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Research Paper

The essential oil of *Croton nepetaefolius* selectively blocks histamine-augmented neuronal excitability in guinea-pig celiac ganglion

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Abstract

Objectives *Croton nepetaefolius* is a medicinal plant useful against intestinal disorders. In this study, we elucidate the effects of its essential oil (EOCN) on sympathetic neurons, with emphasis on the interaction of EOCN- and histamine-induced effects.
Methods The effects of EOCN and histamine were studied in guinea-pig celiac ganglion *in vitro*.
Key findings Histamine significantly altered the resting potential (E_m) and the input resistance (R_i) of phasic neurons (from –56.6 ± 1.78 mV and 88.6 ± 11.43 MΩ, to –52.9 ± 1.96 mV and 108.6 ± 11.00 MΩ, respectively). E_m, R_i and the histamine-induced alterations of these parameters were not affected by 200 μg/ml EOCN. The number of action potentials produced by a 1-s (two-times threshold) depolarising current and the current threshold (I_{th}) for eliciting action potentials (rheobase) were evaluated. Number of action potentials and I_{th} were altered by histamine (from 2.6 ± 0.43 action potentials and 105.4 ± 11.15 pA to 6.2 ± 1.16 action potentials and 67.3 ± 8.21 pA, respectively). EOCN alone did not affect number of action potentials and I_{th} but it fully blocked the histamine-induced modifications of number of action potentials and I_{th}. All the effects produced by histamine were abolished by pyrilamine.
Conclusions EOCN selectively blocked histamine-induced modulation of active membrane properties.
Keywords action potential; *Croton nepetaefolius*; essential oil; neuron excitability; resting potential; sympathetic

Introduction

Croton nepetaefolius Baill. (Euphorbiaceae) is an aromatic bush that grows natively in drought regions ('caatinga') of northeastern Brazil, where it is popularly named 'marmeleiro sabiá'. Teas and infusates of leaves, small branches and of log skin of *C. nepetaefolius* have had widespread use in folk medicine as a carminative agent and to treat intestinal disorders. Leaves and small branches of *C. nepetaefolius* produce a relatively rich essential oil content (1–3% of dry weight), which has 1,8-cineole, methyl-eugenol and xantoxylin as the major constituents.[1]

Laboratory studies have demonstrated a variety of pharmacological effects for the essential oil of *C. nepetaefolius* (EOCN). In the guinea-pig gastrointestinal system, EOCN promotes antispasmodic activity with the interesting property of being more potent in relaxing basal tone than decreasing spontaneous activity.[1] In the cardiovascular system, EOCN decreases blood pressure and heart rate in either conscious or anaesthetised normotensive rats.[2] In conscious DOCA-salt hypertensive rats, EOCN promotes similar effects, except that it is more potent on blood pressure.[3] In both cases, EOCN-induced bradycardia was dependent on the presence of an intact and functional parasympathetic nerve drive to the heart.[2,3] EOCN also causes antiedematogenic and antinociceptive activities in mice. The antinociceptive action is unrelated to opioid receptor activation but seems to result from changes in neural activity.[4] Recently, it has been reported that EOCN and its major constituent, 1,8-cineol, block the compound action potential and decrease excitability in the sciatic nerve.[5]

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EOCN-induced changes in neural activities were mainly inferred from studies examining the effects of EOCN on autonomic nervous system parameters such as alterations in heart rate. In the present study we tested whether EOCN affected autonomic neurons directly or indirectly by modifying the actions of endogenous mediators that control autonomic neuronal excitability. Histamine (HA) is a well-characterised proinflammatory autacoid that modulates the excitability of autonomic neurons. It is widely distributed throughout the mammalian nervous system.[6,7] Additionally, HA has been shown to directly alter the excitability of sympathetic neurons via a postsynaptic effect mediated by H_1 receptors.[8] HA induces membrane depolarisation, increases membrane resistance and causes facilitation of synaptic transmission in sympathetic ganglia (superior cervical ganglion) *in vitro*.[9] Thus, in order to elucidate whether EOCN acts directly or indirectly on autonomic nervous system neurons, we examined the electrophysiological effects of EOCN and HA in celiac neurons, a prevertebral sympathetic ganglion, *in vitro*. The present study demonstrates that EOCN, at concentrations that do not show demonstrable electrophysiological effects on sympathetic neurons, dramatically inhibits selective HA-induced modifications of celiac neuron excitability.

Materials and Methods

Plant material

EOCN was kindly provided by the Department of Organic and Inorganic Chemistry of the Federal University of Ceará. EOCN extraction and analysis were as previously described.[1–3] Briefly, EOCN was extracted from dry leaves by steam distillation and analysed by gas chromatography and mass spectrometry (Hewlett-Packard 6971 GC/MS) and had the following chemical composition (percentage of oil weight): 1,8-cineole (25.4%), methyl-eugenol (14.9%), xantoxylin (10.1%), β-caryophyllene (9.7%), sabinene (5.2%), α-terpineol (5.0%) and other minor constituents. A voucher specimen (Number 3185) of *C. nepetaefolius* is deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará.

Animals

Male guinea-pigs (300–400 g) were obtained from local colonies maintained at the Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, Brazil. They were kept under conditions of constant temperature (22 ± 2°C) with a 12-h light/12-h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health.[10] All procedures described here were reviewed by and had prior approval from local animal ethics committee.

Preparation of tissue

Celiac ganglia from adult guinea-pigs were used throughout this study. Animals were sacrificed by cervical dislocation and exsanguination. Subsequently, their abdominal cavity was opened, the viscera were removed and the celiac ganglion was rapidly identified and removed with the adjacent connective tissue to a dissection chamber containing cold (8–10°C) Locke solution. Fat and connective were carefully removed from the surface of the ganglion with the aid of a dissecting microscope. The ganglia were then cut into approximately 0.6–0.9 mm thick slices, which were transferred to a reservoir containing cold Locke solution and used the same day. For intracellular recording, a ganglion slice was pinned to the silastic-coated floor of the recording chamber and superfused (1 ml/min) with Locke solution that had been equilibrated with 95% O_2 in 5% CO_2 and maintained at room temperature (20–24°C). The recording chamber was fixed to the stage of a compound microscope (20 and 40× magnification).

The recording chamber was designed to allow the level of the perfusate to be lowered to within approximately 100 μm of the surface of the ganglion slice, thus minimising stray capacitance and improving the discontinuous current-injection mode of the amplifier. Reservoirs containing oxygenated Locke solution with various drugs were connected to the inflow line of the recording chamber by three-way valves that could rapidly switch between the main reservoir and test solutions. The effects of HA were determined following a single bath application of the amine. By contrast, the effect of EOCN was based on collection of data points during a sequence of three steps: control measurements, application of EOCN alone and then addition of HA in the presence of EOCN. When pyrilamine was used to inhibit HA-induced stimulation of H_1-receptors, the experiment started with a control Locke solution containing 1 μM pyrilamine.

Electrophysiology

Intracellular recording was accomplished with glass microelectrodes fabricated from borosilicate capillaries (1.0 mm o.d., 0.56 mm i.d.; Sutter Instruments, San Francisco, CA, USA) using a Brown and Flamming puller (Sutter Instruments). The pipettes, which had DC resistance ranging from 70 to 120 MΩ when back-filled with a solution consisting of 3 M KCl and 0.05 M K^+ citrate, were connected via an Ag–AgCl pellet to an Axoclamp-2B amplifier (Axon Instruments, Union City, CA, USA). A hydraulic micromanipulator and microscope was used to impale celiac neurons with the recording glass micropipette. Impalement was facilitated by transiently unbalancing the negative capacitance compensation.

Membrane input resistance was determined by measuring the magnitude of electrotonic voltage transients elicited by a 1-s hyperpolarising constant current pulse delivered at 0.5-Hz intervals. Action potentials were activated by depolarising current pulses varying from 0.02 to 0.5 nA. No procedure, such as current injection, was used to maintain resting potential.

Neurons were acceptable for study if their resting membrane potential (>–45 mV) and input resistance (>40 MΩ) remained stable for 3–10 min after impalement. Action potential characteristics were recorded in bridge mode (filtering at 10 kHz). For most other measurements the amplifier was used in the discontinuous current injection mode (switched; 2.0–5.0 kHz). Headstage (unsampled) voltage was continuously

monitored to ensure that it settled completely prior to sampling. The output of the sample-and-hold amplifier was low-passed filtered at approximately 1/10 the sampling frequency. Current and voltage outputs were displayed on-line with an oscilloscope (model 5111A, Tektronix, Beaverton, OR, USA). Analogue signals, digitised by an analogue-to-digital converter (Digidata 1200, Axon Instruments), were computer-stored for analysis using appropriate software (pClamp6, Axon Instruments). Neuronal cells in celiac ganglia show considerable heterogeneity in action potential waveforms and firing patterns and have been classified as phasic, tonic and long after-hyperpolarisation neurons.[11–13] In the current work we utilised exclusively phasic neurons. These neurons fire one, or only a few, action potentials following a sustained suprathreshold depolarising current pulse, 1 s in duration.

Solutions and chemicals

The composition of the Locke solution was (mM): 136 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.2 $CaCl_2$, 14.3 $NaHCO_3$, 1.2 NaH_2PO_4, 10 dextrose, equilibrated with 95% O_2/5% CO_2 (pH 7.4). EOCN was initially diluted in dimethyl sulfoxide (DMSO) and then directly dissolved in Locke solution, at an amount sufficient to reach the final desired concentration. The DMSO concentration in the Locke solution was less than 0.1%, a concentration that had no discernible effect on the neurophysiological parameters measured. HA dihydrochloride and pyrilamine solutions were prepared daily from concentrated (>10 mM) stock aliquots which were stored frozen (–20°C). All reagents and drugs used in this study were of analytical grade, purchased from Sigma Chemical Co. (St Louis, MO, USA) or Merck (Darmstadt, Germany).

Statistical analysis

All data are expressed as means ± SEM. Significance ($P < 0.05$) was assessed by means of paired Student's *t*-test and one- or two-way analysis of variance (ANOVA), followed by a multiple comparison test where appropriate; *n* values ranged from 7 to 60, unless specified.

Results

Effect of EOCN on passive and active membrane properties and its protective actions on histamine-induced excitatory effects

EOCN (200 μg/ml) had no measurable effect on the two passive membrane properties measured, resting membrane potential or input resistance. HA (10 μM), reversibly and significantly ($P < 0.01$; paired Student's *t*-test), depolarised the resting membrane potential as well as increasing the input resistance of phasic neurons to average values corresponding to −52.9 ± 1.96 mV; $n = 11$ and 108.6 ± 11.00 MΩ, $n = 7$, respectively (control values: −56.6 ± 1.78 mV and 88.6 ± 11.43 MΩ, respectively (Table 1). In the presence of EOCN (200 μg/ml), HA still produced a membrane depolarisation and an increase in input resistance (Table 2).

HA also significantly decreased the action potential amplitude from 79.3 ± 1.07 mV in control, to 75.9 ± 1.04 mV (Table 1). EOCN had no effect on the amplitude of the action

Table 1 Effects of histamine on electrophysiological parameters of phasic neurons of the guinea-pig celiac ganglion recorded *in vitro*

Parameter	Resting potential (mV)	Input resistance (MΩ)	AP amplitude[a] (mV)	Current threshold[b] (pA)	Overshoot[c] (mV)
Control	–56.6 ± 1.78[d] (11)	88.6 ± 11.43 (7)	79.3 ± 1.07 (60)	105.4 ± 11.15 (11)	23.7 ± 1.17 (53)
HA (10 μM)	–52.9 ± 1.96** (11)	108.6 ± 11.00** (7)	75.9 ± 1.04* (60)	67.3 ± 8.21* (11)	23.7 ± 1.13 (53)

HA, histamine. [a]Amplitude of the action potential (AP); [b]threshold current necessary to detonate an action potential; [c]action potential measured from zero mV to peak depolarisation; [d]mean ± SEM. Figures in parentheses are the number of experiments. *, **, significantly different from control ($P < 0.05$ and $P < 0.01$, respectively, paired Student's *t*-test).

Table 2 Effects of the essential oil of *C. nepetaefolius* on the actions of histamine recorded in phasic neurons of the celiac ganglion *in vitro*

Parameter	Resting potential (mV)	Input resistance (MΩ)	AP amplitude[a] (mV)	Current threshold[b] (pA)	Overshoot[c] (mV)
Control	–52.4 ± 2.18[d] (8)	72.9 ± 10.17 (7)	76.7 ± 1.88 (30)	120.0 ± 13.33 (9)	24.9 ± 1.75 (22)
EOCN (200 μg/ml)	–52.7 ± 2.38 (8)	78.6 ± 11.00 (7)	74.1 ± 1.93 (30)	128.9 ± 14.57 (9)	22.6 ± 1.32 (22)
HA (10 μM) + EOCN	–48.2 ± 1.77* (8)	101.4 ± 16.25* (7)	68.4 ± 1.79* (30)	106.7 ± 17.95 (9)	22.4 ± 1.19 (22)

HA, histamine; EOCN, essential oil of *C. nepetaefolius*. [a]Amplitude of the action potential (AP); [b]threshold current necessary to detonate an action potential; [c]action potential measured from zero mV to peak depolarisation; [d]mean ± SEM. Figures in parentheses are the number of experiments. *Significantly different from control ($P < 0.05$, ANOVA and Dunnett's test).

potential nor did it block the HA-induced reduction in the amplitude of the action potential (Table 2).

Action potential overshoot (control: 23.7 ± 1.17) was unaffected by histamine (10 μM) or by EOCN 200 μg/ml (Tables 1 and 2).

EOCN prevents histamine-induced increase in cell excitability

HA (10 μM) significantly decreased ($P < 0.05$; paired Student's *t*-test) the current threshold to produce neuron firing (from 105.4 ± 11.15 pA in control, to 67.3 ± 8.21 pA in the presence of HA; Table 1) and increased the number of action potentials elicited by a depolarising current pulse at twice threshold (from 2.6 ± 0.4 to 6.2 ± 1.2 action potentials; Figure 1). EOCN (200 μg/ml) alone did not alter the current threshold to elicit action potentials, but it significantly ($P < 0.05$; ANOVA and Dunnett's test) prevented the HA-induced (10 μM) alteration of threshold (Table 2). EOCN did not affect the number of action potentials evoked by a two-times threshold depolarising current pulse but it did significantly block ($P < 0.05$; ANOVA and Dunnett's test) the increase of the number of action potentials induced by HA application ($n = 7$; see Figure 1).

We also examined the latency to detonate an action potential in response to current pulses ranging from 20 to 200 pA. First spike latency was characterised as the time between stimulus onset and the occurrence of the first action potential, measured at the most positive peak of the action potential. In control preparations, action potentials did not occur with pulse amplitudes up to 60 pA. Using current pulses ranging from 80 to 200 pA, latency varied between 134.3 ± 14.12 ms and 86.0 ± 4.33 ms, for 80 and 200 pA, respectively (Figure 2). In the presence of HA (10 μM), all neurons tested fired an action potential at a current intensity significantly lower than control neurons (60 pA) ($P < 0.05$, two-way ANOVA). For stimuli of 80 and 200 pA the latency values in the presence of HA ranged from 98.5 ± 3.65 ms to 64.6 ± 6.89 ms, respectively (Figure 2). EOCN (200 μg/ml) alone did not alter spike latency, but it prevented the HA-induced reduction on this parameter (Figure 2).

Histamine-induced electrophysiological effects are due to activation of H_1 histamine receptors

Preparations perfused with a Locke solution containing the H_1-receptor antagonist pyrilamine (1 μM) for at least 5 min, had electrophysiological parameters that were not significantly different from control values (Table 3). The presence of pyrilamine significantly blocked ($P < 0.05$; ANOVA and Dunnett's test) the HA-induced (10 μM) effects on resting potential, input resistance, current threshold and the number of action potentials occurring during the voltage response to a suprathreshold depolarising current pulse (Table 3).

Discussion

The major findings of the present study are that the EOCN, at concentrations that did not reveal any electrophysiological effect on sympathetic neurons, may selectively interfere with the effects of HA, an inflammatory mediator that exerts important electrophysiological activities on sympathetic neurons. Due to the importance of the sympathetic nervous system for the control of visceral[14] and immunological[15] functions, the effects of HA as well as the modification of HA activity produced by EOCN are probably of pathophysiological and therapeutic importance. Although actions of essential oils and of their constituents on nerves have been reported,[16–19] to the best of our knowledge this is the first report showing direct effects of an essential oil on excitability of prevertebral sympathetic neurons.

The sample of EOCN used in this study has 1,8-cineole as its major constituent (~25%). We hypothesise that this terpenoid oxide is probably involved in the effects induced by EOCN on celiac neurons, but its contribution appears just partial. As a matter of fact, we have recently reported that 1,8-cineole blocks the excitability of sympathetic neurons of superior cervical ganglion through two distinguishable mechanisms of action, one of which acts indirectly, via neuronal membrane depolarisation.[20] This effect was observed at 1,8-cineole concentrations higher than that expected to occur with EOCN, which was used herein at 200 μg/ml. As EOCN showed no activity on sympathetic neuronal passive parameters, such as resting membrane potential and input resistance, we may conclude that a depolarising effect induced by 1,8-cineole does not contribute to the effects of EOCN.

Other lines of evidence further support the hypothesis of the involvement of 1,8-cineole in the mediation of the EOCN's effects reported herein: (1) 1,8-cineole blocked compound action potential and decreased excitability in the sciatic nerve,[5] (2) 1,8-cineole inhibited electromechanical coupling in airway smooth muscle,[21] (3) EOCN inhibited HA-induced contractions in both tracheal and intestinal smooth muscle[22,23] and (4) 1,8-cineole fully relaxed guinea-pig isolated trachea precontracted with HA.[21] Data contained in these early studies reveal a promising role for 1,8-cineole on HA-induced effects in sympathetic neurons too. However, further studies will be necessary to address this issue.

The present observations for EOCN are consistent with our previous data on rat sciatic nerves. At concentrations ranging from 500 to 1000 μg/ml, EOCN blocked sciatic action potential generation and conduction, but at 100 to 300 μg/ml it had no measurable effect.[5] The present data also corroborate the inefficacy of 200 μg/ml EOCN on membrane resting potential, which has been reported in smooth muscle cells of guinea-pig ileum.[23] These data suggest that, at concentrations less than 200 μg/ml, EOCN does not directly affect excitable membranes. Nonetheless, EOCN may interfere with the neural activity of autacoids and neurotransmitters, as shown here for HA.

Interestingly, one of the properties of EOCN is that it is a hypotensive agent,[2,3] which is supposedly related to its ability to decrease both the blood pressure and heart rate. Bilateral vagotomy, as well as pretreatment with methylatropine or hexamethonium, reduced the magnitude of the EOCN-induced bradycardia without affecting hypotension, which suggests that its bradycardic activity is dependent on an operational autonomic nervous system, whereas the hypotension is related to a vasodilator action.[3,24] Thus, the present

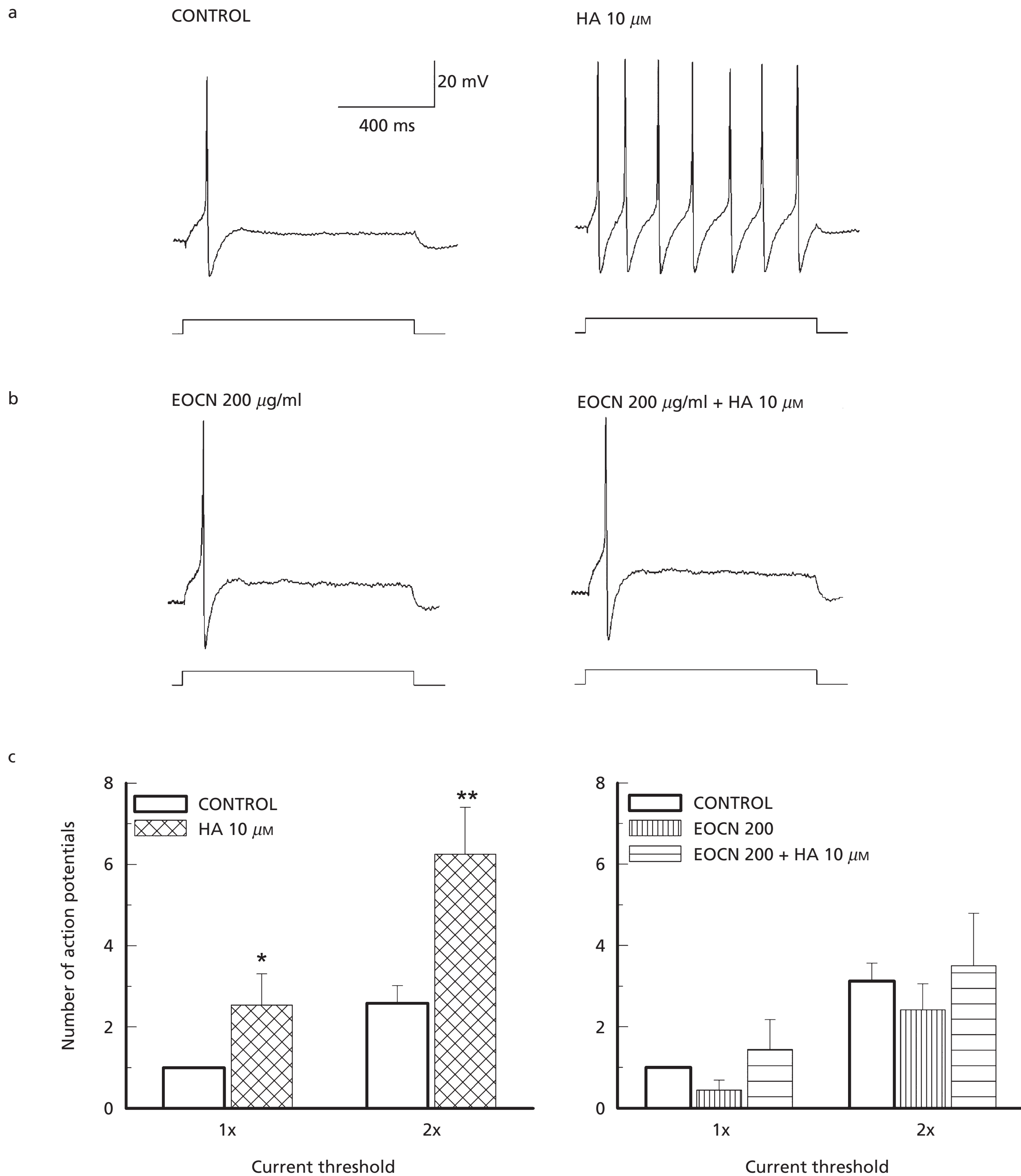

Figure 1 Effect of histamine and EOCN on the number of action potentials in celiac ganglion of guinea-pigs. HA, histamine; EOCN, essential oil of *C. nepetaefolius*. (a) Action potential activity of a phasic neuron in response to a 1-s current pulse with a threshold intensity to trigger an all-or-none spike (lower row of traces) recorded in control Locke solution (CONTROL) and in a Locke solution containing HA (10 μM). Note that in the presence of HA the threshold current pulse now evokes multiple action potentials. Trace recordings were made in the same neuron. (b) Action potential activity of a phasic neuron in response to a 1-s current pulse with intensity just threshold (lower row of traces) recorded in a Locke solution containing EOCN (200 μg/ml), and in a Locke solution containing both EOCN and HA. Note that the excitatory effect of HA is abrogated by the presence of EOCN. Trace recordings were made in the same neuron. (c) Quantification of the number of action potentials (AP) evoked by depolarising current pulses that are threshold (1×) or two-times threshold (2×). Bars are mean ± SEM. ($n = 12$ in all cases for the left graph; $n = 7$ in all cases for the right graph). *,**Significantly different from control ($P < 0.05$ and $P < 0.01$, respectively; ANOVA and Dunnett's test).

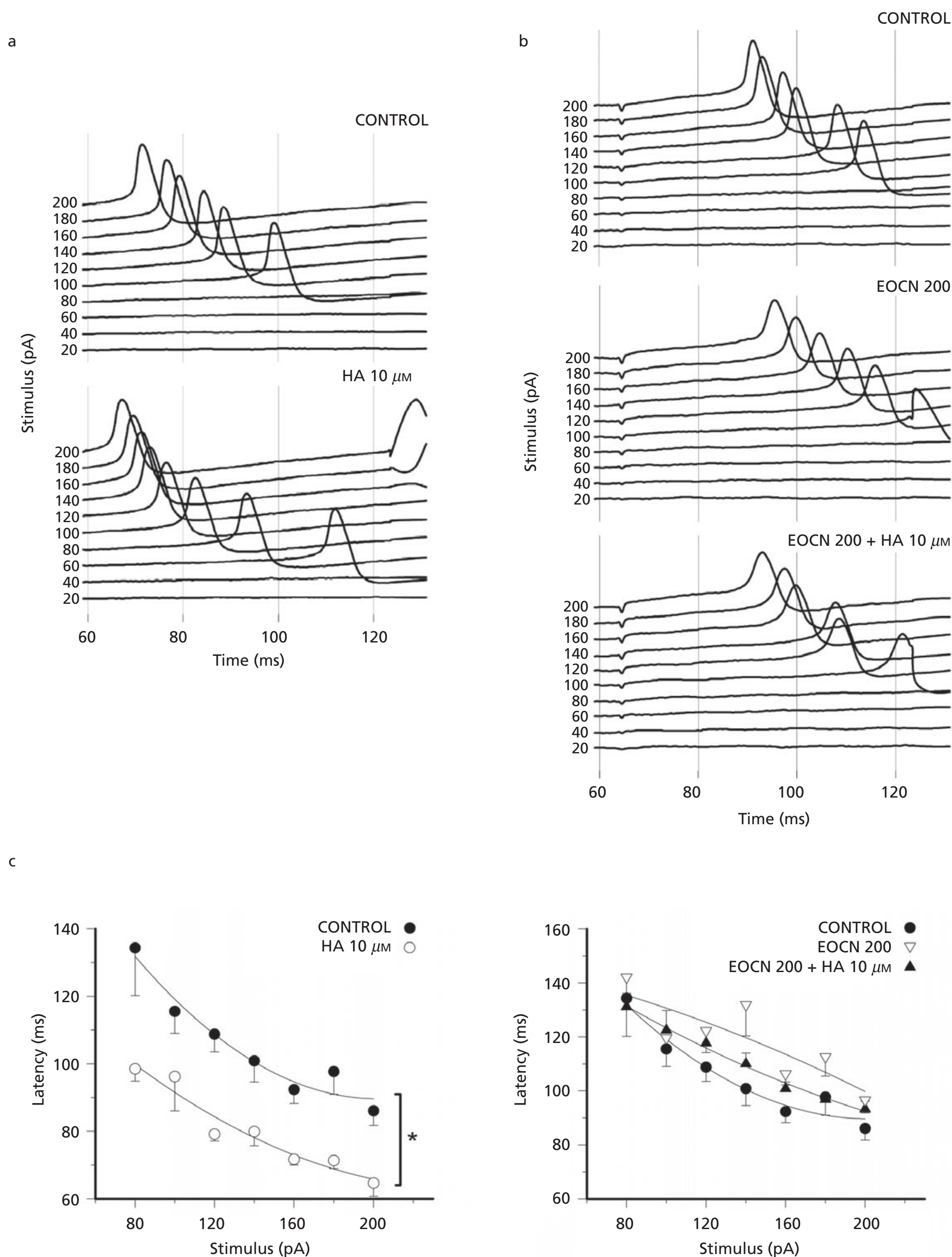

Figure 2 Effect of histamine and EOCN on the latency of the first spike recorded in phasic neurons of the guinea-pig celiac ganglion *in vitro*. HA, histamine; EOCN, essential oil of *C. nepetaefolius*. (a) Typical traces of a series of subthreshold to suprathreshold depolarising current steps in the absence (control, upper trace) and presence of 10 μM histamine (HA 10 μM, lower trace). Incremental 20-pA depolarising current steps (20–200 pA, 1000 ms in duration, 5-s interstep interval) were applied. All recordings were made in the same neuron. (b) Typical traces of a series of subthreshold to suprathreshold depolarising current steps in the absence (control) and presence of 200 μg/ml EOCN (EOCN 200) alone or in the presence of 10 μM histamine (EOCN 200 + HA 10 μM). Incremental 20-pA depolarising current steps (20–200 pA, 1000 ms duration, 5-s interstep interval) were applied. All recordings were made in the same neuron. (c) Plots of stimulus strength versus latency to action potential detonation in control conditions, or with histamine (HA; left-hand plot; $n = 12$), and in control conditions, or in the presence of EOCN, or in the presence of both EOCN and HA (right-hand plot; $n = 7$). Data points are mean ± SEM. *Significantly different from control ($P < 0.05$, two-way ANOVA).

Table 3 Effects of pyrilamine on histamine-induced changes in passive and active membrane properties recorded in phasic neurons of the celiac ganglion *in vitro*

Parameter	Resting potential (mV)	Input resistance (MΩ)	AP amplitude[a] (mV)	Current threshold[b] (pA)	Overshoot[c] (mV)
Control	−56.3 ± 2.9[d]	91.4 ± 13.0	69.8 ± 4.4	100.0 ± 16.3	23.7 ± 3.3
Pyrilamine (1 μM)	−51.0 ± 3.12[e]	103.3 ± 7.60	70.4 ± 5,06	103.3 ± 22.1	30.0 ± 4.64
Pyrilamine + histamine (10 μM)	−51.5 ± 3.64[f]	106.6 ± 13.58	69.4 ± 4,13	88.3 ± 13.27	27.9 ± 3.81

[a]amplitude of the action potential; [b]threshold current necessary to detonate an action potential (AP); [c]action potential measured from zero mV to peak depolarisation; [d]mean ± SEM. $n = 7$ in all cases; [e]no value in pyrilamine was significantly different from control ($P > 0.05$, ANOVA and Dunnett's test); [f]no value in pyrilamine + histamine was significantly different from pyrilamine alone ($P > 0.05$, ANOVA and Dunnett's test).

EOCN-induced electrophysiological activity on sympathetic neurons reinforces the possibility that some in-vivo effect elicited by EOCN, like the previously demonstrated intestinal transit acceleration[1] and the antinociceptive activity,[4] might include a direct neural component of action.

Although the present study did not aim to elucidate the mechanism of action of HA effects, or that of EOCN interference with HA-induced activity, some considerations on their mechanism are appropriate. First, since all HA effects were blocked by pyrilamine, these effects are most likely due to activation of the H_1 receptor. The presence of H_1 receptors has already been reported in sympathetic neurons[8] and its activation promotes the various HA-induced effects, e.g. changes in both presynaptic and postsynaptic properties in sympathetic superior cervical ganglion.[9,25] This receptor is involved in the modulation of sympathetic postganglionic synaptic transmission via a presynaptic action of HA.[26] In AH/type 2 myenteric neurons, HA depolarises the resting membrane potential, increases the input resistance and blocks a post-spike after-hyperpolarisation.[27] We did not test the effects of H_2 or H_3 histamine receptor antagonists, but the question of whether EOCN acts on these receptors deserves further investigation. However, HA receptor-mediated actions of EOCN are unlikely because earlier studies have shown that EOCN inhibits HA-induced contractions in tracheal or ileal smooth muscle with the same potency that it inhibits other contractile agents.[22,23]

EOCN blocked only those HA-induced effects associated with active membrane properties (not those associated with passive membrane properties). However, in the presence of an H_1-receptor antagonist, both active and passive membrane properties altered by HA were blocked. Thus our data suggest that: (1) this EOCN-induced blockade of some HA effects is not a pharmacological antagonism on the H_1 receptor and (2) the actions of EOCN appear to occur at a certain step downstream in the signal-transduction cascade of events following H_1-receptor stimulation, which is selectively related to the control of the neuronal excitability, but without influence on passive properties of the membrane. Jafri *et al.* showed that H_1 receptors may activate two or more second messengers to control the ionic currents.[28]

Bath application of HA produced neuronal depolarisation and an increased input resistance. These effects are similar to previous reports and probably involve an action on different ionic currents, some of them important for determination of resting potential and input resistance.[28] A few studies have examined the ionic mechanisms underlying the HA-induced effects in mammalian neurons, and they have implicated blockade of potassium conductances because they were inhibited by the presence of K^+ channel blockers or because they showed reversal potential close to the reversal potential for K^+ ions.[28–31] In slice preparations of rat brain using cholinergic neurons of the medial septum/diagonal band of Broca, an increase in a tetrodotoxin-insensitive sodium conductance was also reported.[32] Whether, under our experimental conditions, HA is changing potassium or sodium conductances in the neurons of celiac ganglion deserves further investigation, but it is worth noting that whilst HA altered many electrophysiological parameters of celiac neurons, EOCN significantly blocked only those related to neuronal excitability (active membrane properties), such as the number of action potentials and the latency of the first spike in response to a depolarising current pulse. A combination of cell depolarisation and a decrease in membrane conductance can potentially increase neuronal excitability by bringing the membrane potential closer to the spike threshold and increasing its responsiveness to depolarising stimuli.[28]

HA significantly decreased the stimulus strength necessary to elicit an action potential because the number of incremental depolarising current steps necessary to reach the action potential threshold was greater in the control than in the presence of HA and the latency to activate an action potential was shorter in the presence of HA than in the control. Under these conditions, HA is probably acting on a conductance relevant for excitability but not on a conductance associated with resting potential or input resistance. This is not paradoxical because some K^+ conductances, i.e. A and M conductances, are time- and voltage-dependent, and thus they can modulate neuronal excitability without necessarily interfering with resting membrane potential and input resistance.[33,34] Although such interference induced by HA on A or M conductances has not yet been specifically demonstrated in celiac ganglia, we hypothesise that HA may impair activation of K^+ currents that limit the frequency of spike discharge by the somal membrane of guinea-pig celiac neurons and that EOCN is preventing the presumed activity of HA on active membrane properties by interfering with either A-current or M-current. Finally, it appears improbable that inhibition of HA is due to a role of EOCN acting directly as a potassium channel activator

because it is unable to hyperpolarise the transmembrane potential of celiac neurons.

Conclusions

The present study did not intend to validate the widespread use in folk medicine of teas and infusates prepared with parts of *C. nepetaefolius*. A previous study by our laboratory,[1] although it did not directly have that goal, was more directly related to it and demonstrated coherence between the EOCN intestinal effects and folk use of the plant. The present study, however, showed a potential therapeutic application for EOCN unrelated to its use in folk medicine. Should the effect of HA on sympathetic neuron excitability prove to be an activity of physiological or pathophysiological significance, EOCN may have therapeutic application, since it blocks this HA effect without having an effect of its own. This possibility deserves further investigation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Magalhães PJC *et al*. Intestinal myorelaxant and anti-spasmodic effects of the essential oil of *Croton nepetaefolius*, and its constituents cineole, methyl-eugenol and terpineol. *Phytother Res* 1998; 12: 172–177.
2. Lahlou S *et al*. Cardiovascular effects of the essential oil of *Croton nepetaefolius* in rats: role of the autonomic nervous system. *Planta Med* 1999; 65: 553–557.
3. Lahlou S *et al*. Essential oil of *Croton nepetaefolius* decreases blood pressure through an action upon vascular smooth muscle: studies in DOCA-salt hypertensive rats. *Planta Med* 2000; 66: 138–143.
4. Abdon AP *et al*. Antinociceptive effects of the essential oil of *Croton nepetaefolius* on mice. *Braz J Med Biol Res* 2002; 35: 1215–1219.
5. Lima-Accioly PM *et al*. Essential oil of *Croton nepetaefolius* and its main constituent, 1,8-cineole, block excitability of rat sciatic nerve *in vitro*. *Clin Exp Pharmacol Physiol* 2006; 33: 1158–1163.
6. Hill SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol Rev* 1990; 42: 45–83.
7. Schwartz JC *et al*. Histaminergic transmission in the mammalian brain. *Physiol Rev* 1991; 71: 1–51.
8. Whyment AD *et al*. Histamine excites neonatal rat sympathetic preganglionic neurons *in vitro* via activation of H1 receptors. *J Neurophysiol* 2006; 95: 2492–2500.
9. Snow RW, Weinreich D. Presynaptic and postsynaptic effects of histamine and histamine agonists in the superior cervical ganglion of the rat. *Neuropharmacology* 1987; 26: 743–752.
10. National Research Council. Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23). Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences. National Academy Press, Washington, DC, 1996.
11. Weems WA, Szurszewski JH. An intracellular analysis of some intrinsic factors controlling neural output from inferior mesenteric ganglion of guinea pigs. *J Neurophysiol* 1978; 41: 305–321.
12. McLachlan EM, Meckler RL. Characteristics of synaptic input to three classes of sympathetic neurones in the coeliac ganglion of the guinea-pig. *J Physiol* 1989; 415: 109–129.
13. Janig W, McLachlan EM. Characteristics of function specific pathways in the sympathetic nervous system. *Trends Neurosci* 1992; 15: 475–481.
14. Miolan JP, Niel JP. The mammalian sympathetic prevertebral ganglia: integrative properties and role in the nervous control of digestive tract motility. *J Auton Nerv Syst* 1996; 58: 125–138.
15. Friedman EM, Irwin MR. Modulation of immune cell function by the autonomic nervous system. *Pharmacol Ther* 1997; 74: 27–38.
16. Brodin P, Røed A. Effects of eugenol on rat phrenic nerve and phrenic nerve-diaphragm preparations. *Arch Oral Biol* 1984; 29: 611–615.
17. Moreira MR *et al*. Effects of terpineol on the compound action potential of the rat sciatic nerve. *Braz J Med Biol Res* 2001; 34: 1337–1340.
18. Leal-Cardoso JH *et al*. Effects of estragole on the compound action potential of the rat sciatic nerve. *Braz J Med Biol Res* 2004a; 37: 1193–1198.
19. Leal-Cardoso JH *et al*. Effects of the essential oil of *Alpinia zerumbet* on the compound action potential of the rat sciatic nerve. *Phytomedicine* 2004b; 11: 549–553.
20. Ferreira-da-Silva FW *et al*. Effects of 1,8-cineole on electrophysiological parameters of neurons of rat superior cervical ganglion. *Clin Exp Pharmacol Physiol* 2009; 36: 1068–1073.
21. Bastos VP. Ação broncodilatadora e anti-inflamatória do 1,8-cineol em modelo experimental de asma em cobaias. Fortaleza, Ceará: Universidade Federal do Ceará, 2009 (doctoral thesis) [in portuguese]. www.teses.ufc.br/tde_busca/arquivo.php?codArquivo=4075 (accessed 07 April 2010).
22. Magalhães PJC *et al*. Myorelaxant effects of the essential oil of *Croton nepetaefolius* on the contractile activity of the guinea-pig tracheal smooth muscle. *Planta Med* 2003; 69: 874–877.
23. Magalhães PJC *et al*. Antispasmodic effects of the essential oil of *Croton nepetaefolius* on guinea-pig ileum: a myogenic activity. *Fundam Clin Pharmacol* 2004; 18: 539–546.
24. Magalhães PJC *et al*. Vasorelaxation induced by the essential oil of *Croton nepetaefolius* and its constituents in rat aorta are partially mediated by the endothelium. *Fundam Clin Pharmacol* 2008; 22: 169–177.
25. Christian EP *et al*. Endogenous histamine excites neurones in the guinea-pig superior cervical ganglion *in vitro*. *J Physiol* 1989; 409: 297–312.
26. Christian EP, Weinreich D. Presynaptic histamine H1 and H3 receptors modulate sympathetic ganglionic synaptic transmission in the guinea-pig. *J Physiol* 1992; 457: 407–430.
27. Nemeth PR *et al*. Intracellular study of effects of histamine on electrical behaviour of myenteric neurones in guinea-pig small intestine. *J Physiol* 1984; 355: 411–425.
28. Jafri MS *et al*. Histamine H1 receptor activation blocks two classes of potassium current, IK(rest) and IAHP, to excite ferret vagal afferents. *J Physiol* 1997; 503: 533–546.

29. McCormick DA, Williamson A. Modulation of neuronal firing mode in cat and guinea pig LGNd by histamine: possible cellular mechanisms of histaminergic control of arousal. *J Neurosci* 1991; 11: 3188–3199.
30. Reiner PB, Kamondi A. Mechanisms of antihistamine-induced sedation in the human brain: H1 receptor activation reduces a background leakage potassium current. *Neuroscience* 1994; 59: 579–588.
31. Li Z, Hatton GI. Histamine-induced prolonged depolarization in rat supraoptic neurons: G-protein-mediated, Ca^{2+}-independent suppression of K^+ leakage conductance. *Neuroscience* 1996; 70: 145–158.
32. Gorelova N, Reiner PB. Histamine depolarizes cholinergic septal neurons. *J Neurophysiol* 1996; 75: 707–714.
33. Starodub AM, Wood JD. Histamine suppresses A-type potassium current in myenteric neurons from guinea pig small intestine. *J Pharmacol Exp Ther* 2000; 294: 555–561.
34. Guo J, Schofield GG. Histamine inhibits KCNQ2/KCNQ3 channel current via recombinant histamine H(1) receptors. *Neurosci Lett* 2002; 328: 285–288.