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EFFECTS OF ANTAGONISTS ON PUTATIVE HISTAMINE RECEPTORS IN THE FIRST VISUAL NEUROPILE OF THE HOUSEFLY (*MUSCA DOMESTICA*)

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Summary

Intracellular recordings were made from the large monopolar cells (LMCs), which are the cells postsynaptic to photoreceptors, in the housefly *Musca domestica*. A multi-barrelled ionophoretic pipette glued to the recording electrode was used to apply a variety of cholinergic and histaminergic antagonists onto the recorded neurones. All substances which blocked the physiological response to light also antagonized the response to ionophoretically applied histamine, supporting the hypothesis that histamine is the neurotransmitter released by insect photoreceptors. In order of potency, the following drugs were found to block or reduce the LMC's responses to light: benzoquinonium $\geq$ gallamine $>$ ranitidine $\geq$ atropine $\approx$ cimetidine $>$ metiamide $\approx$ SK&F 93479 $\geq$ mepyramine. Mecamylamine, scopolamine, dexetimide, nicotine, mequitazine, chlorpheniramine and clemastine were ineffective.

Two other cholinergic ligands, hexamethonium and decamethonium, were much more potent than even benzoquinonium, but had the effect of facilitating and greatly slowing down the responses to light. Responses evoked by acetylcholine showed a different pharmacology, being blocked by mecamylamine but unaffected by hexamethonium. Despite testing a number of known cholinergic and histaminergic agents, no effective agonist for histamine was found. The results indicate the existence of a novel class of histamine-sensitive receptor with nicotinic features. In addition the unusual effects of a traditional H1 agonist, 2-thiazolylethylamine, suggested the presence of a second, distinct class of histamine receptor.

Introduction

Recently, substantial evidence has accumulated indicating that histamine may be the major neurotransmitter released by photoreceptors in the compound eyes and ocelli of insects: endogenous histamine is present in large amounts in both the retina and the first visual neuropile (lamina) of locusts, cockroaches and tobacco hornworm moths (Elias & Evans, 1983); antibodies raised against histamine label photoreceptor terminals in the fly compound eye and ocellus (Nässel *et al.* 1988);

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retina and optic lobe tissues of the tobacco hornworm moth (Maxwell *et al.* 1978) and the locust (Elias & Evans, 1983) are capable of synthesizing histamine from a radioactive precursor (histidine), and locust optic lobe also has enzymatic pathways for inactivation of histamine (Elias & Evans, 1983); ionophoretically applied histamine mimics the action of the photoreceptor transmitter on post-synaptic cells in the fly lamina (Hardie, 1987) and also the locust ocellus (Simmons & Hardie, 1988); and certain drugs have been identified which block the actions of both exogenously applied histamine and of the photoreceptor transmitter (Hardie, 1987; Simmons & Hardie, 1988).

The first synapse in the insect visual system is the only putative histaminergic system in insects. Indeed, only one other case has been reported in any arthropod, namely in the lobster stomatogastric ganglion (Claiborne & Selverston, 1984). The retina–lamina projection in the insect, and in particular in the fly (e.g. *Musca* and *Calliphora*) has been particularly well studied and intracellular recordings may be made routinely from both photoreceptors and their major postsynaptic elements, the large monopolar cells (LMCs) (e.g. Laughlin & Hardie, 1978; see review by Laughlin, 1981). The photoreceptors respond to light with a depolarization, releasing a neurotransmitter which causes the LMCs to hyperpolarize (see review by Laughlin, 1981) *via* a rapid increase in chloride conductance (Zettler & Straka, 1987). A chloride mechanism has also been implicated in the case of histaminergic synapses in the stomatogastric ganglion (Claiborne & Selverston, 1984).

To date there is only sparse information regarding the pharmacological nature of the histamine-sensitive receptors on the postsynaptic neurones. Elias *et al.* (1984) described binding of [^{3}H]mepyramine (an H1 blocker) in locust lamina, but this was non-specific, possibly cross-reacting with octopamine sites. Atropine was an effective antagonist of synaptic transmission in the fly lamina (Hardie, 1987), and curare blocked transmission in the stomatogastric ganglion (Claiborne & Selverston, 1984) and also in the ocelli of dragonflies (Klingman & Chappell, 1978) and locusts (Ammermüller & Weiler, 1985; Simmons & Hardie, 1988).

In the present study an *in vivo* pharmacological analysis has been performed on the LMCs of the fly lamina by examining the effects of a variety of antagonists on postsynaptic responses to naturally released transmitter and ionophoretically applied histamine. The results show that the LMCs are surprisingly sensitive to a variety of traditional cholinergic agents and indicate the presence of a novel class of receptor.

Materials and methods

Animals

The majority of experiments were performed upon male *Musca domestica* from laboratory cultures but a few experiments were also performed upon female *Calliphora erythrocephala*.

Recording and ionophoresis

The same procedures were used as described in a previous study (Hardie, 1987).

Briefly, intracellular recordings were made from the large monopolar cells (LMCs) in the lamina by advancing a multi-barrelled electrode into the eye of a fly immobilized in wax. The electrode consisted of a triple-barrelled ionophoretic pipette (overall tip diameter 1–3 μm) glued onto the intracellular pipette with a tip separation of less than 5 μm. The close proximity of tips is essential if the ionophoretic barrel is to penetrate the glial sheath which envelops each group of photoreceptors and postsynaptic neurones (collectively known as a cartridge – see review by Shaw, 1984). One barrel of the ionophoretic assembly contained 0·2 mol l^{-1} NaCl and was used as a current balance electrode, the other two contained pharmacological agents. The extremely local ionophoresis means that only relatively small doses are required to produce effects (typically, ionophoretic currents of less than 10 nA were used) and recovery times are fast. Backing currents in the range 1–3 nA were used to minimize leakage.

The following drugs were used: hexamethonium bromide, decamethonium bromide, atropine sulphate, gallamine triethiodide (Flaxedil), acetyl-β-methyl choline, mecamylamine, scopolamine, *d*-tubocurarine chloride, nicotine hydrogen tartrate, carbamyl choline (carbachol), acetylcholine, histamine dihydrochloride, chlorpheniramine maleate, clemastine fumarate (all Sigma); cimetidine hydrochloride (Aldrich); metiamide, promethazine, mepyramine, SK&F 93479, 2-methyl histamine, 3-methyl histamine, 4-methyl histamine, 2-thiazolylethylamine, dimaprit, impromidine (Smith Kline & French); dexetimide (Janssen); mequitazine (May & Baker); ranitidine hydrochloride (Glaxo); benzoquinonium chloride (gift of D. Sattelle, AFRC, Cambridge).

Unless otherwise stated, all drugs were dissolved at 0·1 mol l^{-1} in distilled water, with pH values in the range 3·5–4·5.

Resistance measurements

In certain experiments the resistance of the cell membrane was monitored simply by injecting a train of constant-current pulses. In addition, the resistance changes during the LMC's response to light were measured using a technique first described by van Hateren (1986). The response to a light flash is sampled and digitized on-line in the presence and absence of a small (0·1–0·2 nA) current step. The two voltage traces are subsequently subtracted and, after 50–100 repeats, the result averaged. The resultant voltage signal is divided by the current to obtain the resistance. This method requires only a single electrode, a normal amplifier and a simple computer program. It gives reliable, high time resolution resistance measurements in cells where there are minimal voltage-sensitive conductances, as has been demonstrated over most of the physiological voltage range in LMCs (Laughlin, 1988).

Results

Substances which block the response to light and histamine

In a first series of experiments one barrel of the ionophoretic pipette contained a

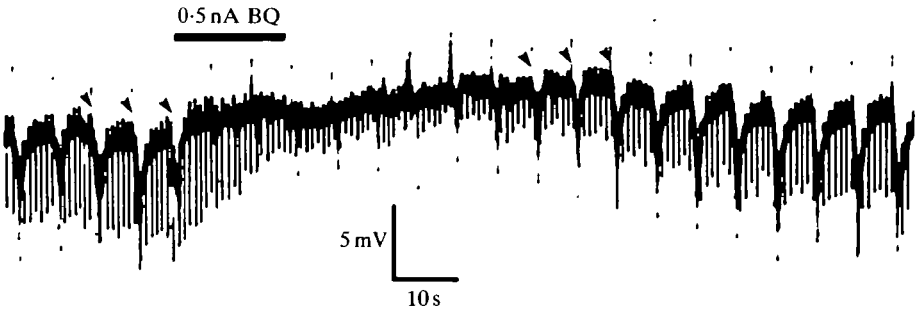

Fig. 1. Sub-threshold light flashes delivered at approx. 0·8 Hz induce rapid hyperpolarizations (vertical lines) in an LMC; ionophoretic pulses of histamine (1 nA for 1 s, delivered every 6 s) cause slower hyperpolarizations (e.g. arrowheads). An ionophoretic current of only 0·5 nA benzoquinonium (BQ) rapidly and reversibly blocks responses to both light and histamine.

putative antagonist whilst the other active barrel contained histamine. Recordings were made of LMC responses both to sub-saturating 50 ms light flashes and also to short pulses of ionophoretically applied histamine (both of which produce hyperpolarizations), and the effects of antagonists observed. All substances which antagonized the response to light also blocked the response to ionophoretically applied histamine. An example is shown in Fig. 1, where benzoquinonium, the most potent antagonist tested, is shown to block reversibly responses to both histamine and light. As discussed earlier (Hardie, 1987), responses to ionophoretically applied histamine were more rapidly blocked by a given antagonist than were responses to light. However, this is easily understood from the geometry of the situation: histamine and the antagonist are released from the same pipette and can thus be expected to have immediate access to the same sites, whereas the photoreceptor–LMC synapses (approx. 1000 per LMC; Nicol & Meinertzhagen, 1982) are more widely distributed over a large number of dendrites.

In the second series of experiments, designed to investigate the relative potency of different antagonists, pairs of antagonists were directly compared on the same cell by applying them alternately from neighbouring barrels of the same pipette. Relative potency was assessed, ideally by comparing the ionophoretic charge required to obtain a similar degree of antagonism of the physiological response (Fig. 2B,C). Alternatively one drug was judged less potent than another when a larger dose consistently resulted in a substantially lower level of antagonism (e.g. Fig. 2A). In addition, Fig. 2C indicates that the antagonism achieved by two different drugs (in this case, the nicotinic agent gallamine and the H2 antagonist, ranitidine) are functionally equivalent. To construct a rank order of potency (Table 1), every drug was compared with, in most cases, at least two other antagonists (one more, and one less potent). Short application regimes (5–20 s) were preferred, since, apart from the limited recording time from these small cells, longer application of benzoquinonium and gallamine usually led to a complete

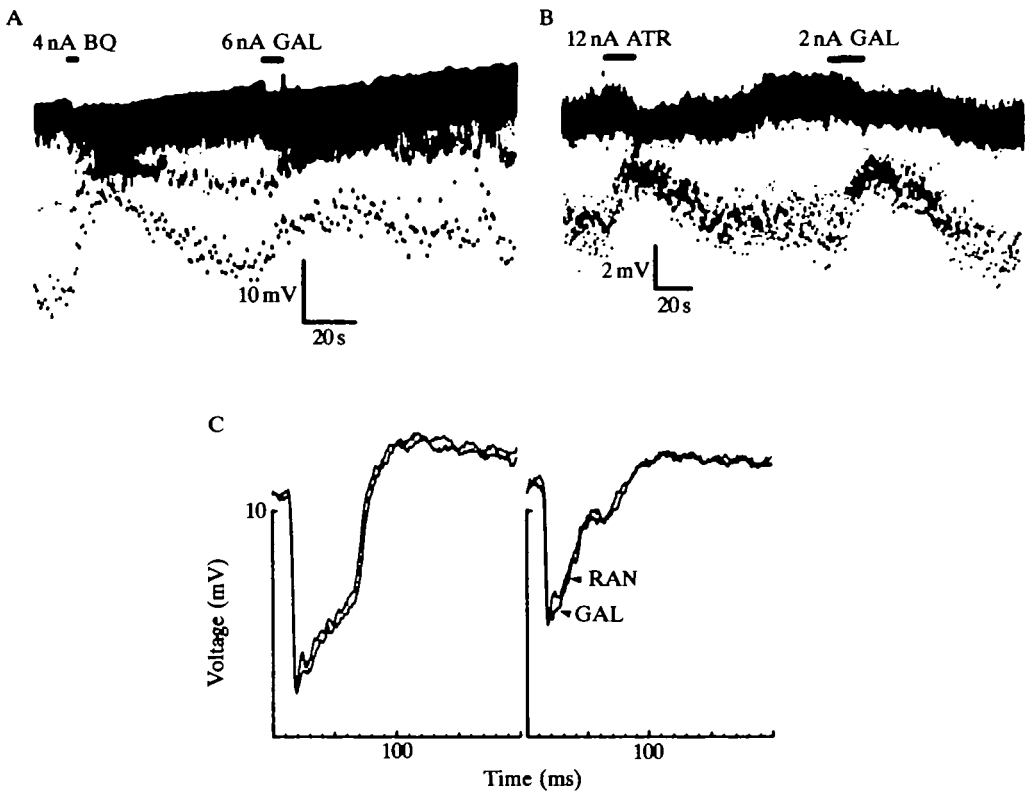

Fig. 2. Examples of data used to construct the pharmacological profile (Table 1). In A and B, only the peaks of the responses to light (approx. 1 flash s^{-1}) can be seen as dark points. (A) 4 nA benzoquinonium (BQ) applied for 4 s induces a more effective block of the light response than 6 nA gallamine (GAL) applied for twice as long (8 s). The ionophoretic currents are actually equivalent since BQ is doubly and GAL trebly charged. (B) Similar degrees of antagonism achieved by 12 nA atropine (ATR) and 2 nA GAL. Since atropine is only singly charged this suggests that gallamine is 18 times more potent. (C) Right, averaged light responses ($N = 5$) during ionophoresis of GAL (2 nA) and ranitidine (RAN) (8 nA) are affected in virtually identical fashion. Left, control responses before and after drug application.

block of the light response (Fig. 3). In most experiments, however, the less potent drug was applied for substantially longer periods (60–90 s) without affecting the apparent rank order. In addition, although a reliable comparison of potencies of drugs ejected from different electrode assemblies is not strictly feasible, owing to wide variability from recording to recording, the same rank order was also obtained by taking the lowest dose required to achieve a substantial (50 % or greater) block of the light response from all experiments (including those in which an agonist was in the other active barrel).

For technical reasons the ionophoretic pipette was limited to three barrels (one of which was used for current balancing), so that it was not feasible to compare the

Table 1. *Pharmacological profile of the LMC light response, drugs listed in order of potency*

Antagonist	Relative molecular mass	Specificity	Relative potency	Trials
Hexamethonium^{2+}	202	*Ng*−	+++++	2(3)
Decamethonium^{2+}	418	*Nn*−	+++++	2(3)
Benzoquinonium^{2+}	617	*Nn*−	++++	3(4)
Gallamine^{3+}	892	*Nn*−	++++	5(8)
Ranitidine^{+}	298	*H2*−	+++	7(11)
Atropine^{+}	289	*M*−	++	13(22)
Cimetidine^{+}	252	*H2*−	++	6(11)
SK&F 93479^{3+}	523	*H2*−	+	2(2)
Metiamide^{+}	244	*H2*−	+	3(5)
Mepyramine^{+}	402	*H1*−	+	2(3)
d-Tubocurarine	682	*Nn*−	? (pressure injection)	2(2)

Hexamethonium and decamethonium were an order of magnitude more potent than all other drugs tested, but their effects were qualitatively different.

Charge, relative molecular mass and traditional specificity of each drug are indicated: (*Nn*, nicotinic neuromuscular; *Ng*, nicotinic ganglionic; *M*, muscarinic; *H1*, *H2*, vertebrate histamine classes; + agonist; − antagonist).

Trials refers to number of successful electrodes (number of cells in brackets) and only for experiments with pairs of antagonists; all drugs were also tested on numerous other occasions with an agonist in the other active barrel.

Metiamide, SK&F 93479 and mepyramine were ineffective in experiments when atropine did antagonize the light response, but were found to reduce responses to light and histamine in other experiments.

Mecamylamine (*Nn*−), scopolamine (*M*−), dexetimide (*M*−), nicotine (*Nn*+), carbachol (*Nn*/*M*+), chlorpheniramine (*H1*−), clemastine (*H1*−) and mequitazine (*H1*−) were all ineffective.

effects of *pairs* of antagonists upon responses to histamine. An alternative strategy – applying antagonists singly in combination with histamine and quantifying the antagonism by normalizing against histamine dose–response curves – had to be abandoned because of variability, at least in part believed to be due to problems of coupling experienced during *simultaneous* injection from both pipettes. Strictly speaking therefore, the profile (Table 1) refers only to physiological responses, and extrapolation to histamine receptors requires the assumption that the physiological responses are mediated by histamine.

The most potent substances tested were the nicotinic ganglionic blocker hexamethonium (see Ascher *et al.* 1979), and the nicotinic neuromuscular blocker decamethonium (see Adams & Sakmann, 1978), but these two substances had complex effects and will be discussed separately below. Of the substances which appeared simply to block responses to light or histamine, the most effective were two other nicotinic agents, benzoquinonium (neuromuscular blocker) and galla-

mine (a widely used ganglionic blocker, also known as Flaxedil). In a direct comparison, benzoquinonium was consistently slightly more effective than gallamine even though its poor solubility meant it had to be ionophoresed from a 30 mmol l^{-1} solution (Fig. 2A). In turn, gallamine was an order of magnitude more effective than the most effective of the established histamine antagonists tested,

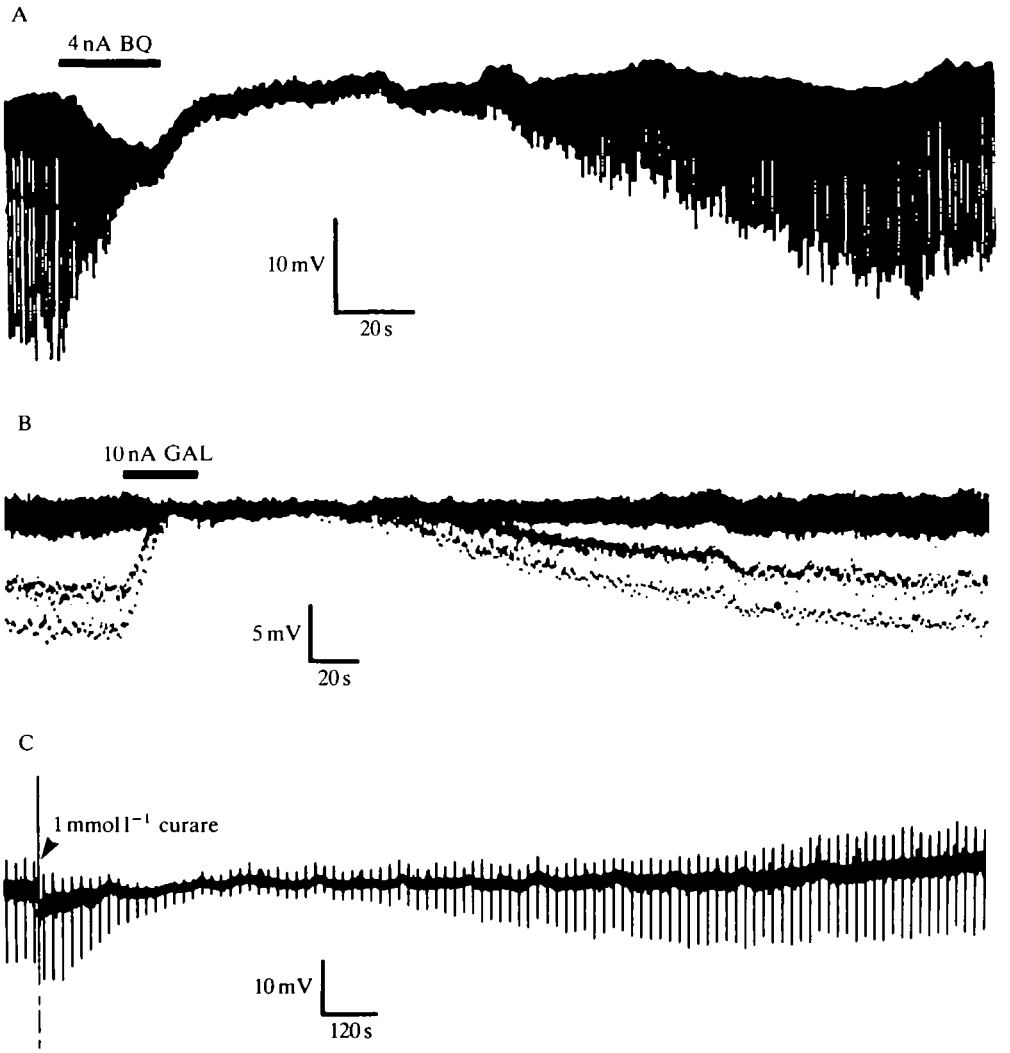

Fig. 3. Complete, reversible antagonism of light responses by (A) 4 nA benzoquinonium (BQ) and (B) 10 nA gallamine (GAL, not recorded with the same electrode). The hyperpolarization observed during the BQ block (A) was not consistently observed and may have been a current artefact. (C) 20 s pressure injection of 1 mmol l^{-1} curare (arrowhead), *via* an independent injection electrode introduced into the lamina neuropile, results in almost complete block of the light response. Note the much longer time course of the effect and recovery compared with BQ and GAL, which were applied ionophoretically.

the H2 blocker ranitidine (Fig. 2C). The muscarinic agent atropine was approximately equipotent with another H2 agent, cimetidine. With all of these more potent agents antagonism and recovery were rapid and it was possible to achieve virtually identical blocks with repeated doses, irrespective of the order of application.

Some examples of the most potent blocks obtained are shown in Fig. 3. Both benzoquinonium and gallamine were capable of completely blocking the light response, the responses recovering completely after 1 or 2 min.

Results with a number of other histamine antagonists were less conclusive. Metiamide and SK&F 93479 (also H2 blockers) had no effect in situations where atropine was weakly effective, but in these experiments, because of blocking, only about twice as much current could be applied as was necessary for the atropine effect. Large doses of both metiamide and SK&F 93479, however, were found to cause partial blocks of the LMC's response to light in other experiments (when histamine was in the other active barrel). Positive results were only achieved with large doses in one cell each in the case of the H1 agents, promethazine and mepyramine, and no antagonism was reliably observed for chlorpheniramine, mequitazine or clemastine (also H1).

When applied ionophoretically, curare had no effect. However, curare is relatively insoluble and was used at concentrations of approx. 10 mmol l^{-1} in 0·2 mol l^{-1} NaCl. Since curare is an effective antagonist in other putative histaminergic systems in arthropods (Claiborne & Selverston, 1984; Simmons & Hardie, 1988) an alternative method of application was attempted, namely pressure injection *via* an independent electrode inserted into the lamina neuropile. When applied in this manner, curare (1 mmol l^{-1} in Ringer) did indeed greatly attenuate the response to light (Fig. 3C) but the different method of application does not allow a direct comparison of potency with the ionophoresed drugs.

Several other ligands were found to be ineffective in situations where either another antagonist, or histamine itself, were effective. These included the cholinergic agents: mecamylamine, scopolamine, dexetimide, nicotine and carbachol. In earlier experiments (R. C. Hardie, unpublished results) several other drugs, including a range of glutamate antagonists and the GABA antagonists bicuculline and picrotoxinin (the latter, however, only sparingly soluble and weakly ionized) were also found to be ineffective in blocking the response to light.

Effects of hexamethonium and decamethonium

Both these agents were at least an order of magnitude more potent than any of the previously named drugs. In fact it was found necessary to dilute the drugs to less than 10 mmol l^{-1} in 0·2 mol l^{-1} NaCl to prevent leakage from the ionophoretic pipette completely poisoning the LMCs. Even so, ionophoretic charges of only 1–10 nC (of which, presumably only about 5% was carried by the drugs) were sufficient to produce marked effects. Of all the agents tried in the fly lamina, hexamethonium and decamethonium were the only agents which produced effects with lower doses than those necessary to obtain a threshold effect from histamine

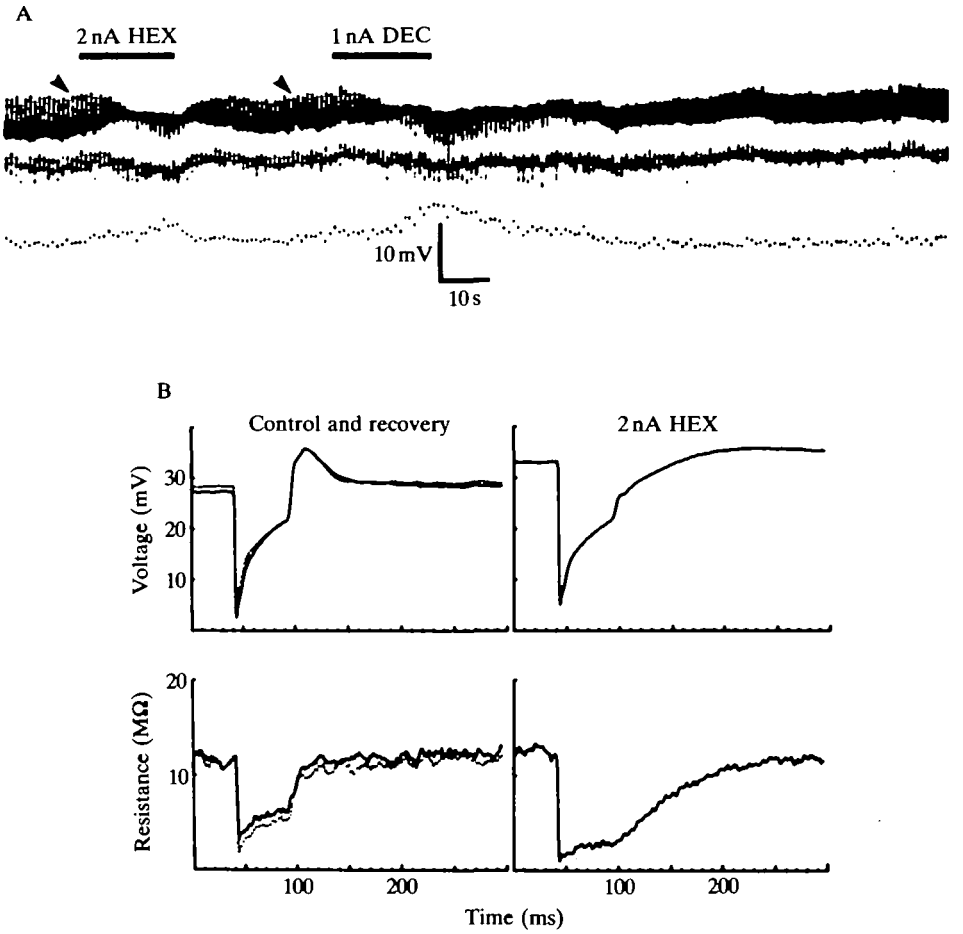

Fig. 4. (A) Both hexamethonium (HEX, 2 nA) and decamethonium (DEC, 1 nA) block the small depolarizing off transient (arrowheads) in an LMC. Recovery from DEC is significantly longer. (B) The loss of the off transient is associated with a prolonged resistance decrease. The upper traces are averaged responses to 50 ms sub-saturating light flashes: left, before (solid) and after (dotted); right, during background ionophoresis of 2 nA HEX. The resistance changes associated with the same responses are shown below (measured using the paradigm described in Materials and methods). In the presence of HEX the resistance decrease during the response is larger and only returns slowly to the dark level at the end of the stimulus. Resistance and voltage scale zeros are arbitrary.

in a neighbouring barrel. The effects of hexamethonium and decamethonium were virtually indistinguishable and the two drugs appeared approximately equipotent. However, since recovery times for decamethonium were somewhat longer (Fig. 4A), the majority of experiments were performed with hexamethonium.

LMCs respond to light flashes with a biphasic response consisting of a hyperpolarizing transient at light-on and a depolarizing transient at light-off (e.g.

Fig. 4B; see review by Laughlin, 1981). The 'on' transient can be directly attributed to transmitter released by the photoreceptors activating a chloride conductance. The 'off' transient is more complex, being caused both by a shut down in release of photoreceptor transmitter, and also by some additional, as yet unresolved, mechanism(s) (S. B. Laughlin & D. Osorio, in preparation).

Although large doses of hexamethonium completely, but reversibly, eliminated any response to short light flashes the initial effect of smaller doses appeared to be a selective block of the LMC off transient (Fig. 4). Since acetylcholine (ACh) has been shown to cause depolarizations of the LMC (Hardie, 1987 and see below), perhaps the most immediate interpretation of this effect would be that hexamethonium is antagonizing a depolarizing conductance mediated by acetylcholine. However, analysis of the underlying resistance changes revealed that the loss of the off transient was, in fact, associated with a larger and more prolonged conductance increase at light-on (Fig. 4B). Furthermore, doses of hexamethonium which blocked the off transient did not block depolarizing responses to ionophoretically applied acetylcholine (see below, Fig. 8A).

Although larger doses of hexamethonium completely blocked the response to light flashes, responses could still be obtained to longer periods of light. These responses, however, were dramatically altered (Fig. 5). Normally the response to such a step is very rapid (time to peak approx. 10 ms) and adapts rapidly, the membrane potential returning to the resting potential within a few 100 ms (e.g. Laughlin & Hardie, 1978, see also Figs 5A, 4B). During background ionophoresis of hexamethonium the LMC responded to the same light step much more slowly (time to peak approx. 1 s) but with a large, maintained hyperpolarization (Fig. 5A). The responses to ionophoretically applied histamine pulses were also greatly facilitated (Fig. 5B); there was little apparent effect on the speed of the response to histamine, as in this case the limiting factor for the time course was probably diffusional access. The effects of hexamethonium on both the light and histamine responses recovered with a similar time course.

Fig. 6A shows how increasing doses of hexamethonium blocked first the off transient of the response to light flashes, then the entire response. In this experiment the LMC was light-adapted by a continuous weak background light, so that the largest dose of hexamethonium caused a hyperpolarization as in Fig. 5A. In dark-adapted cells hexamethonium also often induced a slow depolarization (Fig. 6B).

Effects of agonists

A range of potential agonists was tested, using histamine in the neighbouring barrel for comparison. However, no substance, other than histamine itself, was found with any obvious agonist activity, despite using ionophoretic doses 10–100 times greater than the threshold required to elicit a response to histamine. Substances tested included the cholinergic agonists acetyl-β-methyl choline, nicotine and carbachol, and the histamine agonists 2-methyl histamine, 3-methyl

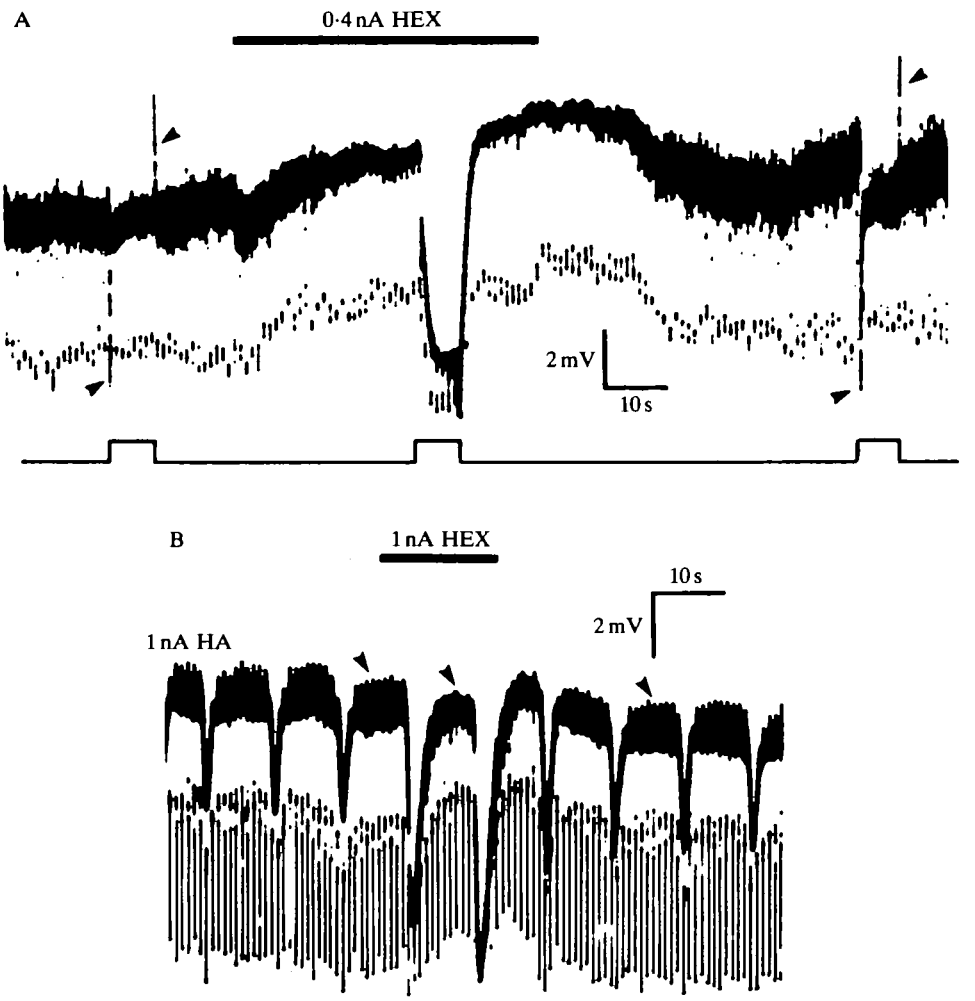

Fig. 5. (A) Responses of an LMC to long light steps (stimulus trace below). In this record *only*, the rapid hyperpolarizations are voltage deflections induced by constant hyperpolarizing current pulses (0·5 nA) and are a measure of the cell's input resistance. Before and after application of hexamethonium (HEX, 0·4 nA) the light response consists of rapid on and off transients (arrowheads) and no maintained response. In the presence of HEX, however, the response is a much slower maintained hyperpolarization. Notice that whereas virtually no resistance change can be detected in the control responses, in the presence of HEX, light induces a large resistance decrease (approx. 15MΩ). (B) Effect of hexamethonium on LMC responses to ionophoretic pulses of histamine (HA, 1 nA for 1 s, delivered every 10 s) and also to light flashes (rapid hyperpolarizations). The small off transient is abolished (arrowheads) and the responses to histamine are greatly facilitated.

histamine, 4-methyl histamine, dimaprit and impromidine (see reviews by Prell & Green, 1986; Schwartz *et al.* 1986). As previously reported (Hardie, 1987), virtually all the classical neurotransmitter candidates, including other biogenic

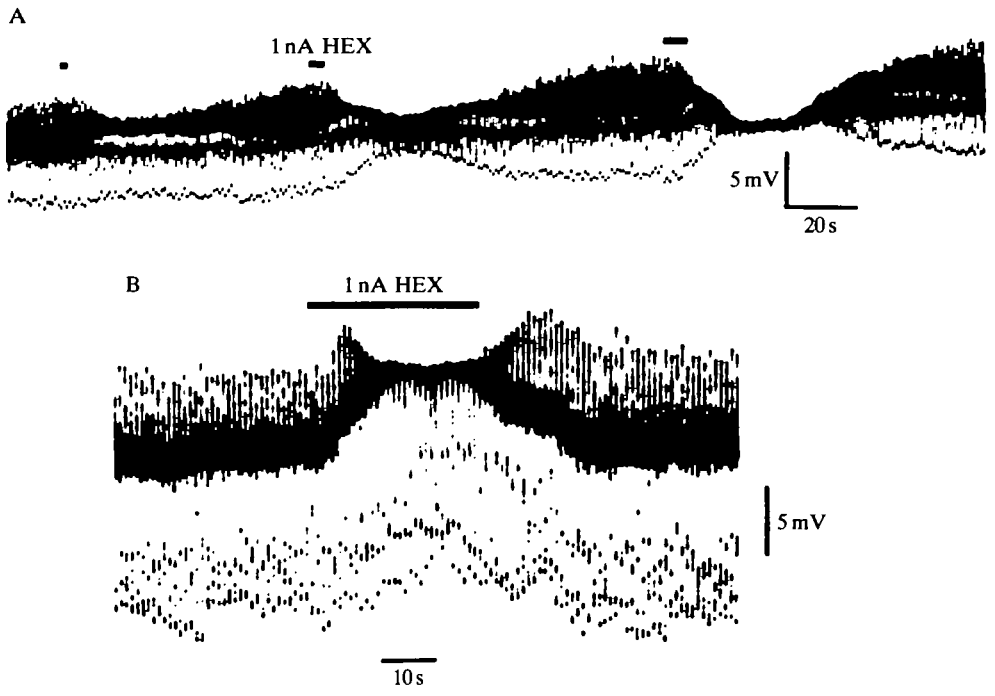

Fig. 6. (A) Responses to incremental light flashes superimposed on a weak background light. Increasing doses of hexamethonium (HEX, 1 nA for 2, 4 and 8 s) block first the off transient and then the whole response. The largest dose also induces a slow hyperpolarization which can be interpreted as a slow maintained hyperpolarization in response to the background light (i.e. similar to the response shown Fig. 5A). (B) In dark-adapted cells hexamethonium (1 nA) often induces a slow depolarization in addition to its effect on the light response.

amines (5-hydroxytryptamine, dopamine, octopamine and noradrenaline), have also been tested on fly LMCs, but none was found to mimic the effect of histamine.

Marked effects on LMCs were observed with 2-thiazolylethylamine (2-TE) which is traditionally an H1 agonist (Schwartz *et al.* 1986). Superficially, 2-TE appeared to antagonize responses to light. However, the antagonism was qualitatively different from the effects of other antagonists, suggesting the involvement of a distinct receptor. When 2-TE was injected with gallamine as a reference in the other barrel of the ionophoretic pipette, the relative potency of 2-TE compared with gallamine varied widely. In many cases, where low doses of gallamine blocked the response to light, 2-TE had no effect, whereas in other cases similar doses of 2-TE produced marked effects. Such variability would be consistent with a different physical location of the two sites of action. Typically, the effect of 2-TE was to reduce the background noise in the LMC (which is presumed to be caused by a tonic release of transmitter) resulting in a small depolarization of the LMC. Thereby, responses to weak light flashes were strongly attenuated, although brighter flashes still elicited normal responses. By compari-

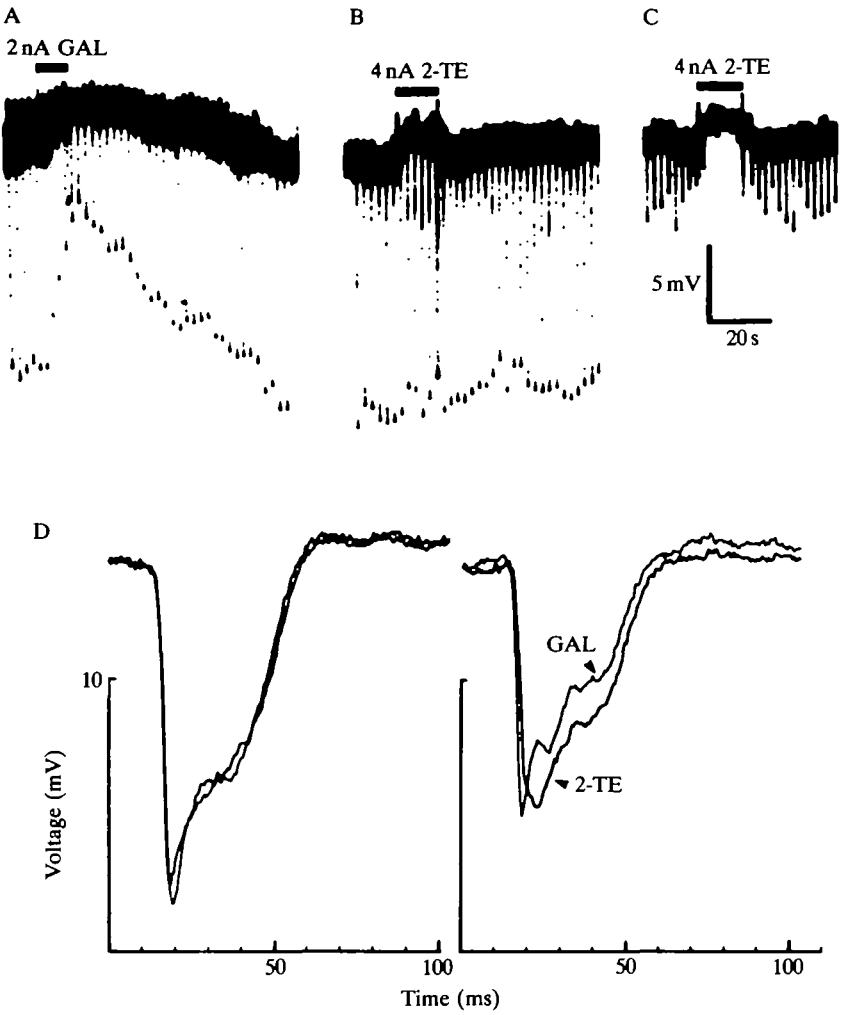

Fig. 7. Effects of the H1 agonist 2-thiazolylethylamine (2-TE) compared with those of gallamine (GAL) in the same cell. (A) 2 nA gallamine rapidly antagonizes responses to light. (B) 2-TE (4 nA) reduces the background noise but responses to bright, subsaturating flashes are unaffected. (C) A similar dose of 2-TE, however, severely attenuates responses to weak flashes. (D) Right, doses of GAL (0·5 nA) and 2-TE (4 nA), adjusted to achieve the same reduction in amplitude of a test flash, affect the response waveform differently; left, control responses before and after drug ionophoresis (compare Fig. 2C). Light responses are averages of five sweeps.

son, the effect of gallamine on the noise was less marked, but responses to even bright (normally saturating) flashes were virtually abolished (Fig. 7). There were significant differences between the responses to light obtained during partial blockades induced by 2-TE and gallamine: in the presence of 2-TE the responses typically had a longer latency and were less phasic than those in the presence of gallamine (Fig. 7D, cf. Fig. 2C).

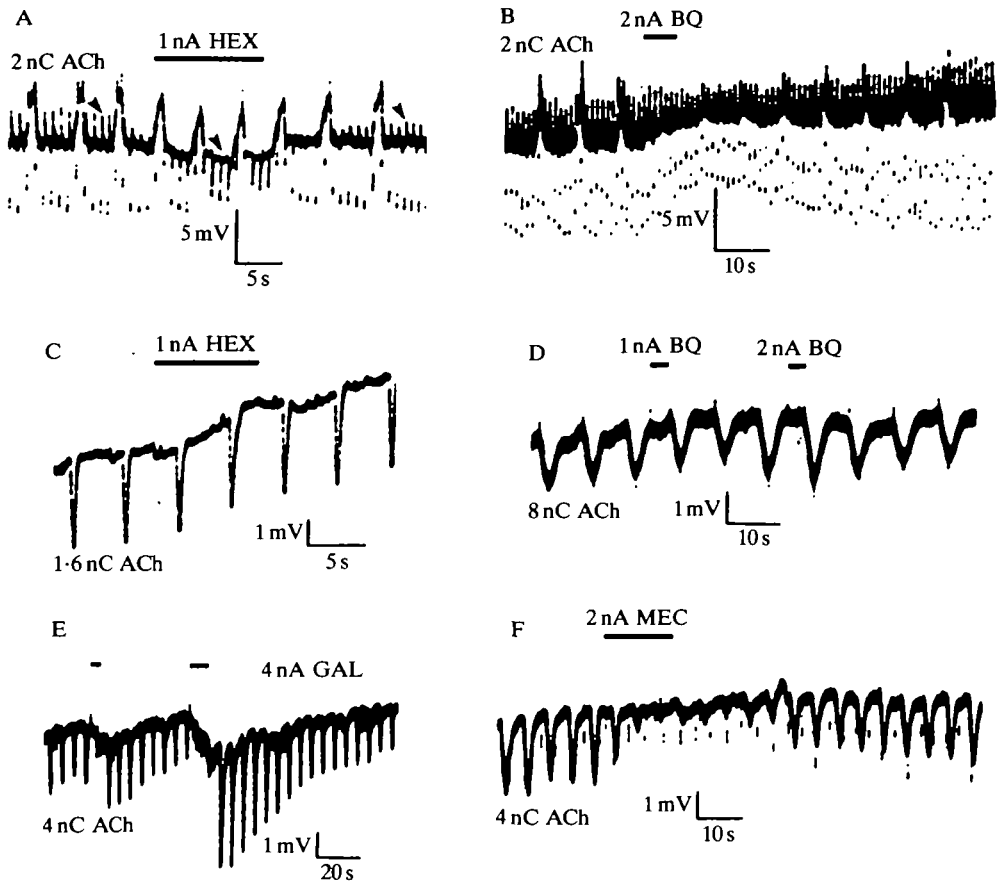

Fig. 8. Pharmacology of acetylcholine-induced responses in the fly lamina. Total ionophoretic charge (nC) of ACh indicated. (A,B). Ionophoretic pulses of ACh (2 nA for 1 s) induce depolarizations in an LMC. These are unaffected by hexamethonium (HEX) (A) although the dose is sufficient to block the off transient (arrowheads), but blocked by benzoquinonium (BQ) (B). (C–F) Hyperpolarizations of the extracellular space induced by ACh pulses are unaffected by hexamethonium (HEX) (C) or benzoquinonium (D), but are blocked by gallamine (GAL) (E) and mecamylamine (MEC) (F). The recovery from gallamine is marked by a large temporary facilitation (cause unknown).

Unfortunately it was not possible to measure any significant resistance changes associated with the effects of 2-TE.

Pharmacology of acetylcholine-induced responses

LMCs also respond sensitively to ionophoretic application of acetylcholine (ACh) (Hardie, 1987), and it was of interest to determine the effects of the cholinergic ligands used in this study on such responses. ACh has at least two kinds of effects in the fly lamina: first, a fast depolarization of the LMCs which may reflect ACh receptors directly on the LMC membrane and, second, a hyperpolar-

Table 2. *Pharmacology of acetylcholine-induced responses*

	LMC	ECS
Hexamethonium	–	–
Benzoquinonium	+	–
Gallamine	?	+
Atropine	+	?
Mecamylamine	+ (1 cell only)	+
Dexetimide	–	–
Scopolamine	–	–

Cholinergic ligands are listed in order of potency of action on the light response.
Effect on acetylcholine-induced depolarization of LMCs (LMC) and acetylcholine-induced hyperpolarization of the extracellular space (ECS) are indicated: + indicates block, – no block, ? not known.

ization of the extracellular space (ECS), the origin of which is unclear. Note, however, that each cartridge in the lamina is, to a large extent, sealed by a glial sheath and, in principle, polarization of the ECS can be caused by current flow across the membrane of any cell in the cartridge (see review by Shaw, 1984). Sometimes ACh also caused a slow noisy depolarization or hyperpolarization in the LMCs and it may be suggested that this is due to transmitter release from the photoreceptors induced by the hyperpolarization of the extracellular space.

It was not practicable to construct a pharmacological profile of ACh responses using triple-barrelled pipettes since only one antagonist could be tried at a time (the other active barrel containing ACh). However, it was clear that the pharmacology of responses to ACh differed from that of responses to light and histamine (Fig. 8). Hexamethonium appeared to be ineffective on either type of ACh response at concentrations which did affect the response to light. Benzoquinonium blocked the depolarization of the LMC but not the extracellular hyperpolarization. Mecamylamine, which had no effect on histamine- or light-induced responses, did block at least the extracellular ACh response. The results are summarized in Table 2.

Discussion

The major aim of this study has been to provide some pharmacological data on putative histamine receptors in the insect visual system. Presently the only available assay has been to observe the effects of a range of antagonists on the physiological responses of LMCs – an approach which assumes that the responses are mediated by histamine. Evidence for this has already been listed in the Introduction, and in the present study it was found that all agents which antagonized the light response also blocked responses to ionophoretically applied histamine, thus further strengthening the hypothesis.

In vivo pharmacological investigations of the central nervous system are always complicated by the problem of quantifying drug concentrations. In the present

study, problems associated with access have largely been obviated by applying the drugs very locally from inside the blood–brain barrier. Ionophoretic doses could be corrected for charge numbers, but not for transport numbers which are mostly unknown. However, the antagonists used were all cationic, were all dissolved at 0·1 mol l^{-1} with similar pH (approx. 4), and were relatively well ionized as judged by the low resistances (40–150 MΩ) of the barrels. A survey of the literature (e.g. Kelly, 1975; Stone, 1985) indicates that transport numbers of a wide variety of ionized agonists and antagonists in aqueous solution generally only vary within a small range (typically greater than 0·1 but less than 0·4). In addition, identical backing currents were applied to both barrels so as to minimize any variability from this source.

For at least the most potent drugs tested (hexamethonium down to atropine in Table 1), the speeds of action were similar and rather fast, a quasi-steady-state block being reached typically in 15–20 s (see Figs). Recoveries were also fast (less than 1 min for smaller doses). This suggests that the rate of action is probably being limited by diffusion rather than, for example, rate of association with a receptor site, but that the various agents had similar diffusional access to receptor sites. (Note, in any case, that the most potent antagonists happened to be the largest – and presumably least diffusible – molecules.) In no experiment did longer application (60–90 s) of the weaker agent lead to a revision of its apparent potency. Although pharmacokinetic factors cannot be ruled out, it would thus seem that the apparent potency may well reflect the actual affinity for the receptor. For substances which showed little or no antagonism under these experimental conditions, it cannot be excluded at this stage that their lack of action reflects, for example, much slower association and dissociation rate constants rather than weak affinity.

Although the pharmacological profile derived in this study should be treated as qualitative, the major finding, that certain nicotinic agents (benzoquinonium and gallamine) were an order of magnitude more potent than any of the histamine antagonists tested, would appear to be firmly based.

The implications of the pharmacological profile for the nature of the receptor and the transmitter will be considered first, and then the unusual effects of hexamethonium and decamethonium will be discussed.

Comparison with other histamine and acetylcholine receptors

Although the most potent histamine antagonists were all H2 agents and, with the exception of SK&F 93479, also showed the same order of potency (ranitidine > cimetidine > metiamide) as would be expected of the vertebrate H2 receptor, histamine receptors in the fly lamina are apparently quite distinct from those characterized in vertebrates since certain cholinergic ligands were much more potent than any histaminergic agent tested. This is not necessarily surprising, however, since the LMC histamine receptors are presumably directly associated with a channel (chloride) whereas all histamine receptors in vertebrates appear to act *via* second messenger systems and thus may be expected to belong to a

different family of proteins (for reviews see Schwartz *et al.* 1986; Prell & Green, 1986). Interestingly, however, vertebrate histamine receptors also show similarities with ACh receptors. Thus a number of H1 agents can also block muscarinic sites and atropine also has moderate actions on H1 receptors (Prell & Green, 1986). In the present case, however, note that nicotinic agents were more effective than muscarinic ones and H2 agents more effective than H1 ones.

The most effective antagonists of the light response were all nicotinic agents; however, neither nicotine itself nor ACh showed any agonist or antagonist activity, and the pharmacology cannot be equated with any insect or vertebrate cholinergic profile. For example, the lack of action of mecamylamine, nicotine or carbachol argues against the possibility of the insect nicotinic receptor whereas the lack of effect of dexetimide and scopolamine argues against insect muscarinic sites (for reviews see Sattelle, 1985; Breer & Sattelle, 1987). The unusual and potent effects of hexamethonium and decamethonium are also particularly distinctive, although probably reflecting interaction with the channel rather than the receptor site (see below).

There also appears to be no direct parallel with histamine receptors on identified neurones in molluscs. In *Aplysia* C2 neurones, histamine has satisfied all the standard criteria for a neurotransmitter and several postsynaptic responses have been identified, each presumably elicited by a distinct histamine receptor (McCaman & Weinreich, 1985). A fast potassium conductance shows some similarity with the LMC's response, in that cimetidine was the only effective antihistamine drug tested. However, the conductance was not affected by curare, hexamethonium or atropine. By contrast, a histamine-activated chloride conductance was blocked by curare but not cimetidine, atropine or hexamethonium (for a review see Walker, 1986).

An interesting possibility is that the histamine-sensitive receptors might correspond to the putative 'mixed' cholinergic receptor isolated from housefly heads and showing similar affinity for both nicotinic and muscarinic ligands (Eldefrawi & O'Brien, 1970; for reviews see Dudai, 1979; Sattelle, 1985). The 'receptor' is characterized by its binding to [^{3}H]decamethonium (a very potent agent in the present study) and also by the very high density of sites. (The LMC histamine receptors can also be expected to be present in very high numbers because of the exceptional concentration of photoreceptor–LMC synapses; Nicol & Meinertzhagen, 1982.) This site is quite distinct from the nicotinic and muscarinic receptors present in the central nervous system of a variety of insects (for a review see Breer & Sattelle, 1987), but none of the physiological cholinergic responses characterized in insects possesses a profile corresponding to that of the putative mixed receptor. There are also inconsistencies with the present profile, in particular nicotine and dexetimide, which both showed strong inhibition of binding activity (Jewess *et al.* 1975), had no measurable effect on the LMC responses to light or histamine; however, binding studies and *in vivo* studies rarely yield identical pharmacological profiles, and it would be worthwhile investigating the similarities further.

There is very little information on putative histamine receptors in other arthropods. One common factor is that they are all sensitive to curare, albeit at relatively high concentrations (Claiborne & Selverston, 1984; Simmons & Hardie, 1988). Furthermore, in at least two cases they activate a chloride conductance (in the locust ocellus the postsynaptic response is also a fast hyperpolarization, but the ionic basis is unknown). In addition the locust ocellar L neurones also respond similarly and very sensitively to hexamethonium (Simmons & Hardie, 1988). More data are required, however, to determine if there really is a common arthropod class of histamine receptor.

The effects of 2-thiazolylethylamine deserve special mention as they possibly indicate the existence of a second class of histamine receptor in the lamina. The data are not sufficient to make any positive conclusions, but either of the following hypotheses would be consistent with the data: (i) 2-TE antagonizes a distinct class of histamine receptor on the LMCs which is normally responsible for mediating responses to tonically released histamine; or (ii) 2-TE inhibits the tonic release of transmitter (presumably histamine) from the photoreceptors by interacting with hypothetical histamine receptors on feedback neurones, or autoreceptors on the photoreceptor terminals.

Interestingly, 2-TE also produces a fairly selective block of one of the several classes of postsynaptic response to histamine in *Aplysia*, namely a slow EPSP (McCaman & Weinreich, 1985).

Identity of the photoreceptor transmitter

The most potent antagonists of the physiological response in the LMCs are traditionally all cholinergic ligands. It is thus pertinent to reconsider if histamine really is the photoreceptor neurotransmitter, or whether acetylcholine might be a candidate. The fact that histamine and not ACh mimics the physiological response (Hardie, 1987) is not conclusive since it could be argued that acetylcholinesterase (AChE) activity is so high that, despite the extremely local ionophoresis, exogenously applied ACh cannot reach its site of action. A similar situation has been reported in *Hermissenda*. Thus the photoreceptors of this mollusc are believed to use ACh as a neurotransmitter, but bath application of ACh is ineffective whereas histamine evokes a response of appropriate polarity, albeit with a different reversal potential to the physiological response (Heldman *et al.* 1979).

Evidence favouring histamine as the major insect photoreceptor transmitter has already been listed in the Introduction; the following considerations all suggest that ACh is *not* the major photoreceptor neurotransmitter in insects. (a) ACh does have large effects on LMCs, but causes them to depolarize (Hardie, 1987; see above). ACh can thus reach its site of action without being inactivated by AChE. ACh also only causes depolarizations when co-ionophoresed with the AChE inhibitor neostigmine (R. C. Hardie, unpublished results). (b) ACh agonists including nicotine, acetyl-β-methyl choline and carbachol, which should be resistant to AChE, also do not cause LMCs to hyperpolarize (see above). (c) The

retina of the tobacco hornworm moth *Manduca* retina is apparently incapable of synthesizing significant quantities of ACh, although histamine is synthesized in large amounts (Maxwell *et al.* 1978). (d) *Drosophila* mutants deficient in ACh synthetic or degradative enzymes show deficiencies in the off transient of the electroretinogram, but not in the on transient, which is believed to represent the activity of the photoreceptor–LMC synapse (Greenspan, 1980; Greenspan *et al.* 1980). (e) In *Drosophila*, antibodies against choline acetyltransferase stain lamina interneurones, but not photoreceptors (Buchner *et al.* 1986; Gorczyca & Hall, 1987). Histamine antibodies, however, do stain photoreceptors in *Calliphora* and *Musca* (Nässel *et al.* 1988).

Effects of hexamethonium and decamethonium

Hexamethonium and decamethonium were consistently more potent than any other drug tested; however, the basis of their action is obscure. Their effects can most simply be described as an overall slowing down of the response, although no precedent for such a dramatic effect appears to exist in the literature. In terms of conductances, the chloride channels appear to open more slowly (response rise time of approx. 1 s compared with normal 10 ms), to remain open longer thus giving rise to facilitation, and to close more slowly – resulting in the disappearance of the off transient. Assuming that the drugs are acting on the receptor molecule, they are presumably doing so at a site other than the histamine binding site – such as the channel. Additional, or alternative actions cannot be excluded, however. For example, the facilitation and abolition of the off transient (Figs 4, 5) might also be explained by interference with the presumptive histamine inactivation mechanism, although this alone cannot explain the dramatic slowing of the response. The slow depolarization (Fig. 6B) also finds no ready explanation in any of the above terms and may indicate action on another receptor (e.g. as a cholinergic agonist) or reflect some indirect effect mediated *via* other interneurones.

Hexamethonium and decamethonium do not appear to act as simple antagonists in other systems either. Thus the action of hexamethonium on ganglionic ACh receptors in both *Aplysia* and vertebrates is also believed to be at the level of the channel, since the antagonism is voltage-sensitive (Ascher *et al.* 1978, 1979), and decamethonium is believed to act both as a partial agonist and also as a channel blocker at the vertebrate endplate (Adams & Sakmann, 1978).

In conclusion, the pharmacological profile presented here is believed to represent that of histamine receptors on LMC postsynaptic membranes in the housefly. The profile, which is only qualitative, has been obtained in an intact preparation and may have to be revised in the light of *in vitro* assays. Nevertheless, the distinctive pharmacology cannot be identified with any functionally characterized receptor, and would seem to indicate the existence of a novel histamine receptor with marked cholinergic (nicotinic) features. The few data available leave open the possibility that the receptor may be common to other arthropod neurones which are postsynaptic to histaminergic cells, namely the ocellar L

neurones and certain neurones of the lobster stomatogastric ganglion. In both of these the receptors are also probably coupled to a chloride channel and are blocked by curare.

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