JOURNAL OF NEUROPHYSIOLOGY
Vol. 75, No. 2, February 1996. *Printed in U.S.A.*

Histamine Depolarizes Cholinergic Septal Neurons

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SUMMARY AND CONCLUSIONS

1. Bath application of 10 μM histamine (HA) resulted in a depolarization or inward current in 58/59 cholinergic neurons located in the medial septum and nucleus of the diagonal band of Broca (MS/DBB) in a slice preparation of rat brain.

2. In bridge mode, the histamine-induced depolarization consisted of both fast and slow phases; inward currents that followed the comparable time course were observed under voltage-clamp conditions. The fast depolarization was associated with variable changes in input resistance, while the slow depolarization always was associated with an increase in input resistance.

3. Both fast and slow responses persisted in the presence of tetrodotoxin (TTX), but only the fast response persisted when transmitter release was abolished by bathing the slice in either a low-Ca^{2+}-, high-Mg^{2+}-containing medium or one containing Cd^{2+}.

4. When ramp voltage-clamp commands were applied during the fast depolarization, the resultant current-voltage (*I-V*) curves did not intersect over the range of membrane potentials from -130 to -30 mV. Ionic substitution experiments suggested that the bulk of the ionic current flowing during the fast depolarization was carried by sodium ions.

5. The *I-V* characteristics of the slow inward current identified it as a reduction in an inwardly rectifying potassium conductance.

6. The fast depolarization was significantly reduced by the H_1 receptor antagonists pyrilamine and promethazine, but not by the H_2 receptor antagonist cimetidine. Neither the H_2 receptor agonist impromidine nor the H_3 receptor agonist R-α-methylhistamine mimicked the response to HA. None of the agonists or antagonists had any observable effect upon the slow depolarization.

7. We conclude that HA directly depolarizes cholinergic MS/DBB neurons by acting as an H_1 receptor, which primarily couples to an increase in a TTX-insensitive Na^+ conductance. Additionally, HA evokes a slow depolarization mediated by a decrease in an inwardly rectifying potassium conductance but is not generated by activation of classically defined HA receptor subtypes.

INTRODUCTION

The cholinergic neurons of the medial septum/diagonal band of Broca (MS/DBB) provide the major extrinsic cholinergic innervation of the hippocampus. Interest in regulation of this pathway stems from the extensive literature suggesting a role for the septo-hippocampal pathway in memory, learning, and arousal (Reiner and Fibiger 1995) and the observation that cholinergic neurons in the basal forebrain are lost in Alzheimer's disease (Coyle et al. 1983). As part of an ongoing effort to understand the functional organization of central cholinergic systems, we have begun to investigate neurotransmitter regulation of MS/DBB cholinergic activity.

The present set of experiments focuses on the effects of histamine (HA) upon MS/DBB cholinergic neurons. HA is well established as a neurotransmitter in the CNS (Schwartz et al. 1991). Neurons containing the requisite cellular machinery for synthesis and release of histamine are located within the tuberomammillary nucleus of the hypothalamus. These neurons give rise to a highly divergent projection system, innervating much of the brain from cortex to spinal cord. An ever-increasing body of evidence indicates that the central histaminergic system plays a role in control of behavioral states (Lin et al. 1988). The key observations are that the activity of histaminergic neurons increases with arousal (Vanni-Mercier et al. 1984) and that systemic administration of H_1 histamine receptor antagonists, commonly known as antihistamines, results in sedation in humans (Nicholson 1983). Given the proposed role of basal forebrain cholinergic neurons in arousal mechanisms (Buzsaki et al. 1988; Semba 1991), one site at which histamine may act to modify behavioral state is the MS/DBB, and indeed the HAergic innervation of the MS/DBB is relatively dense (Inagaki et al. 1988; Panula et al. 1989). In the present set of experiments, we tested the hypothesis that histamine has excitatory effects upon identified cholinergic MS/DBB neurons in a rat brain slice preparation.

METHODS

The methods for preparation of brain slices and whole cell patch-clamp recording from identified cholinergic MS/DBB neurons are identical to those described in Gorelova and Reiner (1996). In a pilot set of experiments, we found that responses to histamine exhibited desensitization (see RESULTS). Therefore, when HA was applied more than once to a neuron, a minimum interval of 45 min between applications was utilized, sufficient to eliminate the confounding effects of desensitization. HA, cimetidine, and R-α-methylhistamine were obtained from Research Biochemicals, pyrilamine, promethazine, and tetrodotoxin (TTX) were from Sigma, and impromidine was a gift from Smith, Kline and French. Statistical comparisons were performed using Student's *t*-test, unless otherwise stated. Measures were accepted as statistically significant if $P < 0.05$.

RESULTS

The effects of HA were studied on 59 cholinergic MS/DBB neurons. Of these, 29 were definitively identified as cholinergic on the basis of colocalization of choline acetyltransferase immunoreactivity, and intracellularly applied biocytin, as described by Gorelova and Reiner (1996). In the remaining 30 neurons, the biocytin-labeled neurons were not recovered and therefore no histological evidence of the cholinergic phenotype was obtained. These neurons were

classified as cholinergic based on the following criteria: anatomic localization to the MS/DBB, the presence of a slow afterhyperpolarization of ≥200-ms duration after a single action potential, and anomalous rectification upon application of hyperpolarizing current steps. In the companion paper (Gorelova and Reiner 1996), we have demonstrated that >96% of neurons exhibiting these properties are cholinergic, and similar results have been obtained by others (Griffith 1988; Griffith and Matthews 1986; Khateb et al 1992). The effects of HA upon immunohistochemically identified neurons were identical to those for which histological evidence was unavailable, and therefore we have pooled the data.

Membrane potential changes

Bath application of HA at 10 μM resulted in a marked membrane depolarization or inward current in 58/59 neurons studied. The response in bridge mode (studied in 41 neurons) typically consisted of two distinct parts: a relatively fast depolarization of 15.5 ± 4.6 mV (mean ± SD, n = 27), which invariably began to recover during histamine application, and a slow depolarization of 5.9 ± 2.5 mV (n = 12), which considerably outlasted HA application (Fig. 1, *A* and *B*). In some cases, the time course of the two responses were clearly separable, whereas in other neurons they overlapped to a considerable degree, precluding accurate measurement of the magnitude of slow depolarization in all cells. Changes in input resistance, measured by delivering small hyperpolarizing current pulses at the resting potential, were variable during the fast depolarization, including increases (Fig. 6*A*), decreases (Fig. 5*A*), and no change (Fig. 2*B*), whereas the slow depolarization always was associated with an increase in input resistance of 13.5 ± 2.4% (n = 6, Fig. 1, *B* and *C*).

Addition of TTX to the bath solution failed to block either response to HA (Fig. 2*A*). However, when the artificial cerebrospinal fluid (ACSF) was changed to one containing either low calcium (0.5 mM) and high magnesium (10 mM) or cadmium (100 μM), HA application produced an insignificant decrease of 7.5 ± 6.5% (n = 4) in the magnitude of the fast depolarization, whereas the slow response was significnatly diminished by 82 ± 6% (Fig. 2*B*, n = 4). These observations suggest that the fast depolarization is due to a direct action of histamine upon cholinergic MS/DBB neurons. With respect to the slow depolarizing response, the data are compatible with the hypothesis that this phenomenon is either due to activation of a calcium-dependent ionic conductance in cholinergic neurons or alternatively is secondary to calcium-dependent release of another neuroactive substance from adjacent cells. Distinguishing between these two hypotheses in an unambiguous fashion is experimentally difficult. Perhaps the best evidence addressing this issue emerged from experiments in which the degree of calcium buffering provided by the patch pipette solution was altered. When we recorded from cholinergic MS/DBB neurons using an internal patch pipette solution with elevated ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA, 11 mM, n = 4) to increase the effectiveness of our intracellular calcium buffering, there were no significant differences in the size of either the fast (16.7 ± 5.8 mV in 11 mM EGTA vs. 15.5 ± 4.6 mV in 0.1 mM EGTA) or the slow (7.5 ± 2.1 mV in 11 mM EGTA vs. 5.9 ± 2.5 mV in 0.1 mM EGTA) HA-mediated depolarization as compared with those neurons studied with low EGTA (0.1 mM, n = 27) internal solution (Fig. 2*C*). These results are consistent with, but do not prove, that the slow depolarization is caused by release of another neuroreactive substance from adjacent cells.

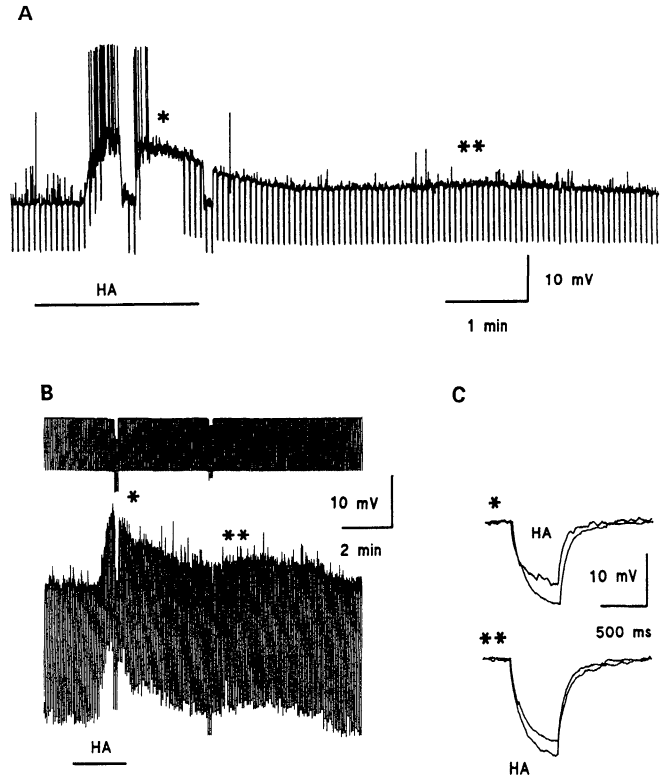

FIG. 1. Excitatory effect of histamine upon medial septum/diagonal band of Broca (MS/DBB) cholinergic neurons. *A*: whole cell patch-clamp recording of membrane potential in bridge mode in response to bath application of 10 μM histamine (HA) induces a membrane depolarization consisting of both fast (*) and slow (**) components. Fast membrane depolarization results in slow rhythmical firing. Note that fast depolarization starts to wane despite the continued presence of histamine. Negative going deflections on trace are voltage transients produced by hyperpolarizing current steps (0.05 nA, 250 ms, 1-s interval) applied to monitor input resistance. Repolarization of the membrane potential to the resting level with intracellular injection of current demonstrated no change in input resistance during fast response. Resting membrane potential was −70 mV. *B*: effect of HA (shown on a different cell) persists in the presence of 300 nM tetrodotoxin (TTX). Resting membrane potential is −60 mV. *C*: superimposition of traces produced by hyperpolarizing current steps under control conditions and during fast (*) and slow (**) depolarizations of the cell shown in *B* demonstrate a decrease and increase in input resistance, respectively.

Ionic basis of responses to HA

To study the ionic conductances underlying the depolarizing responses to HA, the neurons were studied under voltage-clamp conditions. Application of HA induced an inward current with prominent fast and slow components (n = 20, Fig. 3*A*). Ramp voltage clamp commands were applied under three conditions: before application of HA (control), during the fast component of the inward current seen with HA application (HA-fast), and during the delayed, slow component of the inward current seen after HA application

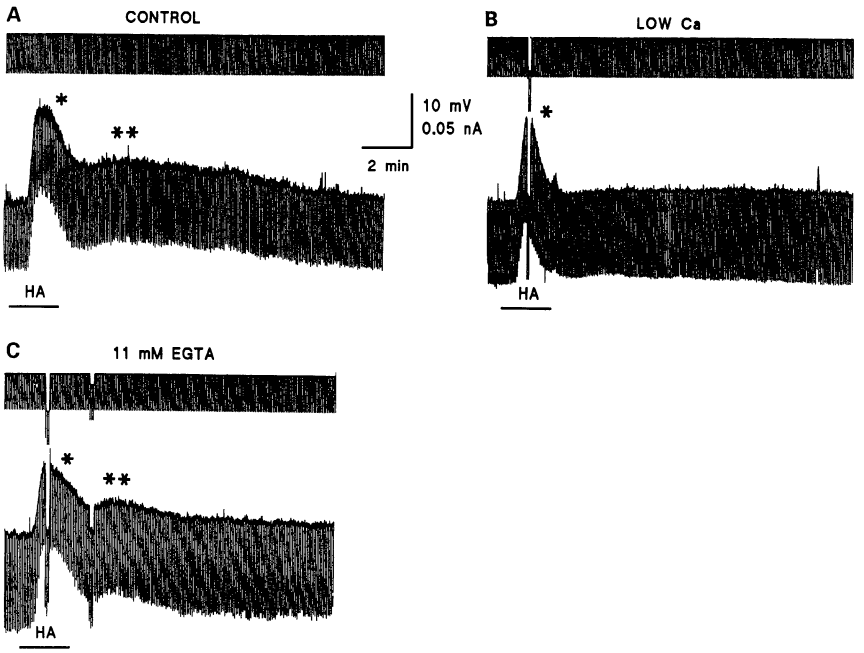

FIG. 2. Effects of manipulating extracellular and intracellular Ca^{2+} upon HA-induced responses. *A*: In TTX (300 nM)-containing artificial cerebrospinal fluid (ACSF), 10 μM HA induced a depolarization consisting of prominent fast (*) and slow components (**). Membrane potential is −68 mV. *B*: after application of ACSF containing low Ca^{2+} (0.5 mM), 10 μM HA evokes only fast (*) membrane depolarization. Time between application of HA is 56 min. Membrane potential, −68 mV. *C*: in a different cell, buffering of intracellular Ca^{2+} by using 11 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) in the internal patch pipette solution did not block either the fast (*) or the slow (**) responses to HA. Membrane potential, −68 mV.

(HA-slow). Analysis of these voltage ramps provides some insight into the current-voltage (*I-V*) relationship of the conductance(s) modified by HA application.

The intersection of the *I-V* curves obtained under control conditions and in the presence of HA should represent the reversal potential of the underlying conductance. For the slow conductance, the reversal potential was −100.5 ± 12.6 mV (Fig. 3*D*, $n = 6$), which is not significantly different from the predicted potassium equilibrium potential of −102 mV. Taken together with the observation that the depolarization is associated with a decrease in conductance, these data suggest that the slow depolarization is due to closing of potassium channels. Subtraction of the *I-V* relationship obtained during HA-slow from that obtained under control conditions revealed that the potassium current suppressed by HA exhibited marked inward rectification (Fig. 3*E*).

The *I-V* relationship obtained during the rapid, desensitizing inward current (HA-fast) did not intersect with that obtained under control conditions over the range of membrane potentials from −130 to −30 mV (Fig. 3*B*). (At more depolarized potentials, the quality of the voltage-clamp recordings deteriorated, and therefore we restricted our analysis to this voltage range.) Subtraction of HA-fast from the current measured under control conditions yielded a U-shaped *I-V* curve, with a maximum around −70 mV (Fig. 3*C*). Because the changes in input resistance associated with HA-fast were so variable between cells, we considered the hypothesis that the U-shaped *I-V* curve might arise via superimposition of an increase in a voltage-independent inward current and a decrease in an inwardly rectifying potassium current. We first considered the possibility that this complex response might be due to contamination of HA-fast by the decrease in potassium conductance induced by HA-slow. Therefore, HA was applied in the presence of either low-calcium (0.5 mM)-, high-magnesium (10 mM)-, or cadmium-containing ACSF. As was observed in bridge mode, under these conditions HA-slow was reduced dramatically in magnitude and only HA-fast remained. However, this manipulation did not change the shape of the *I-V* curve during HA-fast (Fig. 4*C*), suggesting that the apparent voltage dependence was *not* due to contamination by HA-slow. We next added 100 μM $BaCl_2$ to the ACSF. This treatment reduced both inward rectification and HA-slow to the point where they were no longer discernable and altered the shape of the voltage dependence of HA-fast ($n = 3$, Fig. 4*D*). Consistent with this was the observation that the voltage dependence of the barium-sensitive part of the fast inward current, obtained by subtracting the *I-V* curve for HA-fast under control conditions from that obtained in the presence of barium, is inwardly rectifying (data not shown). One interpretation of these data is that HA-fast is due to activation of a voltage-independent inward conductance as well as a decrease in a barium-sensitive inward rectifying conductance. Alternatively, barium may interact with the channel in an allosteric fashion and alter its voltage dependence.

The ionic species mediating HA-fast's inward conductance was investigated using ionic substitution experiments. On the basis of the data described above, it seems clear that the inward current is due to an increase in a conductance whose reversal potential is positive to −30 mV. Under physiological conditions, both calcium- and sodium-mediated conductances should have reversal potentials in this range. Additionally, under our experimental conditions, chloride-mediated conductances may be considered, as their calculated reversal potential would be −42 mV. As was shown above, reducing calcium in the ACSF did not significantly change HA-fast. Shifting the chloride equilibrium potential from −42 to −90 mV by replacing intracellular chloride with gluconate also did not alter HA-fast (data not shown). However, reducing extracellular sodium to 26 mM (thereby changing the calculated sodium reversal potential from 46 to 2 mV) resulted in a dramatic reduction in HA-fast ($n = 4$, Fig. 5). These data are consistent with the hypothesis that

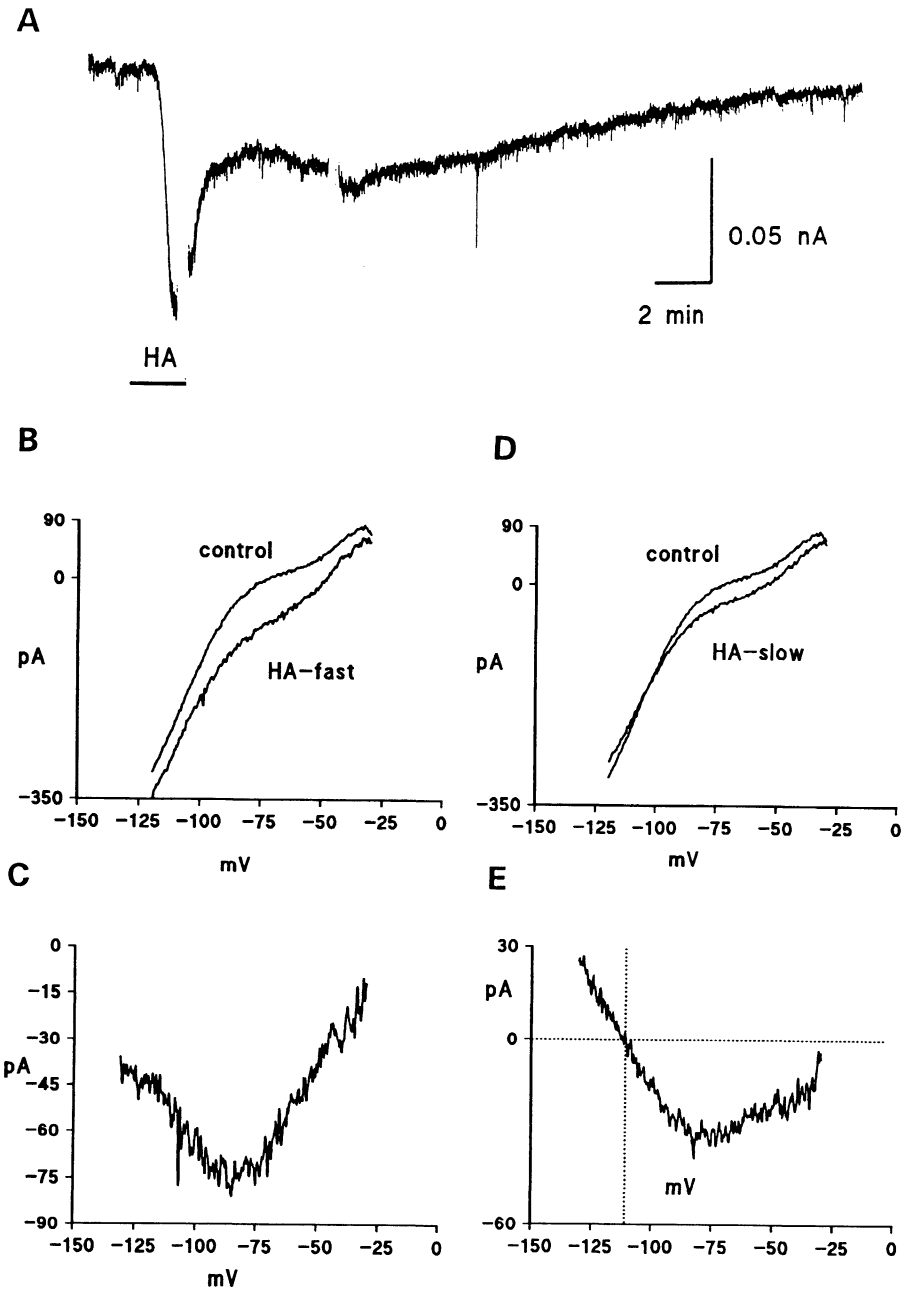

FIG. 3. HA-induced inward currents. *A*: voltage-clamp recording of HA-induced current. Holding potential −70 mV. Application of 10 μM HA (bar) produced an inward current consisting of fast and slow components. Gaps in the current record indicate times when *I-V* ramps were generated. *B*: *I-V* relations obtained before application of HA and at the peak of the fast response to HA (HA-fast). *C*: digital substraction of the *I-V* relation obtained under control conditions from that obtained during HA-fast reveals the voltage dependence of fast inward current induced by HA. *D*: *I-V* relations obtained before application of HA and during the slow response to HA (HA-slow). *I-V* curves cross at approximately −105 mV. *E*: digital substruction of *I-V* relation obtained under control conditions from that obtained during HA-slow demonstrates the voltage dependence of slow inward current induced by HA.

HA-fast is largely due to an increase in sodium conductance. It is worth noting that application of TTX to block voltage-sensitive sodium channels had no obvious effect on HA-fast, save for eliminating the action potentials that were evoked by the depolarization. Thus HA-fast appears to be due to a depolarization of MS/DBB cholinergic neurons mediated by the opening of TTX-insensitive sodium channels. Reducing extracellular sodium also dramatically diminished HA-slow, consistent with the observation that inwardly rectifying potassium conductances are dependent on external sodium (Harvey and Ten Eick 1989).

Histamine receptors

Bath application of the H_1-receptor antagonist pyrilamine at 1 μM (n = 4) or promethazine at 3 μM (n = 2) significantly reduced (19.5 ± 5.3 mV under control conditions vs. 0 mV in presence of antagonist) HA-fast, but did not significantly reduce HA-slow (4.5 ± 0.5 mV under control conditions and 4.1 ± 0.2 mV in the presence of antagonists, Fig. 6*B*). The H_2-receptor antagonist cimetidine failed to block either the fast (14.8 ± 4.1 mV control, 14.8 ± 4.1 mV in the presence of cimetidine) or the slow (8.0 ± 4.0 mV control, 9.5 ± 5.2 mV cimetidine) response at concentrations up to 10 μM (n = 4, Fig. 6*C*). Neither the H_2-receptor agonist impromidine (10 μM) nor the selective H_3-receptor agonist R-α-methylhistamine (10 μM) mimicked either of the depolarizations of MS/DBB cholinergic neurons induced by HA (data not shown). Taken together, these results suggest that the HA-fast is mediated by H_1-receptors, whereas the pharmacology of HA-slow remains to be clarified.

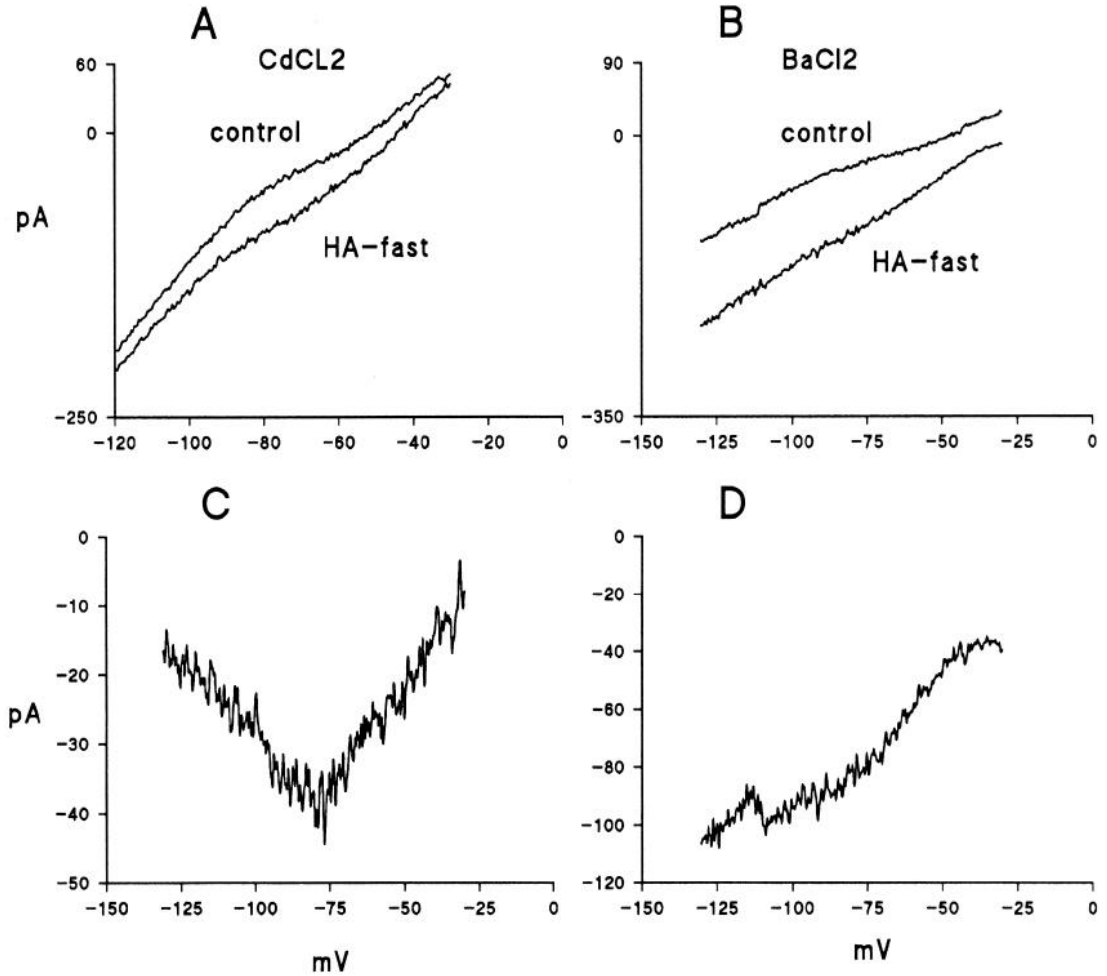

FIG. 4. *I-V* relations of HA-fast in Cd^{2+}- and Ba^{2+}-containing solutions. *A*: *I-V* relations obtained before application of HA and at the peak of the fast response to HA in the presence of 100 μM $CdCl_2$. *B*: *I-V* relations obtained before application of HA and at the peak of fast response to HA in the presense of 500 μM of $BaCl_2$. *C*: digital subtraction of control *I-V* relation from that obtained during HA-fast in the presence of 100 μM $CdCl_2$ reveals the same voltage dependence as in normal ACSF (compare with Fig. 3*C*). *D*: digital subtraction of control *I-V* relation from that obtained during HA-fast in presence of 500 μM $BaCl_2$ reveals a nearly linear voltage dependence.

Functional consequences of HA application

The rapid depolarization induced by histamine acting at H_1 receptors readily brings MS/DBB cholinergic neurons to threshold for firing spontaneous action potentials (Fig. 1). Thus HA can be considered as a potent excitatory input to MS/DBB cholinergic neurons. However, it would appear that this response is transient, insofar as it exhibits desensitization: the excitation diminished in the face of continued bath application of HA (Fig. 1). Although we did not systematically study the issue, repeated applications of HA within 10 min resulted in blunted responses, whereas the

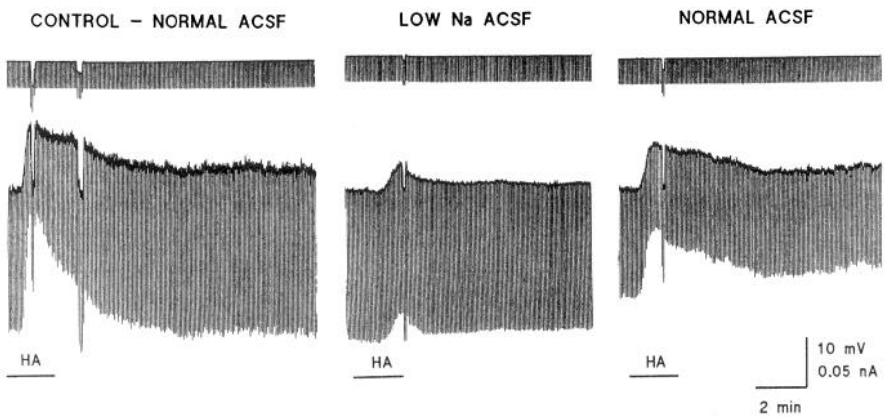

FIG. 5. Effect of manipulating extracellular Na^+ on HA-induced depolarizations. Three fragments of current-clamp recording of the same neuron. Interval between fragments ~45 min. Response of this cholinergic MS/DBB neuron to bath application of 10 μM HA is shown in normal ACSF containing 300 nM TTX (*left*), after reduction of Na^+ in external media to 26 mM (*center*), and after 45 min wash in normal ACSF (*right*).

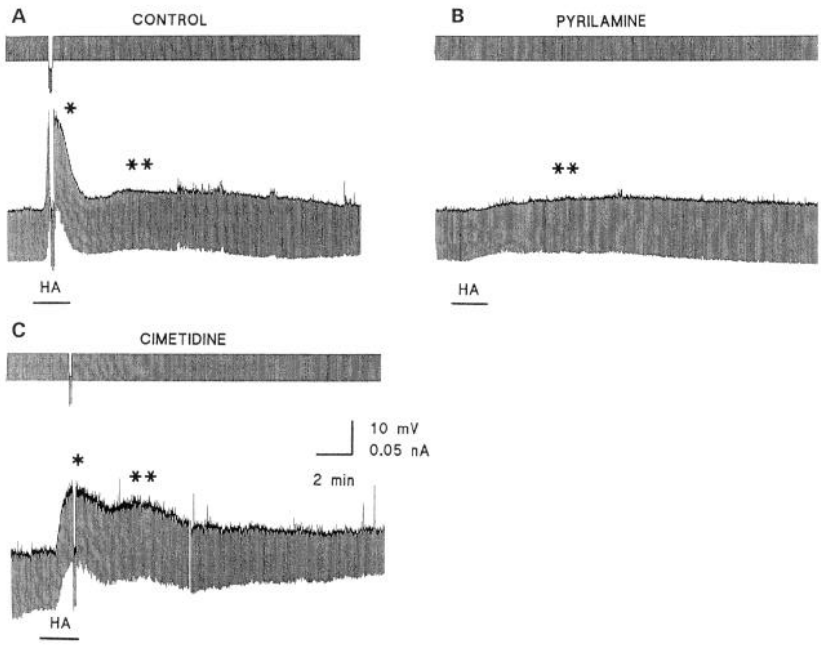

FIG. 6. Pharmacology of depolarizing responses of cholinergic MS/DBB neurons to HA. *A*: in TTX containing ACSF, bath application of 10 μM HA induced both fast (*) and slow (**) depolarizations. *B*: addition of 1 μM pyrilamine completely blocked the fast depolarizing effect of HA, but had no apparent effect upon the slow (**) depolarizing response. *C*: after 45 min of washing, 10 μM cimetidine was applied and the response to HA recorded. Note that both fast (*) and slow (**) depolarization can be observed in presence of cimetidine.

response fully recovered if a 45-min interval between application was provided.

The functional significance of the slow depolarizing response to histamine is more subtle. The size of the depolarization was not sufficient to bring the membrane potential into the range where spontaneous action potentials were evoked. However, a consistent observation was that the number of action potentials induced by a depolarizing current pulse was increased (Fig. 7, *B* and *C*). This was not due to any change in the slow afterhyperpolarization (sAHP; Fig. 7*A*), although transmitter-mediated blockade of the sAHP will alter the firing pattern of cholinergic MS/DBB neurons (Gorelova and Reiner 1996). Rather, it would appear that blockade of inward rectificiation increased the input resistance of the neuron, effectively increasing the potency of depolarization. Thus the slow depolarization excites cholinergic MS/DBB neurons both by changing the membrane potential and by altering intrinsic excitability.

DISCUSSION

The major finding of the present study is that HA excites identified cholinergic MS/DBB neurons. One effect is a powerful depolarization mediated by activation of H_1 receptors leading to an increase in sodium conductance. A second effect of HA application is a slow depolarization due to blockade of inwardly rectifying potassium channels, but the effect appears to be indirect and the HA-receptor subtype that mediates the phenomenon remains unclear. These data add a new dimension to our understanding of the functional effects of HA receptor occupation in the CNS (Haas 1992).

The rapid depolarization of MS/DBB cholinergic neurons appears to be direct. Neither blockade of voltage-dependent sodium channels with TTX, nor blockade of synaptic transmission by impairment of calcium influx prevented the phenomenon. The pharmacology of the effect suggests that it is mediated by the H_1 subtype of histamine receptor, consistent with binding data showing that the septal nuclei contain a high density of H_1 histamine receptors (Pollard and Bouthenet 1992). Taken together, these data strongly suggest that cholinergic MS/DBB neurons express functional H_1 receptors.

A surprising finding was that the H_1-receptor mediated depolarization was due to an increase in sodium conductance. Although excitation mediated by H_1 receptors is not an uncommon observation, the few studies that have examined the ionic mechanisms underlying this phenomenon in mammalian neurons have implicated blockade of voltage-independent potassium channels (McCormick and Williamson 1991; Reiner and Kamondi 1994). However, H_1 receptor activation has been shown to be mediated by an increase in sodium conductance in molluscan neurons (Carpenter and Gaubatz 1975; Gotow et al. 1980), and this appears to be the case in cholinergic MS/DBB neurons as well. The H_1 mediated fast depolarization bears some similarities to the histamine-operated cation channel found in endothelial cells (Yamamoto et al. 1992), in particular with respect to its propensity to exhibit desensitization, although their ionic permeabilities differ.

The slow depolarization of cholinergic MS/DBB neurons appears to be indirect, insofar as it was blocked by manipulations that reduced calcium influx, but was not appreciably affected by changing the calcium buffering capacity of the intracellular milieu. An unusual aspect of the response is that it is mediated by receptors exhibiting a pharmacological profile distinct from that of classically defined H_1, H_2, or H_3 receptors. The depolarization clearly was mediated by a decrease in an inwardly rectifying potassium conductance, an effect that also is seen with application of substance P (Stanfield et al. 1985; Yamaguchi et al. 1990) and neurotensin (Alonso et al. 1994; Farkas et al 1994) to basal forebrain cholinergic neurons in culture. It is tempting to speculate that HA either causes release of one of these neuroactive peptides or activates their receptors. Nonetheless, these data

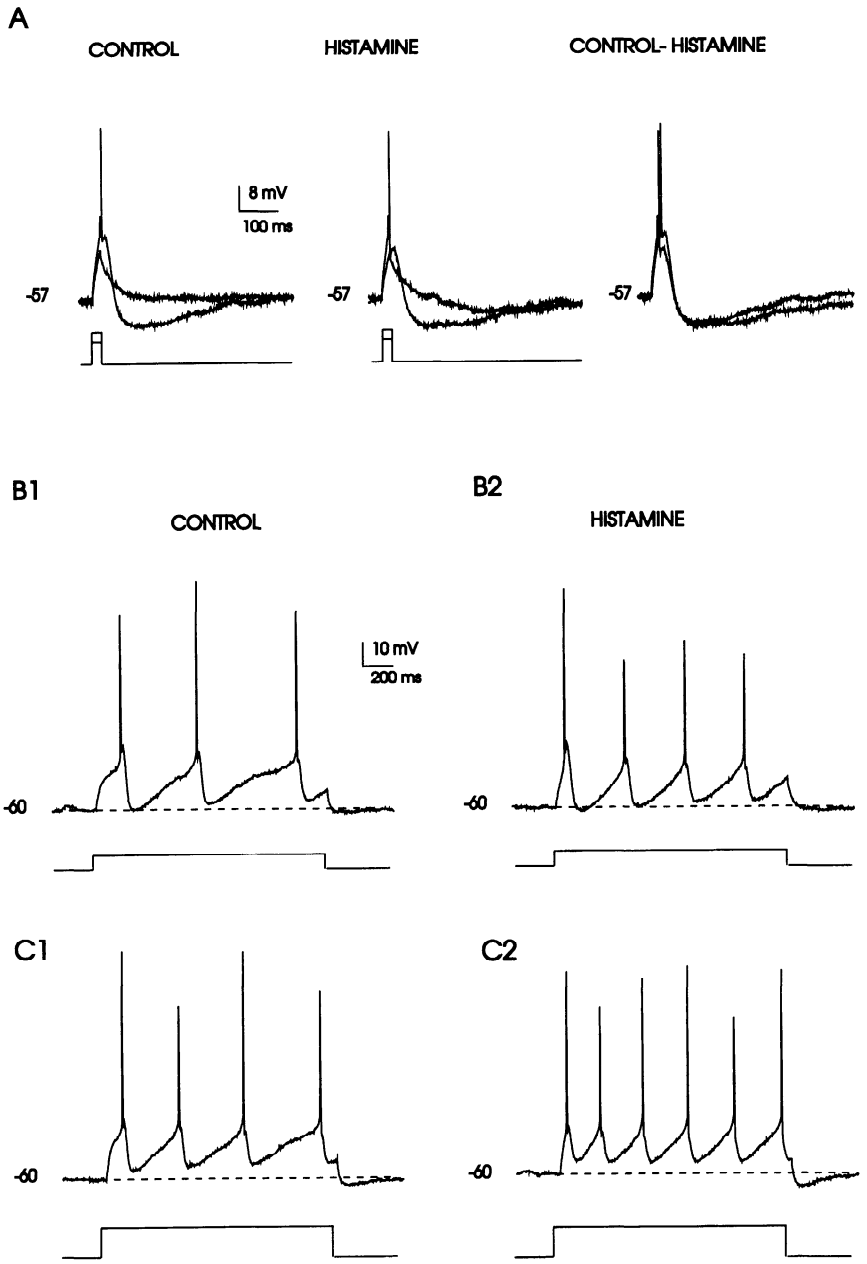

FIG. 7. Effect of HA on slow afterhyperpolarization (sAHP) and repetitive firing of cholinergic MS/DBB neurons. *A*: postspike sAHP from the same cell, before and during the HA-induced depolarization. Spikes were induced by short depolarizing current pulses (10 ms, 0.1 nA). Holding membrane potential was −57 mV. Superimposition of the sAHP before and during HA is shown (*right*). *B* and *C*: repetitive firing of same cell during prolonged depolarization steps of 2 different intensities before (*B1, C1*) and during (*B2, C2*) HA-induced depolarization. Note that HA decreased latency to first action potential and increased number of spikes evoked by identical currents. Depolarizing pulses in both cases were delivered from a holding potential of −60 mV.

indicate a third route by which inwardly rectifying potassium channels may be inhibited in forebrain cholinergic neurons.

Our data confirm and extend earlier results which demonstrated that ionophoretically applied histamine excites septal neurons in vivo (Carette 1978), although neither the receptor subtype nor phenotype of responsive neurons was identified. Moreover, these data provide a cellular explanation for the observation that electrical stimulation of hypothalamic regions containing histaminergic cell bodies evokes a marked elevation of acetylcholine release in the hippocampus in vivo (Mochizuki et al 1994).

Histaminergic neurons are most active during waking and exhibit diminished activity during slow wave sleep and complete silence during REM sleep (Vanni-Mercier et al. 1984). This behavioral neurophysiological profile suggests that release of histamine is at least correlated with behavioral arousal. That the fast depolarizing response to HA exhibits desensitization may suggest that cholinergic MS/DBB neurons would respond most briskly to changes in the activity of histamine neurons, as might be expected to occur during shifts in level of arousal. Numerous investigations have suggested that the cholinergic input to the hippocampus is intimately involved with cognitive functions (Reiner and Fibiger 1995). As such, these findings identify the histaminergic innervation of cholinergic MS/DBB neurons as a candidate substrate for the interaction between arousal and cognition.

The observation that H_1 receptor antagonists induce sedation in humans (Nicholson 1983) has been taken as indicating that HA, acting at H_1 receptors in the brain, plays a key role in control of behavioral state. The locus of such effects have been suggested to include both thalamic relay neurons (McCormick and Williamson 1991) and the pyramidal neu-

rons of the neocortex (Reiner and Kamondi 1994). The present data indicate that the cholinergic neurons of the MS/DBB represent another site at which the sedative effects of H_1 antagonists may operate.

We thank J. Williams and M. von Krosigk for comments on an earlier version of the manuscript and A. Laycock for technical assistance.

This work was supported by Grant MT-90399 from the Medical Research Council of Canada. P. B. Reiner is an MRC Scientist.

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Received 13 December 1994; accepted in final form 21 September 1995.

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