Enhancement by Histamine of NMDA-Mediated Synaptic Transmission in the Hippocampus

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Histamine is a neuromodulator in the brain, and the hippocampus is one of the regions of the brain that is innervated by histaminergic neurons. When applied to cultured hippocampal neurons, histamine selectively increased by up to tenfold the amplitude of the component of synaptic transmission that was mediated by *N*-methyl-D-aspartate (NMDA) receptors. Spontaneous miniature synaptic currents and the current elicited by applied NMDA also were enhanced, indicating that the histamine effect was expressed primarily postsynaptically. These results suggest that histamine may modulate processes involving NMDA receptors, such as the induction of long-term potentiation.

Histamine (HA)-containing nerve cells in the brain are found exclusively in the tuberomammillary nucleus of the hypothalamus, and they project throughout the brain and to all fields of the hippocampus (*1*, *2*). In this respect HA is like other biogenic amines, such as dopamine and serotonin, in that it occurs in neural circuits that would allow centralized modulation of wide areas of cortex. Indeed, HA has been strongly implicated in arousal and attentiveness, to which this pervasive circuitry seems suited (*3*). Because the laying down of memories may be an important component of attentiveness and because the hippocampus participates in the formation of memory (*4*), the question arises as to whether HA might affect hippocampal function. Previous work has shown that HA reduces a calcium-dependent K^+ conductance in pyramidal neurons in areas CA1 and CA3 (*5*). One consequence of this inhibition is that HA may modulate the release of neurotransmitter from presynaptic terminals, as has been suggested on the basis of other experiments (*6*). The purpose of the present experiments was to look for additional kinds of neuromodulation by HA in the hippocampus.

Most of these experiments used synapses that an isolated pyramidal neuron makes with itself ("autapses") (*7*–*9*), because autaptic currents are a convenient model system, being necessarily monosynaptic and homogeneous (*8*). However, all of the main results were confirmed for synaptic currents recorded between pairs of neurons in culture. Superimposed currents recorded before and during external perfusion of the cell with 100 μM HA show that HA increased the amplitude of the slow, NMDA component of autaptic transmission and had no effect on the rapid, non-NMDA component (Fig. 1A). In the presence of 20 μM D-2-amino-5-phosphonovalerate (D-APV) to block NMDA current, HA had no effect on the non-NMDA current that remained (Fig. 1B). Enhancement of the NMDA component of autaptic currents by HA was observed in 119 out of 126 CA1 pyramidal cells; in 7 cells HA had no effect. I also found that type A γ-aminobutyric acid ($GABA_A$)–mediated inhibitory autaptic currents (*8*), voltage-gated Na^+ and K^+ currents, and the passive properties of neurons were all unaffected by HA.

Not only did HA increase the amplitude of the NMDA autaptic current, it also accelerated its rate of decay (Fig. 2A) (*10*). All of the HA-enhanced current [measured in the presence of the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM)] was blocked by D-APV (Fig. 2B), indicating that the enhanced current was probably carried by NMDA channels. The HA enhancement was rapidly reversible (Fig. 2B). Normalized dose-response data, obtained from experiments in which the HA concentration was varied, were well fitted by a single-site binding scheme with a half-maximal HA concentration of 9 μM (*11*). Peak enhancement factors ranged from 1.6 to 10.2 (2.7 ± 1.6; mean ± SD, $n = 10$) at 100 or 200 μM HA, which were saturating concentrations.

Three subtypes of the HA receptor are known—H_1, H_2, and H_3—and all are present in the brain (*2*, *12*). The H_1 and H_2 subtypes were not involved because the HA enhancement (at 100 μM HA) was unaffected by the H_1 antagonists mepyramine (10 μM, $n = 4$) and promethazine (10 μM, $n = 2$) or by the H_2 antagonist cimetidine (1 to 10 μM, $n = 3$). Furthermore, the H_1 agonist 2-thiazolylethylamine (100 μM, $n = 3$) and the H_2 agonists impromidine (10 to 100 μM, $n = 8$) and dimaprit (100 μM, $n = 3$) produced no enhancement of the autaptic current. On the other hand, the H_3 antagonist (and H_2 agonist) impromidine (10 μM, $n = 5$) blocked the HA enhancement but also reduced the size of the NMDA current to about half. In addition, the H_3 agonist *R*-α-methylhistamine (100 μM, $n = 3$) mimicked the enhancing effect of HA. These results suggest that H_3 receptors mediate the HA effect. Surprisingly, however, another H_3 antagonist, thioperamide (10 μM, $n = 4$), was unable to block the HA enhancement in the same cells in which impromidine was effective.

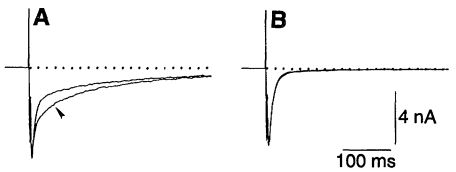

Fig. 1. Selective enhancement by HA of the NMDA component of excitatory autaptic transmission in a hippocampal pyramidal cell. (**A**) Superimposed excitatory autaptic currents from the same neuron in control solution and 4 s later (arrowhead), during application of 100 μM HA. (**B**) Same as (A), but with 20 μM D-APV present throughout. (The traces with and without HA superimpose.) The external solutions all contained 10 μM glycine.

Because NMDA and non-NMDA channels are colocalized at hippocampal synapses (*13*) and because the non-NMDA component of transmission is unchanged by HA, it seemed unlikely that HA functioned by presynaptically increasing transmitter release. Nevertheless, I investigated a presynaptic mechanism by applying 100 μM HA in the presence of phorbol dibutyrate (1 μM) or elevated external Ca^{2+} (20 mM compared with normal 3 mM) to increase the basal release of neurotransmitter from presynaptic terminals (*14*). The amplitudes of NMDA currents were greatly increased by phorbol ester (3.2-fold ± 2.2; mean ± SD, $n = 5$) and high Ca^{2+} (3.7-fold ± 0.8, $n = 4$) treatments alone, but their kinetics were unchanged, in contrast to the effect of HA, for which decay rates were also enhanced. Furthermore, large HA enhancement of NMDA currents (2.1-fold ± 0.5, $n = 9$) still occurred in addition to the increases produced by phorbol dibutyrate and high Ca^{2+}. Thus, enhancement due to HA differed qualitatively from that due to increased release of neurotransmitter, suggesting that HA does not modulate the release process.

If we examine postsynaptic mechanisms of HA enhancement, one possibility is that HA increases NMDA currents by modulating Mg^{2+} block of the NMDA ion channel (*15*). However, this outcome is unlikely because most of the experiments were done in nominally Mg^{2+}-free solutions, and similar enhancement was found with 100 μM HA in solutions containing 0.1 mM Mg^{2+} (2.9-fold ± 1.1; mean ± SD, $n = 8$). Histamine may act by altering the binding of glycine to its site on the NMDA receptor (*16*). Increasing the glycine concentration in the bath from 1 to 10 μM (which is

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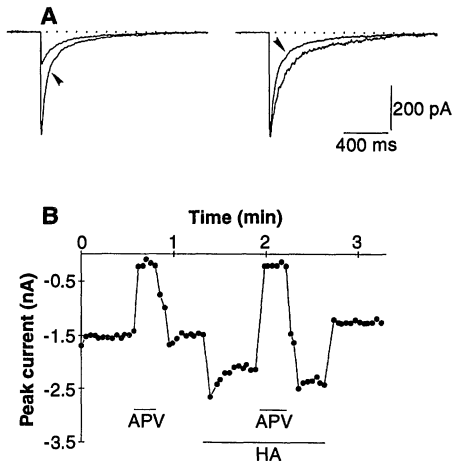

Fig. 2. Some properties of the HA-induced enhancement of the autaptic current. (**A**) (Left) Average NMDA autaptic current recorded before and during (arrowhead) application of 100 μM HA. Ten sweeps, evoked at 10-s intervals, were averaged for each condition. (Right) The control current has been scaled so that its peak amplitude matches that of the HA-enhanced current (arrowhead), to reveal the altered kinetics in HA. Non-NMDA currents and the stimulus artifact were removed by subtraction of a trace recorded in the presence of 50 μM D-APV. The external solutions all contained 10 μM CNQX. (**B**) Plot of the peak amplitude of autaptic current versus time during the experiment. Both D-APV (50 μM) and 100 μM HA were added to the bath solution during the times indicated by the horizontal bars. Currents were evoked at 3-s intervals. The external solutions all contained 10 μM CNQX.

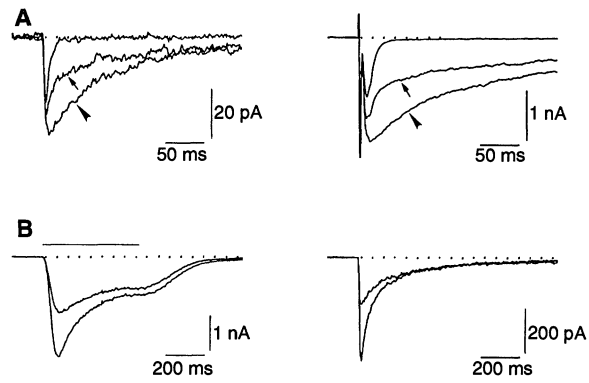

Fig. 3. Evidence for a postsynaptic locus of expression of the HA enhancement. (**A**) (Left) Superimposed averages of 13 to 27 miniatures from one cell in three external solutions: control (arrow), with 100 μM HA (arrowhead), and with 20 μM D-APV (unlabeled). (Right) Superimposed autaptic currents evoked in the same cell in the same three solutions. Currents were evoked at 10-s intervals, and CNQX was not present. (**B**) (Left) Whole-cell currents elicited by rapid 500-ms-long application (indicated by the bar) of 1 mM NMDA in control bath solution (smaller trace) or with 100 μM HA (larger trace). The HA effect was fully reversible. (Right) Superimposed autaptic currents evoked in the same cell, with and without 100 μM HA. The stimulus artifact has been blanked for clarity. Currents were elicited at 10-s intervals, and all solutions also contained 10 μM CNQX.

saturating) increased the amplitude of the NMDA autaptic current 1.8- to 2.2-fold without affecting its kinetics, in contrast with HA enhancement, in which the kinetics were altered. Furthermore, 100 μM HA still caused >1.7-fold enhancement in addition to the enhancement produced by 10 to 50 μM glycine ($n = 5$). These results suggest that HA enhancement and glycine modulation act by way of separate mechanisms.

A standard method for identifying the locus of expression in synaptic plasticity is to look for the phenomenon at the level of spontaneous miniature synaptic currents ("miniatures") (*17*). Because a single miniature is thought to result from the discharge of a relatively fixed unit of neurotransmitter, this procedure provides an assay of changes that are occurring entirely in the postsynaptic membrane. Averages of miniatures showed that HA enhanced the slow, D-APV–sensitive component of the miniatures (Fig. 3A, left panel) without greatly affecting their frequency (*18*). Autaptic currents evoked in the same cell closely resembled the averages of miniatures, apart from being about 50-fold larger (Fig. 3A, right panel). These results suggest that the HA enhancement resides mainly in a postsynaptic modification of NMDA channels. This was confirmed by the rapid application of 1 mM NMDA to a cell in the presence or in the absence of HA (*19*): HA produced similar enhancement of both the NMDA-elicited whole-cell current and the NMDA component of autaptic current measured in the same cell (Fig. 3B).

Pretreatment of cultures with pertussis toxin (200 ng/ml) blocked the inhibition of excitatory autaptic transmission by 100 μM adenosine (*20*) but had no effect on HA enhancement in the same cell ($n = 5$) (*21*). Also, the inclusion of 0.5 to 1 mM guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S) in the pipette solution had no effect on HA enhancement ($n = 3$) (*22*).

These results show that HA potently modulates the gating of NMDA channels, increasing the amplitude and rate of decay of the NMDA component of synaptic transmission. The effect may be mediated by H_3 receptors; however, in view of the lack of block by the H_3 antagonist thioperamide, the possible involvement of another kind of HA receptor cannot be excluded. The lack of effect of pertussis toxin and GTP-γ-S suggests that certain kinds of G protein are not involved in the HA enhancement (*23*), but further evidence is needed. It will be important to determine whether the HA effect is present in outside-out patches, which lack diffusible messenger substances.

By selectively enhancing the NMDA component of neurotransmission, HA should enhance processes in which NMDA currents participate, such as the triggering of long-term potentiation (LTP) (*4*, *24*). Conversely, pathological conditions that deplete HA in the brain might lead to a reduced ability to trigger LTP and so to memory loss. There is increasing evidence, too, for the involvement of NMDA receptors in other forms of computation in the central nervous system (*25*). It is therefore conceivable that HA participates in such high-level functions as the control of arousal and attentiveness (*3*) through its action on the NMDA channel.

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9. Cells were dissociated from region CA1 of newborn rats, and single neurons were grown separately on small spots of collagen and poly-D-lysine for 1 to 3 weeks before use (*8*). Under these conditions neurons formed profuse autaptic connections. Neurons were whole-cell voltage-clamped at the soma at −60 mV (Axopatch-1C, Axon Instruments, Foster City, CA) with patch electrodes that had resistances of 1.5 to 2.5 megohms after filling with internal solution (below). An unclamped "action current" was elicited by a 2-ms depolarization to 0 mV; this produced a spike of capacitative and Na^+ current (visible near the beginning of the traces in Fig. 1, A and B) followed by a large "autaptic current." This current is functionally identical to synaptic currents (between pairs of cells) in culture, on the basis of several criteria (*8*). Patch pipettes contained 125 mM potassium methylsulfate, 5 mM KCl, 5 mM EGTA, 0.5 mM $CaCl_2$, 2 mM Na_2ATP (ATP, adenosine triphosphate), 2 mM $MgCl_2$, 0.4 mM guanosine triphosphate, and 10 mM Hepes, adjusted to pH 7.3 with KOH and to 290 mOsM with sorbitol. In a few experiments the HA effect was observed with the perforated patch configuration and nystatin as the pore-former [R. Horn and A. Marty, *J. Gen. Physiol.* **92**, 145 (1988)]. Control external solutions contained 135 mM NaCl, 5 mM KCl, 3

mM $CaCl_2$, 10 mM glucose, 0.001 to 0.01 mM glycine, and 10 mM Hepes, adjusted to pH 7.3 with NaOH and to 310 mOsM with sorbitol. In some experiments 0.1 mM $MgCl_2$ was added. The neuron was continuously perfused at a rate of about 0.1 ml min^{-1} with control or test solution by means of a sewer pipe arrangement (internal diameter, 500 μm); exchange was complete within 1 s. Currents were evoked at 3- to 10-s intervals, filtered in the range 0.2 to 2 kHz, and digitally sampled at 0.5 to 5 kHz. A 10- to 20-mV hyperpolarizing test pulse was used to monitor neuronal input resistance and access resistance throughout the experiment. Sources of drugs were as follows: HA (Sigma or RBI, Natick, MA); CNQX and D-APV (Tocris Neuramin, Bristol, England); glycine and phorbol dibutyrate (Sigma); cimetidine, dimaprit, thioperamide, and *R*-α-methylhistamine (RBI); 2-thiazolylethylamine and impromidine (SmithKline Beecham, Dandenong, Australia); mepyramine and promethazine (gift of D. R. Curtis). Experiments were done at room temperature (24°C).

10. The decay of the control NMDA current in Fig. 2A was fit with two exponentials (τ = 98 ms, 590 ms here) as previously reported [S. Hestrin, P. Sah, R. A. Nicoll, *Neuron* **5**, 247 (1990)]. However, the HA-enhanced current required at least three exponentials (τ = 19, 75, and 425 ms in this case).
11. I accumulated data by applying HA at 1 to 200 μM concentration to each of 10 cells and measuring the increase in peak amplitude of autaptic currents while blocking non-NMDA currents with 10 μM CNQX.
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18. Miniatures were identified visually from sweeps digitally sampled at 1 kHz after filtering at 500 Hz. The non-NMDA channel antagonist CNQX was omitted from external solutions in these experiments because the rapidly rising non-NMDA component of the miniatures provided a landmark with which to align the miniatures for averaging. For the cell in Fig. 3A, the frequency of miniatures during HA application was 1.18 times that during the control period.
19. Rapid steps into NMDA-containing external solution were made by means of an array of glass tubes (internal diameter, 0.5 mm) attached to a piezoelectric multimorph (Philips). Solution flowed continuously through each of the tubes at about 3 ml min^{-1}, completely bathing the cell and its dendritic tree with the solution of choice. The time for solution changes was less than 30 ms, as determined from the junction potential change measured with a cell-free patch electrode. Enhancement due to HA was seen with the application of either 1 mM NMDA (n = 9) or 30 μM to 1 mM L-glutamate (n = 5).
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21. Cultures were incubated for 18 to 43 hours with pertussis toxin, with 0.1% dimethyl sulfoxide as the vehicle. In control cells, taken from the same culture plating and treated with vehicle alone, 100 μM adenosine produced a complete and reversible block of excitatory autaptic transmission (n = 4).
22. In these experiments the HA effect was monitored for up to 1 hour, and, although the strength of autaptic transmission declined markedly during this time [S. D. Hess, P. A. Doroshenko, G. J. Augustine, *Science* **259**, 1169 (1993)], the relative HA enhancement remained constant. However, there was no independent measure to confirm that GTP-γ-S had reached the postsynaptic terminals.
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A Transglutaminase That Converts Interleukin-2 into a Factor Cytotoxic to Oligodendrocytes

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Regenerating optic nerves from fish produce a factor that is cytotoxic to oligodendrocytes. The cytotoxic factor is recognized by antibodies to interleukin-2 (IL-2) and has the apparent molecular size of a dimer of IL-2. An enzyme, identified as a nerve transglutaminase, was purified from regenerating optic nerves of fish and was found to catalyze dimerization of human IL-2. The dimerized IL-2, unlike monomeric IL-2, is cytotoxic to oligodendrocytes from rat brain in culture. The results suggest that posttranslational modification of a cytokine can alter its activity. Under conditions in which oligodendrocytes inhibit neuronal regeneration, dimerization of IL-2 might provide a mechanism to permit nerve growth.

Axons in the central nervous system of mammals, unlike those of fish, have a poor capacity for regeneration after axonal injury (*1–3*). Injured central nervous system axons do regenerate, however, when grown in a supportive environment, such as in peripheral nervous system grafts (*4*). One of the environmental elements that might be responsible for the poor ability to regenerate in mammals is oligodendrocyte-mediated inhibition of nerve growth (*5*).

Regenerating optic nerves from fish produce a factor cytotoxic to oligodendrocytes that may act to eliminate mature oligodendrocytes (*6–8*). This cytotoxic factor was recognized by antibodies to IL-2 (*8*). We now present data indicating that a dimer of IL-2, produced by a transglutaminase derived from regenerating fish optic nerves, is a factor cytotoxic to oligodendrocytes.

Because the cytotoxic factor from regenerating fish optic nerves was about twice the molecular size of IL-2 derived from fish lymphocytes (*8*), it seemed possible that it could be a dimer of IL-2 produced by a posttranslational modification. We incubated conditioned medium of regenerating fish optic nerves (CM) (*9*) with human IL-2 (hIL-2) and then visualized proteins in the mixture with antibodies to IL-2. A new immunoreactive band (of 30 kD) was observed in addition to the original 15-kD IL-2 monomer (Fig. 1), suggesting that the new immunoreactive band, which was recognized by antibodies to IL-2, might be a dimer of IL-2 and that the CM contains the substances required for the apparent dimerization. Similar results were observed with mouse IL-2. The new band appeared only when Ca^{2+} was added to the preparation. It did not appear in control experiments in which either the CM or IL-2 was present alone.

We considered the possibility that dimerization of IL-2 occurred by means of a Ca^{2+}-dependent enzyme that is selectively synthesized or activated in regenerating nerves after injury. An enzyme of the cross-linking transglutaminase family (*10, 11*) appeared to be a likely candidate. We raised in rabbits antibodies against a 14–amino acid peptide (KKVKYGQCWVFAGV) (*12*) (SWISS-PROT data bank) that contains the active site of transglutaminases and is conserved among tissues and species. Immunoblotting analysis of the CM with affinity-purified antibodies to this peptide (TG-Ab) revealed a 55-kD immunoreactive protein (Fig. 2). We homogenized regenerating or uninjured fish optic nerves immediately after their excision, collected the supernatants after high-speed centrifugation (HSS), and compared them by immunoblotting analysis with the TG-Ab. Densitometric analysis showed that the 55-kD immunoreactive band was three times as intense in regenerating nerves than in uninjured nerves; therefore, regeneration is associated with increased transglutaminase immunoreactivity.

We purified the nerve transglutaminase to apparent homogeneity from HSS or CM of regenerating fish optic nerves by two steps of affinity chromatography, consisting of columns of poly-L-lysine (PLL) and of TG-Ab. The eluates were subjected to gel

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