0026-895X/94/030531-11$03.00/0
Copyright © by The American Society for Pharmacology and Experimental Therapeutics
All rights of reproduction in any form reserved.
MOLECULAR PHARMACOLOGY, 46:531–541, 1994.

MOL PHARM

Subunit-Specific Potentiation of Recombinant *N*-Methyl-D-Aspartate Receptors by Histamine

KEITH WILLIAMS

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084

Received May 3, 1994; Accepted June 20, 1994

SUMMARY

Histamine has numerous functions in the brain and has recently been shown to modulate responses of *N*-methyl-D-aspartate (NMDA) receptors on hippocampal neurons by a mechanism that does not involve classical histamine receptors. In the present work, voltage-clamp recording was used to study the effects of histamine on recombinant NMDA receptors expressed in *Xenopus* oocytes, to determine whether histamine acts directly on NMDA receptors and to investigate the subunit specificity of the effects of histamine. Histamine potentiated responses to NMDA at heteromeric NR1/NR2 receptors containing splice variants of the NR1 subunit (NR1A or NR1E) that lack the amino-terminal insert, together with the NR2B subunit but not the NR2A or NR2C subunit. Stimulation by histamine (EC_{50} = 10 μM) had a novel profile, involving a rapid increase in the magnitude of NMDA-induced currents followed by slow (≈1-min) desensitization to a steady state level. Desensitization of the response to histamine was time dependent and could occur in the absence of receptor activation by NMDA. Stimulation by histamine was dependent on the concentration of agonist used to activate NR1A/NR2B receptors and was seen with high but not low concentrations of NMDA and glutamate. The effect of histamine was not blocked by classical histamine receptor antagonists but was mimicked by the histamine metabolite 1-methylhistamine. At a high concentration (1 mM) histamine produced a voltage-dependent inhibition of NMDA currents at NR1A/NR2B receptors and at receptors (NR1B/NR2B) that are not sensitive to stimulation by histamine. The results suggest that histamine acts directly at a novel recognition site on some subtypes of NMDA receptors to increase their activity.

Histamine has been reported to modulate responses mediated by NMDA receptors (1, 2). At autaptic synapses of cultured hippocampal neurons, histamine was found to selectively increase the NMDA receptor-mediated component of excitatory synaptic currents (1). Histamine was also found to increase the magnitude of NMDA-induced whole-cell currents in isolated hippocampal neurons (2). The effect of histamine was not mimicked by selective agonists for the classical H_1, H_2, or H_3 receptors and was not blocked by H_1 or H_2 receptor antagonists (2). This may suggest the involvement of an atypical histamine receptor or a direct effect of histamine on the NMDA receptor itself. Histaminergic innervation, arising from cell bodies in the anterior hypothalamus, is widespread in the brain and includes cortical and hippocampal areas that are rich in NMDA receptors (3). Histamine could therefore play a role in modulating the function of NMDA receptors *in vivo*.

With the cloning of cDNAs encoding subunits of the NMDA receptor, it has become possible to examine the effects of modulators of the NMDA receptor by using recombinant receptors expressed from particular subunit combinations. Such studies are useful in elucidating the sites and mechanisms of action of endogenous modulators of the receptor. Two families of NMDA receptor subunits have been cloned. The rat brain NMDAR1 (NR1) subunit (the mouse homologue is termed ζ1) (4), which is expressed widely throughout the brain, is transcribed as nine alternatively spliced mRNAs, eight of which are functionally active (5–7). The NR2 family consists of the NR2A, NR2B, NR2C, and NR2D cDNAs cloned from rat brain (8, 9) and the mouse homologues ε1–ε4 (10–12). Although the subunit composition of native NMDA receptors is unknown, they are likely to be hetero-oligomers containing combinations of NR1 and NR2 subunits.

The effects of some modulators of the NMDA receptor are markedly dependent on the subunit composition of the receptor. For example, voltage-dependent block by Mg^{2+} is much weaker at NR1/NR2C receptors than at NR1/NR2A or NR1/NR2B receptors (8–10), and the sensitivity of NR1/NR2B receptors to glycine is 10-fold greater than that of NR1/NR2A receptors (10, 13). A particularly striking example of subunit-dependent properties is seen with polyamines such as spermine. Spermine has multiple effects on the NMDA receptor, includ-

This work was supported by Grants NS30000 and GM34781 from the United States Public Health Service.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; GABA, γ-aminobutyric acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

MOL PHARM

MOLECULAR PHARMACOLOGY

ing "glycine-independent" stimulation, which involves an increase in the magnitude of NMDA-induced whole-cell currents in the presence of saturating concentrations of glycine. This form of stimulation by spermine is seen at receptors containing a variant of NR1, such as NR1A, that lacks the amino-terminal insert (14), and the manifestation of this stimulatory effect is controlled by the type of NR2 subunit present in a heteromeric complex (13). Thus, stimulation by spermine is seen at NR1A/NR2B receptors but not at NR1A/NR2A or NR1A/NR2C receptors (13).

In the present work the effects of histamine on recombinant NMDA receptors expressed in *Xenopus* oocytes have been examined. These experiments were designed to determine whether histamine has a direct effect on the NMDA receptor/channel complex, to examine the subunit specificity of the effects of histamine using receptors composed of various NR1 splice variants and NR2 subunits, and to investigate the mechanism of action of histamine. The results indicate that the stimulatory effect of histamine is probably mediated by a direct action on the NMDA receptor complex. Furthermore, the effects of histamine at heteromeric NMDA receptors are subunit dependent and have similarities to effects seen with spermine. However, stimulation by histamine has a novel and possibly unique profile characterized by a rapidly developing increase in the magnitude of NMDA-induced macroscopic currents followed by partial desensitization over a period of ≈1 min.

Materials and Methods

Oocyte injection and recording. Defolliculated stage V-VI oocytes were prepared from *Xenopus laevis* (Nasco, Fort Atkinson, WI) as described previously (15). For studies with rat brain RNA, oocytes were injected with 200 ng of total RNA prepared from adult rat forebrain as described previously (15). For studies with cloned subunits, capped cRNAs were synthesized from linearized cDNA templates containing NR1 or NR2 clones using a commercially available *in vitro* transcription kit (mMessage mMachine; Ambion Inc., Austin, TX) with appropriate RNA polymerases. Most of the 5′ untranslated region was removed from the NR2A and NR2B clones before synthesis of cRNAs (16). Oocytes were injected with NR1 plus NR2A or NR2B cRNAs in a ratio of 1:5 (1–2 ng of NR1 plus 5–10 ng of NR2). For studies with NR2C, the mouse clone (ε3) was used. Oocytes were injected with 4 plus 20 ng or 4 plus 40 ng of NR1 plus ε3 cRNAs. The resulting NR1/ε3 (NR2C) receptors produced macroscopic currents of magnitudes similar to those seen with NR1/NR2A and NR1/NR2B receptors. We have been unable to obtain comparable levels of expression using the rat NR2C clone. After injection, oocytes were maintained in a saline solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM Na-HEPES, 2.5 mM sodium pyruvate, 50 μg/ml gentamycin, pH 7.5) at 18° for 2–7 days before recording. The saline solution was replaced daily.

Macroscopic currents were recorded with a two-electrode voltage-clamp using an OC-725 amplifier (Warner Instruments, Hamden, CT) or a GeneClamp 500 amplifier (Axon Instruments, Foster City, CA), as described previously (15, 16). Electrodes were filled with 3 M KCl and had resistances of 0.5–1 MΩ (current-injecting electrode) or 1–5 MΩ (voltage-sensing electrode). Oocytes were positioned in a small chamber (≈0.1-ml volume) and continuously superfused (≈5 ml/min) with a Mg^{2+}-free saline solution (96 mM NaCl, 2 mM KCl, 1.8 mM $BaCl_2$, 10 mM Na-HEPES, pH 7.5). Unless otherwise indicated, NMDA was applied in solutions containing 10 μM glycine. The extracellular solution contained $BaCl_2$ rather than $CaCl_2$, to minimize Ca^{2+}-activated Cl^- currents (17). In most experiments oocytes were injected with K-BAPTA (50–100 nl of a 40 mM solution, pH 7.5) on the day of recording, to eliminate a slowly activating Cl^- current that is seen even in the presence of extracellular Ba^{2+} (16, 18). For experiments on oocytes injected with brain RNA the extracellular solution contained $CaCl_2$ and oocytes were not injected with BAPTA.

Data acquisition was carried out using an MP-100 interface with AcqKnowledge software (Biopac Systems, Goletta, CA), on a Macintosh computer (16). The current signal was low-pass filtered (four- or eight-pole Bessel filter) at 10–50 Hz and digitized at 30 Hz to 2 kHz. AcqKnowledge or Axograph (Axon Instruments) software was used for data analysis. Data from concentration-response curves were analyzed using SigmaPlot (Jandel Scientific, Corte Madera, CA) and were fit to the logistic function $I = I_{max}/1 + ([agonist]/EC_{50})^{n_H}$, where I is the response to glutamate or histamine, I_{max} is the maximum response, and n_H is the Hill slope. Values for I_{max}, n_H, and EC_{50} were derived from this procedure.

Materials. NMDA, GABA, and histamine receptor agonists and antagonists were purchased from Research Biochemicals Int. (Natick, MA). AMPA and kainate were purchased from Cambridge Research Biochemicals (Wilmington, DE). Histamine dihydrochloride, glycine, histidine, 3-methylhistamine, and niflumic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Spermine tetrahydrochloride was purchased from Aldrich Chemical Co. (Milwaukee, WI). The rat brain NR1A, NR1B, and NR1E clones (originally termed NMDAR1A, -R1B, and -R1E, respectively) (6) were a gift from Dr. S. Nakanishi (Kyoto University, Kyoto, Japan). The NR2A, NR2B, and NR2C clones (8) were a gift from Dr. P. H. Seeburg (University of Heidelberg, Heidelberg, Germany). The mouse ε3 (NR2C) clone (10) was a gift from Dr. M. Mishina (Niigata University, Niigata, Japan).

Results

Specificity of the effects of histamine. In an initial series of experiments the specificity of the effects of histamine was studied using oocytes injected with rat brain RNA and voltage-clamped at −70 mV. With this approach it was possible to examine the effects of histamine on responses to a number of different amino acid receptor agonists in the same oocytes. Histamine (100 μM) had no effect on responses to GABA, AMPA, or kainate but markedly increased the response to NMDA (Fig. 1, A–D). When histamine was applied during steady state responses to NMDA, there was an initial peak followed by a desensitization of the response to histamine (Fig. 1D, *right*).

In the presence of extracellular Ca^{2+}, part of the response to NMDA in *Xenopus* oocytes involves Ca^{2+}-activated Cl^- conductances initiated by entry of Ca^{2+} through the ion channel of NMDA receptors. In native or brain RNA-injected oocytes a transient outward current involving a Ca^{2+}-activated Cl^- conductance is also seen after step depolarization, due to activation of voltage-dependent Ca^{2+} channels (17, 19, 20). This current, which can be blocked by Cl^- channel blockers such as niflumic acid (17, 20) (Fig. 1E), was unaffected by histamine, suggesting that histamine does not alter the properties of voltage-dependent Ca^{2+} channels or the Ca^{2+}-activated Cl^- conductance (Fig. 1E) and providing another measure of the specificity of histamine at NMDA receptors.

Subunit-specific effects at recombinant NMDA receptors. The effects of histamine were studied at heteromeric NMDA receptors containing different combinations of NR1 and NR2 subunits. To facilitate quantitative studies of the effects of histamine, these experiments were carried out in Ba^{2+}-containing saline using oocytes injected with BAPTA to eliminate Ca^{2+}-activated Cl^- currents. In all oocytes tested, 100 μM histamine did not induce currents when applied in the absence of NMDA and glycine (data not shown). Heteromeric

Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

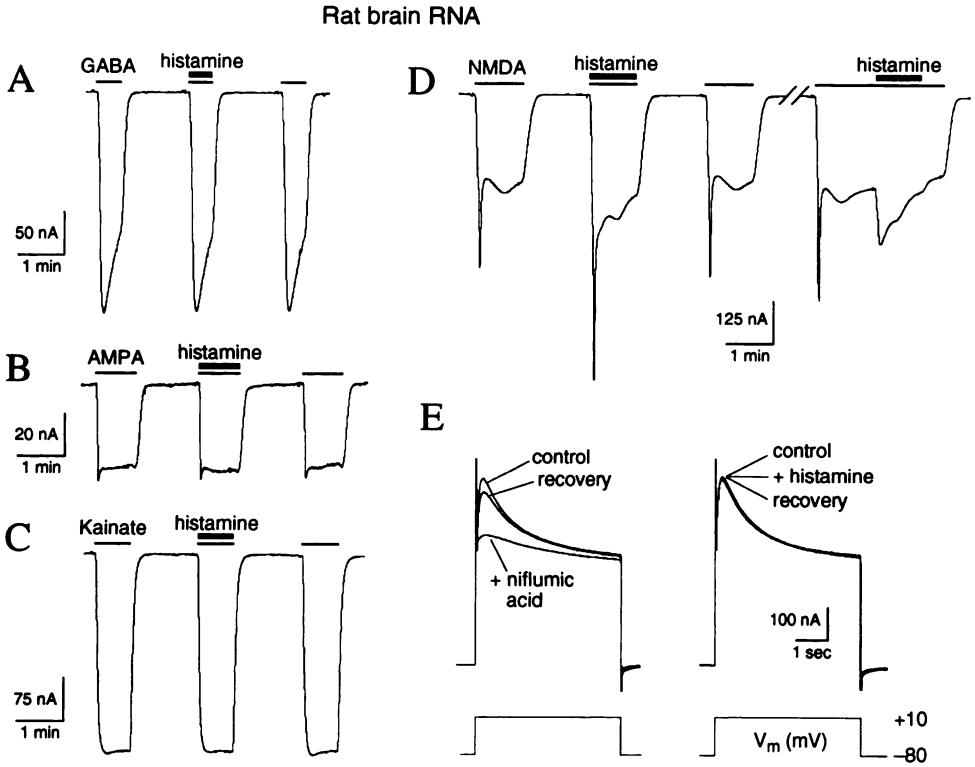


Fig. 1. Specificity of the stimulatory effect of histamine. A–D, The effects of histamine (100 μM) on responses to GABA (A), AMPA (B), kainate (C), and NMDA (D) were measured in an oocyte injected with rat brain RNA and voltage-clamped at −70 mV. The concentration of all agonists was 100 μM, and NMDA was applied in the presence of 10 μM glycine. Similar results were obtained with six oocytes. E, Outward currents after step depolarizations in the absence (*control* and *recovery*) and presence of 100 μM niflumic acid or 100 μM histamine were measured in an oocyte injected with brain RNA. The initial capacitance transients after the voltage steps have been mostly removed for presentation.

NR1/NR2 receptors expressed from each of three NR1 splice variants, NR1A, NR1B, and NR1E, together with each of three NR2 subunits, NR2A, NR2B, and NR2C, were examined (Fig. 2). NR1A, the major splice variant of NR1 in rat forebrain, contains both carboxyl-terminal (3′) inserts but lacks the amino-terminal (5′) insert. NR1B differs from NR1A in that it contains the amino-terminal insert, which has previously been shown to influence sensitivity to polyamines and Zn^{2+} (7, 13, 14), whereas NR1E lacks all three inserts (see Fig. 2D). At receptors containing the NR1A variant, histamine produced a marked stimulation of the response to NMDA at NR1A/NR2B but not at NR1A/NR2A or NR1A/NR2C receptors (Fig. 2A). Histamine had no effect on receptors containing the NR1B variant (Fig. 2B). At receptors containing NR1E the profile was similar to that seen at receptors containing NR1A, with histamine stimulation occurring at NR1E/NR2B but not at NR1E/NR2A or NR1E/NR2C receptors (Fig. 2C).

Thus, stimulation by histamine is seen at receptors containing NR1A or NR1E but not at receptors containing NR1B. Because NR1B includes the amino-terminal insert, whereas NR1A and NR1E do not (Fig. 2D), the results suggest that the amino-terminal insert influences sensitivity to histamine and that stimulation by histamine requires a variant of NR1 that lacks this insert. In contrast, there is no obvious relationship between histamine stimulation and the presence or absence of the two carboxyl-terminal inserts, which are present in NR1A and NR1B but absent in NR1E (Fig. 2D). In addition, NR2 subunits also control the effects of histamine, with stimulation being seen at heteromeric receptors containing NR2B but not at receptors containing NR2A or NR2C (Fig. 2).

Potentiation by histamine was seen at homomeric NR1A receptors but not at homomeric NR1B receptors. With 100 μM histamine, the peak potentiation was 70 ± 7% and steady state potentiation was 26 ± 8% (mean ± standard error, five oocytes) at homomeric NR1A receptors. Thus, the histamine binding site may be located on the NR1A subunit and its properties altered by NR2 subunits in heteromeric NR1A/NR2 complexes.

Effects of histamine on NR1A/NR2B receptors. The effects of histamine were characterized in detail at NR1A/NR2B receptors. At these receptors, histamine produced a rapid increase in the magnitude of NMDA-induced currents that decayed to a steady state level within ≈1 min (Fig. 2A, *center*), similar to the effects seen at receptors expressed from rat brain RNA (Fig. 1D). The stability and reproducibility of the effects of histamine at NR1A/NR2B receptors were determined by measuring responses to NMDA and NMDA plus histamine every 7 min for up to 1 hr. In some oocytes, the control response to NMDA showed a marked run-down over time, whereas in other oocytes there was little or no change in the size of the response to NMDA over time. Irrespective of whether the response to NMDA showed run-down, both the peak and steady state effects of histamine on NMDA-induced currents were stable over time (data not shown).

The effects of histamine on responses to NMDA could be due to a direct action on the NMDA receptor or could be mediated indirectly through activation of histamine receptors endogenous to *Xenopus* oocytes, although there are no published reports of the presence of histamine receptors on *Xenopus* oocytes. To determine whether endogenous histamine receptors may be involved, the effects of various agonists and antagonists selective for H_1, H_2, and H_3 histamine receptors were investigated in oocytes expressing NR1A/NR2B receptors.

The H_2 receptor agonist dimaprit (10 μM) had no effect on responses to NMDA (96 ± 2% of control; six oocytes). The effects of histamine were mimicked by (*R*)-α-methylhistamine but not by N^{α}-methylhistamine (Fig. 3), both potent H_3 receptor agonists. Another H_3 receptor agonist, imetit (10 μM), produced a small inhibition (9 ± 1%; six oocytes) of the re-

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

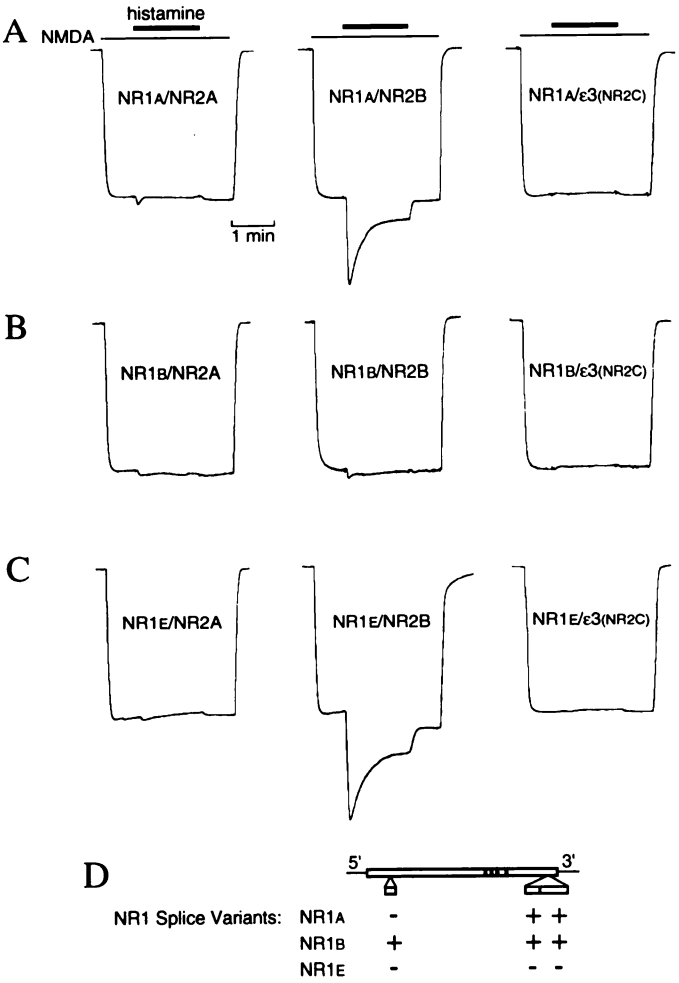


Fig. 2. Subunit-dependent effects of histamine on recombinant NMDA receptors. A–C, The effects of 100 μM histamine on responses to NMDA (100 μM; with 10 μM glycine) were determined in oocytes expressing NR1A/NR2 (A), NR1B/NR2 (B), and NR1E/NR2 (C) receptors and voltage-clamped at −70 mV. Oocytes were injected with NR1 plus NR2A (*left*), NR1 plus NR2B (*center*), and NR1 plus NR2C (*right*) subunit RNAs. In all traces, NMDA and histamine were applied during the times shown by the *horizontal bars above the traces in A*, and inward currents are downward. To facilitate comparison of the effects of histamine, all currents have been normalized to the control response to NMDA, which, in these oocytes, ranged from −120 nA (NR1B/NR2B) to −551 nA (NR1A/NR2A). Similar results were obtained with five to seven oocytes for each combination of subunits, and histamine stimulation was seen at NR1A/NR2B receptors in all batches of oocytes. D, A schematic diagram of the NR1 cDNA is shown to illustrate the absence (−) or presence (+) of the 5′ (amino-terminal) and 3′ (carboxyl-terminal) alternatively spliced inserts in NR1A, NR1B, and NR1E.

sponse to NMDA in oocytes voltage-clamped at −70 mV but had no effect in oocytes voltage-clamped at −25 mV. Histidine (100 μM), the metabolic precursor of histamine, had no effect on responses to NMDA (six oocytes; data not shown). Notably, the histamine metabolite 1-methylhistamine (*tele*-methylhistamine) potentiated NMDA currents and produced a larger effect than histamine (Fig. 3). Thus, 1-methylhistamine may be more potent than histamine itself at NR1A/NR2B receptors. 1-Methylhistamine is not active at H_1, H_2, or H_3 receptors (3). In contrast to 1-methylhistamine, another imidazole ring-methylated derivative, 3-methylhistamine (10 μM), was inactive (Fig. 3). The results shown in Fig. 3 indicate that the effects of histamine have a structural specificity, and they suggest that an unsubstituted primary amino group on the ethaneamine moiety may be necessary for histamine stimulation at NMDA receptors, because stimulation was not seen with N^{α}-methylhistamine. The lack of activity of 3-methylhistamine also suggests that an unsubstituted nitrogen in position 3 (π) is important for histamine activity at NMDA receptors.

The H_1 receptor antagonist mepyramine (1 μM) partially inhibited responses to histamine and also produced a small inhibition of the response to NMDA (Table 1). However, a 10-fold higher concentration of mepyramine (10 μM) did not produce any further antagonism of the response to histamine (Table 1), indicating that mepyramine is not a competitive antagonist of the effect of histamine. The inhibition by mepyramine may represent nonspecific antagonism of NMDA receptors. Furthermore, two other H_1 receptor antagonists, (+)-chlorpheniramine and triprolidine, did not antagonize the effects of histamine at NR1A/NR2B receptors (Table 1). Responses to histamine were not affected by the H_2 receptor antagonist ranitidine or the H_3 receptor antagonist thioperamide (Table 1). Thus, the effects of histamine at recombinant NMDA receptors do not appear to be mediated through classical histamine receptors and may involve a direct action of histamine on the NMDA receptor.

Desensitization of the response to histamine. The response to histamine at NR1A/NR2B receptors is characterized by an initial peak that desensitizes to a steady state level. Desensitization of the response to histamine may be time dependent or may be activity dependent, requiring activation of NMDA receptors by NMDA and glycine. To distinguish between these possibilities, the effects of pre-exposure to histamine in the absence of receptor activation were determined (Fig. 4, A–C). Coapplication of histamine and NMDA produced an increase in the size of the NMDA current, which then decayed to a steady state level (Fig. 4, A–C, *control*). After a 2-min pre-exposure to histamine, the peak response to coapplication of histamine and NMDA was smaller than that seen without pre-exposure, but the steady state effect of histamine was not altered (Fig. 4, A–C, *histamine pre-tx*). Thus, desensitization of the response to histamine is time dependent and can occur without channel activation.

Recovery from desensitization was monitored by applying pulses of histamine, separated by various time intervals, in the presence of NMDA (Fig. 4D). After brief (15-sec) or prolonged (≥90-sec) washout after desensitization by histamine, the peak of the second response to histamine was reduced but the response desensitized to the same level as that seen with the first application of histamine (Fig. 4D). Recovery from desensitization required 2–4 min for full recovery of the peak response to histamine (data not shown).

Voltage dependence. Experiments were carried out to determine whether the effects of histamine on NMDA receptors were dependent on the membrane potential. The effects of histamine (10–1000 μM) were studied in oocytes voltage-clamped at different holding potentials (Fig. 5). Peak and steady state responses to 10 μM histamine were not voltage dependent (Fig. 5A). The steady state response to 100 μM histamine showed a very weak voltage dependence, with the response being reduced at hyperpolarized membrane potentials (Fig. 5B). Responses to 1000 μM histamine showed a marked voltage dependence, with the peak and steady state responses being reduced at hyperpolarized membrane potentials (Fig. 5C).

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

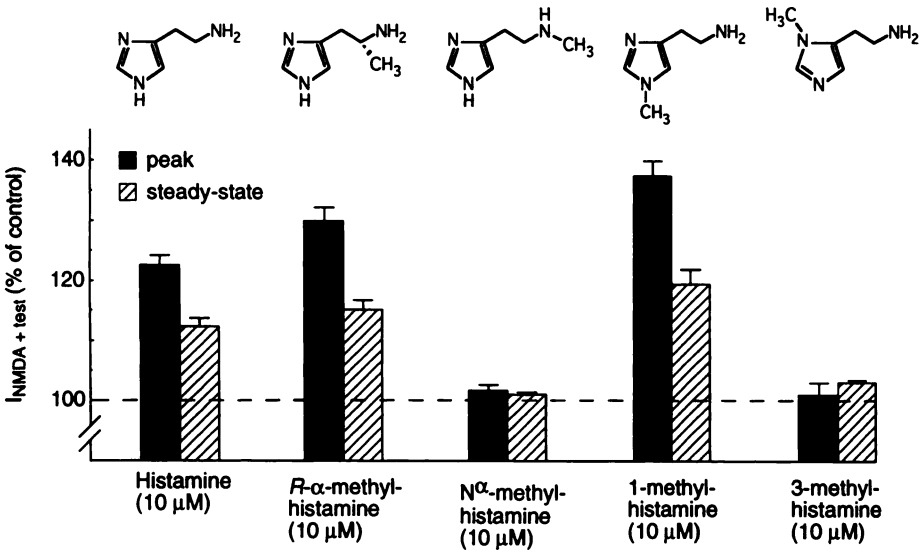


Fig. 3. Structure-activity relationships. The effects of histamine and methylhistamine analogues on responses to NMDA (100 μM; with 10 μM glycine) were determined in oocytes expressing NR1A/NR2B receptors and voltage-clamped at −70 mV. Responses to NMDA measured in the presence of histamine and methylhistamine are expressed as a percentage of the control response to NMDA. Values are means ± standard errors from seven oocytes, except for 3-methylhistamine (six oocytes). The structures of histamine and the methylated derivatives are also shown.

TABLE 1

Effects of histamine receptor antagonists at NR1A/NR2B receptors

Control responses to NMDA plus histamine are the means of responses measured before and after tests in the presence of antagonists. All values are means ± standard errors and are expressed as a percentage of the response to NMDA measured in the absence of histamine and antagonists.

Antagonist	NMDA		NMDA + 100 μM histamine				n^a
			Peak		Steady state		
	Control	+Antagonist	Control	+Antagonist	Control	+Antagonist	
	% of response to NMDA		% of response to NMDA				
Mepyramine, 1 μM (H_1)	100	95 ± 1[b]	150 ± 2	133 ± 2[b]	112 ± 2	105 ± 1[b]	9
Mepyramine, 10 μM (H_1)	100	95 ± 1[b]	148 ± 3	135 ± 2[b]	115 ± 1	105 ± 1[b]	8
(+)-Chlorpheniramine, 1 μM (H_1)	100	100 ± 1	143 ± 3	140 ± 2	112 ± 1	111 ± 1	7
Triprolidine, 1 μM (H_1)	100	100 ± 1	146 ± 3	141 ± 2	109 ± 1	108 ± 1	6
Ranitidine, 1 μM (H_2)	100	100 ± 1	148 ± 2	144 ± 2	110 ± 1	110 ± 1	6
Thioperamide, 1 μM (H_3)	100	99 ± 1	138 ± 3	135 ± 2	111 ± 2	106 ± 2	6

[a] *n*, number of oocytes.
[b] $p < 0.01$ (paired *t* test).

During the steady state phase of the response to histamine, a voltage-dependent inhibition of the NMDA-induced current was seen with 1000 μM histamine (Fig. 5C).

The voltage-dependent inhibition seen with 1 mM histamine was more pronounced on the steady state response than the peak response to histamine (Fig. 5C). To determine whether this may be due to a relatively slow onset of the voltage-dependent block, the effects of 1 mM histamine were determined with voltage jumps after equilibration of histamine with oocytes voltage-clamped at +10 mV (Fig. 6). At this holding potential, NMDA induced a small outward current and the steady state response to NMDA plus 1 mM histamine was similar to the NMDA response (Fig. 6). After a hyperpolarizing step to −100 mV, voltage-dependent inhibition by histamine developed immediately. Thus, in this paradigm, the voltage-dependent block has a very rapid onset, suggesting that the apparent discrepancy seen with peak and steady state effects of 1 mM histamine (Fig. 5C) may be due to the relatively slow solution exchange time of the bath, rather than the kinetics of the voltage-dependent block.

These results indicate that the peak responses, steady state responses, and desensitization of responses seen with low (10–100 μM) concentrations of histamine are not voltage dependent but an additional voltage-dependent inhibition occurs with a high (1000 μM) concentration of histamine. Voltage-dependent inhibition by 1000 μM histamine was also seen at NR1A/NR2A receptors (data not shown) and at NR1B/NR2B receptors (Fig. 7), which are not stimulated by histamine. Voltage-dependent inhibition by histamine is therefore unrelated to the stimulatory effect of histamine and may represent weak nonspecific antagonism.

Concentration-response relationships and agonist dependence. Concentration-response curves for histamine at NR1A/NR2B receptors were determined using oocytes voltage-clamped at −25 mV, to minimize the voltage-dependent inhibition seen with high concentrations of histamine. Both the peak and steady state effects of histamine were concentration dependent (Fig. 8, A and B). The curve for the peak response to histamine had an EC_{50} of 10 μM and a Hill slope close to unity, suggesting that histamine acts at a single site to potentiate responses to NMDA (Fig. 8B). The time constant of desensitization was determined by fitting the declining phase of the histamine response to a single-exponential function (Fig. 8C, *inset*). The rate of desensitization became faster with increasing concentrations of histamine, with a maximal effect at 100–300 μM histamine (Fig. 8C).

The stimulatory effect of histamine was found to be dependent on the concentration of NMDA used to activate NR1A/NR2B receptors. Histamine had little or no effect on responses to 10 μM NMDA but produced a marked potentiation of the

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

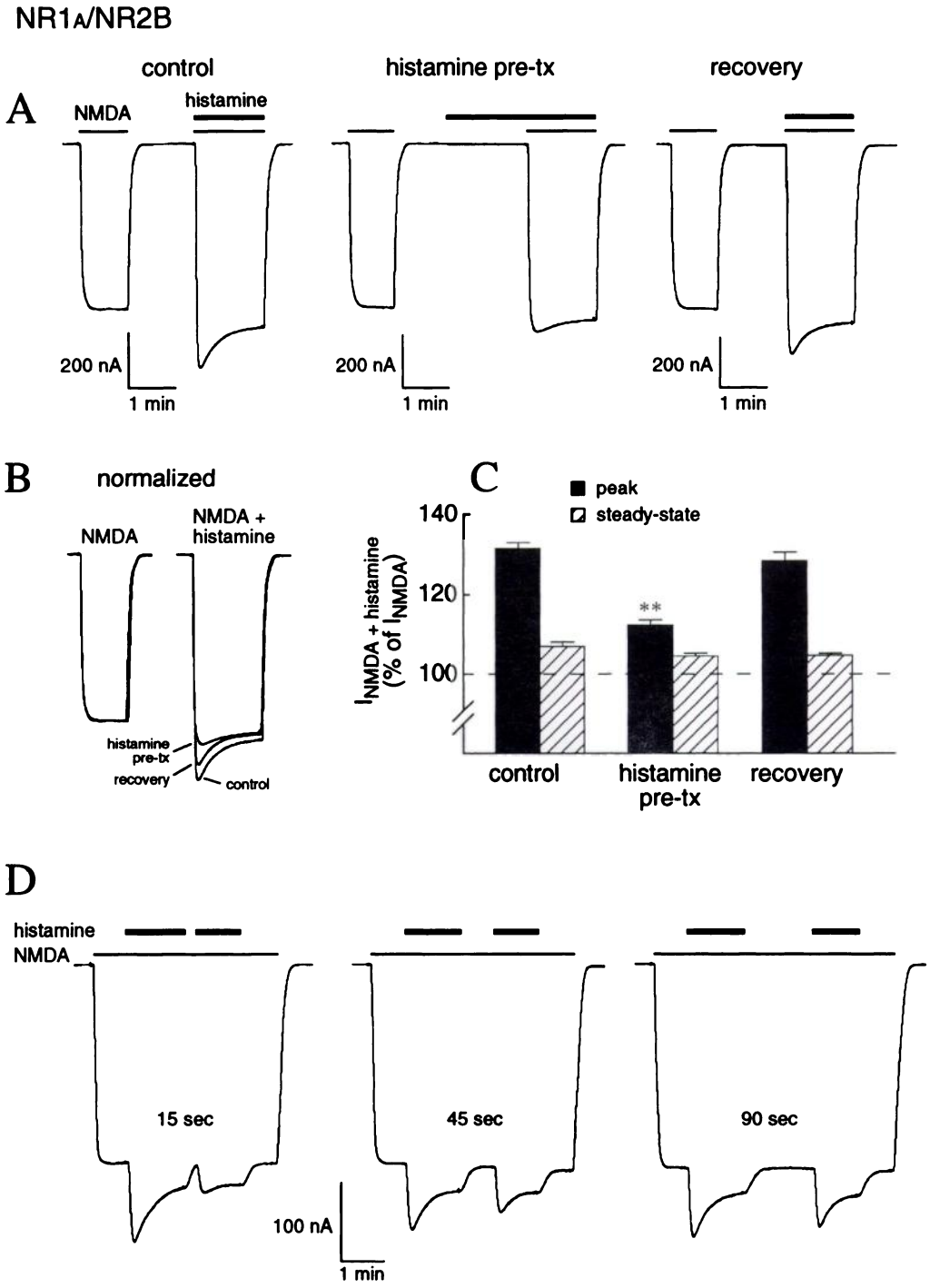


Fig. 4. Onset and recovery of desensitization with histamine. A, The effects of 100 μM histamine on responses to NMDA (100 μM; with 10 μM glycine) were measured without (*control* and *recovery*) and after (*histamine pre-tx*) a 2-min pretreatment of an oocyte with histamine. Note that desensitization of the response to histamine (the decline from peak to steady state) is almost complete after pre-exposure to histamine. B, Responses to NMDA and NMDA plus histamine have been normalized to the NMDA response under each condition. C, Peak and steady state responses to NMDA plus histamine were measured using the protocol described for A and are expressed as a percentage of the response to NMDA under each condition. Values are means ± standard errors from five oocytes. **, $p < 0.01$, compared with control peak (repeated-measures analysis of variance with *post hoc* Dunnett's test). D, The recovery of the response to histamine after desensitization was measured. Currents were recorded from an oocyte expressing NR1A/NR2B receptors and voltage-clamped at −70 mV. NMDA (100 μM; with 10 μM glycine) and histamine (100 μM) were applied during the times shown by the *horizontal bars*. In the three sets of traces, pulses of histamine were separated by intervals of 15, 45, and 90 sec. Similar results were obtained in seven oocytes, and full recovery required 2–4 min between applications of histamine.

response to 100 μM NMDA in the same oocytes. With 10 μM NMDA potentiation by histamine was 3 ± 1% (peak) and 0.7 ± 0.5% (steady state), and with 100 μM NMDA potentiation was 40 ± 6% (peak) and 8 ± 2% (steady state) (mean ± standard error, 10 oocytes). A similar dependence of histamine stimulation on agonist concentration was seen when L-glutamate, rather than NMDA, was used to activate NR1A/NR2B receptors (Fig. 9). Histamine produced a small decrease in the

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

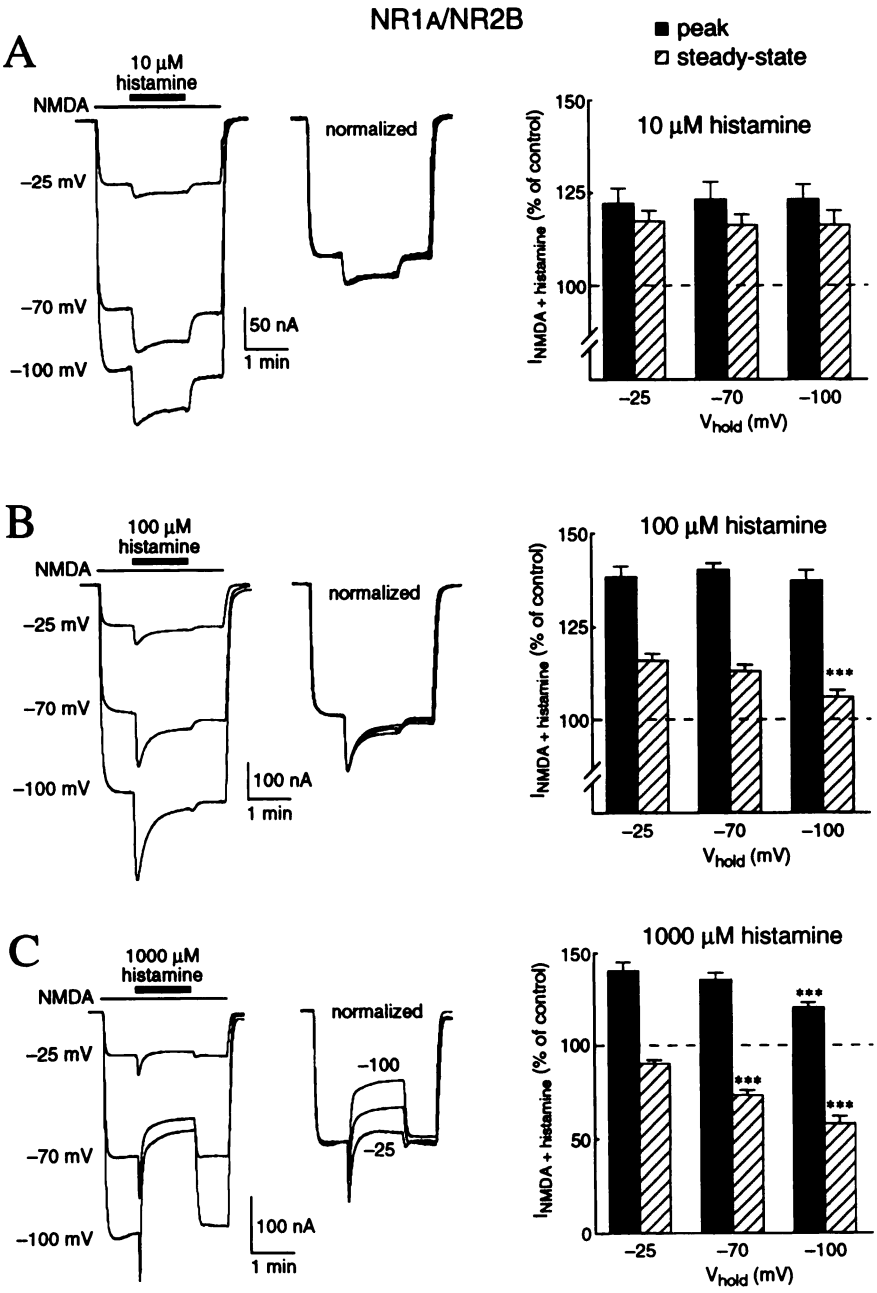


Fig. 5. Voltage dependence of the effects of histamine. The effects of 10 μM (A), 100 μM (B), and 1000 μM (C) histamine on responses to NMDA (100 μM; with 10 μM glycine) were determined in oocytes expressing NR1A/NR2B receptors and voltage-clamped at −25, −70, or −100 mV. *Left*, superimposed traces from oocytes voltage-clamped at each holding potential. *Center*, responses normalized to the control response measured at each holding potential. *Right*, means ± standard errors from five or six oocytes. Peak and steady state responses to NMDA plus histamine are expressed as a percentage of the control response to NMDA at each holding potential. ***, $p < 0.001$, compared with −25 mV (repeated-measures analysis of variance with *post hoc* Bonferroni test for multiple comparisons).

apparent affinity for glutamate at NR1A/NR2B receptors (Fig. 9A). It is possible that a decrease in agonist affinity, which will reduce the response to glutamate or NMDA, counteracts the effects of histamine at low concentrations of agonist.

Histamine stimulation showed only a small dependence on the concentration of glycine used to activate NR1A/NR2B receptors. Histamine increased NMDA currents by 60 ± 5% (peak) and 10 ± 2% (steady state) in the presence of 10 μM glycine and by 84 ± 8% (peak) and 20 ± 5% (steady state) in the presence of 0.1 μM glycine (mean ± standard error, seven oocytes). Control responses to NMDA in these oocytes were 340 ± 55 nA (10 μM glycine) and 35 ± 8 nA (0.1 μM glycine).

Interaction with polyamines. The stimulatory effect of histamine is similar in some respects to stimulation by spermine that is seen in the presence of saturating concentrations of glycine. Stimulation by histamine and by spermine occurs at receptors containing NR1A but not at receptors containing NR1B. Similarly, both agents have stimulatory effects at NR1A/NR2B but not NR1A/NR2A or NR1A/NR2C receptors (13), and the degree of potentiation is dependent on agonist concentration (21). Thus, the effects of histamine and spermine may involve common mechanisms or binding sites on the NMDA receptor. However, unlike histamine stimulation, the effect of spermine on NMDA currents does not desensitize (Fig. 10A) (13, 21).

To investigate possible interactions between histamine and spermine, experiments were carried out to determine whether the effects of these agents were additive and to determine whether spermine can enhance NMDA currents after desensitization of the response to histamine (Fig. 10). To minimize voltage-dependent inhibition by spermine, oocytes were voltage-clamped at −25 mV. At this holding potential the inhibitory

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

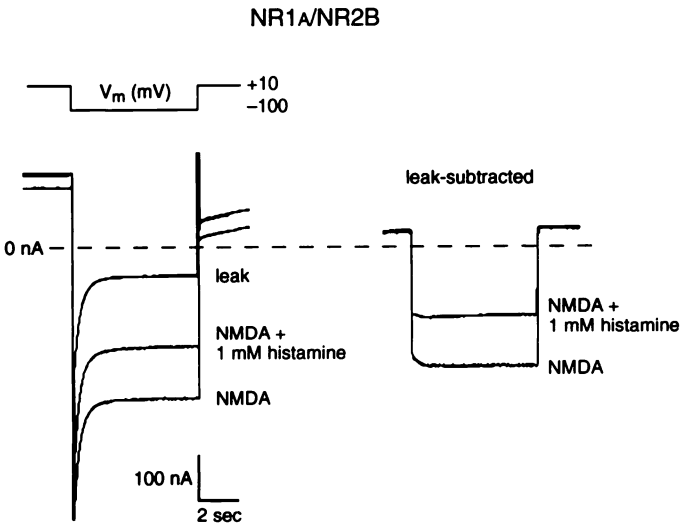
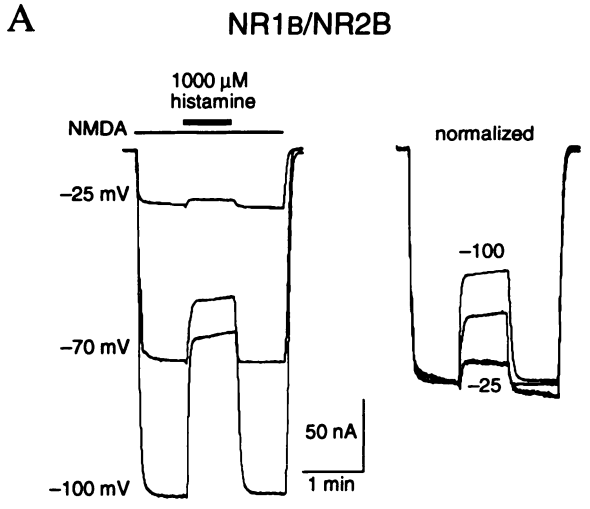


Fig. 6. Voltage-dependent block at NR1A/NR2B receptors. *Left*, hyperpolarizing steps from +10 to −100 mV were applied in the absence (leak current) or presence of NMDA (100 μM; with 10 μM glycine) or NMDA plus 1 mM histamine. *Right*, leak currents have been subtracted from currents measured with NMDA and NMDA plus histamine. The capacitance transients at the beginning and end of the voltage steps have been mostly removed for presentation. Similar results were obtained with four oocytes.



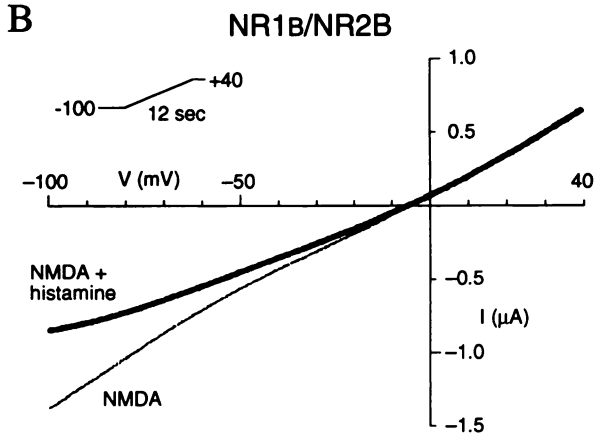


Fig. 7. Voltage-dependent block at NR1B/NR2B receptors. A, The effects of 1000 μM histamine on responses to NMDA (100 μM; with 10 μM glycine) were determined in an oocyte expressing NR1B/NR2B receptors and voltage-clamped at −25, −70, or −100 mV. B, In another oocyte, current-voltage curves were constructed by ramping the command potential from −100 to +40 mV over a period of 12 sec during steady state responses induced by NMDA or NMDA plus histamine. Leak currents have been subtracted. Similar results were obtained in five oocytes.

effect of 100 μM spermine is negligible or absent (13, 21). The protocol for these experiments is illustrated in Fig. 10A. Spermine (100 μM) increased the response to NMDA by 66 ± 5%, and histamine (100 μM) increased the response by 54 ± 4% (peak) and 20 ± 2% (steady state) (Fig. 10B, *a*, *b*, and *c*). When applied in the presence of spermine, histamine produced only a 10 ± 1% (peak) and 7 ± 5% (steady state) further increase in the size of the response (Fig. 10A, *trace d/e*). Thus, the effects of histamine and spermine are not additive. Furthermore, the degree of desensitization of the response to histamine was reduced in the presence of spermine (Fig. 10, C and D). This may suggest that spermine prevents or reduces desensitization of the response to histamine. When the effects of spermine were measured during steady state responses induced by histamine (Fig. 10, A and B, *trace f*), spermine still produced a marked stimulation (50 ± 5%) of the response to NMDA and histamine. Thus, spermine stimulation is not prevented or blocked after desensitization of the response to histamine.

Discussion

Subunit specificity, site of action, and relationship to native NMDA receptors. Two reports have shown that histamine can increase responses mediated by NMDA receptors in cultured or acutely isolated neurons (1, 2). In the present work it was found that a stimulatory effect of histamine is retained at certain subtypes of recombinant NMDA receptors expressed in *Xenopus* oocytes. As was seen in studies of hippocampal neurons (1, 2), the pharmacological profile of the effect of histamine is not consistent with an action at H_1, H_2, or H_3 histamine receptors, although one cannot unequivocally exclude the possibility that histamine acts at an atypical histamine receptor that is endogenous to *Xenopus* oocytes. However, several observations suggest that this is not the case and that histamine acts directly on the NMDA receptor, presumably at an extracellular site, to increase macroscopic NMDA currents. The potency of histamine at recombinant NR1A/NR2B receptors (EC_{50} = 10 μM) is similar to its potency for enhancing NMDA receptor-mediated excitatory postsynaptic currents in hippocampal neurons (EC_{50} = 9 μM) (1). Moreover, the effects of histamine are highly subunit specific, being seen only with certain combinations of subunits coexpressed to form heteromeric NMDA receptors.

Stimulation by histamine was seen only at receptors containing NR2B in combination with a variant of NR1 that lacks the amino-terminal insert (NR1A or NR1E). The observation that histamine potentiates NMDA responses in hippocampal neurons isolated from neonatal or young rats (1, 2) is consistent with the observation that NR2B is the predominant NR2 subunit in developing rat hippocampus and that NR1 variants lacking the amino-terminal insert are the major forms of NR1 in rat hippocampus (22, 23).[1] The NR2A subunit is also ex-

[1] J. Zhong, D. B. Pritchett, K. Williams, and P. B. Molinoff, unpublished observations.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

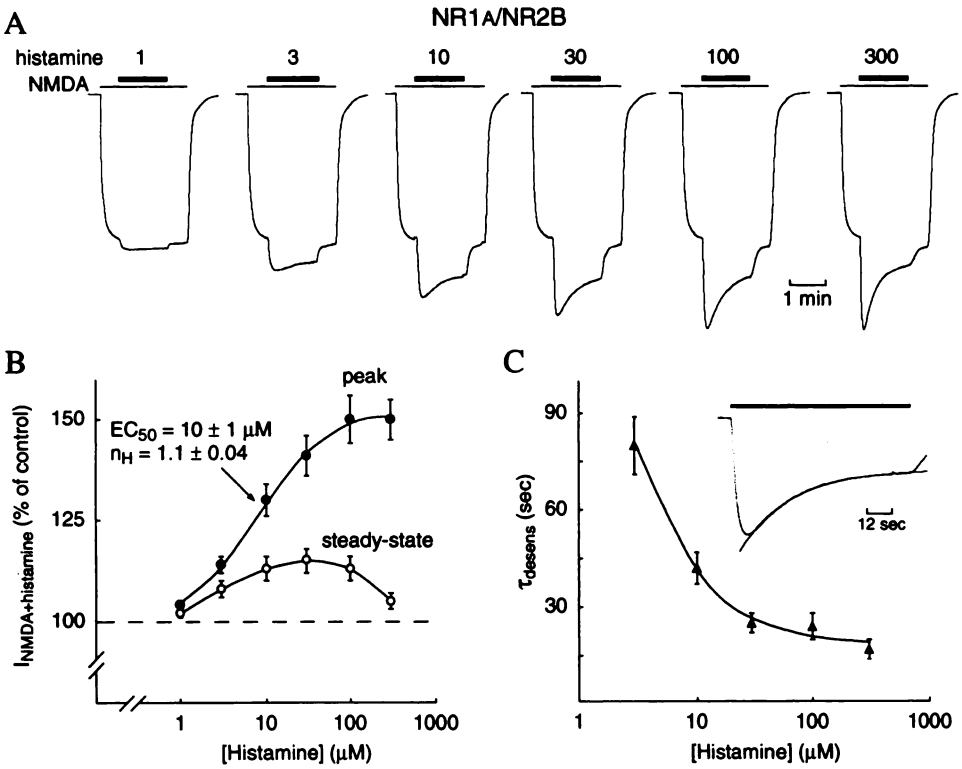


Fig. 8. Concentration-response relationships for histamine. A, The effects of histamine on responses to NMDA (100 μM; with 10 μM glycine) were determined in an oocyte expressing NR1A/NR2B receptors and voltage-clamped at −25 mV. For comparison, responses have been normalized to the control response to NMDA (−267 to −238 nA) measured immediately before application of histamine, because NMDA responses showed a small run-down over time. B, Currents measured in the presence of NMDA plus histamine are expressed as a percentage of the control response to NMDA. Data for peak responses were fit to a logistic function to obtain values for the EC_{50} and Hill slope (n_H). Values are means ± standard errors from 13 oocytes. C, The time constant of desensitization (τ_{desens}) was obtained by fitting the declining phase of the histamine response to a single-exponential function. *Inset*, an example for 100 μM histamine.

pressed in developing rat hippocampus, and its mRNA is present in adult hippocampus at levels similar to those of NR2B (8, 9, 24, 25).[1] We have previously shown that two populations of NMDA receptors, having properties that correspond to those of recombinant NR1A/NR2A and NR1A/NR2B receptors (16), are coexpressed in developing rat forebrain and cultured cortical neurons (15, 26). Expression of different NR2 subunits in cultured neurons is time dependent and is affected by the composition of the culture medium (26). Cell-specific expression of receptors containing NR2A or NR2B may explain the observation reported by Vorobjev *et al.* (2) that histamine stimulation of NMDA responses did not occur on all hippocampal neurons.

The requirement for histamine stimulation of an NR1 variant that lacks the amino-terminal insert is similar to that seen for stimulation by polyamines and Zn^{2+} (7, 13, 14). The amino-terminal insert is located in a putative extracellular region of the NR1 subunit (6, 27). The presence of this insert may interfere with binding of cationic molecules such as histamine, polyamines, and Zn^{2+}. Alternatively, these cations may have a common mechanism of action to alter channel gating that is not manifested in receptors containing NR1 variants with the amino-terminal insert because the presence of the insert itself directly alters channel gating properties. Similarly, different NR2 subunits may alter the binding of histamine or influence channel gating such that histamine stimulation is manifested in receptors containing NR2B but abolished in receptors containing NR2A or NR2C.

At high concentrations of histamine, a voltage-dependent inhibition was observed at NR1A/NR2B receptors. This effect may explain the bell-shaped dose-response curve for histamine reported by Vorobjev *et al.* (2), who found stimulation of NMDA responses at low concentrations of histamine (1–100 μM) and inhibition at high concentrations of histamine (500–1000 μM) in neurons voltage-clamped at −75 mV. Voltage-dependent inhibition, which is also seen at receptors (NR1B/NR2B) that are not sensitive to stimulation by histamine, appears to be unrelated to the stimulatory effect and may represent weak nonspecific antagonism, possibly open-channel block, by histamine.

Mechanism of action and similarities to effects of polyamines. In the presence of a saturating concentration of glycine, stimulation by histamine is similar to stimulation by spermine, in that both agents potentiate responses at receptors containing NR1A but not NR1B in combination with NR2B but not NR2A or NR2C (13). The effects of histamine show other similarities to the effects of spermine. Stimulation by both agents is dependent on the concentration of NMDA or glutamate used to activate NR1A/NR2B receptors (21). In the case of spermine, this appears to be due to a decrease in agonist affinity that masks spermine stimulation at low concentrations of agonist (21). Such a mechanism could also account for the agonist-dependent effects of histamine. If histamine decreases agonist affinity and this involves an increase in the rate of dissociation of glutamate from the receptor, then such an effect could account for the histamine-induced increase in the rate of decay of NMDA receptor-mediated synaptic currents reported by Bekkers (1).

The stimulatory effects of spermine and histamine on NR1A/NR2B receptors were not additive. A similar finding was reported for histamine and polyamines at NMDA receptors on cultured neurons (2). These observations may suggest that histamine and spermine share a common site or mechanism of action. Alternatively, the nonadditivity of these modulators may simply reflect potentiation of NMDA receptors by either agent towards a "maximal" state, which would presumably involve a maximum probability of channel opening. Furthermore, stimulation by spermine was not altered in the presence

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

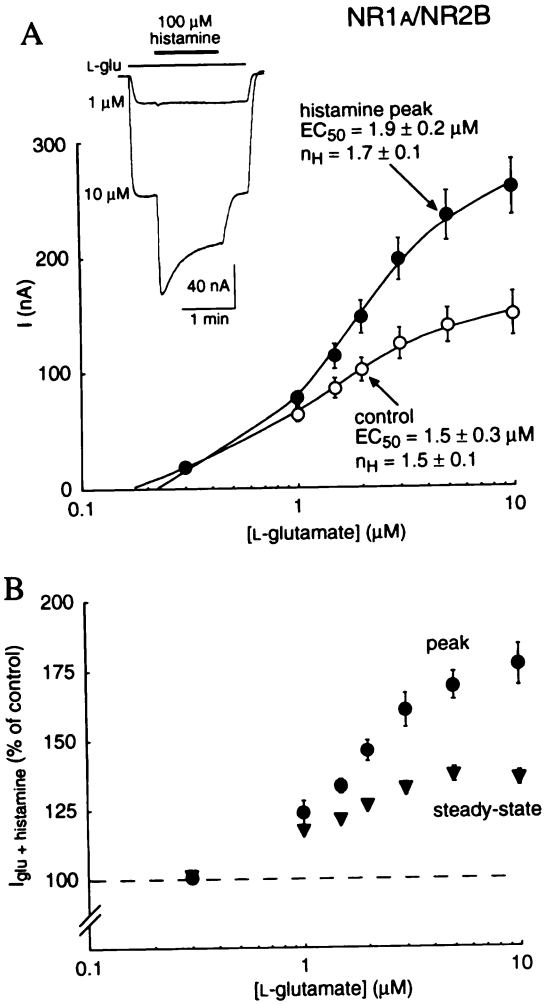


Fig. 9. Dependence of histamine stimulation on agonist concentration. A, The effects of histamine (100 μM) on responses to various concentrations of glutamate were determined in oocytes expressing NR1A/NR2B receptors and voltage-clamped at −25 mV. Data for control responses to glutamate and the peak response to histamine are shown. *Inset*, inward currents induced by 1 and 10 μM glutamate in the absence and presence of histamine in one oocyte. B, Peak and steady state responses to glutamate plus histamine are expressed as a percentage of the control response at each concentration of glutamate. Values are means ± standard errors from five oocytes.

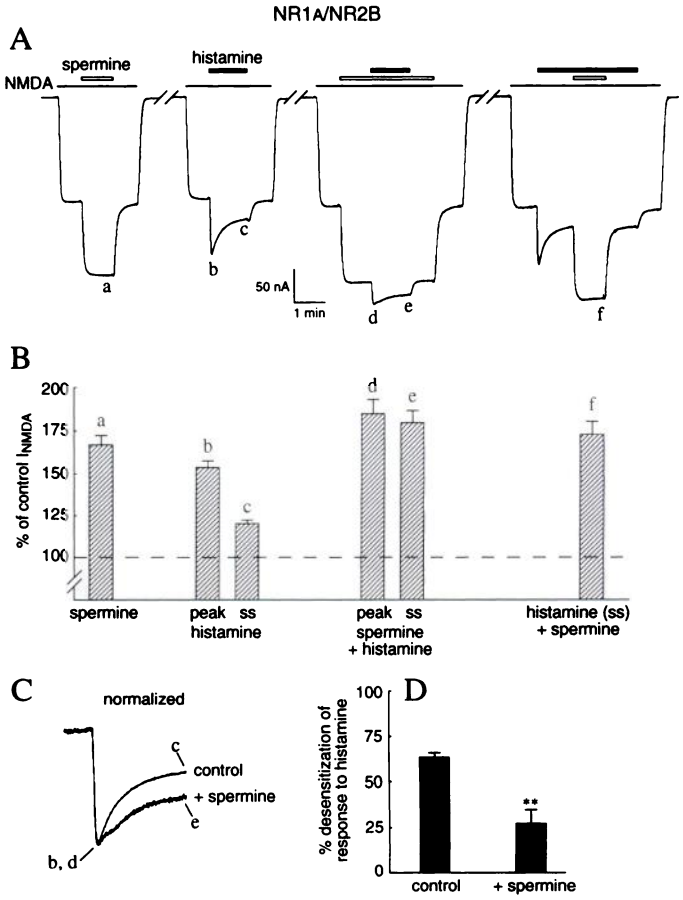


Fig. 10. Effects of histamine and spermine on NMDA receptors. A, Effects of spermine (100 μM), histamine (100 μM), spermine plus histamine, and histamine plus spermine on responses to NMDA (100 μM; with 10 μM glycine) were measured in an oocyte expressing NR1A/NR2B receptors and voltage-clamped at −25 mV. NMDA, spermine, and histamine were applied during the times shown by the *horizontal bars*. Note that the degree of stimulation by histamine measured in the presence of spermine (*trace d/e*) is smaller than that seen in the absence of spermine (*trace b/c*) but that spermine is equally effective when applied in the absence of histamine (*trace a*) or during the steady state (*ss*) response to histamine (*trace f*). B, The effects of spermine and histamine were determined using the protocol shown in A, and all data are expressed as a percentage of the control response to NMDA. Values are means ± standard errors from seven oocytes. *a–f*, Responses measured under various conditions shown in A. C, Responses to histamine measured in the absence (control) (*trace b/c*) and presence (*trace d/e*) of spermine have been normalized to the peak response to histamine. D, The percentage desensitization of the peak response to histamine was calculated as shown in C; thus, a greater desensitization occurs under control conditions than in the presence of spermine. **, $p < 0.01$ (paired *t* test).

of histamine when spermine was applied after desensitization of the histamine response. This suggests that spermine and histamine do not share a common binding site on the NMDA receptor.

Unlike spermine, which produces a sustained enhancement of the response to NMDA, the response to histamine shows marked desensitization. Histamine thus produces a novel and possibly unique form of potentiation of NMDA receptors. It is possible that the fade of the histamine response does not represent "desensitization" but, rather, is due to a separate, slowly developing, noncompetitive antagonism. However, the observation that histamine had no effect on responses to low concentrations of NMDA and glutamate suggests that desensitization of the response to histamine, seen with high concentrations of agonist, does not represent a direct inhibitory effect of histamine. If desensitization were due to a mechanistically distinct inhibitory effect, one would expect to see inhibition of NMDA and glutamate responses by histamine under conditions (low agonist concentration) where stimulation by histamine is absent. Furthermore, desensitization can be induced by preexposure to histamine in the absence of receptor activation, suggesting that desensitization may be due to a conformational change in the receptor that can occur during a closed-channel state. Thus, although desensitization does not represent a direct inhibitory effect of histamine, it can occur in the absence of measurable histamine stimulation and receptor activity.

Histaminergic innervation is widespread in the brain, including cerebral cortical and hippocampal areas (3). All of the three known subtypes of histamine receptors (H_1, H_2, and H_3) are found in the brain and presumably mediate many of the physiological effects of histamine in the central nervous system (3, 28). The present work, together with previous results describing

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012

effects of histamine at native NMDA receptors (1, 2), suggests that endogenous histamine may modulate the properties of some subtypes of NMDA receptors *in vivo*. In this context, the observation that 1-methylhistamine also markedly enhances the activity of NMDA receptors is relevant. 1-Methylhistamine is the major metabolite of histamine in the central nervous system and is present in very low levels in cerebrospinal fluid (3). Levels of 1-methylhistamine are increased after treatment with monoamine oxidase inhibitors. It is conceivable that 1-methylhistamine could act as a modulator of NMDA receptors and that the levels and activity of 1-methylhistamine could be altered in some therapeutic settings. The central histaminergic system is known to be involved in arousal and wakefulness (3) and could conceivably influence synaptic plasticity processes involving NMDA receptors in both the mature and developing nervous system. The specificity and novel mechanism of action of histamine at NMDA receptors also provide another handle for molecular studies designed to probe the structural and functional properties of NMDA receptors and the role of different forms of NMDA receptors in synaptic transmission and plasticity.

Acknowledgments

The author is grateful to Lena Zappia for technical assistance, to Drs. Perry Molinoff and Marc Dichter for comments on the paper, to Dr. S. Nakanishi for the NR1 clones, to Dr. P. H. Seeburg for the NR2A and NR2B clones, and to Dr. M. Mishina for the ε3 clone.

References

1. Bekkers, J. M. Enhancement by histamine of NMDA-mediated synaptic transmission in the hippocampus. *Science (Washington D. C.)* **261**:104–106 (1993).
2. Vorobjev, V. S., I. N. Sharonov, I. B. Walsh, and H. L. Haas. Histamine potentiates *N*-methyl-D-aspartate responses in acutely isolated hippocampal neurons. *Neuron* **11**:837–844 (1993).
3. Schwartz, J.-C., J.-M. Arrang, M. Garbarg, H. Pollard, and M. Ruat. Histaminergic transmission in the mammalian brain. *Physiol. Rev.* **71**:1–51 (1991).
4. Yamazaki, M., H. Mori, K. Araki, K. J. Mori, and M. Mishina. Cloning, expression and modulation of a mouse NMDA receptor subunit. *FEBS Lett.* **300**:39–45 (1992).
5. Moriyoshi, K., M. Masu, T. Ishii, R. Shigemoto, N. Mizuno, and S. Nakanishi. Molecular cloning and characterization of the rat NMDA receptor. *Nature (Lond.)* **354**:31–37 (1991).
6. Sugihara, S., K. Moriyoshi, T. Ishii, M. Masu, and S. Nakanishi. Structures and properties of seven isoforms of the NMDA receptor generated by alternative splicing. *Biochem. Biophys. Res. Commun.* **185**:826–832 (1992).
7. Hollmann, M., J. Boulter, C. Maron, L. Beasley, J. Sullivan, G. Pecht, and S. Heinemann. Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. *Neuron* **10**:943–954 (1993).
8. Monyer, H., R. Sprengel, R. Schoepfer, A. Herb, M. Higuchi, H. Lomeli, N. Burnashev, B. Sakmann, and P. H. Seeburg. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science (Washington D. C.)* **256**:1217–1221 (1992).
9. Ishii, T., K. Moriyoshi, H. Sugihara, K. Sakurada, H. Kadotani, M. Yokoi, C. Akazawa, R. Shigemoto, N. Mizuno, M. Masu, and S. Nakanishi. Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. *J. Biol. Chem.* **268**:2836–2843 (1993).
10. Kutsuwada, T., N. Kashiwabuchi, H. Mori, K. Sakimura, E. Kushiya, K. Araki, H. Meguro, H. Masaki, T. Kumanishi, M. Arakawa, and M. Mishina. Molecular diversity of the NMDA receptor channel. *Nature (Lond.)* **358**:36–41 (1992).
11. Meguro, H., H. Mori, K. Araki, E. Kushiya, T. Kutsuwada, M. Yamazaki, T. Kumanishi, M. Arakawa, K. Sakimura, and M. Mishina. Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature (Lond.)* **357**:70–74 (1992).
12. Ikeda, K., M. Nagasawa, H. Mori, K. Araki, K. Sakimura, M. Watanabe, Y. Inoue, and M. Mishina. Cloning and expression of the ε4 subunit of the NMDA receptor channel. *FEBS Lett.* **313**:34–38 (1992).
13. Williams, K., A. M. Zappia, D. B. Pritchett, Y. M. Shen, and P. B. Molinoff. Sensitivity of the *N*-methyl-D-aspartate receptor to polyamines is controlled by NR2 subunits. *Mol. Pharmacol.* **45**:803–809 (1994).
14. Durand, G. M., M. V. L. Bennett, and R. S. Zukin. Splice variants of the *N*-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proc. Natl. Acad. Sci. USA* **90**:6731–6736 (1993).
15. Williams, K., S. L. Russell, Y. M. Shen, and P. B. Molinoff. Developmental switch in the expression of NMDA receptors occurs *in vivo* and *in vitro*. *Neuron* **10**:267–278 (1993).
16. Williams, K. Ifenprodil discriminates subtypes of the *N*-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol. Pharmacol.* **44**:851–859 (1993).
17. Leonard, J. P., and S. R. Kelso. Apparent desensitization of NMDA responses in *Xenopus* oocytes involves calcium-dependent chloride current. *Neuron* **2**:53–60 (1990).
18. Boton, R., N. Dascal, B. Gillo, and Y. Lass. Two calcium-dependent chloride conductances in *Xenopus laevis* oocytes permeabilized with the ionophore A23187. *J. Physiol. (Lond.)* **408**:511–534 (1989).
19. Barish, M. E. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. *J. Physiol. (Lond.)* **342**:309–325 (1983).
20. White, M. M., and M. Aylwin. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. *Mol. Pharmacol.* **37**:720–724 (1990).
21. Williams, K. Mechanisms influencing stimulatory effects of spermine at recombinant *N*-methyl-D-aspartate receptors. *Mol. Pharmacol.* **46**:161–168 (1994).
22. Nakanishi, N., R. Axel, and N. A. Shneider. Alternative splicing generates functionally distinct *N*-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* **89**:8552–8556 (1992).
23. Standaert, D. G., C. M. Testa, J. B. Penny, Jr., and A. B. Young. Alternatively spliced isoforms of the NMDAR1 glutamate receptor subunit: differential expression in the basal ganglia of the rat. *Neurosci. Lett.* **152**:161–164 (1993).
24. Watanabe, M., Y. Inoue, K. Sakimura, and M. Mishina. Developmental changes in distribution of NMDA receptor channel subunit mRNAs. *NeuroReport* **3**:1138–1140 (1992).
25. Monyer, H., N. Burnashev, D. J. Laurie, B. Sakmann, and P. H. Seeburg. Developmental expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**:529–540 (1994).
26. Zhong, J., S. L. Russell, D. B. Pritchett, P. B. Molinoff, and K. Williams. Expression of mRNAs encoding subunits of the *N*-methyl-D-aspartate receptor in cultured cortical neurons. *Mol. Pharmacol.* **45**:846–853 (1994).
27. Anantharam, V., R. G. Panchal, A. Wilson, V. V. Kolchine, S. N. Triestman, and H. Bayley. Combinatorial RNA splicing alters the surface charge on the NMDA receptor. *FEBS Lett.* **305**:27–30 (1992).
28. Hill, S. J. Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol. Rev.* **42**:45–83 (1990).

Send reprint requests to: Keith Williams, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104–6084.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org at Zhejiang University on December 1, 2012