Journal of Physiology (1992), **457**, *pp.* 407–430
With 10 *figures*
Printed in Great Britain

PRESYNAPTIC HISTAMINE H_1 AND H_3 RECEPTORS MODULATE SYMPATHETIC GANGLIONIC SYNAPTIC TRANSMISSION IN THE GUINEA-PIG

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(*Received 26 September 1991*)

SUMMARY

1. To study the effects of histamine on the efficacy of sympathetic ganglionic synaptic transmission, extracellular recordings of the postganglionic compound action potential (CAP) and intracellular recordings of excitatory postsynaptic potentials (EPSPs) elicited by preganglionic electrical stimulation were obtained from isolated guinea-pig superior cervical ganglia (SCG).

2. In different preparations, superfusion with histamine (0·1–100 μM) either potentiated or depressed the postganglionic CAP elicited by electrical stimulation of the cervical sympathetic trunk (0·2–3·0 Hz). The direction of response produced by histamine did not depend on stimulation frequency or histamine concentration; potentiation and depression both showed concentration dependence over the range of histamine concentrations tested.

3. Experiments employing a variety of histamine receptor agonists or antagonists revealed that histamine-induced potentiation of the postganglionic CAP could be attributed to histamine H_1 receptor activation, and depression to H_3 receptor activation.

4. Histamine similarly potentiated or depressed the intracellularly recorded EPSP. However, these opposite effects occurred at different synapses. In agreement with the studies on the postganglionic CAP, histamine H_1 antagonists prevented histamine-induced potentiation of the EPSP and H_3 receptor antagonists prevented histamine-induced depression.

5. Direct quantal analyses of histamine-induced synaptic potentiation and depression were implemented to determine the pre- and postsynaptic components of these effects. Quantal size was estimated by measuring the amplitude of spontaneous miniature EPSP amplitudes. Histamine-induced potentiation and depression of the evoked EPSP were found to be accompanied by increased or decreased quantal content respectively, and unchanged quantal size, providing evidence that presynaptic mechanisms were involved in mediating both effects.

6. Some guinea-pigs were actively sensitized to ovalbumin. Subsequent exposure

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of the isolated SCG from these animals to the sensitizing antigen produced changes in the EPSP amplitude that correlated significantly to the response produced by exogenously applied histamine at the *same* synapse.

7. The correspondence between the effects of specific antigen challenge and exogenous histamine on evoked EPSPs at a synapse provides evidence that endogenous histamine released during an immunological response to antigen challenge can activate histamine H_1 and H_3 receptors to modulate synaptic efficacy in sympathetic ganglia.

INTRODUCTION

Histamine, an autacoid distributed widely throughout the peripheral autonomic and enteric nervous systems (Kwiatkowski, 1943; von Euler, 1949, 1966), has been shown to modulate ganglionic synaptic transmission in both an excitatory and depressant manner. Some evidence has emerged that either of these opposing effects can be elicited within the same tissue, depending on stimulus conditions. For example, in the rabbit superior cervical ganglion (SCG) histamine depresses ganglionic synaptic transmission to single preganglionic stimuli but facilitates synaptic transmission elicited by 2 Hz stimulation (Brimble & Wallis, 1973). Other studies have documented opposing effects on ganglionic synaptic transmission depending on the histamine concentration. Low histamine concentrations elicit facilitatory effects, and higher concentrations produce depressant effects in both the cat (Brezenoff & Gertner, 1972) and the bullfrog (Yamada, Tokimasa & Koketsu, 1982) SCG. These opposing effects of histamine have been attributed to activation of different histamine receptor subtypes (Marshall, 1981). Histamine-induced facilitation of synaptic transmission in sympathetic ganglia of several species has been demonstrated to be mediated via histamine H_1 receptors (Trendelenberg, 1957; Brimble & Wallis, 1973; Yamada *et al.* 1982; Lindl, 1983; Snow & Weinreich, 1987), and histamine-induced synaptic depression has been linked to H_2 histamine receptor activation (Brimble & Wallis, 1973; Yamada *et al.* 1982; Snow & Weinreich, 1987).

Until recently, these studies using pharmacological antagonists have focused on effects mediated via activation of two different histamine receptor subtypes, the histamine H_1 and H_2 receptors. A novel subtype of histamine receptor, the H_3 histamine receptor, has recently been discovered. This histamine receptor was initially characterized in rat brain (Arrang, Garbarg & Schwartz, 1983), where it was shown to exert an auto-inhibitory effect on histamine release. Subsequently, indirect evidence has suggested that functional H_3 histamine receptors are present on non-histaminergic neurons in enteric ganglia (Tamura, Palmer & Wood, 1988), on sympathetic perivascular nerve terminals (Ishikawa & Sperelakis, 1987), on parasympathetic nerve endings (Ichinose, Stretton, Schwartz & Barnes, 1989), and on sensory peptidergic nerve endings in the airway (Ichinose, Belvisi & Barnes, 1990). In these preparations, activation of histamine H_3 receptors has been associated with presynaptic inhibition of transmitter release. However, none of the studies have demonstrated directly that H_3 histamine receptor activation mediates effects at a presynaptic locus, either by way of biochemical measurement of transmitter release from presynaptic nerve terminals, or by electrophysiological quantal analysis.

Our laboratory has identified previously several distinct postsynaptic electrophysiological effects mediated by histamine released *endogenously* by an antigenic stimulus in the guinea-pig SCG (Christian, Undem & Weinreich, 1989). These effects were shown to result from histamine H_1 receptor activation. In an attempt to explore more fully the effects of *endogenous* histamine in sympathetic ganglia, we have endeavoured in the present study to characterize the modulatory effects of histamine on ganglionic synaptic transmission. We have used available histamine receptor agonists and antagonists in combination with direct quantal analysis to determine the pre- and postsynaptic loci of the effects mediated by the activation of various histamine receptor subtypes. Our results demonstrate that functional inhibitory presynaptic histamine H_3 receptors, and facilitatory presynaptic histamine H_1 receptors modulate synaptic transmission in the guinea-pig SCG. In addition, both of these receptor subtypes are significant functionally in that they are activated by histamine released endogenously by an antigenic stimulus. A preliminary report of this work has been presented (Christian & Weinreich, 1990).

METHODS

Preparation of tissue

Adult male guinea-pigs (250–600 g) were used in the study. Animals were killed by a sharp blow to the head and exsanguinated. Most of the animals used were actively sensitized to ovalbumin as previously described (Weinreich & Undem, 1987). Briefly, sensitization involved three intraperitoneal injections of ovalbumin (10 mg kg^{-1}) on alternating days. Animals were killed 21–45 days following the final injection. No significant differences were noted between non-sensitized and sensitized animals with regard to any of the passive or active membrane properties examined or with regard to the effects of histamine receptor agonists and antagonists on ganglionic synaptic transmission. Therefore, comparisons between non-sensitized and ovalbumin-sensitized guinea-pigs are not given further attention in this report.

The SCG and associated major nerve trunks (i.e. the cervical sympathetic trunk and the superior postganglionic trunk; see Purves (1975)) were dissected free bilaterally and immediately submerged in ice-cold (5 °C) Locke solution (composition (mM): 136 NaCl; 5·6 KCl; 14·3 $NaHCO_3$; 1·2 NaH_2PO_4; 2·2 $CaCl_2$; 1·2 $MgCl_2$; 11·5 dextrose; 0·03 choline chloride), equilibrated by bubbling continually with 95% O_2–5% CO_2 (pH 7·2–7·4).

Within 10 min–4 h of initial dissection, ganglia were trimmed of excess adhering tissues, split longitudinally and pinned flat with stainless steel pins to the Sylgard (Dow Corning Co., Midland, MI, USA) floor of a recording chamber (total volume ~ 0·5 ml). The ganglia were superfused (2–5 ml min^{-1}) with oxygenated Locke solution that was heated to 35–37 °C at the inlet of the chamber. Temperature was monitored continuously by a microthermistor (Thermometrics Corp., Edison, NJ, USA) placed within 5 mm of the ganglion. The chamber was mounted on a fixed-stage microscope where the preparation was transilluminated and viewed with conventional (40 ×) or Hoffman (250 ×) optics. Tissue equilibrated in the recording chamber for at least 60 min before recording procedures commenced.

Extracellular methods

Recording procedure. Extracellular recording and stimulating bipolar suction electrodes were fabricated from capillary glass (1·2 mm o.d., 0·68 mm i.d.; WPI Corp, Sarasoto, FL, USA). The cut end of the cervical sympathetic nerve trunk was placed in a suction electrode 1–3 cm from the body of the ganglion for preganglionic nerve stimulation. Preganglionic stimuli, generated by a Grass Model 88 stimulator (Grass Instrument Co., Quincy, MA, USA), were monophasic square current pulses 500 μs in duration, 0·2–5·0 Hz frequency, and of variable intensity (see below). Evoked compound postganglionic action potentials (CAPs) were recorded from a suction electrode placed on the superior postganglionic nerve 0·3–1·0 cm from the ganglion body. The recording suction

electrode was connected to the input stage of an AC-coupled differential preamplifier (WPI Corp.; model DAM-5A). Data were filtered at 1 kHz, converted to digitized data files with pClamp acquisition software (Axon Instruments, Foster City, CA, USA), and analysed on-line with a customized software program (see below).

In most experiments, the intensity of the preganglionic stimulus was reduced to produce a CAP of approximately one-half the maximum amplitude attainable. Alternatively, the magnitude of the postganglionic CAP was reduced in some experiments by adding hexamethonium at 100–300 μM, a concentration range that has been shown to depress ganglionic transmission selectively without affecting membrane properties of postsynaptic neurons (Lees & Nishi, 1972). This method has been employed previously to study potentiation of sympathetic ganglionic synaptic transmission (Briggs, Brown & McAfee, 1985). Similar results were obtained with both methods of increasing the size of the subliminal fringe.

Data analysis. Digitized CAP episodes were ensemble averaged usually as 1 min averages with the pClamp software. The acquisition computer was connected to a second PC computer via a two-node network for the purpose of monitoring the CAPs on-line. A monitoring/analysis program ('CAP', written in collaboration with BME Systems, Baltimore, MD, USA; 'CAP' is available upon request from D.W.) enabled quantitative assessment of the peak-to-peak amplitude and integral of responses during the experiment. Peak amplitude and the integral of the CAP were determined between its initial rising phase, and a point where it returned to within 20% of baseline. Data files of these values were used to generate graphics displays, and produce figures with a Hewlett-Packard (Boise, ID, USA) Laser Jet II printer.

Both peak-to-peak and integral values of the CAP were affected similarly by histamine, ovalbumin antigen, and other drug treatments. In most experiments peak-to-peak values were used as an index of the number of synaptically activated postganglionic neurons. In some experiments the peak-to-peak amplitude of the CAP was more variable than the integral values during the control and the post-drug periods. This condition may reflect asynchronous discharge, and when this occurred, we considered the changes only in the integral values in our measurements. The CAP amplitudes or integrals obtained during various drug treatments were normalized as a percentage of control values, and mean values compared using one-way analysis of variance and Newman–Keuls individual comparisons. A P value < 0.05 was taken to indicate a significant difference between means.

Intracellular methods

Recording procedure. Intracellular microelectrodes were fabricated from capillary glass (1·2 mm o.d., 0·68 mm i.d.; WPI Corp.) on a Flaming and Brown Model P-87 puller (Sutter Instruments, San Francisco, CA, USA). Electrodes were back-filled with a solution of 1 M potassium acetate, 2 M KCl (electrode DC resistance: 30–90 MΩ). To prevent the occurrence of Na^+-mediated action potentials at the peak of large excitatory postsynaptic potentials (EPSPs), the electrode in some experiments was filled with 3 M potassium acetate containing 150 mM QX-314 (Gift from Astra Pharmaceutical Co., Worchester, MA, USA; see Connors & Prince, 1982). Neurons recorded with QX-314-containing electrodes did not differ apparently in their responses to histamine agonists and antagonists from neurons recorded with pipettes containing 1 M potassium acetate, 2 M KCl electrolyte.

Neurons were impaled using conventional methods in random locations in the ganglion. The signal from the electrode was amplified with an Axoclamp 2 electrometer (Axon Instruments). Recordings were made only after the membrane potential stabilized for at least 5 min, and if at that time, membrane potential was more negative than −50 mV, input resistance was > 25 MΩ, and action potential overshoot (above 0 mV) was > 10 mV. EPSP responses were recorded using the discontinuous current clamp mode (switched voltage recording–current injection; 3·0–4·5 kHz) of the Axoclamp amplifier. The headstage (unsampled) voltage was monitored continuously to assure that the electrode capacitance charged to an asymptotic level prior to voltage sampling. Membrane voltage and current were viewed continuously and recorded on videocassette tape for off-line analyses. Experimental protocols were electronically controlled and data acquired on-line by a personal computer using pClamp software, and a TL-1 interface (Scientific Solutions, Solon, OH, USA).

To elicit postsynaptic EPSPs, either the cervical sympathetic trunk or the superior postganglionic trunk was stimulated electrically (0·5–4·0 Hz) with a suction electrode placed on the nerve trunk 0·5–3·0 cm from the point of entry to the ganglion. Stimulus intensity was adjusted so

that > 90% of the EPSPs did not exceed threshold for an action potential (or QX-314 was used in the electrolyte). In most experiments approximately one-half of the cross-sectional area of the cervical sympathetic nerve trunk was severed transversely with iris spring scissors between the stimulating electrode and the ganglion, as an alternative method of reducing the number of

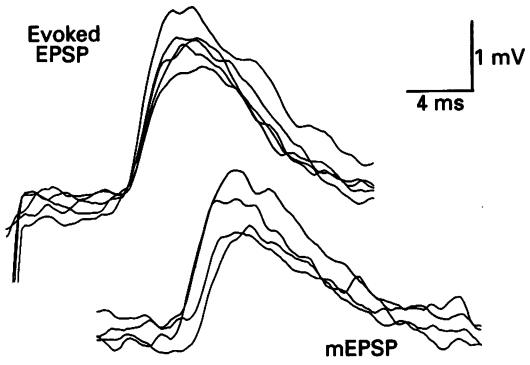

Fig. 1. Spontaneous miniature EPSPs (mEPSP) and EPSPs evoked by a presynaptic stimulus (Evoked EPSP) recorded intracellularly from a neuron in the SCG. Responses were recorded during stimulation of the cervical sympathetic trunk at 3·0 Hz. The four overlaid mEPSPs were collected during the intervals between recovery of an evoked EPSP and the next stimulus artifact (see Methods). The five overlaid stimulus-evoked EPSPs (stimulus artifact at left of traces) were the smallest EPSPs evoked to fifty consecutive stimuli over the same time period in which the mEPSPs were collected during the interstimulus intervals. Note the similarities in rise and decay times, and amplitudes of the mEPSPs and stimulus-evoked EPSPs.

converging synaptic inputs on the neurons. The rising phase of EPSPs was always carefully monitored. Experiments were analysed only if EPSPs showed a constant latency and monophasic rise time throughout the protocol. In most cases a constant-current hyperpolarizing transmembrane step was injected across the membrane following recovery of each evoked EPSP to estimate total cellular input resistance.

Data analysis. Intracellularly recorded EPSPs were analysed for drug-induced changes in mean amplitude. Blocks of fifty successive EPSPs were filtered (500 Hz–1 kHz), digitized, (1–2 kHz), and ensemble averaged with pClamp software. The baseline-to-peak amplitudes of the averaged blocks of EPSPs were measured and plotted over time, using the 'CAP' software program described above. The steady-state voltage transients elicited by transmembrane constant current steps were analysed in a similar manner to estimate concurrent changes in total input resistance. Membrane potential was also monitored and plotted over time. The percentage of control values for EPSPs and input resistance were obtained by using at least two successive averaged points during drug superfusion when the effect had reached a steady state and comparing these measurements to the steady-state control value. The mean percentage of control EPSP amplitudes obtained in the different conditions were compared using a one-way analysis of variance, and Newman–Keuls individual comparison tests. A value of $P < 0{\cdot}05$ was taken to indicate a significant difference between means.

Quantal analysis. In experiments employing presynaptic stimulation at $\geqslant 3$ Hz, spontaneous miniature EPSPs (mEPSPs) occurred in the interstimulus intervals. These experiments were subjected to procedures to estimate the mean quantal content and size, as follows. The mEPSPs were sampled from VCR tape using a window discriminator in conjunction with a delayed sampling circuit. Data were only sampled for mEPSPs in the interval between full recovery of each evoked EPSP and onset of the next stimulus artifact. Each discriminated mEPSP and a 20 ms baseline interval preceding the point where the mEPSP crossed the discrimination window were digitized (1–2 kHz) into pClamp files. The waveform characteristics of the sampled mEPSPs and evoked

EPSPs were compared directly. The mEPSPs were found to be nearly identical in rise and decay times and amplitudes to the smallest grouping of evoked EPSPs when the events were directly overlaid (Fig. 1). Several experiments were rejected from quantal analyses because of lack of correspondence between the mEPSP and small evoked EPSP waveforms. We thus assumed on the basis of similar waveforms that the mEPSPs and evoked EPSPs were generated at the same synapse. Peak amplitudes of mEPSPs were measured and binned into histograms. The mean amplitude and its variance, as well as goodness of fit of the binned amplitudes of mEPSPs to a Gaussian distribution was determined with pStat software (Axon Instruments). In some neurons a small proportion of spontaneous 'giant' mEPSPs occurred (i.e. amplitude > twofold that of overall mean mEPSP amplitude). These giant mEPSPs were excluded from the analysis.

The EPSPs evoked by presynaptic stimulation were digitized (1–2 kHz) into pClamp files and amplitude–frequency distributions subsequently determined by a customized software program. Blocks of at least 150 sequentially collected EPSPs were measured for each distribution. Responses that reached threshold and were distorted by a regenerative action potential were automatically excluded from the analysis. The program determined the mean evoked EPSP amplitude, its variance, and the number of failures. No correction was made for non-linear summation (cf. McLachlan, 1978). Mean quantal content (m) was estimated using the equation:

$$m = \bar{X}/\bar{x}$$

where $\bar{X}$ is the mean measured evoked EPSP amplitude, and $\bar{x}$ is the mean measured mEPSP amplitude. The standard error of m was estimated by:

$$\text{S.E.M.}_{m^2} = \frac{S^2}{\bar{x}^2 N} + \frac{m^2\sigma^2}{\bar{x}^2 M}$$

where σ^2 and M are, respectively, the variance and number of the mEPSPs samples, and S^2 and N are respectively the variance and number of evoked EPSPs sampled.

Evoked EPSP amplitudes in several experiments were binned into amplitude–frequency histograms and tested for goodness of fit to theoretical simple binomial or Poisson distributions. Binomial and Poisson models for derivation of predicted values were determined as previously described (Sacchi & Perri, 1971; McLachlan, 1978). Binomial parameters p and n were estimated using equations (14) and (15), and their standard errors calculated using equations (19) and (20), respectively, from McLachlan (1978). Goodness of fit was determined by comparing observed and predicted bin sizes using the χ^2 statistic (see McLachlan, 1975).

Preparation and delivery of drug solutions

Histamine dihydrochloride, ovalbumin (chick egg albumin, Type V), and pyrilamine maleate were obtained from Sigma Chemical Corp., St Louis, MO, USA. Burimamide and cimetidine were gifts from Smith, Kline and Beecham Corp., Philadelphia, PA, USA. Thioperamide and (*R*)-α-methylhistamine were gifts from Schering Corp., Bloomfield, NJ, USA. Drug solutions were prepared daily from concentrated stock aliquots, which were stored frozen (−20 °C). The SCG was exposed to drugs by quickly switching the input line of a peristaltic pump to a beaker of oxygenated superfusate containing a known concentration of drug (extracellular recordings), or by diverting three-way valves connected to drug reservoirs on the inflow line to the recording chamber (intracellular recordings). In both types of recording studies, the chamber volume was ~ 0·5 ml, and flow rates ranged from 2 to 5 ml min^{-1}. For studies employing histamine receptor antagonists, the SCG was always exposed to the antagonist for > 5 min prior to testing with histamine in the presence of the antagonist.

RESULTS

Potentiation and depression of synaptic transmission by histamine

The effects of histamine on the postganglionic CAP elicited by presynaptic stimulation of the cervical sympathetic trunk (0·2 Hz) was examined in ninety-three different SCG preparations prior to treatment with any histamine receptor antagonists. The magnitude of the evoked postganglionic CAP was taken as an index

of the number of ganglionic cells firing. In agreement with previous reports in other species (Brimble & Wallis, 1973; Snow & Weinreich, 1987), histamine exposure (1–100 μM) either increased or decreased, in a reversible fashion, the magnitude of the postganglionic CAP in different SCG preparations (Fig. 2). In seventy-nine of the

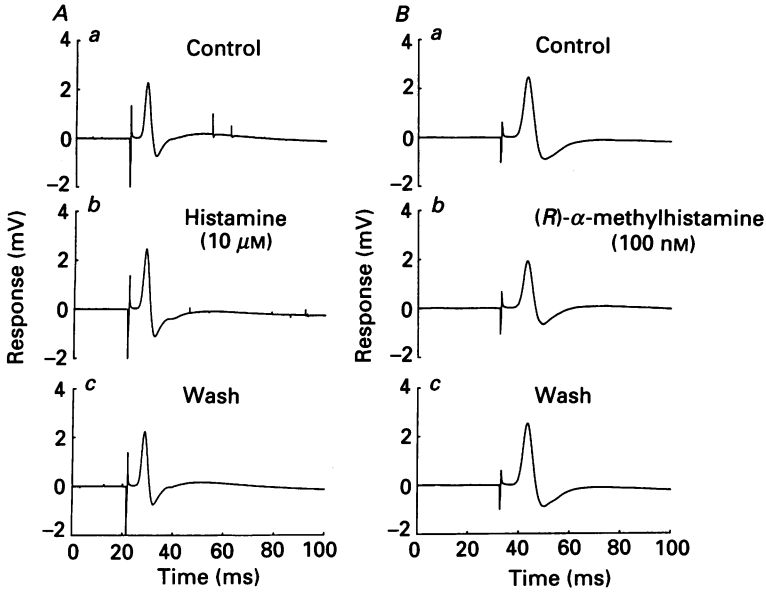

Fig. 2. Effects of histamine and (*R*)-α-methylhistamine on the postganglionic compound action potential (CAP) recorded in two different (*A* and *B*) SCG. Each trace shown is an ensemble average of twelve successive CAPs recorded from the superior postganglionic nerve in response to stimulation of the cervical sympathetic trunk at 0·2 Hz. Stimulus artifact is initial deflection on each trace. In each experiment traces in *a–c* were collected sequentially prior to (*a*, Control), during exposure to each indicated treatment (*b*), and after 10 min of re-exposure to normal Locke solution (*c*, Wash). *A*, experiment in which superfusion with histamine-containing Locke solution reversibly potentiated the peak-to-peak amplitude of the CAP. *B*, experiment in which superfusion with the histamine H_3 receptor agonist, (*R*)-α-methylhistamine, reversibly depressed the CAP amplitude.

ninety-three preparations histamine (10 μM) potentiated the response, while in the remaining fourteen the same concentration of histamine depressed the evoked CAP (Table 1). The mean control amplitude of the CAP averaged from the preparations showing potentiation did not differ from the mean control amplitude averaged from preparations showing depression.

No general environmental or experimental factors could be identified that correlated to the direction of effect that histamine produced on the evoked CAP in different ganglia. Many variables were examined with regard to treatment of the animals and handling of the tissue, including: (1) source of the animals (two different suppliers), (2) housing facilities (animals were housed in two different institutions during the course of the experiments), (3) animal weights, (4) ovalbumin sensitization

(see Methods), (5) time required to dissect and isolate the tissue, (6) time of maintenance *in vitro* prior to recording, and (7) seasonal effects or other clustered effects (e.g. animal batch differences).

Differences in stimulation protocols were examined for potential relationships to the direction of response elicited by histamine in an SCG. Stimuli were delivered at

TABLE 1. Effects of histamine receptor antagonists on histamine-induced changes in ganglionic synaptic transmission

	Treatment	Percentage of control CAP	n
A	Potentiation		
	Hist	$+29 \pm 1$	79
	Hist + Pyril	-14 ± 1*	8
	Hist + Cimet	$+28 \pm 2$	5
	Hist + Burim	$+34 \pm 3$	4
B	Depression		
	Hist	-22 ± 1	14
	Hist + Burim	$+17 \pm 1$**	12
	Hist + Cimet	-25	2
	Hist + Pyril	-27 ± 3	4
	Hist + Pyril + Cimet	-20 ± 3	7

The postganglionic compound action potential (CAP) was measured at peak effect, usually 2–5 min after addition of histamine (Hist; 10 μM), and normalized as a percentage of control value. Values are means ± S.E.M. for the number of experiments shown in column (n). A, effects of histamine receptor antagonists (Pyril: pyrilamine, 1 μM; Burim: burimamide, 1–50 μM. Cimet: cimetidine, 50 μM) on histamine-induced responses in ganglia where the initial response to a 5 min application of 10 μM histamine was potentiation. After wash-out of the histamine response, ganglia were equilibrated for > 5 min with indicated antagonist, and then again exposed to 10 μM histamine for 5 min, in the presence of the antagonist. *The CAP was depressed significantly by histamine in the presence of pyrilamine, compared to the other treatments ($P < 0.001$; one-way analysis of variance; Newman–Keuls individual comparisons). B, effects of histamine antagonists in ganglia where the initial response to 10 μM histamine was depression of synaptic transmission. **The CAP was potentiated significantly by histamine in the presence of burimamide, compared to the other treatments ($P < 0.001$).

half-maximal and supramaximal intensities in many recording protocols; similar relative changes in the postganglionic CAP were induced by histamine at both stimulus intensities. Stimulus frequency, likewise, did not correlate to the direction of response obtained; histamine-induced potentiation and depression of the postganglionic CAP, and of intracellularly recorded EPSPs (see below) were both observed over the different preparations to stimulus frequencies ranging from 0·2–5·0 Hz.

The possibility that the direction of the histamine-induced effect on synaptic transmission depended on histamine concentration was examined in seven experiments. Potentiation and depression both showed concentration dependency, in that progressively greater effects were induced by increasing histamine concentrations in the range 0·1–100 μM. No reversal of the initial histamine effect on the CAP within an experiment was ever observed over this range of concentrations. In subsequent extracellular experiments employing histamine receptor antagonists, and in intracellular experiments below, histamine was superfused at 10 μM, a

concentration that produced approximately 70% of the maximal effect in a concentration–response analysis of the CAP (see Fig. 3 and below).

Histamine receptor subtypes mediating effects on postganglionic population responses

Various histamine H_1, H_2 and H_3 receptor antagonists were used to determine whether histamine-mediated potentiation and depression of synaptic transmission could be differentiated pharmacologically (Table 1). Antagonists were evaluated for their ability to prevent histamine-induced changes of the CAP, which were established prior to exposure to the antagonist. To validate this experimental design, the desensitization properties of histamine-induced effects on the CAP were first assessed. Two successive histamine applications within ~ 15 min at a submaximal concentration (10 μM) produced changes in the CAP that differed by $< 5\%$ ($n = 6$ ganglia). Continuous superfusion for > 10 min with a supramaximal histamine concentration (100–300 μM) produced overt desensitization ($\sim 50\%$ reduction in the peak response) in only one of six preparations. Thus changes in previously established histamine (10 μM)-induced effects on the CAP in the presence of histamine receptor antagonists were interpreted to result from actions of the antagonist, rather than desensitization of the response to histamine itself.

In ganglia where histamine (10 μM) potentiated the CAP amplitude (Table 1A), a subsequent challenge with histamine in the presence of the H_1 histamine antagonist, pyrilamine (1 μM), produced either a blockade of the potentiation, or a reversal to depression of the CAP. In contrast, the H_3 antagonists, burimamide (1 μM; Schwartz, Arrang & Garbarg, 1986) or thioperamide (0·1 μM; Arrang, Garbarg, Lancelot, Lecomte, Pollard, Robba, Schunack & Schwartz, 1987), either enhanced the histamine-induced potentiation or were without effect. The histamine H_2 receptor antagonist, cimetidine (50 μM), did not affect histamine-induced potentiation of the CAP in any experiment. In ganglia where the predominant effect of histamine on the CAP was depression (Table 1B), subsequent challenge with histamine in the presence of the H_3 antagonist, burimamide (1 μM), often produced a reversal to potentiation of the CAP. In contrast, histamine-mediated depression in these ganglia was not affected by the presence of the H_1 antagonist, pyrilamine, or the H_2 receptor antagonist, cimetidine. Taken together these data supported the conclusions that: (1) histamine-induced potentiation of the postganglionic CAP is mediated via histamine H_1 receptors, (2) histamine-induced depression of the CAP is mediated via histamine H_3 receptors, and (3) a functional antagonism of these opposing receptor-mediated effects exists at the level of the population synaptic input–output relation (i.e. as measured by the CAP) in the SCG.

Figure 3 shows a concentration–response relationship for the effects of histamine on the amplitude of the evoked CAP in the absence and in the presence of 1 μM burimamide. Histamine (0·1–100 μM) produced a concentration-dependent increase in the magnitude of the CAP ($EC_{50} = 5{\cdot}28 \pm 0{\cdot}18$ μM; $n = 7$), with a maximal effect occurring at about 50 μM. The addition of burimamide (1 μM) to the superfusate produced a leftward shift of about one-half of a log unit in the histamine concentration–response relationship ($EC_{50} = 5{\cdot}87 \pm 0{\cdot}25$ μM; $n = 5$), but the concentration necessary to produce a maximal response was not affected measurably. These data support a conclusion that the absolute magnitude of histamine-induced

potentiation of the CAP (in absence of antagonists) may be underestimated by the functional antagonism produced through H_3 histamine receptor activation.

Intracellular studies of histamine-mediated effects at single synapses

Intracellular studies of the effects of histamine on the mean EPSP amplitude in the absence of histamine receptor antagonists corroborated the findings obtained from

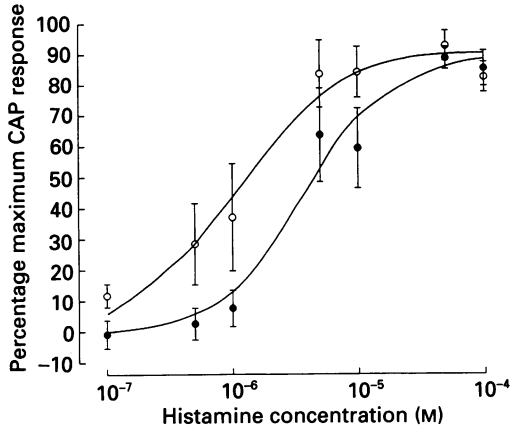

Fig. 3. Mean log concentration–response effects of histamine for potentiating the amplitude of the postganglionic compound action potential (CAP) recorded from the superior postganglionic nerve of the SCG in the absence of antagonists (●; $n = 7$), and in the presence of the histamine H_3 receptor antagonist, burimamide (10^{-6} M; ○; $n = 5$). CAPs were evoked by 0·2 Hz stimulation of the cervical sympathetic trunk. Histamine was superfused at progressively higher concentrations in a cumulative manner, and the CAP amplitude allowed to reach a steady-state level at each concentration (~ 5 min), prior to introducing the next higher histamine concentration. For each experiment, the CAP amplitude at each concentration was determined from an ensemble average of twelve responses, and these amplitudes were then normalized as percentage of the maximal amplitude (y-axis) reached to a single histamine concentration (taken to be 100%). Each point on the graph represents the composite mean ±S.E.M. percentage of control from all experiments performed under the condition. Continuous lines describe the Hill function ($f = 100/(1 + (EC_{50}/\text{conc})^{n^H})$ that was iteratively fitted to the data points under each condition, where EC_{50} is the histamine concentration producing one-half maximal effect, conc is the histamine concentration, and n^H is a coefficient. Pretreatment with burimamide shifted the histamine concentration–response relationship to the left by ~ 0·5 log units (see text for further explanation).

studies of the extracellular population response. Potentiation, depression, and lack of effect on the mean EPSP amplitude were all observed, depending on the synapse recorded (Fig. 4). Figure 4*A* illustrates an experiment where the EPSPs elicited by stimulation of the cervical sympathetic trunk were clearly potentiated in amplitude, while Fig. 4*B* shows results from a different preparation, in which histamine depressed the amplitude of EPSPs elicited by stimulation of the superior postganglionic nerve trunk. Over nine different experiments without antagonists, the mean EPSP were clearly potentiated by histamine (10 μM) in four neurons from four preparations (148±19% of control), depressed in four neurons from four different

preparations (77 ± 11 % of control) and unaffected in one remaining neuron (102 % of control). Thus histamine, in the absence of antagonists, affected the efficacy of synaptic transmission in a manner that was consistent with the excitatory and depressant changes observed at the level of the population (CAP) response.

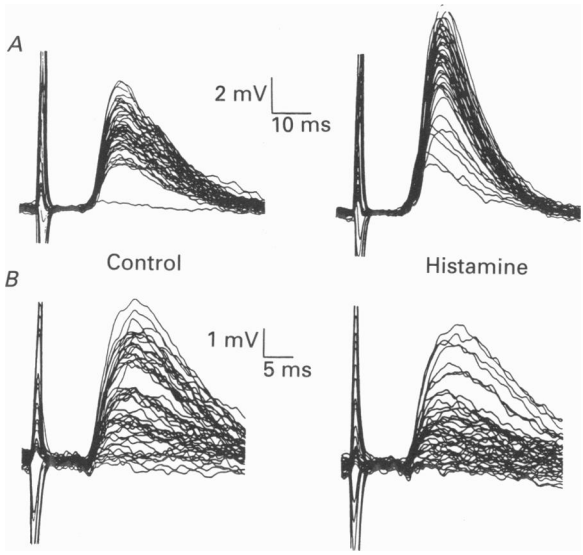

Fig. 4. Effects of histamine on individual stimulus-evoked EPSPs recorded in two neurons from different (*A* and *B*) SCG. Each panel shows fifty sequentially obtained overlaid EPSP responses recorded intracellularly following stimulation of the cervical sympathetic trunk (2·0 Hz; *A*) or the superior postganglionic nerve (3·0 Hz; *B*). Stimulus artifacts are to the left of traces. In each experiment EPSPs were collected prior to (left panels; Control), and during exposure to histamine (10 μM; right panels). *A*, experiment in which superfusion with histamine increased the amplitude of the mean evoked EPSP compared to the control condition. *B*, experiment in which superfusion with histamine decreased the amplitude of the mean EPSP compared to the control condition.

A series of experiments were performed to study the effects of histamine receptor agonists and antagonists on histamine-induced changes in the EPSP (Fig. 5), and results were again compatible with those obtained at the level of the population (CAP) response. Histamine added in the presence of the histamine H_3 antagonists, burimamide or thioperamide (Arrang *et al.* 1987), or in the combined presence of H_2 and H_3 antagonists produced either a potentiation or was without effect; no substantial ($> 5\%$) depression of the mean EPSP was observed in any single experiment ($n = 15$). In contrast, histamine in the presence of the H_1 antagonist, pyrilamine, or the H_3 agonist, (*R*)-α-methylhistamine (Arrang *et al.* 1983, 1987), depressed significantly the mean EPSP amplitude compared to the other treatments (Fig. 5). These observations of the effects of histamine receptor antagonists on the histamine-mediated modulation of EPSPs support the conclusion at the level of the single synapse that histamine-induced potentiation of synaptic transmission is mediated via H_1 receptors and depression via H_3 receptors.

Role of postsynaptic mechanisms in histamine-mediated changes

We have demonstrated previously several distinct histamine-mediated postsynaptic effects in the guinea-pig SCG, including a depolarization of the membrane potential, accompanied usually by an increase in input resistance (Christian *et al.*

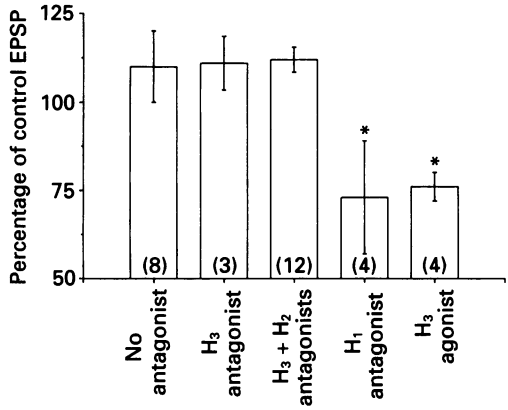

Fig. 5. Summary of results of experiments testing effects of histamine in the presence and absence of selective receptor antagonists on the amplitude of EPSPs recorded intracellularly from SCG neurons. Either the cervical sympathetic trunk or the superior postganglionic nerve was stimulated (0·2–4·0 Hz) in different experiments. Each bar, with exception of the rightmost bar, represents the mean ±S.E.M. (n = number of experiments in parentheses at bottom of bars) percentage of the pre-histamine EPSP obtained during superfusion with histamine (10 μM). Experiments represented by each bar were performed in the continual presence of the histamine receptor antagonist(s) indicated on the x-axis: No antagonist, histamine only; H_3 antagonist, 10 μM burimamide, or 0·01–0·1 μM thioperamide; H_3+H_2 antagonists, 50 μM burimamide, or 1 μM burimamide + 1 μM cimetidine; H_1 antagonist, 1 μM pyrilamine. The rightmost bar represents the percentage of control effects of the H_3 agonist, (*R*)-α-methylhistamine (0·1 μM), compared to a control condition where no histamine receptor agonist or antagonist was present. The percentage of control EPSP was obtained for each experiment by comparing the mean amplitude of $\geqslant$ 100 successive EPSPs collected during the pre-histamine period to the mean amplitude of the same number of successive EPSPs obtained during the subsequent addition of histamine (or (*R*)-α-methylhistamine). Histamine in the presence of the histamine H_1 antagonist, and the H_3 agonist alone significantly depressed the EPSP ($P < 0·05$; one-way analysis of variance; Newman–Keuls individual comparisons) compared to the other treatments.

1989). These changes were found to be mediated by histamine H_1 receptors, and thus could be hypothesized to account for the observed potentiation of synaptic transmission in the present study. We therefore endeavoured to determine the contribution of these postsynaptic effects to changes in synaptic efficacy. To test for the possible contribution of histamine-induced changes in membrane potential in experiments where potentiation of the EPSP was studied, the membrane was manually voltage clamped, when necessary, to within ±2 mV of resting membrane potential during exposure to histamine. In experiments studying EPSP depression, histamine H_1-mediated depolarization of the membrane potential (see Christian *et al.*

1989) was prevented pharmacologically by the presence of the histamine H_1 antagonist, pyrilamine (1 μM), or by use of the H_3 agonist, (*R*)-α-methylhistamine (0·1 μM). Both potentiation (140±12% of control; n = nine neurons in seven preparations), and depression (74±8% of control; n = eight neurons in eight

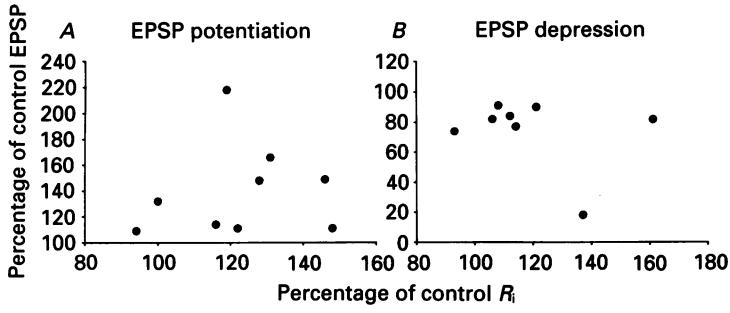

Fig. 6. Relationship between histamine effects on input resistance and the EPSP recorded intracellularly from neurons in the SCG. Experiments are sorted based on whether histamine (10 μM) potentiated the mean EPSP to $\geqslant$ 110% of its control amplitude (*A*), or depressed the mean EPSP to $\leqslant$ 90% of its control amplitude (*B*). Some experiments were conducted in the presence of various histamine H_1, H_2, or H_3 receptor antagonists at the concentrations specified in Fig. 5. Each point shows the results of one experiment. The percentage of control input resistance (R_i; x-axis) was obtained for each neuron by comparing the mean steady-state amplitude of $\geqslant$ 100 electrotonic voltage transients evoked by a transmembrane current step (−100 pA, 200 ms) during a control period to the mean of the same number of voltage transients obtained during superfusion with histamine. The percentage of control EPSP (y-axis) was derived by comparing the mean of $\geqslant$ 100 sequential EPSP amplitudes obtained in the control period to the same number of EPSP amplitudes obtained during histamine treatment. Measurements of the mean R_i and EPSP were made in the same time interval for each neuron under each condition. Histamine-induced changes in the EPSP amplitude were not correlated significantly to changes in input resistance for either EPSP potentiation or depression.

preparations) of the mean EPSP amplitude were observed in the absence of membrane potential changes, arguing that these EPSP effects did not necessarily depend on a histamine-induced depolarization of membrane potential.

To evaluate a possible contribution of histamine-induced changes in input resistance, the extent of correlation between histamine-induced changes in the EPSP amplitude and changes in input resistance was examined (Fig. 6). Correlational analyses revealed no significant relationships between histamine-induced changes in input resistance and EPSP amplitude for either EPSP potentiation (Fig. 6*A*), or EPSP depression (Fig. 6*B*). These data therefore support a conclusion that the known postsynaptic effects of histamine on input resistance cannot *themselves* account for the histamine-induced modulatory effects on the amplitude of the EPSP.

Role of presynaptic mechanisms in histamine-mediated changes

To assess more rigorously the locus of histamine-mediated potentiation and depression of EPSPs, direct quantal analysis was employed. Figures 7 and 8 demonstrate the results of two quantal analyses, obtained from two separate

experiments where histamine respectively potentiated and depressed significantly the mean EPSP amplitude. Quantal size, as measured by the mean amplitude of mEPSPs (insets), was not significantly affected by exposure to histamine in either case, whereas quantal content increased during EPSP potentiation (Fig. 7), and

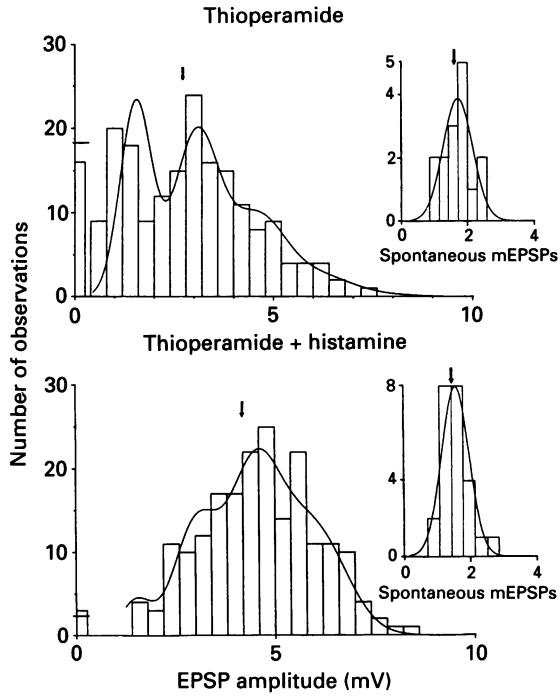

Fig. 7. Quantal analysis of histamine-induced potentiation of the EPSP in an SCG neuron. Data are from an experiment where EPSP responses were recorded intracellularly to stimulation of the superior postganglionic nerve (3·0 Hz) in the presence of the histamine H_3 receptor antagonist, thioperamide (10 nM; upper panel), and subsequently in the presence of histamine (10 μM) and thioperamide (lower panel). The thioperamide was present to block histamine-mediated depression. Each panel shows the amplitude–frequency distribution histogram of 200 sequentially evoked EPSPs (failures in bin at far left), and each inset shows the amplitude–frequency histogram of spontaneous mEPSPs (axis labels same as for evoked EPSP histogram) collected in the interstimulus intervals between the evoked EPSPs. Arrow over each histogram denotes observed mean amplitude. The predicted binomial distribution of EPSP amplitudes based on the mEPSP mean and variance is superimposed (continuous line) on each histogram of evoked EPSPs. Each mEPSP histogram is fitted iteratively to a Gaussian distribution (continuous line). The quantal content (m) $\pm$S.E.M. determined directly from the data in each analysis (see Methods), and the values of parameters p and n determined from each predicted binomial distribution are as follows. Thioperamide: m, $1{\cdot}76 \pm 0{\cdot}14$; p, $0{\cdot}45 \pm 0{\cdot}07$, n, $3{\cdot}88 \pm 0{\cdot}72$. Thioperamide plus histamine: m, $2{\cdot}91 \pm 0{\cdot}16$; p, $0{\cdot}74 \pm 0{\cdot}03$; n, $3{\cdot}93 \pm 0{\cdot}26$. Histamine-mediated potentiation of the EPSP amplitude was accompanied by an increase in m and p (see text).

decreased during EPSP depression (Fig. 8). In both of these experiments, the mEPSP amplitude–frequency histograms were fitted to a Gaussian distribution, and the evoked EPSP amplitude–frequency histograms were fitted to a simple binomial

model. Histamine-mediated potentiation of the EPSP (Fig. 7) was associated with an increase in parameter p, and no change in n, while the reverse was true for depression (Fig. 8). In conclusion, the results of these quantal analyses show that the potentiation and depression of the EPSP observed to histamine in these two

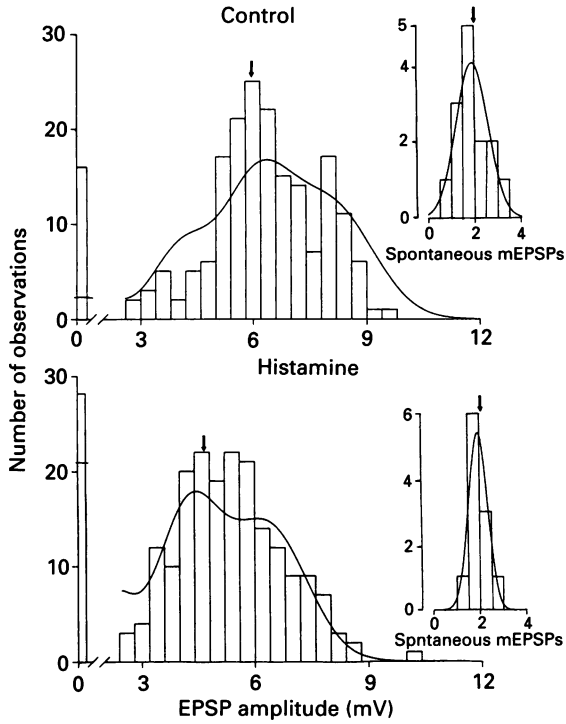

Fig. 8. Quantal analysis of histamine-induced *depression* of the EPSP in an SCG neuron. Data are from an experiment where EPSP responses were recorded intracellularly in response to stimulation of the superior postganglionic nerve (4·0 Hz) during superfusion with normal Locke solution (upper panel), followed by superfusion with histamine (10 μM; lower panel). Data are presented in a format identical to Fig. 7. Histamine-mediated depression of the EPSP amplitude was accompanied by a decrease in m and n (see text). Values for m, p and n are as follows. Control: m, $2{\cdot}97 \pm 0{\cdot}24$; p, $0{\cdot}78 \pm 0{\cdot}04$; n, $3{\cdot}77 \pm 0{\cdot}26$. Histamine: m, $2{\cdot}25 \pm 0{\cdot}23$; p, $0{\cdot}76 \pm 0{\cdot}05$; n, $2{\cdot}95 \pm 0{\cdot}26$.

experiments were both mediated at a presynaptic locus, possibly via two different aspects of the release mechanism.

Table 2 summarizes the results of nine quantal analyses performed at synapses where histamine produced a significant alteration of the mean EPSP amplitude. These data support further the conclusion derived from the examples in Figs 7 and 8, that histamine-mediated potentiation and depression of the EPSP are both mediated via effects on presynaptic release of transmitter. In three of the four experiments where histamine potentiated the EPSP amplitude (Table 2A), quantal size was not significantly affected, while quantal content (m) was increased by a mean of $34 \pm 13\%$. The single experiment (No. 3) that contrasted to the others (i.e.

significantly increased quantal size with unchanged quantal content) may represent a case where postsynaptic changes are responsible for the increase in the mean EPSP amplitude. In the five experiments where histamine reduced the synaptically evoked EPSP amplitude (Table 2B), this depression was always accompanied by a decrease in quantal content (mean = 60 ± 14% decrease) and no significant effect on quantal

TABLE 2. Quantal analysis of histamine effects at SCG synapses

Exp. No.	Treatment	Mean mEPSP (mV)	Mean EPSP (mV)	m
A. Potentiation				
1	Control	1·1 ± 0·1 (5)	2·8 ± 0·1 (200)	2·5 ± 0·1
	Hist	1·0 ± 0·1 (4)	3·2 ± 0·1 (200)*	3·2 ± 0·1
2	Burim	1·6 ± 0·4 (11)	5·2 ± 0·1 (243)	3·3 ± 0·1
	Burim + Hist	1·6 ± 0·1 (7)	5·9 ± 0·1 (242)**	3·7 ± 0·2
3	Cimet + Burim	1·3 ± 0·1 (7)	2·0 ± 0·1 (300)	1·6 ± 0·3
	Cimet + Burim + Hist	1·7 ± 0·1 (4)*	2·8 ± 0·1 (300)**	1·7 ± 0·2
4	Thioper	1·7 ± 0·1 (15)	2·4 ± 0·1 (186)	1·8 ± 0·1
	Thioper + Hist	1·5 ± 0·1 (26)	3·9 ± 0·2 (198)**	2·9 ± 0·2
B. Depression				
1	Control	1·6 ± 0·1 (8)	2·0 ± 0·1 (197)	1·3 ± 0·1
	Hist	1·6 ± 0·2 (6)	1·1 ± 0·2 (200)*	0·7 ± 0·1
2	Control	2·0 ± 0·2 (14)	6·0 ± 0·2 (200)	3·0 ± 0·2
	Hist	2·1 ± 0·2 (11)	4·7 ± 0·1 (200)**	2·2 ± 0·2
3	Control	1·7 ± 0·1 (14)	6·6 ± 0·1 (200)	3·9 ± 0·2
	Hist	1·9 ± 0·1 (7)	6·1 ± 0·1 (200)*	3·2 ± 0·2
4	Pyril + Cimet	1·6 ± 0·3 (12)	3·5 ± 0·1 (192)	2·2 ± 0·2
	Pyril + Cimet + Hist	1·7 ± 0·5 (8)	0·8 ± 0·1 (192)**	0·5 ± 0·3
5	Control	1·7 ± 0·4 (37)	2·3 ± 0·1 (150)	1·4 ± 0·1
	α-MeHist	1·6 ± 0·1 (13)	1·6 ± 0·1 (150)*	1·0 ± 0·1

Mean ± S.E.M. amplitude values of spontaneous mEPSPs and evoked EPSPs averaged from number of events in parentheses under indicated treatment: histamine (Hist, 10 μM); burimamide (Burim, 1·50 μM); cimetidine (Cimet, 50 μM); thioperamide (Thioper, 0·1 μM); pyrilamine (Pyril, 1 μM); (*R*)-α-methylhistamine (α-MeHist, 10 nM). * indicates that mean values obtained under two experimental treatments differed at $P < 0.05$; ** at $P < 0.001$ level (independent t tests). Mean quantal content (m) ± S.E.M. determined for each treatment as described in Methods. Each of nine experiments shown was performed on a different ganglion. Experiments are sorted by whether the mean evoked EPSP was potentiated (A), or depressed (B) following the addition of histamine. Note that the mean quantal content was increased in three of the four experiments (Nos. 1, 2 and 4) where potentiation of the EPSP was observed (A), and decreased in all five experiments where depression of the EPSP was observed (see text for further explanation).

size. Thus these data support the conclusion that histamine produced depression of the EPSP via a presynaptic mechanism.

Effects of endogenously released histamine on synaptic efficacy

Our laboratory has demonstrated previously that sufficient quantities of *endogenous* histamine are released by an antigenic stimulus in antigen-sensitized SCG to produce effects on the postganglionic CAP (Weinreich & Undem, 1987), and on active and passive electrophysiological membrane properties of SCG neurons

(Christian *et al.* 1989). We therefore attempted to determine here whether antigenically released *endogenous* histamine could produce effects at ganglionic synapses similar to those produced by exogenously applied histamine.

Ten SCG from ten guinea-pigs sensitized to ovalbumin antigen were exposed to histamine and subsequently to ovalbumin (10 μg ml^{-1}; this concentration was

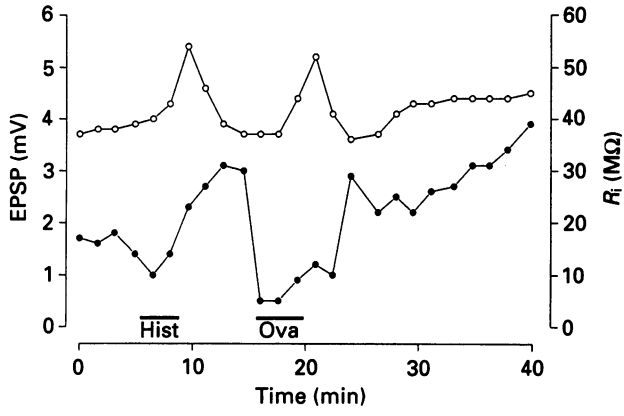

Fig. 9. Time course of effect of histamine (Hist; 10 μM) and specific antigen (ovalbumin; Ova; 10 μg ml^{-1}) challenge on the mean EPSP amplitude (●) and input resistance (R_i; ○) recorded intracellularly from a neuron in an antigen-sensitized SCG. EPSP responses to stimulation of the cervical sympathetic trunk (1·0 Hz), and electrotonic voltage transients to a transmembrane current step (−100 pA, 200 ms) to estimate R_i were recorded continuously. Blocks of fifty sequential EPSPs and fifty voltage transients were each ensemble averaged to yield the mean values represented by each point. Both histamine and antigen reversibly increased R_i. Histamine increased the mean EPSP reversibly, whereas the EPSP increase accompanying antigen exposure did not reverse for the duration of the experiment.

determined previously to release maximal quantities of endogenous histamine, see Christian *et al.* 1989) during stable recordings of EPSPs. Figure 9 illustrates the results of one experiment where *exogenously* applied histamine increased the amplitude of the mean EPSP and the input resistance of the neuron. Both parameters recovered to their pre-histamine control values within 7 min of wash-out with normal Locke solution. Subsequent exposure to ovalbumin also increased both the EPSP amplitude and input resistance to levels comparable to those produced by exogenous histamine. However, 20 min after the initial exposure to ovalbumin, input resistance still remained slightly elevated, and the mean EPSP amplitude was increased substantially above its pre-antigen level. Therefore, the EPSP was altered directionally in a similar way by histamine and antigen treatments, but the time course of recovery of the two effects differed markedly.

Previous biochemical measurements have shown that the efflux of *endogenous* histamine from the SCG reaches a peak within 1 min of antigen exposure, and declines to unmeasurable levels within 10 min (Christian *et al.* 1989). Therefore, to compare antigen-induced effects on the EPSP to those elicited by *exogenously* applied histamine, ovalbumin effects on the EPSP were only studied within the first

5 min of exposure of the tissue to this antigen. Histamine and ovalbumin were superfused in the presence of burimamide (1–50 μM) in three experiments, and in the presence of pyrilamine (1 μM) in two others. Responses of the EPSP to exogenous histamine in these experiments were as described above (i.e. potentiation and

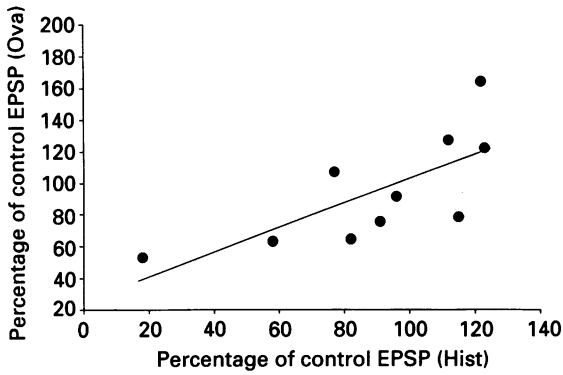

Fig. 10. Relationship between the effects produced on the mean EPSP amplitude by histamine (Hist; 10 μM; x-axis) and by antigen (ovalbumin; Ova; 10 μg ml^{-1}; y-axis) challenge in neurons from antigen-sensitized SCG. Each point represents one experiment, where the EPSP was first monitored during histamine superfusion, during a recovery period, and subsequently during antigen treatment. EPSPs were recorded to stimulation of either the cervical sympathetic trunk or superior postganglionic nerve (0·5–4·0 Hz). Histamine H_1, H_2, H_3 receptor antagonists were present in some experiments at the concentrations specified in Fig. 5. In each experiment the percentage of control EPSP to each treatment was derived by comparing the mean amplitude of $\geqslant 100$ sequential EPSPs obtained immediately preceding the treatment to the same number obtained during the treatment. EPSP sampling during antigen exposure was limited to the first 5 min of exposure, when endogenously released histamine is known to reach peak concentrations in the tissue (see text). Regression analysis revealed a significant linear correlation (continuous line; $r = 0{\cdot}78$, $P = 0{\cdot}007$) between the EPSP changes recorded during the two treatments.

depression respectively; see Fig. 5). Importantly, in these five experiments with histamine receptor antagonists, as well as in the five other experiments without antagonists, ovalbumin treatment produced similar directional peak effects on EPSP amplitudes to those produced by exogenous histamine in the same neuron (Fig. 10). The percentage change produced in the mean EPSP amplitude by histamine in the ten experiments correlated linearly to that produced by ovalbumin (linear regression analysis: $r = 0{\cdot}78$; $P = 0{\cdot}007$; see Fig. 10). These data thus support a conclusion that the effects on mean EPSP amplitude produced within 5 min of ovalbumin treatment can be accounted for primarily by the actions of *endogenously* released histamine.

DISCUSSION

Several previous reports have demonstrated that histamine can both potentiate and depress sympathetic ganglionic synaptic transmission via histamine H_1 and H_2 receptor subtypes (Brimble & Wallis, 1973; Yamada *et al.* 1982; Snow & Weinreich,

1987). Recently, a third histamine receptor subtype, the histamine H_3 receptor, has been recognized in several neuronal tissues other than sympathetic neurons (Arrang *et al.* 1983; Ishikawa & Sperelakis, 1987; Tamura, Palmer & Wood, 1988; Ichinose *et al.* 1989, 1990). These reports have provided evidence associating activation of the histamine H_3 receptor with presynaptic inhibition of transmitter release in various central and peripheral nervous tissues. The present study makes three new important contributions to these previous findings. First, the pharmacological evidence presented demonstrates the existence of a functional histamine H_3 receptor that inhibits the efficacy of sympathetic ganglionic transmission. Second, the evidence obtained by way of a direct quantal analysis demonstrates that the histamine H_3-mediated depression of synaptic transmission occurs at a presynaptic locus. Finally, the effects produced by ovalbumin challenge in antigen-sensitized SCG provide evidence that histamine H_1 and H_3 receptors coupled to these modulatory effector mechanisms are activated by *endogenous* histamine released as a consequence of immunological reactions.

Mediation of synaptic depression by two different histamine receptor subtypes?

Previous reports have attributed histamine-mediated depression of synaptic transmission at different synapses in the peripheral nervous system primarily to activation of the H_2 receptor subtype. For example, histamine has been shown to depress sympathetic ganglionic synaptic transmission via histamine H_2 receptors in bullfrog (Yamada *et al.* 1982), rabbit (Brimble & Wallis, 1973), and rat (Lindl, 1983; Snow & Weinreich, 1987). Histamine-induced depression of synaptic transmission, mediated by pre- and postsynaptic H_2 receptors has been reported in neurons in the guinea-pig submucous plexus (Tokimasa & Akasu, 1989), although no pharmacological studies of histamine receptor subtypes at sympathetic ganglionic synapses are available.

The relatively recent characterization of the histamine H_3 receptor subtype and the development of potent and selective histamine H_3 receptor antagonists (Arrang *et al.* 1983, 1987; Arrang, Schwartz & Schunack, 1985; Schwartz, Arrang & Garbarg, 1986) have led to a growing number of reports documenting depressant effects of histamine in the peripheral nervous system, mediated by histamine H_3 receptor activation (Ishikawa & Sperelakis, 1987; Tamura *et al.* 1988; Ichinose *et al.* 1989, 1990). The present work evaluates histamine-induced modulation of sympathetic ganglionic transmission pharmacologically using several of the most potent and selective histamine receptor agonists and antagonists available (i.e. (*R*)-α-methylhistamine and thioperamide). The results show through direct quantal analyses that histamine H_3 receptors decrease acetylcholine release at sympathetic ganglionic synapses in the guinea-pig. The extracellular and intracellular recording experiments provide mutual evidence that histamine H_2 receptor activation does not contribute to these effects.

The advent of histamine H_3-selective antagonists may warrant a re-examination of some of the conclusions obtained in earlier studies where histamine H_3 receptor activation was not considered. In this regard, it is interesting to note that several H_2 agonists and antagonists have been shown to be antagonists at the histamine H_3 receptor (for review see Timmerman, 1990). If one were to accept the seemingly

unlikely assumption that no histamine H_3 component is present in tissues where H_2-mediated depression has been reported previously (see above), the results together would suggest that substantial phylogenetic diversity exists for histamine receptor subtypes mediating a similar effect (i.e. synaptic inhibition) within analogous tissues of different species. If this were the case, conclusions about histamine receptor-mediated functions based on pharmacological findings in a single species may lead to unwarranted conclusions when extrapolated to other species.

Functionally antagonistic effects of histamine on ganglionic synaptic transmission

Both extracellular recordings of population postganglionic responses and intracellular recordings of EPSPs at single synapses provided evidence for dual modulatory effects of histamine at synapses in sympathetic ganglia. Histamine application in the absence of antagonists potentiated the population CAP in the majority of ganglia (i.e. $\sim 85\%$; Table 1), but a component of synaptic depression was often revealed in CAP recordings when the potentiating action of histamine was pharmacologically blocked by H_1 receptor antagonists (see Table 1). In intracellular studies histamine-mediated potentiation and depression of EPSPs were both observed at different synapses, and the two opposing effects seemed to have about an equal likelihood of occurrence in the absence of histamine receptor antagonists (four cases of each observed in a total of nine different experiments). Thus an apparent discrepancy exists regarding the relative proportions of potentiation and depression observed between the extracellular and intracellular experiments. One possible explanation underlying this discrepancy may be related to the complexity of neuronal cytoarchitecture, and the high degree of convergence of synaptic inputs that is known to exist in the guinea-pig SCG (Purves, 1975; Njå & Purves, 1977). Possibly the synaptic inputs potentiated by histamine are more efficacious at integrating output from the postsynaptic neuron than those that are depressed, due to the spatio-temporal arrangement of these synapses. Our study did not address this issue, which would require quantitative time course comparisons of the rising and falling phases of the EPSP waveforms from different synapses. Irrespective of the reason(s) underlying this discrepancy, however, our results do provide conclusive evidence both at the level of the population input–output relation of the ganglion, and at single synapses that histamine H_1 and H_3 receptors are present mutually in the SCG, and each is coupled to an opposing mechanism that modulates synaptic transmission.

The presence of these functionally antagonistic mechanisms raises two more provocative questions. First, what is the basis for the variability that was found with regard to the predominance of one or the other histamine effect on the population response in the SCG from different animals? Second, are the opposing effects mediated by histamine both manifested at single synapses, or is a given synapse predisposed to only potentiation or depression? As already mentioned (see Results), we were not able to correlate the differential expression of histamine-induced potentiation or depression of the CAP in an SCG to any obvious environmental or experimental variables. At the level of single synapses, one possibility not pursued in the present work, is that the direction of the histamine effect on a given ganglionic neuron may relate to specific target organ innervation or physiological function of

that neuron. Future studies recording intracellularly from neurons whose physiological targets are known may provide insights into this possibility.

Whether histamine can mediate *both* potentiation and depression at a *single* synapse was not addressed systematically in the present work; in the majority of experiments testing antagonists, the tissue was exposed to the antagonist from the onset of the experiment. However, the effects of histamine were studied at several synapses prior to and in the presence of histamine receptor antagonists without observing a clear reversal of one type of effect to the other (i.e. potentiation to depression, or vice versa). These initial observations would therefore suggest that single synapses are predisposed to only one histamine-mediated modulatory action, but future experiments will be necessary to establish this more conclusively.

Presynaptic locus of histamine H_3-mediated effects

Several previous reports indicate that activation of histamine H_3 receptors is coupled to presynaptic inhibitory effects in different nervous tissues (Arrang *et al.* 1983; Ishikawa & Sperelakis, 1987; Ichinose *et al.* 1989, 1990). However, the present results provide, to our knowledge the first direct demonstration, using a rigorous quantal analysis technique, for a presynaptic locus of histamine H_3-mediated depressant effects. We measured directly mean quantal size by sampling spontaneous mEPSPs, and calculated mean quantal content from the stimulus-evoked EPSPs occurring in the same time intervals as the mEPSPs. We verified that both the mEPSPs and evoked EPSPs were generated at the same synapse based on the similarity of their waveforms (Fig. 1). Thus our finding that decreases in quantal content and unchanged quantal size accompanied evoked EPSP depression in five out of five experiments (Fig. 8; Table 2B) showed unequivocally that this depression occurred at a presynaptic locus. In one of these cases depression was produced by H_3 receptor agonists, and in another case histamine produced depression in the presence of histamine H_1 and H_2 receptor antagonists, demonstrating pharmacologically the involvement of histamine H_3 receptors. Yamada *et al.* (1982) applied a similar quantal analysis technique to histamine responses in the bullfrog SCG and found that histamine-induced potentiation and depression were mediated respectively through presynaptic histamine H_1 and H_2 receptors.

The quantal analysis technique also showed that histamine produces synaptic potentiation through activation of presynaptic histamine H_1 receptors (Fig. 7; Table 2A) in three of four experiments. The single experiment where increased quantal size, and unchanged quantal content accompanied EPSP potentiation (Table 2A, Experiment No. 3) may reflect possible involvement of postsynaptic mechanisms at some synapses. A postsynaptic mechanism producing synaptic potentiation is consistent with our previous observations that histamine has direct effects on membrane properties of some guinea-pig SCG neurons (Christian *et al.* 1989). Quantal analyses experiments from a larger sampling population of synapses may clarify whether postsynaptic mechanisms are primarily responsible for histamine-induced potentiation at a minority of synapses, as is suggested by this single experiment. The present work clearly establishes a significant contribution by presynaptic mechanisms.

The possibility that histamine may activate two distinct presynaptic receptor

subtypes, each producing an opposite effect on transmitter release is intriguing, particularly with regard to the coupling mechanisms involved in the two processes. In two experiments the distribution of evoked EPSP amplitudes could be described by a simple binomial distribution, in agreement with previous reports where transmitter release at some SCG synapses was fitted successfully by a binomial model (McLachlan, 1975; Bennett, Florin & Pettigrew, 1976; Birks & Isacoff, 1988). Of these two cases, histamine potentiated EPSPs and increased p values in one (Fig. 7), and depressed EPSPs and decreased n values in the other (Fig. 8). These preliminary findings are thus compatible with histamine exerting receptor-mediated effects on two separate presynaptic mechanisms that produce opposing effects on transmitter release.

Modulation of synaptic efficacy by endogenous histamine

We have previously shown that ganglionic histamine is released by an antigenic stimulus in sufficient quantities to affect specific electrophysiological properties of ganglionic neurons and increase their excitability (Christian *et al.* 1989). We have also provided several lines of evidence that this histamine is derived from ganglionic mast cells (Undem, Hubbard, Christian & Weinreich, 1990). Here we have extended these findings by presenting evidence that the effects produced by antigen challenge on ganglionic synaptic transmission within 5 min of exposure in sensitized SCGs mimic the potentiating or depressant effects of exogenously applied histamine. Ichinose *et al.* (1990) have recently shown that antigen-induced bronchoconstriction in the guinea-pig airway may be modulated in an inhibitory manner via histamine H_3 receptor activation.

The present work provides further evidence for the involvement of histamine H_3 receptors in immune–nervous system interactions. Considering our previous findings that specific antigen challenge releases peak quantities of endogenous histamine within 5 min of exposure (Christian *et al.* 1989), the most prudent explanation for the present observations is that the initial antigen-induced modulation of EPSP amplitude is mediated through activation of histamine H_1 and H_3 receptors (potentiation and depression, respectively) by the endogenously released autacoid. Thus these findings are significant in that they implicate a physiological role of histamine H_1 and H_3 receptors in mediating communication between the immune system and sympathetic nervous system.

The authors wish to thank Drs Elspeth McLachlan and Donald McAfee for their constructive suggestions on an earlier draft of this manuscript. We also thank Mr Glen Taylor and Ms Karen Naper for assistance with constructing figures and editing the manuscript. This work was supported by NIH Grant NS25598 to D.W.

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