spontaneous contractions in the presence of TTX was observed (AUC from 74.1±10.4 to 63.5±10.8 mNxmin; Fig. 3C & 3D; n=17, $P<0.005$). In the presence of TTX, there was a

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dose-dependent reduction on AUC in murine colonic muscles by increasing concentrations of histamine (Fig. 3E; n=4, $P<0.05$).

Effects of histamine on membrane potential in simian and murine colonic muscle

We performed intracellular microelectrode recordings from simian and murine colonic longitudinal muscles to examine the effects of histamine on membrane potential. Histamine (10 μmol L^{-1}) caused sustained depolarization of simian colonic muscle that was reversible upon washout (Fig. 4A, n=5, 16±4mV; $P<0.01$). TTX (1 μmol L^{-1}) did not alter this effect (Fig. 4B). In contrast, histamine (10 μmol L^{-1}) caused a transient hyperpolarization of murine colonic muscles from -45.5±0.6 mV to -50.6±1.8mV (Fig. 4 C, n=9; $P<0.05$). Furthermore hyperpolarization induced by histamine was sustained in the majority of cases (9 of 11) following pretreatment with TTX (1 μmol L^{-1}) (ΔmV=- 4.8±0.6mV, $P<0.005$, Fig. 4D).

Effects of histamine receptor agonists on simian colonic electrical and mechanical activity

H1, H2 and H4 receptor transcripts were found in simian colonic muscle and SMC (see Fig. 1A & 1D). Therefore, we investigated the effects of specific H receptor agonists on contractions. The H1 agonist, HTMT (10-50 μmol L^{-1}), had only minor effects on contractility. However, higher concentrations of HTMT (100 μmol L^{-1}) increased contractile force (41±14%) in the presence of TTX (1 μmol L^{-1}) (Fig. 5A & B, n=4, $P<0.05$). The H2 receptor agonist, dimaprit (50 and 500 μmol L^{-1}), transiently decreased the force of contractions (-52±12%, and -42±15%, respectively (Fig. 5C & D, n=4). The H4 agonist, MHDC, at lower concentrations (50 μmol L^{-1}) had no significant effect on contractility (P=0.1, n=7). However, a higher concentration of MHDC (250 μmol L-1) caused a dramatic increase in contractile force (195±41%, n=4, $P<0.05$), an effect similar to that seen with application of histamine (Fig. 5E & F). H4 agonist (MHDC) can also activate H2 receptors.[16] Therefore to exclude possible effects of the H4 agonist on H2 receptors, we performed experiments in the presence of the H2 inhibitor, Zolantidine (10 μmol L^{-1}). Zolantidine pretreatment augmented the response to MHDC (250 μmol L^{-1}, 19-fold increase in AUC, Fig. 5G & H) compared to without H2 antagonist (3-fold increase in AUC, Fig. 5F).

In order to confirm the absence of H3 receptors in simian colonic tissue (see gel in Fig. 1A), we examined the effects of the H3 agonist, (R)-α-methylhistamine (1μM), on contractility. These experiments revealed no significant change in contractile force upon application of this agonist (data not shown, n=4).

Histamine activated K_{ATP} whole-cell currents in murine colonic SMC

Histamine induced hyperpolarization in murine colonic smooth muscle, which could be due to activation of K^+ conductance(s) and/or inhibition of non-selective cation channels (NSCC). The only H receptor expressed in colonic muscles couples through G_s to activate production of cAMP, which has been linked to activation of several K^+ conductances in canine colonic circular smooth muscle.[17] Therefore, we performed perforated whole-cell patch clamp experiments to resolve the mechanism of hyperpolarization in murine colonic muscles. Isolated cells were held at -80mV and the external solution (CaPSS) was replaced with high K^+ solution (HK; 140 mmol L^{-1} K^+, see Materials and methods section). Under these conditions histamine (10 μmol L^{-1}) activated inward currents. We tested whether Gd^{3+} (10 μmol L^{-1}), a blocker of NSCC, blocked currents activated by histamine. Gd^{3+} had no effect on the inward currents activated by histamine (Fig. 6A, n=4). These data suggested the conductance activated by histamine was likely to be a K^+ conductance. Therefore, we tested blockers of small conductance K^+ (SK) channels (apamin) and K_{ATP} channels (glibenclamide) on the conductance activated by histamine-activated by histamine. Apamin

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(300 nmol L^{-1}) did not affect the conductance activated by histamine (Fig. 6B, n=4), but glibenclamide (10 μmol L^{-1}) reduced histamine-activated currents (Fig. 6C, n=4).

Our cellular experiments suggest that the hyperpolarization of murine colonic muscles (Fig. 4C and 4D) by histamine might be mediated by activation of K_{ATP} channels. Thus, we tested the effects of histamine in muscles pretreated with glibenclamide. Addition of the K_{ATP} blocker caused depolarization (indicating basal activation of K_{ATP} channels as previously reported in murine colonic smooth muscle).[18] Addition of histamine in the presence of glibenclamide had no effect on membrane potential (Fig. 6D). These data suggest that the hyperpolarization and reduced contractility in murine colonic smooth muscle may be through activation of the $H2/G_s$ pathway which increases protein kinase A (PKA) levels and activates K_{ATP} channels.

Histamine activated NSCC whole-cell currents in simian colonic SMC

Unlike murine smooth muscle, histamine induced depolarization and increased contractility in simian colonic smooth muscle which may be the result of activation of NSCC and/or Cl^- currents. Therefore, we performed whole-cell patch clamp experiments to determine the mechanism of depolarization in simian colonic muscles. The internal Cl^- concentration was 30 mmol L^{-1} in Cs^+-rich solution (see Materials and Methods section) and the bath solution was CaPSS. Therefore, E_{Cl} was set at -40mV. At a holding potential of -80mV, perfusion of histamine (10 μmol L^{-1}) activated inward currents (n=4). Subtraction of currents evoked by a single ramp depolarization from -80 to +80mV before (*a*) and after (*b*) histamine application, revealed a histamine-sensitive current that reversed at 0mV (Fig. 6E). Histamine-activated currents were inhibited by the NSCC non-specific blocker, Gd^{3+} (10 μmol L^{-1}) (Fig. 6F, n=4). Therefore, histamine-induced depolarization is due to activation of NSCC but not Cl^- conductance.

Discussion

In the present study we examined the effect of the major inflammatory mediator histamine, on electro-mechanical responses in colonic smooth muscle. In particular, we compared histamine receptor expression and effects of histamine receptor activation in murine and simian colonic muscles and identified several important differences between these two species. In murine colonic tissue, only the H2 receptor was expressed and histamine resulted in hyperpolarization and decreased spontaneous contractility. Conversely, simian colonic tissue expressed H1, H2 and H4 receptors and application of histamine or H1 and H4 specific agonists resulted in contraction. Histamine activated NSCC in simian colonic SMC which may underlie the depolarization seen at the tissue level.

In inflammatory bowel disease (IBD), alterations in colonic excitability have been frequently attributed to dysfunction of the enteric and/or central nervous system.[19-21] Inflammation results in marked mast cell accumulation in rat rectal mucosa and human colon tissue.[22-25] Furthermore, the cellular contents of mast cells, including tumor necrosis factor-α and histamine, dramatically increase in patients with ulcerative colitis.[23-25] In human colitis, mast cell infiltration progresses transmurally to the smooth muscle layers of the colon, thus mast cell mediators may have direct effects on smooth muscle excitability.

H1, H2, H4 and minimal levels of H3 receptors are expressed in the human GI tract, though little is known about the functional role of H receptor subtypes in the colon.[7] There is no report if the expression of H receptors in simian and murine colon are similar to expression patterns in humans therefore justifying the extrapolation of data from these models to human GI pathophysiology. Therefore, we examined the expression of H receptors in murine and simian colon using molecular studies and found there were different expression patterns. In

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simian colonic longitudinal smooth muscle, H1, H2 and H4 receptors were expressed in a similar pattern to human muscles. H3 receptor expression was not detected in simian tissue and SMC. This could be due to the extremely low expression of this receptor. In human colonic tissue, H3 receptor was expressed infrequently and at low levels compared with other H receptors.[7] In murine colonic longitudinal tissue, only the H2 receptor was found to be expressed. Thus, from the perspective of histamine receptors and anticipated effects, the simian is potentially, but the mouse is not, a suitable model to study histaminergic influences in human colonic muscles.

Histamine can have diverse effects in different tissues due to expression of specific H receptors and activation of distinct intracellular pathways.[26] H1 receptors are coupled to $G_{q/11}$ proteins which activates phospholipase C and the phosphatidylinositol 4,5-bisphosphate signaling pathway.[27] They are also linked to $G_{i/o}$ proteins which results in decreased production of cAMP.[28] H4 receptors are coupled to $G_{i/o}$ proteins that inhibit adenylate cyclase activity and therefore decrease cAMP production.[4] In the gastrointestinal tract, synergistic stimulation of muscarinic receptors coupled to $G_{i/o}$ and $G_{q/11}$ proteins is required to activate NSCC, resulting in membrane depolarization. Subsequent activation of voltage-dependent Ca^{2+} channels and an increase in Ca^{2+} influx results in smooth muscle contraction thus promoting intestinal motility.[29] A similar phenomenon could underlie histamine-induced depolarization and contraction in simian colonic smooth muscle since H1 and H4 receptors are coupled to $G_{q/11}$ and $G_{i/o}$ respectively. In equine tracheal SMC, intracellular Ca^{2+} release through stimulation of the H1/$G_{q/11}$ pathway was necessary for histamine-induced activation of NSCC. More importantly, H1 coupled $G_{i/o}$ proteins were essential for activation of these NSCC as dialysis of anti-$G_{\alpha i}$/$G_{\alpha o}$ antibodies blocked the effect of histamine.[28] In the present study, when the effects of H1 and H4 agonists are compared in simian colonic smooth muscle, the contractile response to the H4 agonist, MHDC, closely resembles that induced by histamine. These data suggest that stimulation of the H4/ $G_{i/o}$ pathway and subsequent activation of specific NSCC linked with these G-proteins underlie the major effects of histamine in simian colonic excitability. Furthermore it has been reported that H4 receptors have a higher affinity for histamine than the H1 receptor.[30] Molecular identification of the NSCC targeted by histamine in simian colonic muscle will require further investigation and efforts will lie in examination of members of the Transient Receptor Potential Canonical (TRPC) family since some are reported to be regulated by $G_{i/o}$ and $G_{q/11}$ pathways.[31]

In murine colon, histamine application induced transient hyperpolarization. We hypothesized that activation of the H2 receptor could result in activation of K^+ channels to account for this effect. H2 receptors are positively coupled to adenylate cyclase via G_s, which is a potent stimulant of cAMP production and results in activation of PKA.[32] Perforated patch clamp experiments revealed that histamine activated K_{ATP}-sensitive currents. In addition, pretreatment with the K_{ATP} blocker, glibenclamide, prevented histamine-induced hyperpolarization in colonic smooth muscle. Therefore, histamine exerts its effects in murine colonic smooth muscle via stimulating the H2/G_s/cAMP pathway resulting in activation of K_{ATP} channels through increases in PKA.

In the present study, electrical and contractile responses to histamine were different in murine and simian colonic tissue. Histamine caused a transient decrease in the force of contractions in murine colon and furthermore this transient decrease became sustained following inhibition of neuronal influences by pretreatment with TTX. Although TTX does not block all neural responses, in particular those at nerve varicosities, these data suggest indirectly that there is a histamine-sensitive neuronal component that contributes to the contractile response to histamine in murine colonic smooth muscle under physiological conditions. Wood and colleagues have documented multiple actions of histamine on the

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intestinal myenteric and submucosal plexuses in several different animal species including a suppressing effect of histamine on sympathetic inhibitory input to submucosal secretomotor neurons via presynaptic H3 receptors in guinea pig small intestine.[33, 34] Furthermore, in human small intestine and colon, histamine causes excitation of submucosal plexus neurons.[6] In contrast, the excitatory effects of histamine on simian colons in the presence and absence of TTX were not different suggesting that these responses may be due to stimulation of cells other than neurons (such as SMC, interstitial cells of Cajal etc.). H1 and H4 agonists increased simian colonic contractile force whereas the H2 agonist had the opposite effect. There was no significant difference in expression levels of H1, H2 and H4 receptors in simian colonic longitudinal tissue suggesting that the contractile effects caused by co-stimulation of H1 and H4 receptors by histamine appear to dominate the inhibitory effects of H2 activation.

In conclusion, our data demonstrate that the expression of H receptors and responses to histamine in relation to colonic contractility and membrane potential are different between mouse and monkey. Since H receptor expression in simian colon is closer to that reported in human[7], we feel that it is a more suitable model to predict the effects of the inflammatory mediator, histamine, on colonic motility in humans.

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Abbreviations

AUC	area under the curve
DSS	dextran sulphate sodium
GBC	glibenclamide
GI	gastrointestinal
H	histamine
HTMT	histamine-trifluoromethyl-toluidine
IBD	inflammatory bowel disease
MHDC	4-methylhistamine dihydrochloride
NSCC	non-selective cation channels
PKA	protein kinase A
SK	small conductance K^+ channel
SMC	smooth muscle cells
TTX	tetrodotoxin

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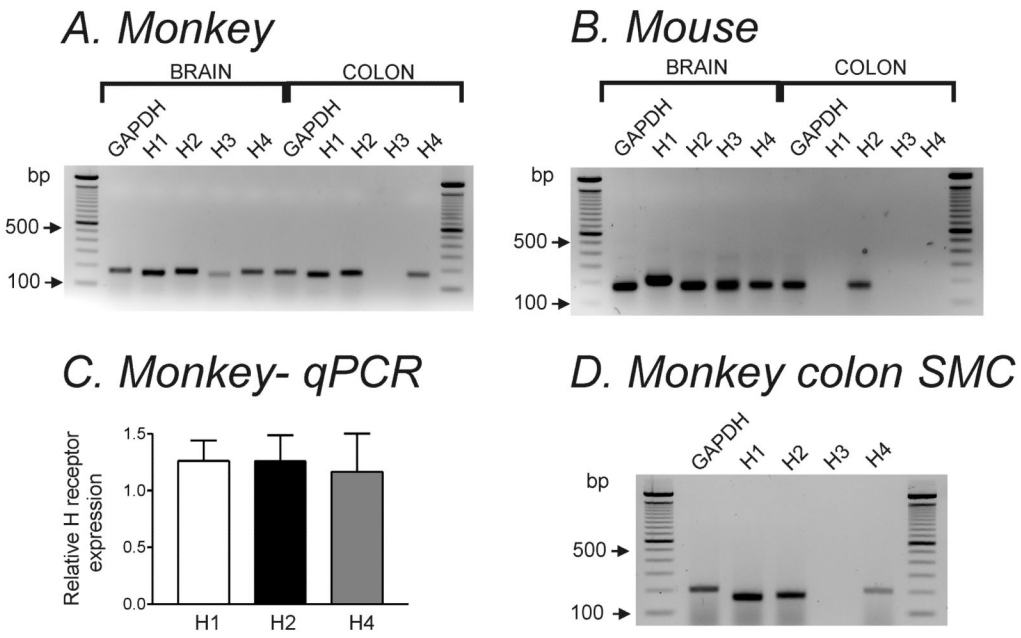

Figure 1. H receptor expression was different in simian and murine colonic longitudinal muscle

Representative agarose gels of RT-PCR products of H1- H4 receptors in (*A*) simian and (*B*) murine colonic tissue. RT-PCR was performed with H receptor isoform specific primers. GAPDH was used for control. (*C*) Summary graph of real-time quantitative PCR (qPCR) analysis of H1, H2 and H4 receptors in simian colonic tissue. Expression of each H receptor isoform was normalized relative to GAPDH expression. (*D*) Representative agarose gel of H1-H4 receptor transcripts in simian colonic SMC (n=6).

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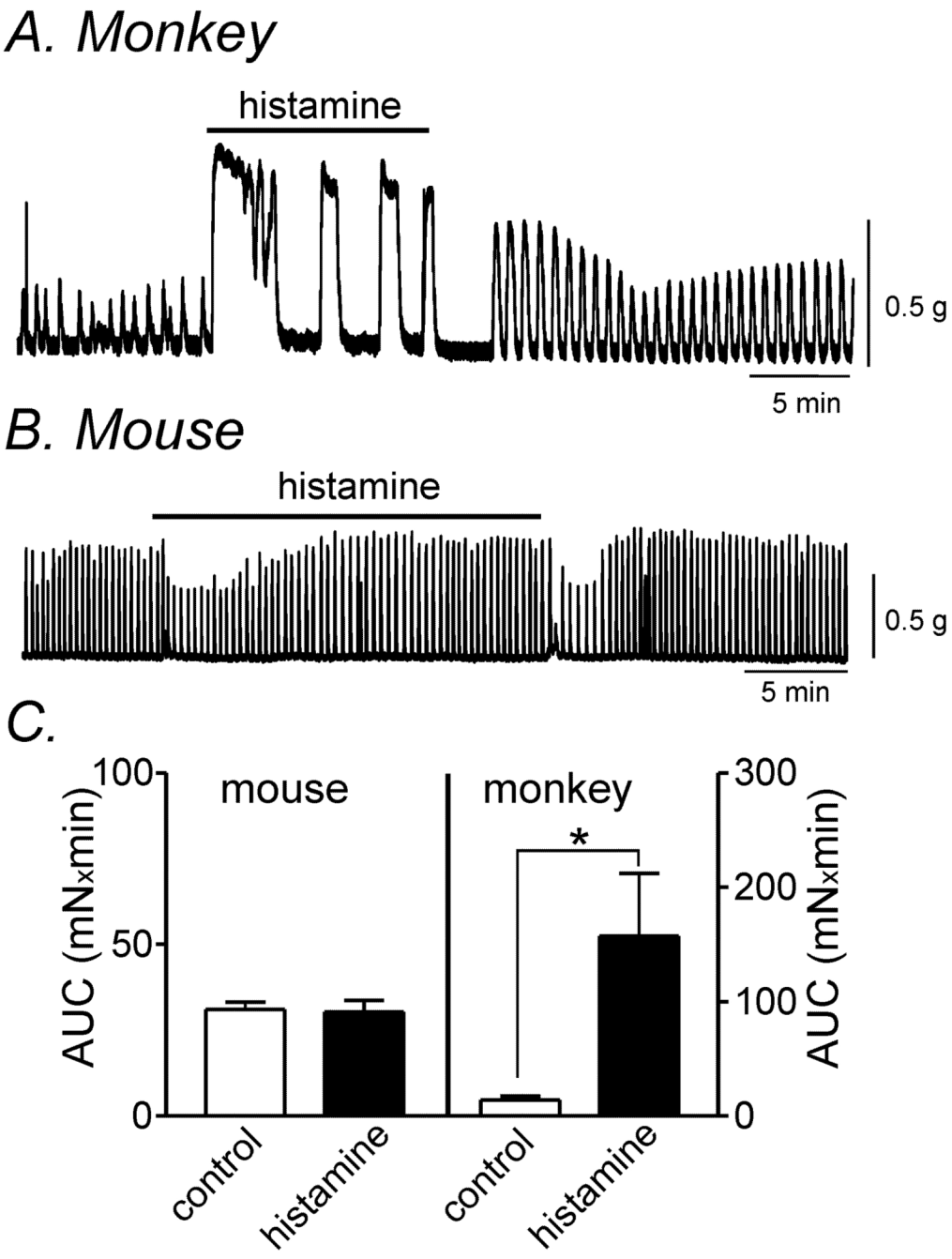

Figure 2. Histamine altered contractility in simian and murine colonic longitudinal smooth muscle

(A) Representative mechanical trace illustrating that histamine (10 μmol L^{-1}) caused an increase in contractile force in simian colonic muscle. (*B*) Application of histamine (10 μmol L^{-1}) resulted in a transient decrease in the force of contractions in murine colonic smooth muscle in 8 out of 15 tissues tested. (*C*) Summary data of area under the curve (AUC) for 5 min recording before and after histamine treatment from murine (n=15) and simian colonic smooth muscle (n=4). * denotes $P<0.05$.

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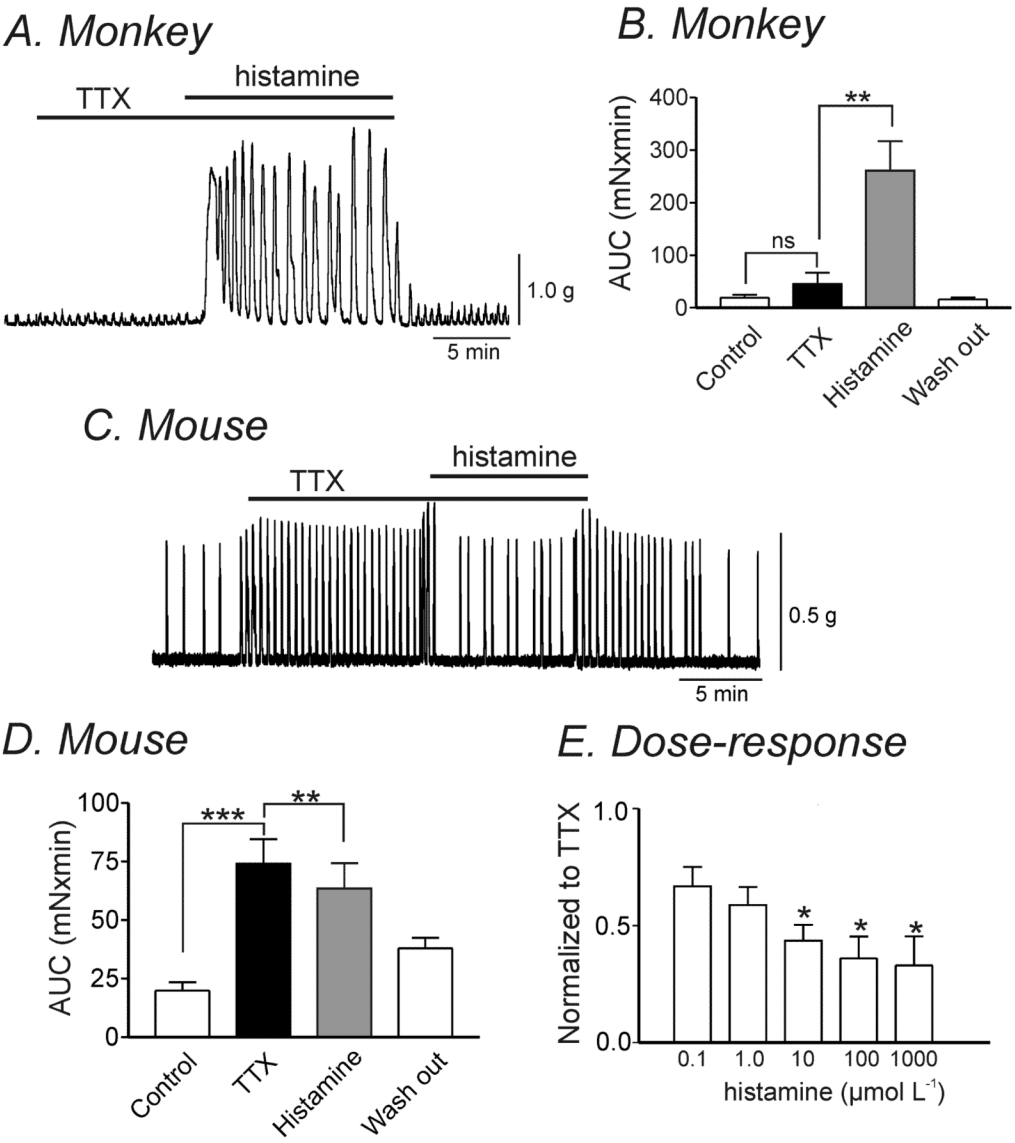

Figure 3. Histamine-evoked changes in contractility in simian and murine colonic longitudinal smooth muscle in the presence of TTX

(A) Representative mechanical trace showing that in the presence of TTX (1 μmol L^{-1}), exposure to histamine (10 μmol L^{-1}) increased contractile amplitude in simian colonic smooth muscle. (*B*) Graph summarizing the significant increase in AUC by histamine in the presence of TTX in simian colonic tissue (n=9, ***P*<0.005). (*C*) In murine colonic smooth muscle, histamine caused a sustained decrease in contractile force in the presence of TTX (1 μmol L^{-1}). (*D*) Graph summarizing the significant decrease in AUC by histamine in the presence of TTX in murine colonic muscle (n=17, ***P*<0.005, ****P*<0.001). (*E*) Summary graph illustrating dose-dependent effects of histamine on murine colonic AUC normalized to control (TTX presence) (n=4, * *P*<0.05).

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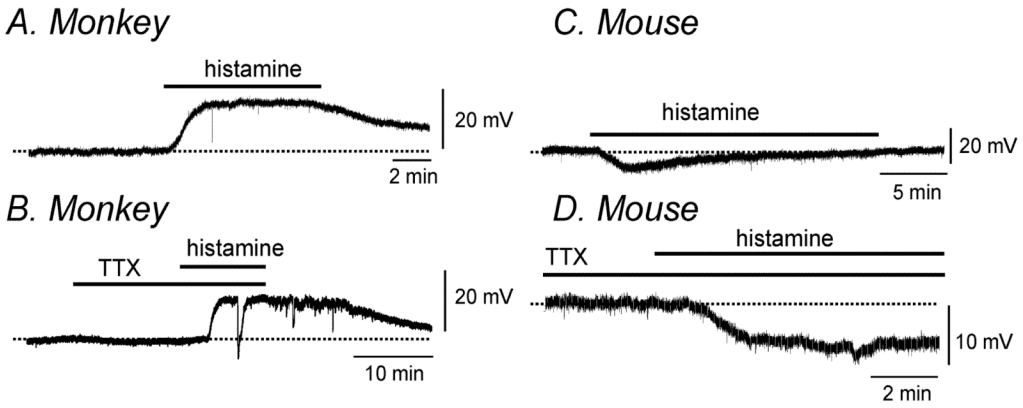

Figure 4. Histamine altered membrane potential in simian and murine colonic longitudinal smooth muscle

Representative traces illustrating that histamine (10 μmol L^{-1}) caused depolarization in the absence (*A*) and presence (*B*) of TTX (1 μmol L^{-1}) in simian colonic smooth muscle. In contrast, in murine colonic smooth muscle, representative traces show that histamine (10 μmol L^{-1}) induced transient hyperpolarization in the absence (*C*) and sustained hyperpolarization in the presence (*D*) of TTX (1 μmol L^{-1}).

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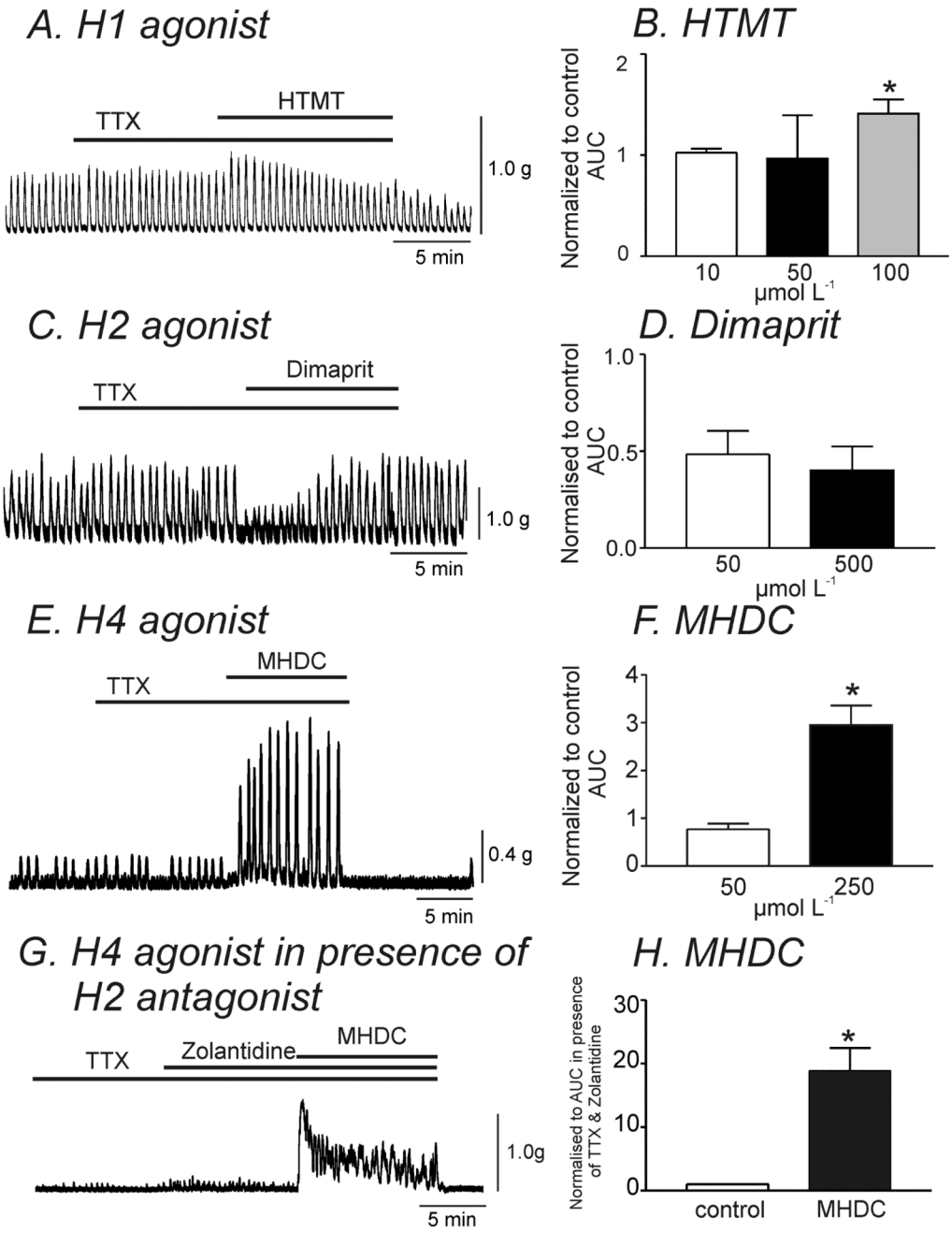

Figure 5. Specific H receptor agonists had different effects on contractility in simian colonic longitudinal smooth muscle

(*A* & *B*) Representative trace illustrating that application of the H1 agonist, HTMT (100 μmol L^{-1}), caused a transient increase in contractile force. (*B*) AUC during application of HTMT was normalized to the control AUC in the presence of TTX (1 μmol L^{-1}). HTMT (100 μmol L^{-1}) transiently increased AUC (n=4). (*C* & *D*) The H2 agonist (dimaprit, 50 μmol L^{-1}) transiently decreased the amplitude of spontaneous contractions but not significantly (n=4). (*E* & *F*) Representative trace illustrating that the H4 agonist, MHDC (250 μmol L^{-1}) caused a dramatic increase in contractile force (n=4). (*G*) In the presence of the H2 antagonist, Zolantidine (10 μmol L^{-1}), the contractile response to MHDC (250 μmol

L^{-1}) was augmented. (*H*) Summarized data showing the effect of MHDC (250 μmol L^{-1}) normalized to control AUC (in presence of TTX and Zolantidine) (n=4). * denotes $P<0.05$.

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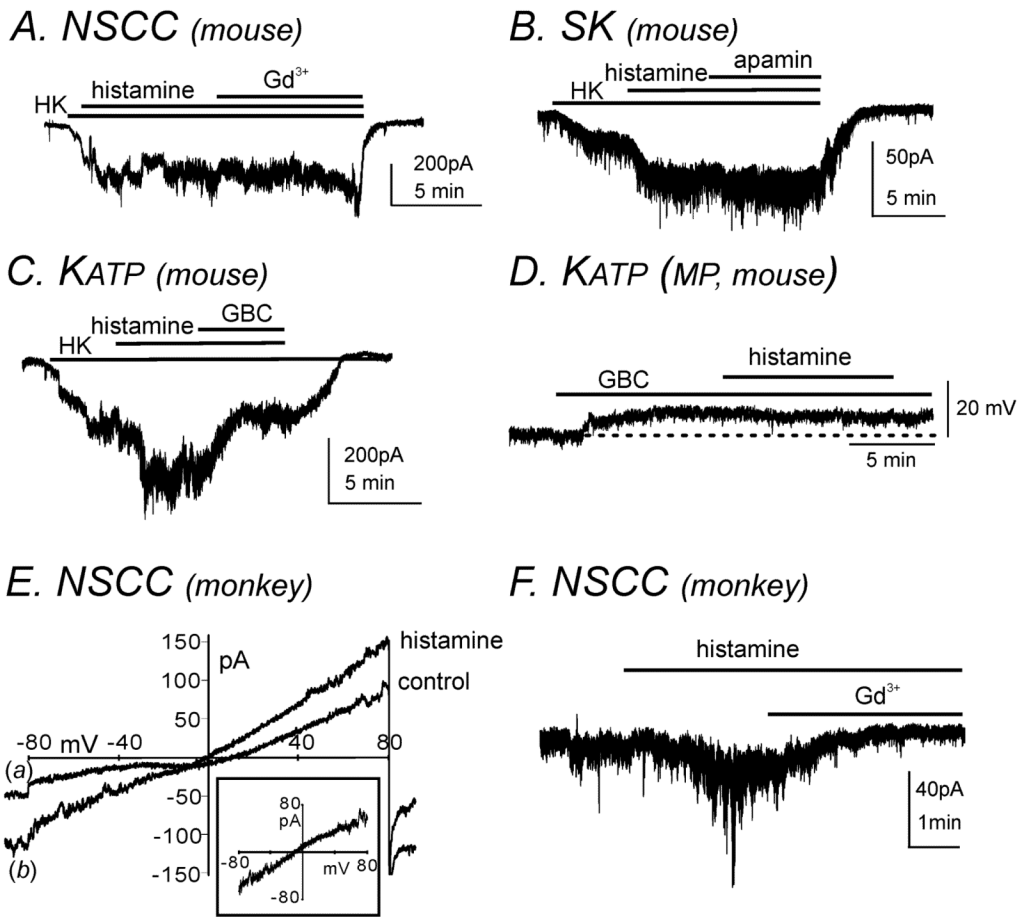

Figure 6. Histamine activated K_{ATP} currents in murine colonic longitudinal SMC and NSCC in simian colonic longitudinal SMC

(*A*) Murine colonic SMC was held at a holding potential of -80mV under perforated whole-cell configuration. CaPSS was replaced with high K^+ solution (HK; 140 mmol L^{-1} K^+, externally). Application of histamine (10 μmol L^{-1}) activated inward currents which were not inhibited by the NSCC blocker, Gd^{3+} (10 μmol L^{-1}). (*B*) Addition of the small-conductance Ca^{2+}-activated K^+ (SK) channel blocker, apamin (300 nmol L^{-1}), did not affect histamine- activated inward currents. (*C*) The K_{ATP} channel blocker, glibenclamide (GBC, 10 μmol L^{-1}), inhibited histamine-activated inward currents. (*D*) Application of histamine (10 μmol L^{-1}) had no significant effect on membrane potential in the presence of GBC (10 μmol L^{-1}) in murine colonic smooth muscle using intracellular microelectrode recordings. Dotted line denotes the resting membrane potential. (*E*) Simian colonic SMC was held at -80mV under whole-cell configuration. Histamine (10 μmol L^{-1}) activated inward currents during ramp depolarization from -80 to +80 mV (see line (*b*) compared with control, line (*a*)). Subtracted (*a*-*b*) histamine-sensitive currents reversed at approximately 0mV (subtracted current in inset, E_{Cl}= -40mV). (*F*) Histamine-activated currents were inhibited by Gd^{3+} (10 μmol L^{-1}).