Pergamon

0306-4522(93)E0010-N

Neuroscience Vol. 59, No. 3, pp. 579–588, 1994
Elsevier Science Ltd

0306-4522/94 $6.00 + 0.00

MECHANISMS OF ANTIHISTAMINE-INDUCED SEDATION IN THE HUMAN BRAIN: H_1 RECEPTOR ACTIVATION REDUCES A BACKGROUND LEAKAGE POTASSIUM CURRENT

P. B. REINER* and A. KAMONDI

Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, BC V6T 1Z3 Canada

Abstract—Antihistamines, more formally termed H_1 receptor antagonists, are well known to exert sedative effects in humans, yet their locus and mechanism of action in the human brain remains unknown. To better understand this phenomenon, the effects of histamine upon human cortical neurons were studied using intracellular recordings in brain slices maintained *in vitro*. Bath application of 50 μM histamine induced a depolarization which could be attributed to reduction of a background voltage-independent "leakage" potassium current: the depolarization was associated with an increase in apparent input resistance, under voltage clamp its reversal potential approximated the potassium reversal potential, and the histamine-induced current exhibited little voltage dependence. The pharmacology of the histamine-induced depolarization of human cortical neurons was studied by use of both agonists and antagonists. Depolarizing responses were blocked by the H_1 antagonist mepyramine, but not by the H_2 antagonist cimetidine nor the H_3 antagonist thioperamide. The H_3 receptor agonist R-α-methyl-histamine did not mimic the effects of histamine. Thus, histamine depolarizes human cortical neurons via action at an H_1 receptor.

These effects of neuronal histamine upon cortical neurons are likely to affect synaptic transmission in several ways. The depolarization *per se* should increase the likelihood that excitatory synaptic potentials will evoke an action potential. The increase in whole-cell input resistance evoked by H_1 receptor activation should make the cell more electrotonically compact, thereby altering its integrative properties. We hypothesize that these mechanisms would allow histamine, acting at cortical H_1 receptors, to enhance behavioral arousal. During waking when histamine release is highest, blockade of H_1 receptors by systemically administered H_1 receptor antagonists would be sedating.

Histamine satisfies many of the criteria for neurotransmitter status in the human brain. A key observation is that histamine and its synthetic enzyme L-histidine decarboxylase are distributed in a non-uniform manner throughout the human brain.[7,33] The general organization of the central histaminergic system in the human seems similar to that of the better-studied rat, with somata in the tuberomammillary nucleus of the hypothalamus[2] and axons projecting throughout the brain including the cerebral cortex.[52] Potassium-evoked release of histamine has been observed in human brain,[5] and all three subtypes of histamine receptors (H_1, H_2 and H_3) are present.[5,9,25,38] Although histamine-mediated synaptic responses have not been documented, the amine has been shown to have pronounced electrophysiological actions in the human brain.[17,41] Thus, it seems reasonable to conclude that histamine is a neurotransmitter in the human brain.

A growing body of evidence suggests that the central histaminergic system is involved in the neuronal control of behavioral states.[32] Of particular interest is the effect of histamine acting at H_1 receptors. Systemic administration of H_1 receptor antagonists, commonly known as antihistamines, results in sedation in humans.[46] However, difficulty has arisen in developing plausible hypotheses regarding the sedative actions of H_1 receptor antagonists because of the lack of good animal models: the sedative effects of antihistamines are not evident in any laboratory animal studied to date, including mice, rats, guinea-pigs, hamsters, dogs, cats and squirrel monkeys.[45] There is also a marked species difference in the distribution of H_1 receptors in the brain.[9] Because the highest densities of H_1 receptors in the human brain are found in the cerebral cortex, we hypothesized that the sedative effects of H_1 receptor antagonists in humans might be due to blockade of cortical H_1 receptors. We now report that histamine depolarizes human cortical neurons *in vitro* via blockade of a voltage-independent potassium conductance, and that this effect is mediated by activation of H_1 receptors.

EXPERIMENTAL PROCEDURES

Human neocortical tissue was obtained from patients undergoing surgical treatment for intractable epilepsy. Only

*To whom correspondence should be addressed.
Abbreviations: ACSF, artificial cerebrospinal fluid.

tissue which would routinely be removed during the course of the surgical procedure was used, and the use of this tissue for experimental purposes did not alter the size of the resected area. The protocol for these experiments was approved by the University of British Columbia Clinical Screening Committee for Research Involving Human Subjects. A small piece of the temporal gyrus was removed by the neurosurgeon (Dr Barrie Woodhurst) and immersed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF). Subsequent neuropathological examination of neighboring regions of the cortex revealed no gross or microscopic pathology. Following immersion in ACSF, the block of cortical tissue was trimmed into several smaller pieces, and transported to the laboratory where it was cut into 300-μm-thick slices on a vibratome. Slices were stored in oxygenated ACSF at room temperature for at least 1 h prior to recording. During the experiment, a single slice was transferred to the recording chamber where it was continuously superfused with warmed (30°C) ACSF which contained (in mM): 2.5 KCl, 126 NaCl, 2.4 $CaCl_2$, 1.3 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 11 glucose; when saturated with 95% O_2–5% CO_2 the pH of the solution was 7.4.

Intracellular recordings were carried out using micropipettes pulled from 1.2 mm (o.d.) glass blanks and filled with 2 M KCl. Electrodes exhibited resistances between 35 and 80 MΩ. Recordings were carried out using an Axoclamp 2A amplifier controlled by a laboratory computer utilizing the pCLAMP suite of programs. For experiments in which single-electrode voltage clamp was used, 300 nM tetrodotoxin was added; switching frequencies ranged from 4 to 8 kHz, and gains of 0.5–1 nA/mV were used. Headstage output was continuously monitored on an independent oscilloscope to ensure that the voltage at the electrode had settled prior to the sample-and-hold measurement. Leakage and capacitative currents were not subtracted. Data were independently digitized at 49 kHz and stored on videotape for off-line analysis. Data are presented as mean ± S.D., and n refers to the number of cells tested.

Drugs used in these experiments included tetrodotoxin from Calbiochem, and carbachol, cimetidine, histamine, mepyramine, R-α-methyl-histamine and thioperamide from Research Biochemicals.

RESULTS

Electrophysiological properties of human cortical neurons

The data are based upon stable intracellular recordings obtained from 39 presumed pyramidal neurons in layers II–V of the middle temporal gyrus. Slices from 13 patients were studied; only one cell was studied per slice. Pyramidal neurons were tentatively identified by their electrophysiological characteristics[12,44] as shown in Fig. 1B. The average resting membrane potential for this population of neurons was −64 ± 3.4 mV. Input resistance, estimated by

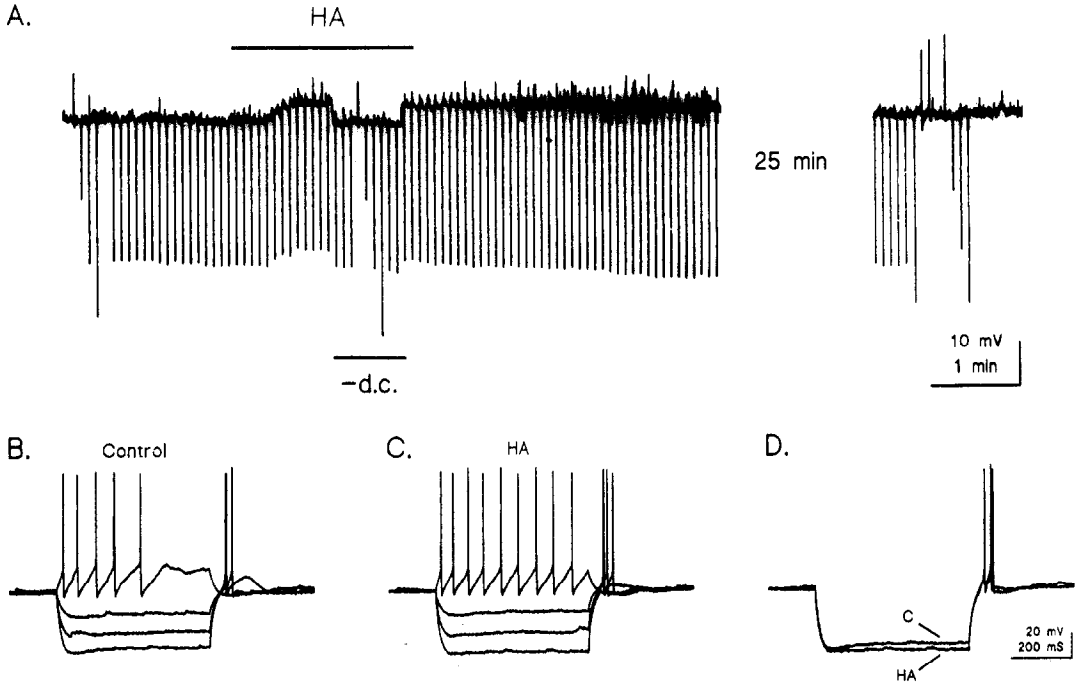

Fig. 1. Histamine depolarizes human neocortical neurons. (A) Chart record demonstrating a typical response to bath application of 50 μM histamine (indicated by the bar). Hyperpolarizing pulses of 400 ms duration and 0.2 nA amplitude were applied every 5 s to monitor input resistance. During the depolarization induced by histamine (HA), the neuron was returned to the resting potential of −67 mV by injection of hyperpolarizing d.c. current (−d.c.). After washing for 25 min, both the resting membrane potential and input resistance returned to normal. (B) Traces illustrating the response of the neuron to current pulses of 0.1, −0.1, −0.2, and −0.3 nA under control conditions. (C) Same paradigm as in (B) during application of anodal current to return the membrane potential to control levels during application of histamine. Spike-frequency adaptation is blocked by application of histamine. (D) Superimposition of the membrane response to −0.3 nA hyperpolarizing current pulses under control (C) conditions and after application of histamine (HA). The larger membrane deflection during histamine application indicates that input resistance has increased.

applying small hyperpolarizing current pulses sufficient to produce ~10 mV of hyperpolarization relative to the resting membrane potential, was 66 ± 4.4 MΩ. This is probably an underestimate of the true input resistance, as cortical pyramidal neurons invariably expressed the hyperpolarization-activated cation current, I_h, as previously reported.[12] Although we made no systematic attempt to distinguish between 'regular-spiking' and 'bursting' neurons, all presumed pyramidal neurons exhibited a rebound depolarization following appropriate hyperpolarizing current pulses (Figs 1 and 3). Only one cell exhibited behavior which could be termed epileptic; it was excluded from analysis. While we have no way of determining whether the other cells were normal in all regards, their behavior was strikingly similar to that seen in recordings from rodent neocortical neurons.[44]

Ionic currents evoked by histamine in human cortical neurons

Upon application of 50 μM histamine for 2 min, 18/23 neurons exhibited a depolarization of 6.9 ± 1.8 mV (Fig. 1A); the effect dissipated over 10–20 min and was usually repeatable after 30 min with little evidence of desensitization. When the neuron was manually returned to the resting membrane potential by injection of hyperpolarizing d.c. current, the apparent input resistance was greater than that seen under control conditions in 8/11 neurons (control = 71.4 ± 5.3 MΩ, histamine = 78 ± 4.7 MΩ; Fig. 1D). These data suggest that histamine depolarizes human cortical neurons by reducing an ionic conductance.

To determine whether the effect was due to activation of receptors on the recorded neuron or upon another neuron presynaptic to that being recorded, the experiments were repeated while perfusing the slice in the presence of 300 nM tetrodotoxin to block voltage-dependent sodium channels. Under these conditions histamine still induced a depolarization or, under voltage clamp, an inward current (cf. Fig. 2, $n = 6$). Thus, histamine appears to depolarize human cortical neurons by direct action at a post-synaptic receptor.

The next set of experiments were carried out using discontinuous single-electrode voltage clamp to further characterize the ionic current modulated by histamine. In the presence of 300 nM tetrodotoxin to block voltage-dependent sodium channels, steady-state I–V curves were generated, either by a series of voltage steps (Fig. 2A–C) or via a smoothly ramped voltage command (Fig. 2D). The results of both manipulations were similar, and several observations are noteworthy. First, under voltage clamp histamine induced an inward current at the holding potential of −70 mV. The intersection of the I–V curves generated in the presence and absence of histamine was −105 ± 3.2 mV (Fig. 2C–D, $n = 3$), very near to the predicted potassium equilibrium potential of −110 mV, assuming an intracellular K^+ concentration of 140 mM. (Because these experiments were carried out using electrodes filled with 2 M KCl, the chloride equilibrium potential would have been considerably more positive than the resting potential.) Thus, histamine appears to depolarize human cortical neurons by reducing a resting potassium current.

Additional properties of the resting potassium current modulated by histamine could be deduced by subtraction of the control I–V curve from that obtained in the presence of histamine (Fig. 2E). This histamine-induced current exhibited little voltage dependence over the range of −130 to −50 mV. By virtue of its apparent reversal potential at the potassium equilibrium potential and voltage independence, the current which was reduced by histamine in human cortical neurons was identified as the ligand-gated leakage potassium current I_{KL}.

Both acetylcholine and serotonin have been reported to reduce the time- and voltage-dependent potassium current I_M in human cortical neurons.[20,41] There is some suggestion of I_M suppression by histamine in Fig. 2B–C, but this was not systematically investigated. The effect which we have focused upon is clearly not due to suppression of I_M, as the increase in resistance was manifest at potentials negative to −60 mV where I_M is not active.[20,41] An additional effect observed in several cells was a reduction in the time-dependent inward rectification seen during application of hyperpolarizing current pulses, which is presumably mediated by the cation current I_h (cf. Fig. 1D). Although we did not investigate this phenomenon in detail, at the resting potential a reduction in I_h this would serve to diminish the depolarization induced by histamine. Thus, histaminergic modulation of I_h cannot account for the observed depolarization of human cortical neurons.

Pharmacological characterization of the histamine-induced depolarization

The subtype of histamine receptor responsible for the depolarization of human cortical neurons was studied by use of both agonists and antagonists. We began with H_2 receptors. In rat, guinea-pig and human cortical neurons occupation of H_2 receptors, acting via production of cAMP, reduces a calcium-activated potassium current resulting in blockade of spike frequency adaptation.[16–18,41,53] We confirmed that histamine reduces spike frequency adaptation in human cortical neurons ($n = 3$, Fig. 1B–C). As can be seen in Fig. 3a–c, prior superfusion of the slice with 50 μM of the H_2 receptor antagonist cimetidine readily prevented blockade of adaptation by histamine. Notably, the histamine-induced depolarization was unaffected ($n = 4$, Fig. 3A). Thus the depolarizing effect of histamine is not due to activation of H_2 receptors.

We next turned our attention to the H_3 receptor. The selective H_3 receptor agonist R-α-methylhistamine[4] did not mimic the depolarizing effect of histamine upon human cortical neurons, even when

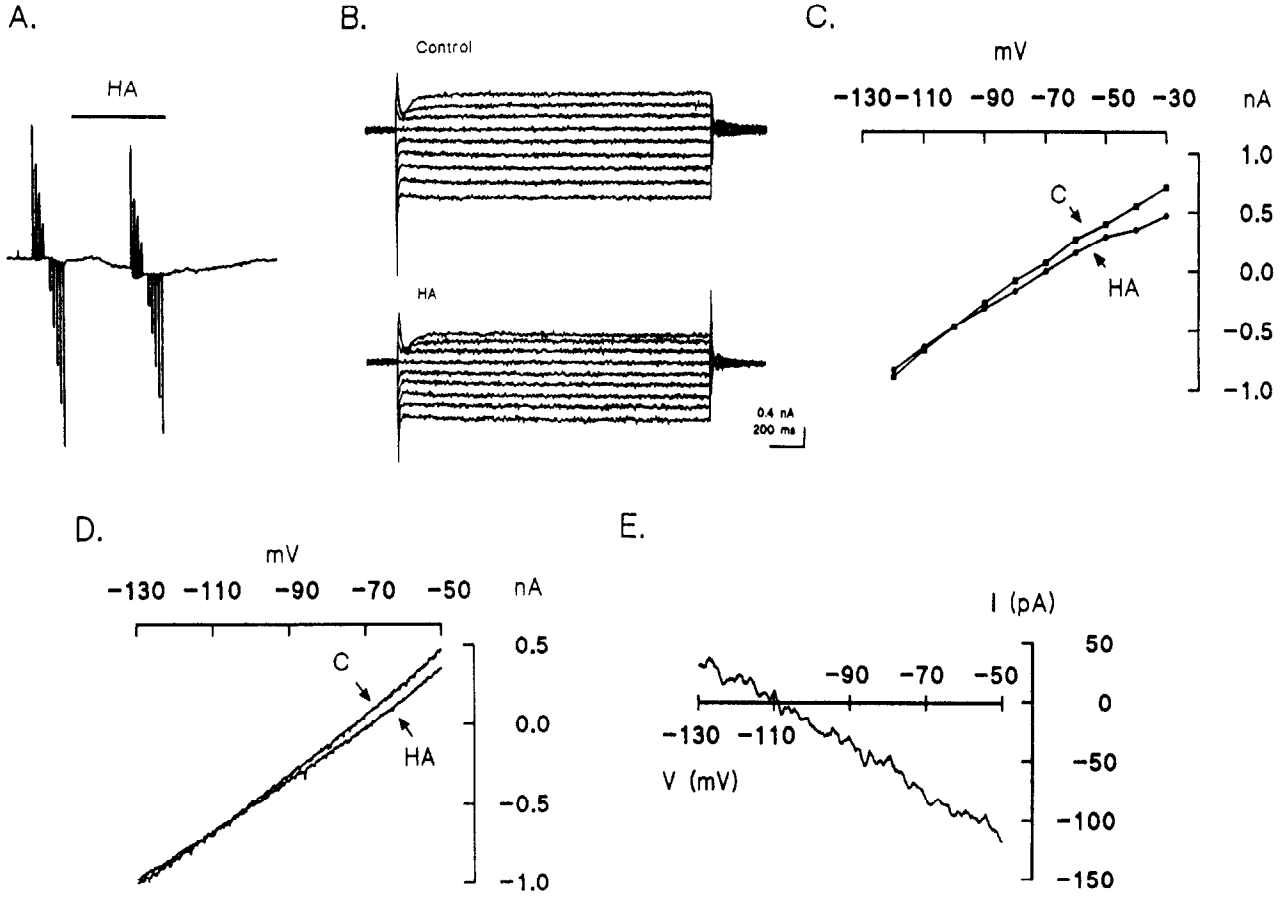

Fig. 2. Ionic currents evoked by histamine in human cortical neurons. (A) Chart record of a cell which was studied under voltage clamp conditions during application of histamine. The cell was held at −60 mV and voltage steps to potentials ranging from −130 to −30 mV applied every 5 s before and during application of histamine, which induced an inward current. (B) Expanded current traces from (A) under control conditions and in the presence of histamine. (C) Steady-state I–V plot of the data in (B) demonstrates that histamine evoked an inward current which reversed sign near −100 mV. (D) In a different cell, 13 s duration voltage ramp commands from −130 to −50 mV were executed before and after application of histamine. The resultant I–V curves cross at −109 mV. (E) Subtraction of control current from that recorded in histamine in the cell shown in (D) demonstrates that the current evoked by histamine has little voltage dependence.

applied at a concentration of 50 μM (Fig. 3C, $n = 5$). Thioperamide, an H_3 receptor antagonist, did not block the effect of histamine when applied at 100 nM ($n = 3$, Fig. 3D), although it did slightly attenuate the histamine-induced depolarization at 1 μM ($n = 2$). Since the K_D of thioperamide at the H_3 receptor is around 6 nM and equimolar application of R-α-methyl-histamine did not mimic the effect of histamine, the partial blockade by 1 μM thioperamide is likely to be a non-specific effect. Taken together, these data suggest that the histamine-induced depolarization is not mediated by H_3 receptors.

In the final set of experiments, pharmacological blockade of H_1 receptors with the selective antagonist mepyramine (50 μM) reversibly blocked the histamine-induced depolarization (Fig. 3A–B, $n = 5$). Mepyramine was chosen as an H_1 antagonist because of its low affinity for aminergic and cholinergic receptors.[21] In keeping with this, in two cells we found that the cholinergic agonist carbachol still depolarized human cortical neurons after histamine responses were blocked by mepyramine (data not shown). These data are consistent with the hypothesis that the depolarization of human cortical neurons by histamine is due to activation of H_1 receptors.

The most important feature of the histamine-induced depolarization is illustrated in Fig. 3b. At the peak of the histamine effect, depolarizing current pulses evoke a train of many more action potentials than seen under control conditions. This is not due to action at H_2 receptors, as spike-frequency adaptation is not affected (Fig. 3c). Rather, it is due to both the change in membrane potential which brings the neuron closer to threshold and the increase in whole-cell input resistance (Fig. 3d). The resultant increase in neuronal excitability is a critical feature of the action of histamine on human cortical neurons.

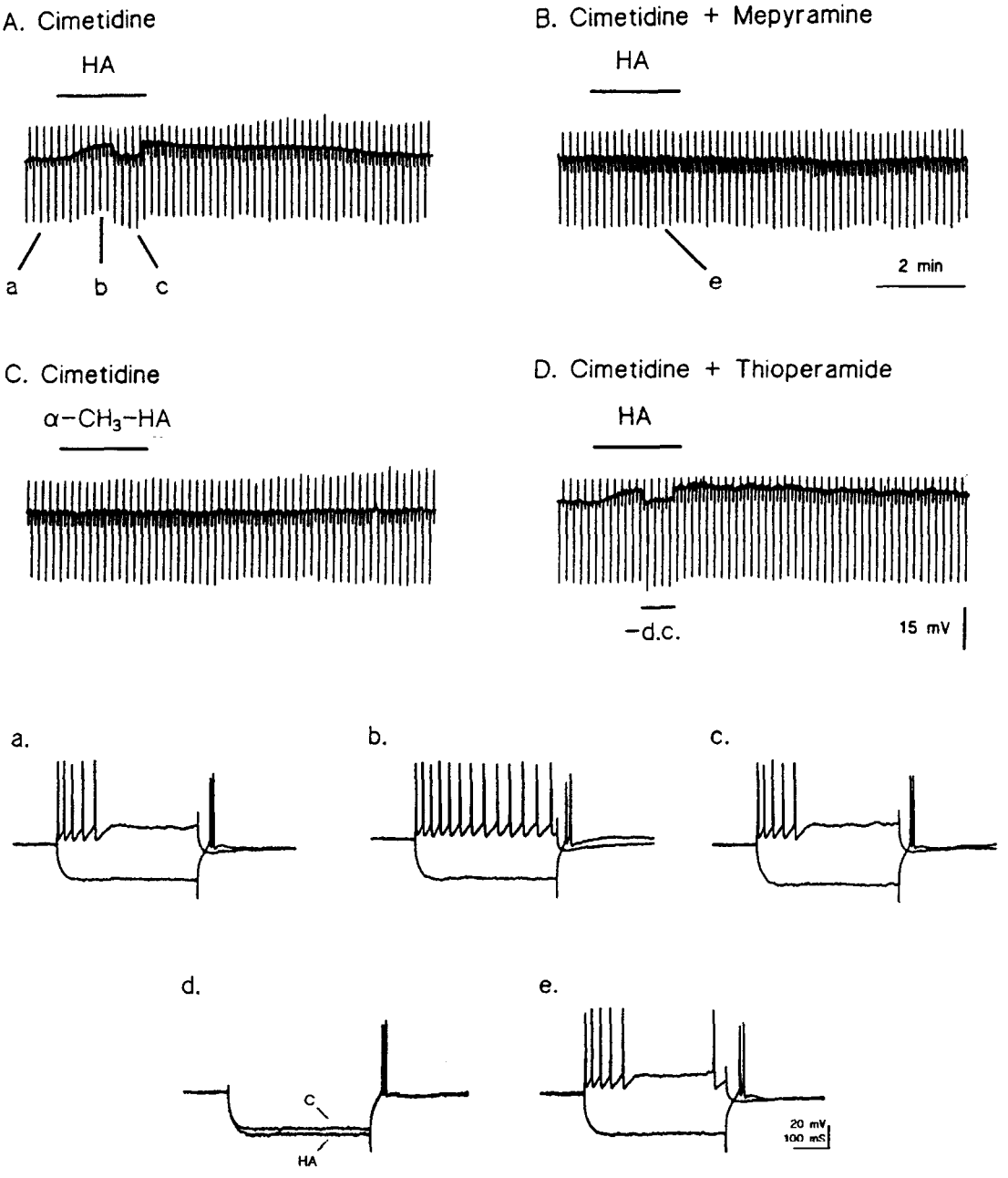

Fig. 3. Pharmacology of the depolarization of human cortical neurons by histamine. (A) Chart record of the response to 50 μM histamine (HA) in the presence of 50 μM cimetidine, an H_2 receptor antagonist. In this and subsequent figures, alternate hyperpolarizing and depolarizing current pulses of -0.2 and 0.1 nA, respectively, were applied every 5 s. At the peak of the histamine-induced depolarization, the membrane potential was returned to the resting potential of -63 mV by injection of hyperpolarizing d.c. current. Expanded sweeps demonstrating the response of the neuron during control conditions (a), during the peak of the histamine-induced depolarization (b), and following manual return of the membrane potential to the resting level (c) at the times indicated by the lines are shown at the bottom of the figure. In the presence of the H_2 antagonist cimetidine, histamine does not alter spike-frequency adaptation although the depolarization is unaffected. There is an apparent increase in input resistance as shown in trace (d), which is a superimposition of the responses shown in (a) and (c) to hyperpolarizing current pulses under control conditions (C) and during application of histamine (HA). (B) Following washout of the histamine-induced effect, the slice was bathed in 50 μM mepyramine in addition to 50 μM cimetidine for 5 min. Bath application of histamine had no effect on membrane potential. The line marked (e) indicates the trace which is shown in expanded fashion at the bottom of the figure. As can be seen, the neuron was fully capable of firing normal action potentials during application of the H_1 antagonist mepyramine. (C) Twenty minutes after removal of mepyramine from the solution bathing the slice, 50 μM R-α-methylhistamine was applied. There was no response to application of this H_3 receptor agonist. (D) Ten minutes of bath application of 100 nM thioperamide, an H_3 receptor antagonist, in addition to 50 μM cimetidine did not block the histamine induced depolarization. All traces from the same neuron.

DISCUSSION

The major findings of the present study are two-fold. First, histamine depolarizes human cortical neurons via blockade of a voltage-insensitive 'leakage' potassium current, I_{KL}. The second major finding is that the histamine-induced depolarization is due to activation of H_1 receptors. The data have considerable implications for the neuronal control of behavioral states and the role of neurotransmitter histamine in human cortical function.

H_1 receptors mediate histamine's action in human cerebral cortex

The pharmacology of the depolarizing response to histamine suggests that it is mediated by H_1 receptors: (i) the effect is blocked by the H_1 receptor antagonist mepyramine; (ii) the depolarization is unaffected by the H_2 receptor antagonist cimetidine; and (iii) the effect is not mimicked by the H_3 receptor agonist R-α-methyl-histamine, nor is it blocked by the H_3 receptor antagonist thioperamide. Although the concentration of mepyramine needed to block the functional response of H_1 receptors in human cortical neurons is considerably higher than its K_D in human brain,[66] such discrepancies are common in the literature. Moreover, this concentration of mepyramine is similar to that required to block functional responses in *Xenopus* oocytes transfected with mRNA of the cloned H_1 receptor.[65] Thus, we are confident that the results reported here represent functional activation of H_1 receptors.

The present data represent the first electrophysiological characterization of H_1 receptor occupation in the human brain. Previous studies with human brain tissue have demonstrated actions at both H_2 and H_3 receptors. In hippocampal and cortical pyramidal neurons, histamine acting at H_2 receptors blocks spike-frequency adaptation,[17,41] while occupation of H_3 receptors reduces potassium-evoked transmitter release from histaminergic axon terminals in human cortex.[5] Both effects have been previously documented in rat and guinea-pig cortex.[6,18] To our knowledge, depolarizing actions due to activation of H_1 receptors have never been previously reported in cortical tissue.

H_1-receptor-mediated depolarizations are clearly not restricted to human cortical neurons: wherever H_1 actions have been noted in mammalian brain, the effect is excitatory. Thus neurons in the supraoptic nucleus, tuberal hypothalamus, arcuate nucleus, suprachiasmatic nucleus, pre-optic nucleus, pedunculopontine tegmental nucleus, medial pontine reticular formation, and dorsal lateral geniculate nucleus have all been shown to be excited by histamine acting at H_1 receptors.[3,13,14,19,24,26,42,57,61] Indeed, there are no published reports of inhibitory H_1 receptor-mediated actions in the mammalian central nervous system.

Depolarization by blockade of a leakage potassium current

The mechanism by which histamine depolarizes human cortical neurons appears to be by reducing the voltage-independent 'leakage' potassium current, I_{KL}. The evidence supporting this conclusion includes: (i) the depolarization is accompanied by an increase in apparent membrane resistance; (ii) the steady-state I–V curves obtained under control conditions and in the presence of histamine intersect near the predicted potassium equilibrium potential; and (iii) the inward current evoked by histamine is linear over the physiological range of membrane potentials.

The first evidence that a neurotransmitter could depolarize cortical neurons by decreasing a resting potassium conductance emerged from the prescient work of Krnjevic *et al.*[29] Utilizing intracellular recordings from cat cortical neurons *in vivo*, they demonstrated that local ionophoresis of acetylcholine resulted in a depolarization which was accompanied by an apparent increase in membrane resistance. Although the ionic current responsible for the depolarization was not known at the time, recent *in vitro* studies using voltage clamp techniques clearly demonstrate that muscarinic receptor activation of cortical neurons results in a depolarization accompanied by a reduction of I_{KL}.[8,10,36]

Agonist modulation of I_{KL} is increasingly being recognized as a general mechanism of transmitter action. Muscarinic receptors, α_1 adrenergic, H_1 histaminergic, 5-HT_2 serotonergic, and metabotropic glutamate receptors have all been shown to reduce I_{KL} in central neurons.[8,10,14,15,36,39,40,42,43,58] Although all of these receptors are known to couple to phospholipase C activation, the second messenger involved in I_{KL} modulation is not known with certainty.[56]

From a functional point of view, H_1-receptor-mediated modulation of I_{KL} represents a powerful mechanism for controlling cortical neuronal activity. Because the underlying leakage potassium conductance is active at all membrane potentials within the physiological range, H_1 receptor activation will result in a depolarization regardless of the prevailing membrane potential of the neuron. Moreover, by increasing the overall resistance of the neuron, the cell will become more electrotonically compact, thereby enhancing the influence of dendritic synaptic events upon the soma. Thus, not only will the H_1-receptor-mediated depolarization increase the excitability of cortical neurons by bringing the membrane potential closer to threshold, but it will also alter the integrative properties of the neuron.

Control of arousal

One of the most compelling reasons for studying the central histaminergic system is the well-known observation that systemic administration of H_1 receptor antagonists ('antihistamines') results in sedation in humans.[46] Because the human cerebral cortex

expresses such high numbers of H_1 receptors, we hypothesize that the sedative effects of H_1 receptor antagonists in the human brain are based upon blockade of the histamine-induced depolarization.

Histaminergic neurons, like noradrenergic and serotonergic neurons, exhibit a stereotypical state-related behavioral neurophysiological profile. Experiments in behaving cats have shown that these neurons fire regularly at moderate rates during waking, at somewhat reduced rates during slow-wave sleep, and become silent during rapid-eye movement sleep.[62] Assuming that the behavior of histamine neurons in humans is similar to that of the cat, one would predict that during waking there would be tonic release of histamine in the cerebral cortex. By depolarizing cortical neurons via H_1 receptor activation, natural occupation of cortical H_1 receptors by histamine released from the axon terminals of hypothalamic histamine neurons may be an important mechanism of cortical arousal.

The anatomy of the central histaminergic system is consistent with such a role. The essential features of the system are preserved in all mammalian species:[1,2,31,50,64] histamine-synthesizing neurons are found only within the region of the tuberomammillary nucleus in the caudal hypothalamus, which is compact in rats but more diffuse in other species. Emanating from this relatively small cluster of neurons is an extensive network of histaminergic axons ramifying widely throughout the brain, including direct projections to the cerebral cortex.[28,51,52,63] Taken together, the anatomy, physiology and pharmacology of the central histaminergic system seem ideally suited to play a significant role in the neuronal control of behavioral states.

The key question with respect to human neurobiology regards the locus and mechanism of action of the sedation induced by H_1 antagonist antihistamines. It is clear that the site of sedative action of antihistamines is within the central nervous system, as H_1 receptor antagonists which do not cross the blood–brain barrier are non-sedating.[37,47] Unfortunately, the selectivity of H_1 receptor antagonists is not ideal; many bind (albeit with lower affinity[30]) and antagonize[54] muscarinic, adrenergic and serotonergic receptors to varying degrees. The critical issue is one of affinity. Therapeutic doses of H_1 receptor antagonists are too low to antagonize non-H_1 receptors to any appreciable degree, while they readily block central H_1 receptors.[55] Even more compelling are studies of the responses of humans to administration of stereoselective enantiomers of chlorpheniramine and dimethindene, H_1 antagonists exhibiting marked sedative side-effects. Enantiomers which exhibit high affinity for H_1 receptors are sedating; those which have low affinity for H_1 receptors are not.[48] Most importantly, both sedating and non-sedating enantiomers maintain their (relatively low) affinity for aminergic receptors. Thus the sedative effects of H_1 receptor antagonists correlate well with their potency at central H_1 receptors, but not at any other receptor studied to date.

Further clues can be inferred from comparative studies of H_1 receptor antagonists. The sedative effect of antihistamines readily observed in humans is not seen in any animal species studied including mice, rats, guinea-pigs, hamsters, dogs, cats and squirrel monkeys.[45] While high doses of H_1 receptor antagonists exert modest sedative effects on sleep–walking parameters of rats some 12 h after administration, they reduce total sleep time during the first 6 h.[27] Both the time course and dosage regimen discount the notion that this represents a plausible animal model of the human situation. Such observations are in keeping with the marked species differences in the distribution of H_1 receptors in the brain. In humans, the cortex exhibits the highest density of H_1 receptors in the brain; within the cortex, the temporal lobe is particularly enriched.[9,25] In guinea-pig, the highest receptor densities are found in cerebellum, while in rats H_1 receptors are distributed relatively uniformly.[9,22,23,60] Taken together, these data suggest that in humans the sedative effects of antihistamines are mediated by blockade of cortical H_1 receptors.

Knowing that antihistamines cause their sedation via a central effect at H_1 receptors in humans, one would like to determine the cellular and molecular mechanisms underlying these effects. Three hypotheses have been proposed: (i) synergism between H_1 receptors and adenylyl cyclase; (ii) H_1 receptor-mediated depolarization of thalamic neurons; and (iii) H_1 receptor-mediated depolarization of human cortical neurons.

The synergism between H_1 receptors and those receptors positively coupled to adenylyl cyclase is an example of heterosynaptic facilitation: although H_1 receptor activation has no effect on cAMP levels alone, when adenylyl cyclase is activated concurrent with H_1 receptors, production of cAMP is greatly enhanced.[49] Such conditional enhancement of cAMP production is now thought to involve modulation of activated adenylyl cyclase by the $\beta\gamma$ subunits of G-proteins.[11,59] In cortical neurons, activators of adenylyl cyclase reduce spike-frequency adaptation by blockade of a calcium-activated potassium current, thereby selectively enhancing responses to excitatory inputs without necessarily affecting the resting membrane potential.[18,34,35] In theory, such a process would be enhanced by H_1 receptor activation by neuronal histamine, and conversely, would be blunted by H_1 receptor blockade. In this way, H_1 receptor blockade may contribute to the sedative effects of antihistamines.

In cat and guinea-pig thalamic relay neurons, H_1 receptor occupation results in a depolarization which biases these cells towards single-spike mode.[42] Because this mode of firing is thought to underly the desynchronized EEG of waking, it seems plausible that H_1-receptor-mediated arousal may result from the change in thalamic firing pattern induced by

histamine. Conversely, blockade of this depolarization by H_1 receptor antagonists may also contribute to the sedative effects of antihistamines. Although the human thalamus exhibits only low levels of H_1 receptor binding,[9,25] it is not clear how many receptors are required for such effects to be manifest.

In the present study, we have shown that H_1 receptor activation consistently induces a depolarization of human cortical neurons. By depolarizing cortical neurons, H_1 receptor activation brings them closer to threshold and increases their likelihood of firing in response to excitatory synaptic inputs. Moreover, the increase in whole-cell resistance induced by reducing a steady-state leakage current would alter the integrative properties of cortical neurons, making synaptic inputs to the dendrites more effective. Thus, this simple change in membrane potential efficiently increases the excitability of the cerebral cortex. Both because of the consistency of the response and the high density of H_1 receptors in the human cerebral cortex, we hypothesize that depolarization via reduction of I_{KL} represents an important cellular mechanism of cortical arousal.

Acknowledgements—We thank Andy Laycock for superb technical support, Dr Barrie Woodhurst for his neurosurgical skills, and the patients for their cooperation. Supported by grants from the Medical Research Council of Canada and the Alzheimer Society of British Columbia PBR is a Scholar of the MRC. Current Address for Anita Kamondi: Department of Neurology, University of Pecs, Pecs, Hungary.

REFERENCES

1. Airaksinen M. S. and Panula P. (1988) The histaminergic system in the guinea pig central nervous system: an immunocytochemical mapping study using an antiserum against histamine. *J. comp. Neurol.* **273,** 1–24.
2. Airaksinen M. S., Paetau A., Paljarvi L., Reinikainen K., Riekkinen P., Suomalainen R. and Panula P. (1991) Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains. *Neuroscience* **44,** 465–481.
3. Armstrong W. E. and Sladek C. D. (1985) Evidence for excitatory actions of histamine on supraoptic neurons *in vitro*: mediation by an H_1-type receptor. *Neuroscience* **16,** 307–322.
4. Arrang J. M., Garbarg M., Lancelot J. C., LeComte J. M., Pollard H., Robba M., Schunack W. and Schwartz J.-C. (1987) Highly potent and selective ligands for histamine H_3-receptors. *Nature* **327,** 117–123.
5. Arrang J.-M., Devaux B., Chodkiewicz J.-P. and Schwartz J.-C. (1988) H_3-receptors control histamine release in human brain. *J. Neurochem.* **51,** 105–108.
6. Arrang J.-M., Garbarg M. and Schwartz J.-C. (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **302,** 832–837.
7. Barbin G., Palacios J. M., Garbarg M., Schwartz J.-C., Gaspar P., Javoy-Agid F. and Agid Y. (1980) Histidine decarboxylase in the human brain: properties and localization. *J. Neurochem.* **35,** 400–406.
8. Benson D. M., Blitzer R. D. and Landau E. M. (1988) An analysis of the depolarization produced in guinea-pig hippocampus by cholinergic receptor stimulation. *J. Physiol. Lond.* **404,** 479–496.
9. Chang R. S. L., Tran V. T. and Snyder S. H. (1979) Heterogeneity of histamine H_1-receptors: species variations in [^{3}H]mepyramine binding of brain membranes. *J. Neurochem.* **32,** 1653–1663.
10. Constanti A. and Sim J. A. (1987) Calcium-dependent potassium conductance in guinea-pig olfactory cortex neurons *in vitro*. *J Physiol (Lond)* **387,** 173–194.
11. Federman A. D., Conklin B. R., Schrader K. A., Reed R. R. and Bourne H. R. (1992) Hormonal stimulation of adenylyl cylcase through G_i-protein $\beta\gamma$ subunits. *Nature* **356,** 159–161.
12. Foehring R. C., Lorenzon N. M., Herron P. and Wilson C. J. (1991) Correlation of physiologically and morphologically identified neuronal types in human association cortex *in vitro*. *J. Neurophysiol.* **66,** 1825–1837.
13. Geller H. M. (1981) Histamine actions on activity of cultured hypothalamic neurons: evidence for mediation by H_1 and H_2-histamine receptors. *Devl Brain Res.* **1,** 89–101.
14. Gerber U., Greene R. W., McCarley R. W. and Haas H. L. (1990) Excitation of brain stem neurons by noradrenaline and histamine. *J. basic clin. Physiol. Pharmac.* **1,** 71–76.
15. Greene R. W., Gerber U. and McCarley R. W. (1989) Cholinergic activation of medial pontine reticular formation neurons in vitro. *Brain Res.* **476,** 154–159.
16. Haas H. L. and Greene R. W. (1986) Effects of histamine on hippocampal pyramidal cells of the rat *in vitro*. *Expl Brain Res.* **62,** 123–130.
17. Haas H. L., Greene R. W., Heimrich B. and Xie X. (1988) Long-term potentiation in slices from human hippocampus. In *Synaptic Plasticity in the Hippocampus* (eds Haas H. L. and Buzsáki G.), pp. 78–79. Springer-Verlag, Berlin.
18. Haas H. L. and Konnerth A. (1983) Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. *Nature* **302,** 432–434.
19. Haas H. L., Wolf P. and Nussbaumer J.-C. (1975) Histamine: action on supraoptic and other hypothalamic neurones in the cat. *Brain Res.* **88,** 166–170.
20. Halliwell J. V. (1986) M-current in human neocortical neurons. *Neurosci. Lett.* **67,** 1–6.
21. Hill S. J. (1990) Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmac. Rev.* **42,** 45–83.
22. Hill S. J., Emson P. C. and Young J. M. (1978) The binding of [^{3}H]mepyramine to histamine H_1 receptors in guinea-pig brain. *J. Neurochem.* **31,** 997–1004.
23. Hill S. J. and Young J. M. (1980) Histamine H_1 receptors in the brain of the guinea-pig and the rat: differences in ligand binding properties and regional distribution. *Br. J. Pharmac.* **68,** 687–696.
24. Jorgenson K. L., Kow L.-M. and Pfaff D. W. (1989) Histamine excites arcuate neurons in vitro through H_1 receptors. *Brain Res.* **502,** 171–179.
25. Kanba S. and Richelson E. (1984) Histamine H_1 receptors in human brain labelled with [^{3}H]doxepin. *Brain Res.* **304,** 1–7.

26. Khateb A., Serafin M. and Muhlethaler M. (1990) Histamine excites pedunculopontine neurones in guinea pig brainstem slices. *Neurosci. Lett.* **112,** 257–262.
27. Kiyono S., Seo M., Shibagaki M., Watanabe T., Maeyama K., Wada H. (1984) Effects of diphenhydramine, histamine H_1-receptor antagonist, on rat sleep-waking parameters. *Neuroscience* **10,** 241–248.
28. Kohler C., Swanson L., Haglund L. and Wu J.-Y. (1985) The cytoarchitecture, histochemistry and projections of the tuberomamillary nucleus in the rat brain. *Neuroscience* **16,** 85–110.
29. Krnjevic K., Pumain R. and Renaud L. (1971) The mechanism of excitation by acetylcholine in the cerebral cortex. *J. Physiol., Lond.* **215,** 247–268.
30. Kubo N., Shirakawa O., Kuno T. and Tanaka C. (1987) Antimuscarinic effects of antihistamines: quantitative evaluation by receptor-binding assay. *Jap. J. Pharmac.* **43,** 277–282.
31. Lin J.-S., Luppi P. H., Salver D., Sakai K. and Jouvet M. (1986) Neurones immunoreactifs a l'histamine dans l'hypothalamus chez le chat. *C. R. Lebd Sćeanc. Acad. Sci., Paris* **303,** 371–376.
32. Lin J. S., Sakai K. and Jouvet M. (1988) Evidence for histaminergic arousal mechanisms in the hypothalamus of cat. *Neuropharmacology* **27,** 111–122.
33. Lipinski J. F., Schaumburg H. H. and Baldessarini R. J. (1973) Regional distribution of histamine in human brain. *Brain Res.* **52,** 403–408.
34. Madison D. V. and Nicoll R. A. (1982) Noradrenaline blocks accomodation of pyramidal cell discharge in the hippocampus. *Nature* **299,** 636–638.
35. Madison D. V. and Nicoll R. A. (1986) Cyclic adenonsine 3′,5′-monophosphate mediates β-receptor actions of noradrenaline in rat hippocampal pyramidal cells. *J. Physiol. Lond.* **372,** 245–259.
36. Madison D. V., Lancaster B. and Nicoll R. A. (1987) Voltage clamp analysis of cholinergic action in the hippocampus. *J. Neurosci.* **7,** 733–741.
37. Mann K. V., Crowe J. P. and Tietze K. J. (1989) Nonsedating histamine H_1-receptor antagonists. *Clin. Pharmac.* **8,** 331–344.
38. Martinez-Mir M. I., Pollard H., Moreau J., Arrang J. M., Ruat M., Traiffort E., Schwartz J.-C. and Palacios J. M. (1990) Three histamine receptors (H_1, H_2, and H_3) visualized in the brain of human and non human primates. *Brain Res.* **526,** 322–327.
39. McCormick D. A. (1992) Cellular mechanisms underlying cholinergic and noradrenergic modulation of firing mode in cat and guinea pig dorsal lateral geniculate nucleus. *J. Neurosci.* **12,** 278–289.
40. McCormick D. A. and Wang Z. (1991) Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J. Physiol. Lond.* **442,** 235–255.
41. McCormick D. A. and Williamson A. (1989) Convergence and divergence of neurotransmitter action in human cerebral cortex. *Proc. natn. Acad. Sci. U.S.A.* **86,** 8098–8102.
42. McCormick D. A. and Williamson A. (1991) Modulation of neuronal firing mode in cat and guinea pig LGNd by histamine: possible cellular mechanisms of histaminergic control of arousal. *J. Neurosci.* **11,** 3188–3199.
43. McCormick D. A. and von Krosigk M. (1992) Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. *Proc. natn. Acad. Sci. U.S.A.* **89,** 2774–2778.
44. McCormick D. A., Connors B. W., Lighthall J. W. and Prince D. A. (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J. Neurophysiol.* **54,** 782–806.
45. Melville K. J. (1973) Antihistamine drugs. In *Histamine and Antihistamine* (ed. Schacter M.), pp. 156–157. Pergamon Press, New York.
46. Nicholson A. N. (1983) Antihistamines and sedation. *Lancet* **II,** 211–212.
47. Nicholson A. N. (1987) New antihistamines free of sedative side-effects. *Trends pharmac. Sci.* **8,** 247–249.
48. Nicholson A. N., Pascoe P. A., Turner C., Ganellin C. R., Greengrass P. M., Casy A. F. and Mercer A. D. (1991) Sedation and histamine H_1-receptor antagonism: studies in man with the enantiomers of chlorpheniramine and dimethindene. *Br. J. Pharmac.* **104,** 270–276.
49. Palacios J. M., Garbarg M., Barbin G. and Schwartz J.-C. (1978) Pharmacological characterization of histamine receptors mediating the stimulation of cyclic AMP accumulation in slices from guinea pig hippocampus. *Molec. Pharmac.* **14,** 971–982.
50. Panula P., Yang H.-Y. T. and Costa E. (1984) Histamine-containing neurons in the rat hypothalamus. *Proc. natn. Acad. Sci U.S.A.* **81,** 2572–2576.
51. Panula P., Privola U., Auvinen S. and Airaksinen M. S. (1989) Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience* **28,** 585–610.
52. Panula P., Airaksinen M. S., Pirvola U. and Kotilainen E. (1990) A histamine-containing neuronal system in human brain. *Neuroscience* **34,** 127–132.
53. Pellmar T. C. (1986) Histamine decreases calcium-mediated potassium current in guinea-pig hippocampal CA1 pyramidal cells. *J. Neurophysiol.* **55,** 727–738.
54. Phillis J. W., Tebecis A. K. and York D. H. (1968) Histamine and some antihistamines: their actions on cerebral cortical neurones. *Br. J. Pharmac. Chemother.* **33,** 426–440.
55. Quach T. T., Duchemin A. M., Rose C. and Schwartz, J.-C. (1979) In vivo occupation of cerebral histamine H_1 receptors evaluated with [^{3}H]mepyramine may predict sedative properties of psychotropic drugs. *Eur. J. Pharmac.* **60,** 391–392.
56. Reiner P. B. and Wang X.-P. (submitted) Noradrenergic modulation of leakage potassium channels in rat thalamic neurons.
57. Stehle J. (1991) Effects of histamine on spontaneous electrical activity of neurons in rat suprachiasmatic nucleus. *Brain Res.* **130,** 217–220.
58. Stevens D. R., McCarley R. W. and Greene R. W. (1992) Serotonin$_1$ and serotonin$_2$ receptors hyperpolarize and depolarize separate populations of medial pontine reticular formation neurons *in vitro*. *Neuroscience.* **47,** 545–553.
59. Tang W.-J. and Gilman A. G. (1991) Type-specific regulation of adenylyl cyclase by G protein $\beta\gamma$ subunits. *Science* **254,** 1500–1503.
60. Tran V. T., Chang R. S. L. and Snyder S. H. (1978) Histamine H_1 receptors identified in mammalian brain membranes with [^{3}H]mepyramine. *Proc. natn. Acad. Sci. U.S.A.* **75,** 6290–6294.
61. Tsai C. L., Matsumura K., Nakayama T., Itowi N., Yamatodani A. and Wada H. (1989) Effects of histamine on thermosensitive neurons in rat preoptic slice preparations. *Neurosci. Lett.* **102,** 297–302.

62. Vanni-Mercier G., Sakai K. and Jouvet M. (1984) Neurones specifiques de l'eveil dans l'hypothalamus posterieur du chat. *C. R. Lebd Scéanc. Acad Sci., Paris* **298,** 195–200.
63. Vincent S. R., Hokfelt T., Skirboll L. and Wu J.-Y. (1983) Hypothalamic gamma-aminobutyric acid neurons project to the neocortex. *Science* **220,** 1309–1311.
64. Watanabe T., Taguchi Y., Shiosaka S., Tanaka J., Kubota H., Terano Y., Tohyama M. and Wada H. (1984) Distribution of the histaminergic neuron system in the central nervous system of rats: a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res.* **295,** 13–25.
65. Yamashita M., Fukui H., Sugama K., Horio Y., Ito S., Mizuguchi H. and Wada H. (1991) Expression cloning of a cDNA encoding the bovine histamine H_1 receptor. *Proc. natn. Acad. Sci. U.S.A.* **88,** 11,515–11,519.
66. Yanai K., Watanabe T., Yokoyama H., Hatazawa J., Iwata R., Ishiwata K., Meguro K., Itoh M., Takahashi T., Ido T. and Matsuzawa T. (1992) Mapping of histamine H_1 receptors in the human brain using [^{11}C]pyrilamine and positron emission tomography. *J. Neurochem.* **59,** 128–136.

(*Accepted* 7 *September* 1993)