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ENDOGENOUS HISTAMINE EXCITES NEURONES IN THE GUINEA-PIG SUPERIOR CERVICAL GANGLION *IN VITRO*

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SUMMARY

1. Intracellular recordings were obtained from neurones in the guinea-pig superior cervical ganglion (SCG) *in vitro* to study the electrophysiological effects of endogenously released histamine.

2. Guinea-pigs were actively sensitized to the specific antigen, ovalbumin. SCG removed from these animals rapidly released a significant proportion of their endogenous histamine stores into the extracellular space upon exposure to the sensitizing antigen. Several observations indicated that the released histamine was derived from ganglionic mast cells.

3. The electrophysiological effects produced by antigen challenge in a neurone mimicked qualitatively and quantitatively those effects produced by exogenously applied histamine in the same neurone. Under current clamp the membrane effects of antigen and histamine included a transient depolarization, an increase in input resistance and transient blockade of a long-duration component of the spike after-hyperpolarization. In voltage clamp histamine and antigen produced an inward current and decreased membrane conductance.

4. Histamine H_1, but not H_2 or H_3 receptor antagonists prevented the membrane depolarization to both histamine and antigen treatments.

5. These convergent biochemical, physiological and pharmacological data demonstrate that a sufficient quantity of endogenous histamine is released by an antigenic stimulus in SCG from sensitized guinea-pigs to affect specific electrophysiological characteristics of neurones. Histamine may thus be involved in mediating interactions between the mammalian immune system and the peripheral sympathetic nervous system.

INTRODUCTION

Histamine has long been known to be present within the mammalian peripheral autonomic nervous system (Kwiatkowski, 1943; Euler, 1949, 1966). One major storage pool for histamine in autonomic ganglia and peripheral nerves is the mast cell (Torp, 1961; Lindl, Behrendt, Heinl-Sawaja, Teufel & Cramer, 1974; Weinreich,

1985). There is also evidence supporting the existence of minor non-mast cell-associated histamine pools, such as within neurones themselves (Green, 1964; Gross, Guo, Levi, Bailey & Chenouda, 1984; Häppölä, Soinila, Päivärinta, Panula & Eränkö, 1985; Weinreich, 1985). A physiological role for endogenous histamine in autonomic and enteric ganglia has been suggested indirectly by studies demonstrating that exogenous histamine can affect neuronal excitability and synaptic efficacy via specific histamine receptor subtypes (Brimble & Wallis, 1973; Lindl, 1983; Nemeth, Ort & Wood, 1984; Villena, Montoya, Roa, Jofre & Goset, 1986; Snow & Weinreich, 1987). However, the above studies fail to address the fundamental question of whether endogenous histamine is released in amounts sufficient to produce electrophysiological effects on ganglionic neurones.

Our laboratory has recently demonstrated (Weinreich & Undem, 1987) that a significant amount of the endogenous ganglionic histamine, as well as other endogenous mast cell-derived mediators, are released rapidly from the guinea-pig superior cervical ganglion (SCG) *in vitro* by a specific antigenic stimulus. Moreover, this work provides evidence based on extracellular recordings that histamine modulates synaptic transmission upon its release. Here, we have investigated effects of endogenously released histamine on the electrophysiological characteristics of post-ganglionic neurones in the guinea-pig SCG with intracellular recording methods.

In order to demonstrate a role for endogenous histamine, experiments focused on satisfying specific criteria which have previously been defined for neurotransmitter identification (Paton, 1958; Werman, 1966). These include: (1) release of the endogenous substance by an appropriate stimulus, (2) physiological mimicry between the exogenously applied and endogenously released substance, and (3) pharmacological antagonism of responses produced by the endogenous substance with appropriate receptor antagonists. Our results show that endogenous histamine in the guinea-pig SCG satisfies all of these criteria, thus clearly establishing a role for endogenous histamine in affecting excitability of these neurones. Results are discussed in the context of histamine having a neuroregulatory role in the guinea-pig SCG and being a potential mediator of interactions between the immune and nervous systems. A preliminary report of this work has been presented (Christian, Undem & Weinreich, 1987).

METHODS

Sensitization of animals to antigen and preparation of tissue

Adult male guinea-pigs (300–600 g) were actively sensitized to ovalbumin (chicken egg albumin, ovalbumin grade V; Sigma Chemical Co., St Louis, MO, USA), as described previously (Weinreich & Undem, 1987). Animals were injected intraperitoneally with three doses of ovalbumin (10 mg/kg) on alternating days. Twenty-one to 45 days after the last injection, animals were killed by a sharp blow to the head and exsanguinated. The SCG were dissected free and immediately submerged in ice-cold (5 °C) or room temperature (23 °C) Locke solution (composition in mM: 136 NaCl; 5·6 KCl; 14·3 $NaHCO_3$; 1·2 NaH_2PO_4; 2·2 $CaCl_2$; 1·2 $MgCl_2$; 11 dextrose; 0·03 choline chloride), equilibrated continuously with 95% O_2, 5% CO_2 (pH 7·2–7·4). Ganglia were used in experiments either on the day of dissection or stored in Locke solution (5 °C) overnight for use the following morning; storage did not affect measurably the electrophysiological or pharmacological characteristics.

SCG were trimmed of excess connective tissue, split longitudinally and pinned flat to the Sylgard (Dow Corning Co., Midland, MI, USA) floor of a recording chamber with stainless-steel pins. Ganglia were superfused (2–5 ml/min) with oxygenated Locke solution that was maintained at

34–36 °C with a Peltier device (Cambion Corp., Cambridge, MA, USA) located at the entrance of the chamber. A thermistor (Thermometrics Corp., Edison, NJ, USA) positioned ~ 0·5 cm from the ganglion monitored temperature. The chamber was affixed to the stage of a compound microscope equipped with Hoffman optics (400 ×). SCG were allowed to equilibrate in the recording chamber for at least 60 min before experiments commenced.

Histamine release studies

Three to four SCG were pinned to the floor of the recording chamber to insure that released histamine in the superfusate would achieve an adequate concentration for chemical detection. Superfusate leaving the chamber was diverted into 10 ml tubes with a gauze wick. Samples were collected for consecutive 1 min periods from 1 min prior to challenge with ovalbumin antigen (10 μg/ml) to 15 min after the superfusate was switched to the ovalbumin-containing solution. Ganglia were then transferred to a test-tube containing 2 ml of 0·4 N-perchloric acid, and placed in a boiling water bath for 15 min to liberate residual histamine for measurement. The histamine concentration in a 500 μl aliquot from each sample was determined by automated fluorometry as described by Siraganian (1974) within 48 h of each experiment; the detection limit of this system for histamine was 10 pmol/ml. Histamine content was calculated for the total volume of each sample, and then summed for all samples taken during antigen challenge to determine the total amount of antigen-induced histamine release.

Electrophysiological studies

Intracellular microelectrodes were fabricated from capillary glass (1·2 mm o.d., 0·68 mm i.d.; WPI Corp., New Haven, CT, USA) on a Brown and Flaming puller (Sutter Instr., San Francisco, CA, USA) and back-filled with a solution of 1 M-potassium acetate, 2 M-KCl. Electrode impedance ranged from 38 to 94 MΩ for current-clamp recordings and from 28 to 65 MΩ for voltage-clamp recordings. Electrical activity from the microelectrode was amplified with an Axoclamp-2 electrometer (Axon Instr., Burlingame, CA, USA). Neuronal impalement was facilitated by transiently increasing negative capacitance. Once the impalement stabilized (usually within 3–7 min), a neurone was only studied further if the resting membrane potential was more negative than −50 mV, the input resistance > 30 MΩ, and the action potential overshoot (above 0 mV) > 10 mV. The discontinuous (switched; 3·0–4·8 kHz) current injection mode of the amplifier was used for both current-clamp and voltage-clamp applications. Headstage (unsampled) current was monitored continuously. Current and voltage outputs were viewed on-line and recorded on videocassette tapes for off-line analyses.

Electrophysiological data (membrane current and voltage) were analysed and reproduced for figures by photographing them from chart paper. Additionally, analog-to-digital data epochs stored in a PDP 11/23 computer (Digital Equipment Corp., Cambridge, MA, USA) were displayed with a digital oscilloscope and reproduced for figures with an *X–Y* plotter. Data are expressed as mean ± S.E.M. and range unless otherwise noted.

Preparation and delivery of drug solutions

Histamine dihydrochloride, ovalbumin, promethazine hydrochloride and pyrilamine maleate were obtained from Sigma Chemical Corp., St Louis, MO, USA. Burimamide was a gift from Smith, Kline and French, Philadelphia, PA, USA. The same lot number of ovalbumin used to sensitize an animal was always used in experiments on SCG from that animal. Drug solutions were prepared daily for experiments from concentrated (≥ 10 mM) stock aliquots of drugs which were stored frozen. Reservoirs containing oxygenated solutions of superfusate with various drugs were connected to the inflow line to the recording chamber by three-way valves which could rapidly divert flow from the main reservoir to these solutions.

RESULTS

Endogenous histamine release in SCG

The adult guinea-pig SCG contains 97–361 pmol (210 ± 11 pmol; $n = 36$) of histamine (Weinreich, 1985; Weinreich & Undem, 1987). In the present study, SCG removed from animals actively sensitized to the specific antigen ovalbumin, rapidly

released a significant amount of histamine into the superfusate upon exposure to this antigen (10 μg/ml; Fig. 1). At this concentration, antigen was found in pilot studies to release maximal amounts of histamine, and was thus used in all experiments described here. The mean amount of histamine released per ganglion by antigen was 119 ± 27 pmol ($n = 4$ experiments). Histamine was undetectable in the superfusate

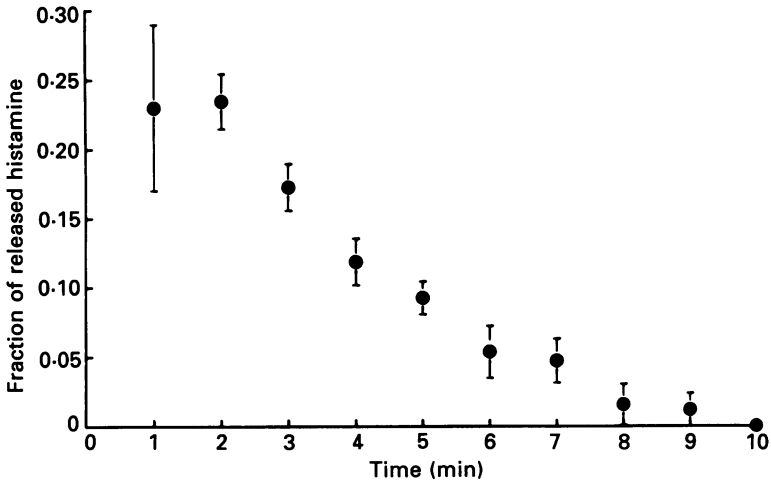

Fig. 1. Effect of specific antigen challenge on endogenous histamine release from antigen-sensitized SCG. Data points represent means (error bars = $\pm$S.E.M.) from four experiments. In each experiment, three to four SCG obtained from animals sensitized to antigen (ovalbumin) were pinned to the floor of the chamber used for intracellular recording. Locke solution (35–37 °C) superfused (3–5 ml/min) the ganglia and was collected as consecutive 1 min samples as it exited the chamber. Histamine content in each sample was calculated as described in Methods. No histamine was detectable (< 10 pmol/ml) in 1 min samples collected prior to antigen challenge. At time zero (x-axis) superfusate was switched to Locke solution containing 10 μg/ml antigen. Histamine contained in each subsequent sample was normalized as a fraction of the total histamine released (y-axis) from the ganglia over 15 min of continuous antigen challenge. Histamine content peaked within 2 min and declined to an undetectable level within 10 min of exposure to antigen.

(< 10 pmol/ml) prior to antigen challenge, reached a peak value within 2 min of ovalbumin challenge, and declined exponentially to an undetectable level within 10 min ($n = 4$; Fig. 1). Antigen could only evoke histamine release once in a preparation; subsequent challenges with ovalbumin after a wash period (0·5–3 h) with antigen-free Locke solution produced no further detectable release ($n = 5$). Finally, depolarizing ganglionic neurones by superfusion with Locke solution containing 55 mM-K^+ failed to release detectable histamine into the superfusate ($n = 4$).

Similarity of electrophysiological effects produced by histamine and antigen

Specific antigen challenge with ovalbumin mimicked closely several electrophysiological effects of exogenously applied histamine on SCG neurones from animals that had been sensitized to this antigen. Current-clamp and voltage-clamp studies revealed that these substances affected several distinct active and passive membrane properties of these neurones.

Effects on passive membrane properties

Preliminary experiments revealed that histamine at 10 μM produced a sub-maximal depolarization of SCG neurones that were responsive to this amine. This concentration was thus used in experiments described below. Histamine depolarized twenty-six out of thirty-six neurones tested as exemplified in Fig. 2. This effect was

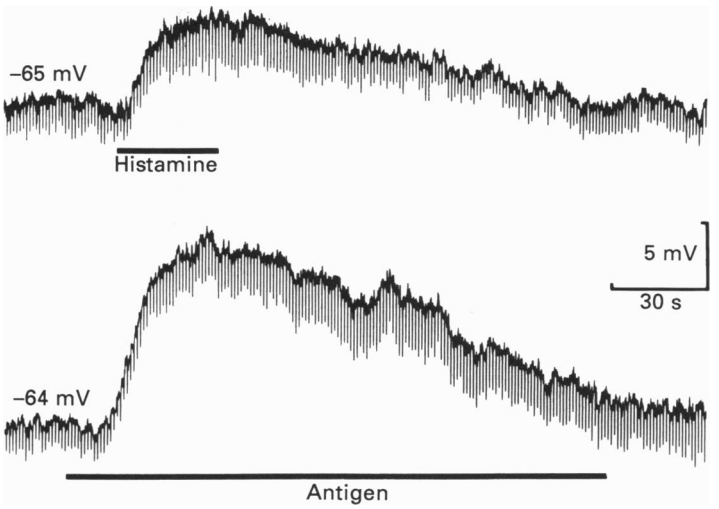

Fig. 2. Effects of histamine and antigen challenges on membrane potential and input resistance of a neurone from an antigen-sensitized SCG. Data shown are intracellularly recorded voltage records obtained during exposure (bars) to 10 μM-histamine (upper trace)- and 10 μg/ml antigen (ovalbumin; lower trace)-containing superfusate. Resting membrane potential is indicated at left of each trace. Negative-going deflections on traces are electrotonic voltage transients produced by hyperpolarizing current steps (50 pA, 160 ms, 1 s intervals) to estimate total input resistance. Histamine and antigen both depolarized the membrane and increased the input resistance of this neurone. Resistance did not change measurably over this range of membrane potentials in the absence of histamine or antigen (not shown).

most often (see below) accompanied by an increase in total input resistance and recovered upon wash with drug-free Locke solution. Treatment with ovalbumin antigen also depolarized neurones and usually increased their input resistance (Fig. 2). The steady-state amplitudes of the depolarizations achieved during histamine and antigen exposures were quite variable in different neurones, ranging from 1 to 12 mV ($2{\cdot}9 \pm 0{\cdot}5$ mV; $n = 26$), and from 1 to 16 mV ($4{\cdot}2 \pm 0{\cdot}7$ mV; $n = 27$), respectively. However, the sensitivity of a *given* neurone to the two manipulations was similar: the magnitudes of the histamine- and antigen-induced depolarizations in thirty-six neurones tested with both substances were highly correlated (correlation analysis: $r = 0{\cdot}88$; analysis of variance on the correlation: $P < 0{\cdot}001$; Fig. 3). Histamine repeatedly hyperpolarized one neurone, and in this case antigen treatment also elicited a transient membrane hyperpolarization (see Fig. 3). Thus the effect of antigen treatment on membrane potential of a neurone was predictable directly from the effect of exogenously applied histamine on this property.

The depolarization produced by antigen (Fig. 2) had several properties in common with antigen-induced histamine release (Fig. 1). First, the duration of the overall depolarization and repolarization of the membrane potential during maintained ovalbumin exposure (5·34 ± 0·65 min; 1·7–8·2 min; $n = 10$ experiments; e.g. Fig. 2) resembled that measured for the rise and decay of biochemically measured histamine in the superfusate following antigen challenge (Fig. 1). In addition, secondary anti-

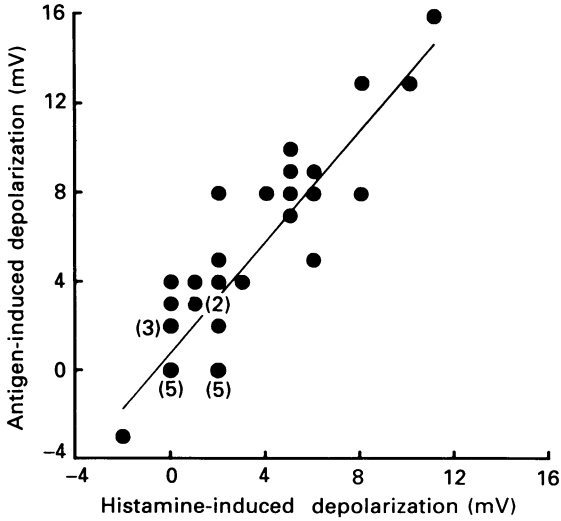

Fig. 3. Relationship between amplitudes of membrane depolarizations produced by histamine and by antigen in thirty-six individual neurones (thirty-six animals) from antigen-sensitized SCG. Co-ordinates corresponding to each filled circle denote steady-state amplitudes of histamine (10 μM; x-axis)-induced and antigen (ovalbumin; 10 μg/ml; y-axis)-induced depolarizations in a given neurone. Numbers in parentheses beside certain filled circles indicate number of overlayed values those symbols represent. Line on plot was fitted by correlation analysis. Amplitudes of depolarizations produced in a neurone by histamine and by antigen were highly correlated ($r = 0{\cdot}88$; $P < 0{\cdot}001$; see text).

gen challenge, following a 10–30 min wash period after the initial challenge, never affected membrane potential ($n = 11$ experiments). Finally, antigen never affected membrane potential in SCG neurones ($n = 5$) from five non-sensitized animals. Therefore the antigen-induced depolarization occurred only under circumstances where released histamine would have been predicted from the biochemical studies (see above) to be present.

Input resistance was monitored throughout the histamine-induced depolarization in twenty-five neurones and in thirteen of the same cells during subsequent exposure to antigen (e.g. Fig. 2). Both treatments affected membrane resistance, although the magnitude and direction of the change varied in different neurones (Table 1). Resistance changes did not appear to result from non-linearity in membrane resistance during the depolarization, because the current–voltage relation determined for many of these neurones was approximately linear throughout the range of membrane potentials encompassed by the depolarization. Neurones which did not clearly depolarize (< 2 mV depolarization) to histamine and antigen are excluded

from analyses of membrane resistance, because it was not notably affected in these cases.

That resistance changes varied directionally (i.e. increased, decreased or unaffected) in different cells suggests that histamine may affect several conductance mechanisms in SCG neurones (see below and Discussion). In addition, a hypothesis that antigen-induced resistance changes are mediated through histamine would predict that both treatments produce similar changes within an individual neurone.

TABLE 1. Effects of histamine and antigen on membrane resistance

	Increased resistance (%)	Decreased resistance (%)	No effect (< 5% change)
Histamine ($n = 25$)	125 ± 4 ($n = 17$) (106–167)	81 ± 1 ($n = 3$) (77–85)	($n = 5$)
Ovalbumin ($n = 13$)	130 ± 6 ($n = 8$) (106–173)	66 ($n = 1$)	($n = 4$)

Mean ± S.E.M. and range (in parentheses) from indicated number of neurones (n) of percentage change in total input resistance (relative to control) observed at the steady-state peak of histamine (10 μM)- and antigen (ovalbumin; 10 μg/ml)-induced depolarizations (> 2 mV). All data are from neurones in antigen-sensitized ganglia. Input resistance was estimated by measuring the steady-state value of the voltage transients produced by 50–100 pA, 160 ms hyperpolarizing current steps. Data are grouped in columns based on the direction of input resistance change observed: > 5% increase, > 5% decrease, < 5% change. The direction of change induced by histamine corresponded to that produced by antigen in twelve of the thirteen neurones tested with antigen (see text).

A close correspondence did in fact exist between the direction of membrane resistance changes accompanying the histamine- and antigen-induced depolarizations in twelve of thirteen neurones where this was evaluated. The eight neurones which increased resistance and the one which decreased resistance during antigen treatment (Table 1) showed the *same* directional changes in resistance during exposure to exogenous histamine. In four neurones antigen challenge depolarized the membrane without affecting input resistance (Table 1); application of histamine to three of these neurones also produced a depolarization without altering resistance. In the fourth case, histamine increased membrane resistance in contrast to the lack of effect of antigen.

One notable exception to the resemblance between histamine and antigen effects on input resistance was in the overall time course of increases in resistance. The increase in resistance produced by exogenous histamine in seventeen neurones reverted to resting value during repolarization of the membrane upon histamine washout (percentage change in membrane resistance upon repolarization: 105 ± 2%; 96–117%). In contrast, in the eight neurones where antigen produced increases in resistance, these increases tended to persist after the resting membrane potential recovered (percentage change in membrane resistance upon repolarization: 125 ± 8%; 100–160%; $n = 8$; e.g. Fig. 2). This disparity in the durations of histamine- and antigen-induced input resistance changes could be due to other mediators that are released in concert with histamine during antigen-induced responses in the SCG (see Table 1, Weinreich & Undem, 1987).

Voltage-clamp studies of histamine and antigen effects on passive membrane properties

Single-electrode voltage-clamp recordings were used to measure directly the membrane currents induced by exogenous histamine and by antigen. Two different voltage-clamping protocols were used. Neurones were either clamped near their resting potential and repeated rectangular hyperpolarizing voltage command steps

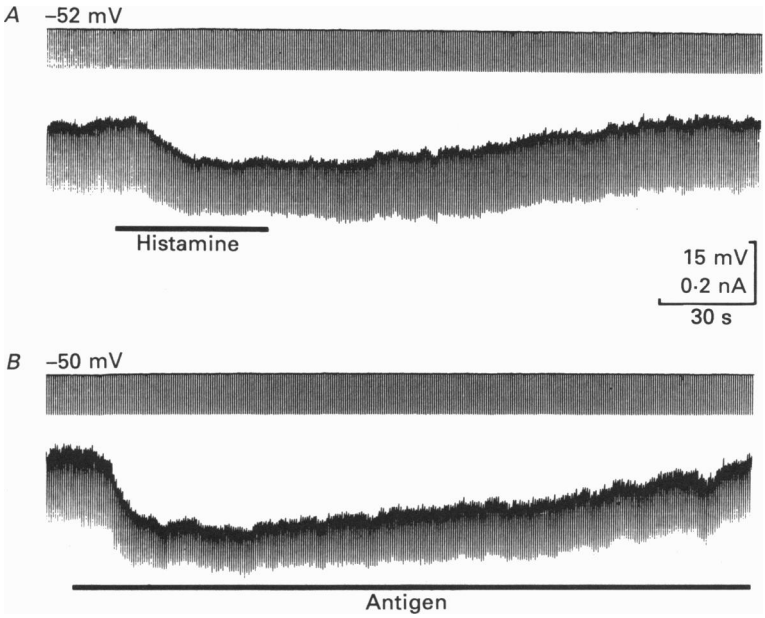

Fig. 4. Effects of histamine and antigen on membrane current and conductance in a neurone from an antigen-sensitized SCG. Each panel shows intracellularly recorded voltage-clamped membrane potential (upper traces; holding potential indicated at left) and voltage-clamp current (lower traces). Negative-going deflections on voltage traces are hyperpolarizing voltage step commands (10 mV, 150 ms, 520 ms intervals) to monitor membrane conductance. *A*, exposure to 10 μM-histamine in superfusate (bar) produced an inward current and decreased membrane conductance. *B*, 10 μg/ml ovalbumin antigen (bar) also elicited an inward current and decreased membrane conductance. These effects recovered during continued exposure to antigen.

(usually 10 mV, 150 ms) were employed to monitor conductance. Alternately, hyperpolarizing voltage ramps (1 s duration) were applied from a depolarized holding potential before and during the course of histamine- and antigen-induced membrane currents. The latter method permitted us to assess the characteristics of these membrane currents over a wide range of membrane potentials, and thus gave us a maximal amount of data for the one antigen-induced response that could be studied in a given preparation (see above).

Histamine produced an inward current (134 ± 21 pA; 20–350 pA) in twenty neurones voltage-clamped near their resting membrane potential (Fig. 4*A*). Repeated hyperpolarizing command steps used in seventeen of these experiments (in the other

three experiments only ramp commands were used; see below) revealed a conductance decrease in eleven of the seventeen neurones at the peak of the inward current (percentage decrease: $76 \pm 3\%$; 60–94%; Fig. 4*A*). Of the remaining six neurones, conductance increased in two (112 and 133%), and remained unchanged ($< 5\%$) in the other four cases. Eight of the twenty neurones tested with histamine in the above experiments were subsequently exposed to ovalbumin antigen. This also produced an inward current (207 ± 51 pA; range 30–530 pA; Fig. 4*B*). Repeated hyperpolarizing voltage command steps (10 mV, 150 ms) used in three of the experiments revealed a conductance decrease in two cases (57 and 82%; Fig. 4*B*), and no change ($< 5\%$) in the third during the inward current. These data demonstrate that the conductance changes observed during histamine and antigen challenges are not merely voltage-dependent effects of the depolarization, *per se*.

Voltage ramp commands were used to investigate the characteristics of histamine- and antigen-induced membrane currents in six experiments. Results of these studies further support the idea that histamine and antigen probably affect multiple conductances in SCG neurones (Fig. 5). An inward current was observed in all six neurones during both histamine (123–350 pA) and antigen (125–540 pA) exposure. In four of the experiments, voltage ramp commands applied during the steady-state peak of the inward current revealed a decrease in conductance (Fig. 5*A*), relative to control for both histamine and ovalbumin. In two of these cases, the currents recorded during the histamine and antigen exposures crossed their respective pre-treatment currents between -80 and -102 mV, while in the other two the currents merged between -77 and -94 mV, but never crossed. These results suggest that a decreased resting K^+ conductance contributed to the apparent inward current in the above four neurones. In the remaining two experiments, membrane conductance *increased* during the histamine- and antigen-induced inward currents (Fig. 5*B*). In these cases, currents converged, but never fully crossed at depolarized levels (-50 to -60 mV), suggesting that a conductance change to an ion species other than K^+ may have been responsible. Importantly, whichever effect exogenous histamine produced in a given neurone, it was mimicked closely by antigen exposure (Fig. 5), providing additional evidence of correspondence between the effects of the two treatments.

Effects on active membrane properties

Previous work has revealed that a subpopulation of neurones (i.e. $\sim 18\%$) in the guinea-pig SCG possess an unusually long-duration spike after-hyperpolarization (AHP) component (i.e. > 1 s), that can be selectively and reversibly abolished by bath-applied histamine (Christian & Weinreich, 1988). These observations therefore provided an additional test of the electrophysiological correspondence between antigen and histamine: ovalbumin treatment should mimic this particular histamine effect in SCG neurones from guinea-pigs sensitized to this antigen. The data in Fig. 6 show the results of one experiment which confirmed this prediction. Histamine, as previously reported, selectively and reversibly blocked the long-duration AHP in these neurones (Fig. 6*A–C*) without affecting the shorter duration AHP. Subsequent treatment with antigen also selectively abolished the long-duration AHP (Fig. 6*D*). These effects were consistent; histamine completely blocked the long-duration AHP in all eight neurones tested (AHP amplitude pre-histamine: $10 \pm 1{\cdot}5$ mV; post-

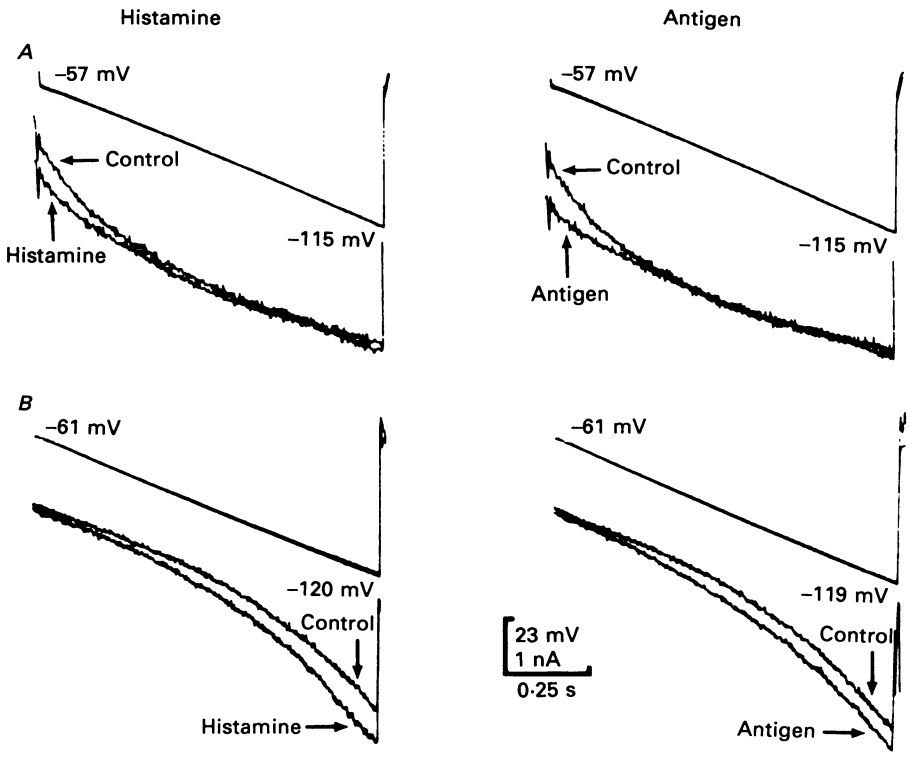

Fig. 5. Effects of histamine and antigen treatments on current–voltage characteristics of two neurones (*A* and *B*) in different antigen-sensitized SCG. Each panel shows intracellularly recorded voltage ramp commands (upper traces; holding potential indicated at each extreme of ramp) and corresponding membrane currents (lower traces). Voltage ramps and corresponding currents obtained during a control period are overlayed with those obtained during the steady-state peak of inward currents generated by exposure to histamine (10 μM) or ovalbumin antigen (10 μg/ml) in the superfusate. *A*, currents obtained during voltage ramps reveal that histamine and antigen both decreased membrane conductance and produced inward currents over a similar range of membrane potentials in this neurone. Note that currents sampled during both histamine and antigen barely reverse to outward direction at the most hyperpolarized extreme of the voltage ramps. *B*, currents obtained during voltage ramps reveal that histamine and antigen both *increased* conductance and produced inward currents over a similar range of membrane potentials in this neurone.

histamine: $0 \pm 0{\cdot}2$ mV). Similarly, antigen blocked this potential in five of five neurones (AHP amplitude pre-antigen: $9 \pm 2{\cdot}5$ mV; post-antigen: 0 ± 0 mV). Four of these five neurones exposed to antigen had also been tested with histamine. Thus antigen challenge also mimicked this specific effect of exogenous histamine. Aside from this consistent effect on the long-duration AHP, neither histamine nor ovalbumin antigen measurably affected either the action potential amplitude, duration and overshoot above 0 mV, or AHP components with a duration of < 500 ms.

Pharmacological antagonism of histamine and ovalbumin responses

Various H_1, H_2 and H_3 histamine receptor antagonists were used to test whether antigen-induced changes in passive membrane properties resulted from endogenously released histamine. Due to the infrequent occurrence of SCG neurones exhibiting long-duration AHPs (~ 18% of neurones sampled; Christian & Weinreich, 1988), we did not examine its pharmacological properties here. Only neurones showing a

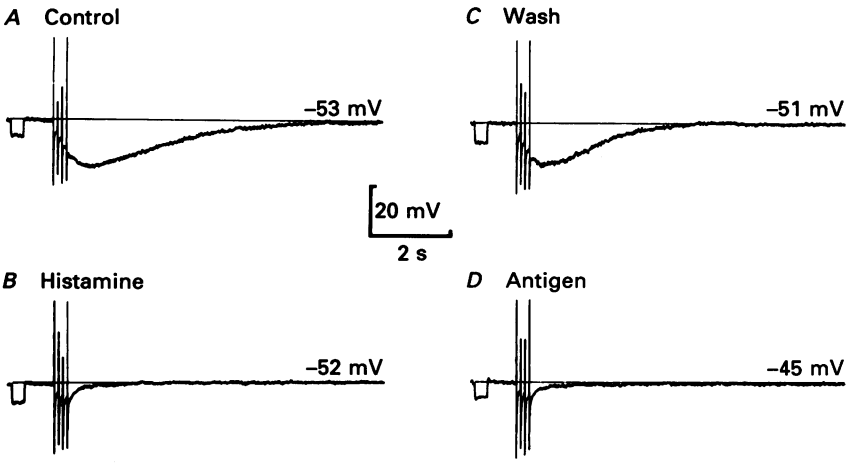

Fig. 6. Effects of histamine and antigen challenges on the long-duration AHP in a neurone from an antigen-sensitized SCG. In each intracellularly recorded voltage trace (membrane potential indicated at right), initial hyperpolarizing deflection was produced by a constant-current step (100 pA, 300 ms) to estimate input resistance, and four action potentials produced by four depolarizing current steps (5 nA, 2 ms, 100 ms inter-step interval) to elicit long-duration AHP. Action potentials and short-duration AHPs are truncated at variable levels due to low frequency (200 Hz) at which data were digitized. *A*, control long-duration spike AHP prior to drug exposure. *B*, blockade of long-duration AHP by exposure to 10 μM-histamine in superfusate for 1 min. *C*, recovery of long-duration AHP upon wash with drug-free Locke solution for 3 min. *D*, blockade of long-duration AHP by exposure to antigen (ovalbumin; 10 μg/ml)-containing superfusate for 1 min. The membrane depolarization (7 mV) occurring during antigen treatment does not account for blockade of the long-duration AHP; membrane was depolarized to ~-45 mV with constant current prior to antigen exposure and this enhanced amplitude of long-duration AHP (not shown).

significant depolarizing response (> 5 mV) to exogenously applied histamine were tested for antigen effects in the presence of antagonists – in these neurones antigen would have been predicted to produce a substantial depolarization in the absence of antagonist, based on the correlation indicated above (see Fig. 3). At the concentrations used, none of the antagonists employed had any measurable effect on membrane potential, input resistance or spike amplitude and duration.

The histamine H_1 receptor antagonists, pyrilamine (1 μM; $n = 4$ experiments) and promethazine (1 μM; $n = 2$ experiments), blocked depolarizing responses to both histamine and antigen as summarized in Fig. 7. The possible involvement of H_2 and H_3 histamine receptors in histamine- and antigen-mediated depolarizations was also examined (Fig. 7). Previous studies have demonstrated that burimamide has a high

affinity for H_3 receptors (rat cerebral cortex; apparent dissociation constant (K_B) = 0·07 μM; Arrang, Garbarg & Schwartz, 1983), and a lower affinity for H_2 receptors (guinea-pig atrium; K_B = 8 μM; Black, Duncan, Durant, Ganellin & Parsons, 1972). Pre-treatment with burimamide at 50 μM, a concentration that would thus be expected to displace histamine (10 μM) binding significantly at both H_2 and H_3 receptors, did not block histamine- or ovalbumin-induced depolarizations (n = 2; Fig. 7). The above results demonstrate that the histamine- and the antigen-induced depolarizations have the same pharmacological properties; both appear to be mediated by H_1 histamine receptor activation.

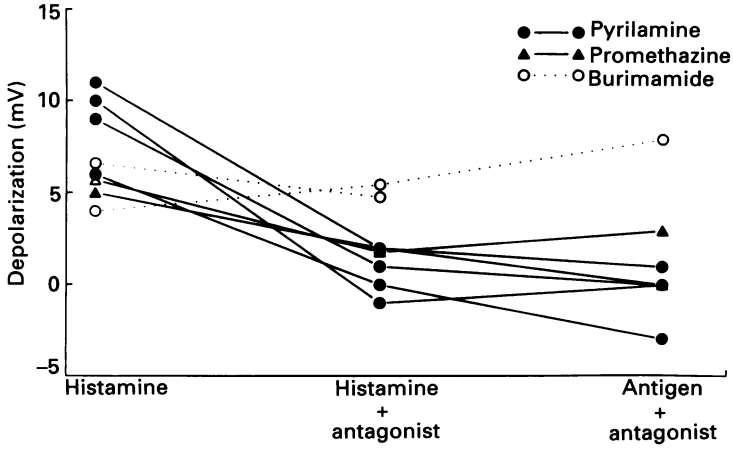

Fig. 7. Summary of experiments testing effects of various classes of histamine receptor antagonists on histamine- and antigen-induced depolarizations in neurones from antigen-sensitized SCG. Each set of connected points represents results obtained from a single intracellularly recorded neurone during sequential exposure (x-axis) to superfusate containing histamine (10 μM), histamine (10 μM) in the presence of the indicated antagonist (H_1 antagonists: pyrilamine, 1 μM, promethazine, 1 μM; H_2 and H_3 antagonist: burimamide, 50 μM), and antigen (10 μg/ml) in the presence of the same concentration of the indicated antagonist. Ganglia were always pre-treated with Locke solution containing antagonist for > 3 min prior to histamine or antigen challenge in presence of the antagonist. Points denote amplitude of steady-state depolarization (y-axis) from resting potential observed to each treatment.

DISCUSSION

We showed previously (Weinreich & Undem, 1987), and confirmed here, that a specific antigenic stimulus can rapidly release a large fraction (~ 35%) of the histamine contained in the SCG from guinea-pigs previously sensitized to the antigen. In the present work we have demonstrated that antigen releases a sufficient amount of endogenous histamine to affect specific electrophysiological properties that would be expected to increase the excitability of mammalian peripheral sympathetic neurones. These data thereby support the general conclusion that antigenic stimulation of the immune system can directly affect sympathetic nervous system function.

Evidence supporting a neuroregulatory function for endogenous histamine

Several lines of evidence (see below) indicate that the histamine released by an antigenic stimulus was probably derived from mast cells. Designating histamine as a neurotransmitter in this situation is therefore inappropriate, because this implies nerve-to-nerve communication via discrete histaminergic synapses. None the less, our results fulfill the following criteria which are consistent with a 'neuroregulatory' role for endogenous histamine in this instance: (1) significant quantities of histamine were *released* by a physiological stimulus (i.e. specific antigen challenge), (2) several distinct electrophysiological actions of exogenously applied histamine were closely *mimicked* by specific antigen challenge, and (3) the transient membrane depolarizations produced by exogenously applied histamine and by antigen challenge could be *pharmacologically antagonized* by histamine H_1 (but not H_2 or H_3) receptor antagonists. These criteria are analogous to those classically used for neurotransmitter identity (Paton, 1958; Werman, 1966), and support the conclusion that endogenous histamine released by a specific antigenic stimulus can affect the electrophysiological properties of SCG neurones. Histamine could therefore be involved in immune system regulation of peripheral sympathetic neuronal excitability. This may be of particular relevance to the pathophysiology of allergic and other inflammatory diseases.

Cellular source of endogenous histamine

Several distinct observations made here and in previous studies lead to the conclusion that the histamine released by antigen was derived from ganglionic mast cells. First, the finding that 55 mM-K^+ did not evoke measurable histamine release argues that the large amount of histamine released by antigen challenge did not come from a neuronal source. Second, that measurable histamine release could only be elicited by antigen once from a preparation coincides with the fact that mast cells rapidly desensitize to a specific antigen upon the initial exposure (Lichtenstein & Osler, 1964). Third, previous results obtained under similar conditions have established that SCG obtained from guinea-pigs not sensitized to ovalbumin antigen fail to release measurable amounts of histamine following ovalbumin challenge (Weinreich & Undem, 1987). Likewise, SCG from ovalbumin-sensitized guinea-pigs do not release histamine when exposed to non-sensitizing antigens (i.e. bovine serum albumin, human serum albumin). Thus antigen-induced histamine release is specific to the sensitizing antigen. Fourth, the number of mast cells stainable with Toluidine Blue decreases $> 50\%$ in sensitized SCG challenged with antigen *versus* control Locke solution (Weinreich & Undem, 1987). Finally, the proportion of total ganglionic histamine released by antigen ($\sim 35\%$ of the total stores) exceeds that which can be accounted for by all non-mast cell histamine (Häppölä *et al.* 1985; Weinreich, 1985). The above findings, taken together, constitute strong evidence that in SCG from guinea-pigs sensitized to ovalbumin antigen, challenge with this antigen *in vitro* leads to the activation of ganglionic mast cells.

The observation that virtually all neurones that were sensitive to exogenous histamine were also responsive to antigen challenge (Fig. 3) is interesting

physiologically, considering the low ratio of mast cells to principal neurones in the guinea-pig SCG (~ 700 mast cells, Weinreich (1985); and ~ 57000 neurones, Purves (1975)). This suggests that the histamine (and other mediators) released from mast cells in the SCG can affect not only adjacent neurones, but also those relatively distant from the release sites. This apparently large sphere of influence of relatively few mast cells is consistent with the prior demonstration from our laboratory with extracellular recording methods (Weinreich & Undem, 1987) that antigen treatment can produce substantial changes in the overall input–output relationship of the SCG (i.e. long-term potentiation of the postganglionic compound action potential). Thus immunologically activated mast cells should be considered amongst possible integrative mechanisms of peripheral sympathetic function.

Although we activated mast cells with an immunological stimulus in this study, a variety of endogenous neurotransmitters and neuropeptides can release histamine from mast cells in other tissues (Foreman & Jordan, 1983; Bani-Sacchi, Barattini, Bianchi, Blandina, Brunelleschi, Fantozzi, Mannaioni & Masini, 1986; Leff, Stimler, Muhoz, Shioya, Tallet & Dame, 1986; Bienenstock, Tomioka, Matsuda, Stead, Quinonez, Simon, Coughlin & Denburg, 1987). The ability of these substances to act as mast cell secretagogues is dependent on the source of the mast cells, since considerable heterogeneity exists between mast cells from different tissues (Befus, Bienenstock & Denburg, 1986). In this regard, virtually nothing is known about the pharmacological regulation of mast cells in the peripheral sympathetic nervous system. None the less, the idea that mast cells could be regulated by peripheral neurones coupled with the results presented here raise the possibility that feed-back loops could occur between mast cells and neurones within the SCG or other autonomic ganglia.

Mechanism of histamine-mediated effects on SCG neurones

Endogenously released or exogenously applied histamine produced the following effects in most neurones that are consistent with reduction of a resting outward K^+ current: (1) a membrane depolarization and associated increase in input resistance (current-clamp records), (2) an apparent inward current with an associated decrease in conductance (voltage-clamp records), (3) a null or reversal potential for this current ranging from -77 to -102 mV, and (4) consistent blockade of a long-duration spike AHP, that is probably mediated by an increased K^+ conductance (Christian & Weinreich, 1988).

Exogenously applied histamine has previously been shown to depolarize hippocampal neurones (Segal, 1980, 1981), and to block a Ca^{2+}-mediated K^+ conductance (Haas & Konnerth, 1983; Pellmar, 1986) that underlies a long-duration AHP (Haas & Konnerth, 1983) in these cells. In enteric neurones from the guinea-pig small intestine, exogenously applied histamine also produces a depolarization, conductance decrease and blockade of long-duration AHPs (Nemeth *et al.* 1984).

Our results suggest that endogenous or exogenous histamine may affect multiple ionic conductances in SCG neurones. Two neurones studied with voltage ramps, in contrast to the prevalent response involving a decreased conductance and inward current (Fig. 5*A*), exhibited an increased conductance during an inward current (Fig. 5*B*). The observed or extrapolated reversal potentials of the inward currents

occurring during these different responses suggest that histamine may exert its effects through decreasing a K^+ conductance in some neurones and increasing a conductance to some alternative ion species (e.g. Na^+ or Ca^{2+}) in others. In neurones where the membrane depolarized without an apparent change in resistance, two or more ionic mechanisms may possibly have been affected simultaneously to produce offsetting (i.e. increased and decreased) conductance changes, which summed to contribute to the depolarization.

The concept of a single neuroactive substance affecting multiple ionic conductances in ganglionic neurones is not unprecedented. Evidence for activation of diverse response mechanisms to exogenously applied substances has been reported in the bull-frog sympathetic ganglion (Kuba & Koketsu, 1976; Kuffler & Sejnowski, 1983; Tsuji, Minota & Kuba, 1987), as well as in neurones in the guinea-pig mesenteric ganglion (Dun & Minota, 1981; Minota, Dun & Karczmar, 1981). The present results provide evidence that such diversity in response mechanisms can occur to an endogenously released mediator (i.e. histamine) as well.

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