0026-895X/91/010045-07$03.00/0

MOLECULAR PHARMACOLOGY, 40:45-51

Effects of Bromowillardiine and Willardiine on Non-*N*-Methyl-D-aspartate Receptors in Postnatal Rat Hippocampal Neurons

CHARLES F. ZORUMSKI, LIU LIN THIO, and DAVID B. CLIFFORD

Departments of Psychiatry (C.F.Z., L.L.T.), Neurology (D.B.C.), and Anatomy and Neurobiology (C.F.Z., L.L.T.), Washington University Medical School, St. Louis, Missouri 63110

Received November 26, 1990; Accepted March 20, 1991

SUMMARY

The physiology and pharmacology of willardiine and bromowillardiine, structural analogues of quisqualate, were studied in cultured postnatal rat hippocampal neurons using whole-cell voltage-clamp techniques. These agonists appear to act at a shared non-*N*-methyl-D-aspartate (non-NMDA) receptor-channel complex and gate nonselective cationic currents. Willardiine currents desensitize rapidly and to a much greater degree than bromowillardiine currents. In addition, the brominated compound produces steady state currents that are 5 times larger than those produced by willardiine at saturation. Bromowillardiine is also a more efficacious excitotoxin, producing about 3-fold greater acute neuronal damage than willardiine at saturating concentrations. These results suggest that agonist structure affects the ability of non-NMDA agonists to induce desensitization and add support to the hypothesis that receptor desensitization serves to limit acute excitotoxicity in cultured neurons.

In vertebrate central neurons, responses mediated by the ionotropic non-NMDA class of glutamate receptors desensitize rapidly when activated by certain excitatory amino acids. Previous studies have shown that glutamate, quisqualate, and AMPA produce responses that decline to a steady state level with a time constant ranging from 10 to 80 msec in whole-cell experiments. In contrast, responses gated by kainate and domoate exhibit a sustained current during prolonged administration, with little evidence of desensitization (1–4).

These observations suggest either that the two classes of non-NMDA agonists act at separate receptor-channel complexes or that the receptor exhibits agonist-specific desensitization. The issue of whether kainate and quisqualate share a common receptor-channel complex has been addressed in several preparations. Based on physiological competition and cross-desensitization experiments in cultured hippocampal and spinal cord neurons (2, 5, 6), as well as *Xenopus* oocytes injected with rat brain mRNA (7, 8), it appears that these agents act at a common receptor-channel complex, suggesting the existence of a single ionotropic non-NMDA receptor. Furthermore, recent studies using cloned AMPA receptors demonstrate currents activated by kainate, quisqualate, and AMPA and interactions among these agonists (9, 10). Binding studies have indicated that separate quisqualate and kainate receptors exist in the nervous system (11), but in many areas these agonists show significant cross-sensitivity for the other receptor (12), making it unclear whether there is more than one non-NMDA receptor.

Willardiine and its brominated derivative, 5-bromowillardiine, are structural analogues of quisqualate that are reported to induce markedly different physiological responses (13). Willardiine is thought to be a specific and relatively potent agonist at quisqualate receptors (14), whereas bromowillardiine produces responses similar to kainate in several preparations (11, 13). In immature rat dorsal root C fibers, bromowillardiine is 3 times more potent than kainate in inducing depolarizations, whereas willardiine is ineffective (15).

The reasons for the differences in physiological responses between these structural analogues are uncertain. Given the observation that bromowillardiine is more "kainate-like" and willardiine is "quisqualate-like," differences in the ability to induce desensitization may account for the increased effectiveness of the brominated compound. Additionally, if these agents act at the same receptor-channel complex but differ in the ability to induce desensitization, chemical structure may determine whether a non-NMDA agonist can induce the conformational changes underlying desensitization. In this study, we have examined the physiology, pharmacology, and toxicity of willardiine and bromowillardiine in cultured postnatal rat hip-

This work was supported in part by Physician Scientist Awards MH00630 and MH45493 and fellowships from the Klingenstein Foundation and the Bantly Foundation.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-4-methylisoxazolepropionic acid; HEPES, *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; APV, 2-amino-5-phosphonovalerate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

pocampal neurons, with emphasis on identifying differences in efficacy, potency, and desensitization.

Materials and Methods

Cell culture. Hippocampal neurons were prepared from 1–3-day-old albino rat pups, using previously described methods (16). Hippocampi were rapidly dissected and incubated for 30 min in Leibovitz L-15 medium containing 1 mg/ml papain and 0.2 mg/ml fatty acid-free bovine serum albumin. Single neurons were gently dissociated by trituration in Eagle's minimal essential medium containing 5% (v/v) fetal calf serum, 5% horse serum, 400 μM glutamine, 50 μg/ml streptomycin, 50 units/ml penicillin, and 17 mM glucose. The resultant cell suspension was plated in 35-mm collagen-coated tissue culture dishes (Falcon), at an approximate density of 300,000 cells/dish, and incubated at 37° in a 5% CO_2 humidified atmosphere. Cytosine arabinoside (10^{-5} M) was added 72 hr after plating, to inhibit glial proliferation. All media, serum, and antibiotics were purchased from GIBCO (Grand Island, NY).

Electrophysiology. Voltage-clamp recordings were obtained, using gigaseal recording techniques (17), from cells maintained in culture for 3–7 days. Over this time, hippocampal neurons typically have input resistances of >500 MΩ and membrane-charging curves for 87% of neurons are described by a single-exponential process. For recording purposes, the growth medium was replaced with a salt solution containing (in mM): 140 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.3. Tetrodotoxin (0.5–1 μM) was added to decrease spontaneous synaptic activity and to improve the spatial voltage clamp.

Recording electrodes were pulled from fiber-filled borosilicate glass capillaries (WPI) on a Sutter P-87 pipette puller. These pipettes were fire polished to obtain 5–8-MΩ recording electrodes. Pipette solutions routinely contained (in mM): 140 CsCl, 4 NaCl, 10 HEPES, 2 $MgCl_2$, 5 EGTA, and 0.5 $CaCl_2$, pH 7.3. In some experiments, 140 mM cesium acetate was substituted for CsCl, to alter the chloride reversal potential, and the Mg^{2+} was omitted.

Whole-cell currents were recorded using a List EPC-7 patch-clamp amplifier. Signals were filtered at 1–3 kHz and recorded on a Gould 220 chart recorder. Filtered currents were also digitized (pCLAMP, version 5.5; Axon Instruments) and stored on disk for off-line analysis. In some analyses, currents were fit to exponential equations using a least-squares minimization or Gauss-Newton algorithm (pCLAMP or ASYSTANT; MacMillan Software). Desensitization was measured as percentage of decline:

$$\%\ \text{of decline} = \frac{I_{max} - I_{plateau}}{I_{max} - I_0} \times 100$$

where I_0 is the zero current level, I_{max} is the peak observed current, and $I_{plateau}$ is the observed steady state current after desensitization. All values are mean ± standard error.

Drug application. Excitatory amino acid agonists were dissolved in the bath solution and applied by close-range pressure ejection from glass pipettes positioned about 5 μm from the cell soma (2). This drug delivery system allows close positioning near neurons and reproducible application without discernable drug leakage. Using this system, currents gated by saturating concentrations of kainate take 71 ± 7 msec ($n = 26$) to achieve half-maximal amplitude. This reflects, in part, the speed of drug delivery, as well as the recovery of any receptors desensitized at baseline (1). In comparison, currents gated by quisqualate, willardiine, and bromowillardiine achieve half-maximal amplitude in 16 ± 2 msec ($n = 27$), 15 ± 1 msec ($n = 38$), and 21 ± 3 msec ($n = 29$), respectively.

Bromowillardiine and willardiine were dissolved in 1 N NaOH, whereas CNQX was dissolved in dimethylsulfoxide. Drug solutions were diluted to final concentration with bath solution at the time of experiment, with the pH adjusted to 7.3. Bromowillardiine and willardiine were purchased from Tocris Neuramin, whereas CNQX, D-APV, and quisqualate were purchased from Cambridge Research Biochemicals. Other agonists and salts were obtained from Sigma Chemical Company.

Toxicity studies. For studies of excitotoxicity, neurons were plated in 35-mm culture dishes that had been modified using a Mecanex BB-Form-2 dish imprinter to facilitate identification of fields. All toxicity experiments were performed on cultures maintained for >10 days. Dishes were rinsed thoroughly with the bath solution described above. Willardiine or bromowillardiine was applied for 20 min in the bathing solution. After agonist exposure, cultures were stained with 5 μM propidium iodide for 5 min. Propidium iodide is a dye that is excluded from living cells but crosses the membrane of dead or dying cells, where it interacts with DNA to impart a red fluorescence (18, 19). Cell damage was measured as the percentage of neurons stained with propidium iodide. Ten representative fields were counted in each culture. Statistical differences were determined using two-tailed t tests.

Results

When rapidly administered to postnatal rat hippocampal neurons that are voltage clamped at −50 mV, willardiine and bromowillardiine produce inward currents. Responses produced by 1 mM willardiine exhibit rapid desensitization, declining with a time constant of 35 ± 2 msec to a plateau level that is 50 ± 2% of the observed peak response ($n = 24$) (Fig. 1B). In contrast, responses mediated by 1 mM bromowillardiine exhibit considerably less desensitization (8 ± 2% decline, $n = 22$, $p < 0.001$) (Fig. 1A). Although the rapidity of desensitization leads to an underestimate of the true peak current and, hence, an underestimate of the actual percentage of decline in current, bromowillardiine produces steady state currents that are 5-fold greater than those produced by 1 mM willardiine, suggesting that there is a significant difference in desensitization between these agents.

The differences in desensitization and steady state current amplitudes between willardiine and bromowillardiine are similar to differences between quisqualate and kainate acting at non-NMDA receptors in cultured postnatal rat hippocampal neurons, with quisqualate currents exhibiting 72 ± 4% decline with a time constant of 49 ± 7 msec ($n = 10$). Kainate currents show no desensitization during administrations of 10–30 sec (Fig. 1C). Steady state currents produced by a saturating concentration of quisqualate are 24 ± 2% ($n = 25$) of those produced by a comparable concentration of kainate.

Willardiine and bromowillardiine activate currents in a dose-dependent fashion. Based on physiological dose-response data (Fig. 2), bromowillardiine is about 6 times more potent than willardiine (EC_{50} values for peak currents are 31 μM versus 173 μM). For both agonists, the Hill coefficient is similar (bromowillardiine, 1.21; willardiine, 1.16). At saturating concentrations, steady state currents produced by bromowillardiine are 510 ± 50% of those produced by willardiine ($n = 14$), indicating that at steady state the brominated compound is a more effective agonist as well. By comparison, steady state responses produced by a saturating concentration of bromowillardiine are 83 ± 4% of those produced by a similar concentration of kainate ($n = 8$), reflecting at least in part in desensitization produced by the brominated compound. The steady state currents activated by willardiine are 16 ± 5% ($n = 17$) of those produced by kainate.

Both willardiine and bromowillardiine activate currents via non-NMDA receptors. Similar to other agents acting at these receptors (20), current-voltage (I-V) curves for peak currents in the presence of extracellular magnesium are nearly linear

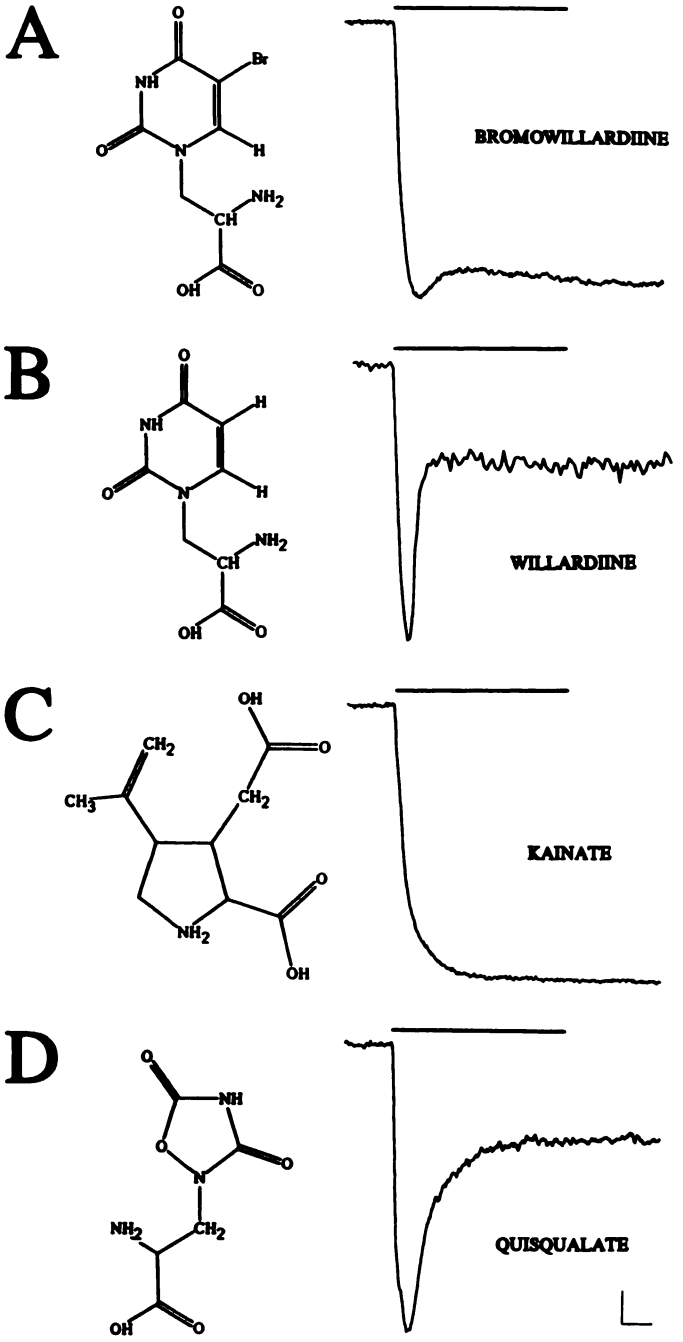


Fig. 1. Bromowillardiine and willardiine produce different degrees of desensitization. Neurons were voltage-clamped at −50 mV and exposed to 500-msec pressure applications of bromowillardiine (A) and willardiine (B). For comparison, responses to kainate (C) and quisqualate (D) are also shown. The agonist concentration was 1 mM for all except quisqualate, which was applied at 100 μM. The chemical structures of these agents are shown next to their respective physiological trace. *Bars above the current traces*, duration of agonist application. *Calibration bar*, A, 60 pA; B, 30 pA; C, 70 pA; D, 60 pA × 100 msec.

over the range of −85 to +50 mV, with reversal potentials of +1 ± 2 mV for willardiine (n = 3) and +4 ± 2 mV for bromowillardiine (n = 3) (Fig. 3). The reversal potentials are unaffected by changes in the chloride equilibrium potential, suggesting that the channels are cation selective.

CNQX, a competitive antagonist at non-NMDA receptors (21), competitively inhibits currents gated by both willardiine and bromowillardiine, with similar antagonist affinities (Fig. 4). The calculated IC_{50} for the CNQX inhibition of peak willardiine currents is 2.6 ± 0.1 μM, whereas it is 2.8 ± 0.2 μM for bromowillardiine. Currents activated by these agonists are insensitive to inhibition by D-APV, a competitive antagonist of NMDA receptors. Currents activated by willardiine and bromowillardiine in the presence of 100 μM D-APV are 100 ± 3% (n = 5) and 95 ± 3% (n = 5) of control, respectively (p = not significant).

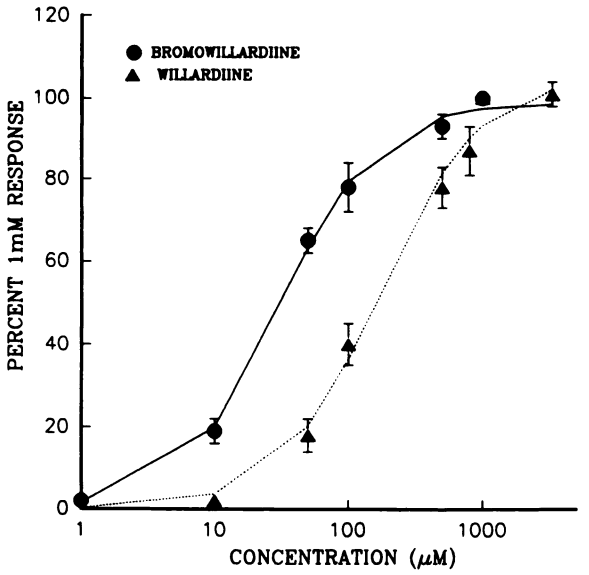


Fig. 2. Bromowillardiine and willardiine gate currents in a dose-dependent fashion. Neurons were voltage-clamped at −50 mV and exposed to 500-msec applications of agonists at various concentrations. Each neuron was exposed to three agonist concentrations, and peak responses were normalized with respect to the response at 1 mM. The data points represent mean ± standard error. These data were fit to a dose-response equation: response = $response_{max} \times ([A]^n/([A]^n + EC_{50}^n))$, where $response_{max}$ is the maximal response to the agonist, [A] is the agonist concentration, EC_{50} is the concentration producing a 50% maximal response, and N is the Hill coefficient (4). The equation was fit using a least squares minimization routine, allowing all three parameters to vary. The best fit curves are displayed. For bromowillardiine, $response_{max}$ = 99%, EC_{50} = 31 μM, and n = 1.21. For willardiine, $response_{max}$ = 106%, EC_{50} = 173 μM, and n = 1.16.

These physiological and pharmacological data suggest that willardiine and bromowillardiine act selectively at non-NMDA receptors. We have also observed that these agents are likely to share a common receptor-channel complex. When non-NMDA receptors are desensitized by exposure to willardiine, there is a concomitant decrease in the response to bromowillardiine (Fig. 5A). During a 500-msec agonist application, the peak current induced by bromowillardiine is diminished after pretreatment with willardiine. In contrast, when willardiine is administered to neurons pretreated with bromowillardiine, there is a rapid decrement in current, reflecting the lower efficacy and more complete desensitization produced by willardiine (Fig. 5B). When the two agonists are administered simultaneously to neurons, at saturating concentrations, the resulting current is always intermediate between the responses to the agonists alone, again suggesting that these agents share a common receptor-channel complex (Fig. 5C). The current obtained during coadministration of agonists at saturating concentrations is 97 ± 3% of the mean response to each agonist alone (n = 11). In other experiments, the effect of 100 μM

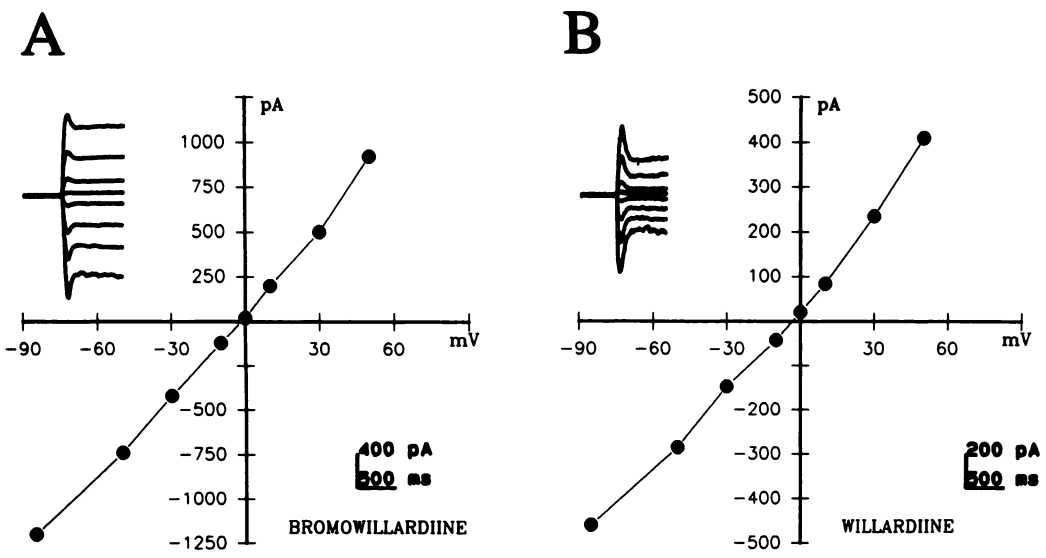


Fig. 3. Bromowillardiine and willardiine have linear current-voltage relationships. Neurons were voltage-clamped at various membrane potentials over the range of −85 to +50 mV and were administered 500-msec applications of 1 mM bromowillardiine (A) or 1 mM willardiine (B). Peak currents are plotted as a function of the holding potential. *Insets*, raw data used to construct the plots.

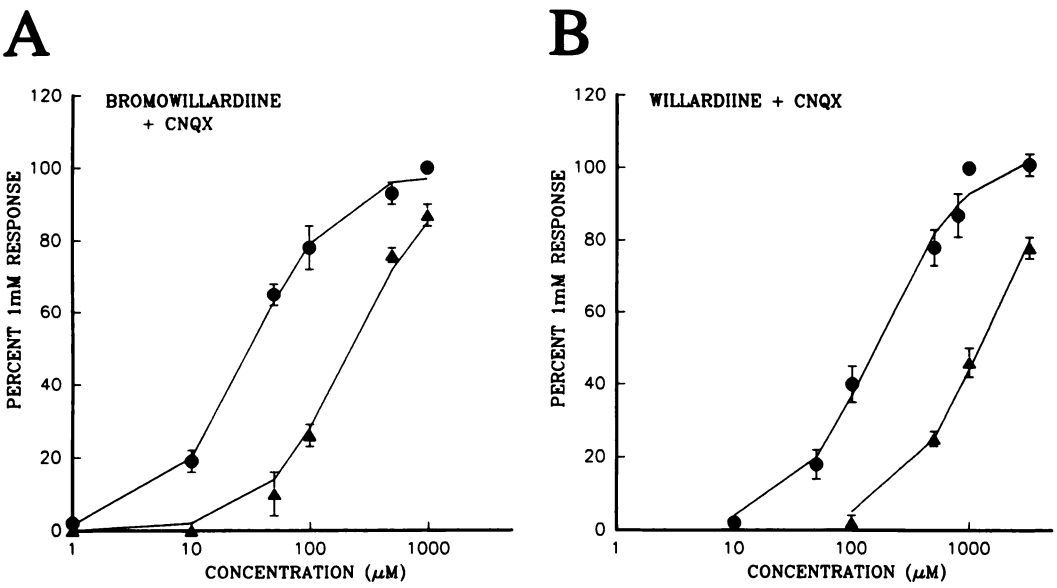


Fig. 4. CNQX is a competitive inhibitor of bromowillardiine (A) and willardiine (B). Neurons were voltage-clamped at −50 mV and administered 500-msec applications of various concentrations of agonists alone (●) and in combination with 10 μM CNQX (▲). Results were normalized with respect to the peak response to the agonist alone at 1 mM. The control curves were fit to the dose-response data described in the legend to Fig. 2. The curves in the presence of CNQX represent the fit to a competitive antagonism model: response = $\text{response}_{\text{max}_{\text{control}}} \times \{[A]'/([A]' + (EC_{50}' \times (1 + [I]'/IC_{50}')))\}$, where $\text{response}_{\text{max}_{\text{control}}}$, EC_{50}, and n are values for the agonist alone (Fig. 2), $[A]$ is the agonist concentration, $[I]$ is the concentration of CNQX, and IC_{50} is the concentration of antagonist producing 50% inhibition. The data shown represent the best fit of the data to this equation, using a least squares minimization routine. The IC_{50} was 2.8 ± 0.2 μM and 2.6 ± 0.1 μM for CNQX against bromowillardiine and willardiine, respectively. Noncompetitive or uncompetitive inhibition models inadequately described the experimental data.

willardiine on the bromowillardiine dose-response curve was examined using a coapplication paradigm. These experiments demonstrated a parallel rightward shift in the dose-response curve, suggesting a competitive interaction between the two agents (Fig. 6). As has been reported previously, we have found similar interactions between kainate and quisqualate in postnatal rat hippocampal neurons (2, 5, 6).

The differences in steady state efficacy between willardiine and bromowillardiine also extend to their actions as excitotoxins. Previously, we have found that agonists producing rapid desensitization at non-NMDA receptors exhibit significantly less acute excitotoxicity than nondesensitizing agonists (22). Similar differences in acute excitotoxicity are seen with willardiine and bromowillardiine (Fig. 7). When neurons are exposed to physiologically saturating concentrations of agonists for 20 min, a significantly greater proportion of neurons are damaged by the brominated compound (63 ± 2% versus 24 ± 7%, $n = 3$, $p < 0.05$).

Discussion

These studies indicate that willardiine and bromowillardiine act at a common non-NMDA excitatory amino acid receptor-

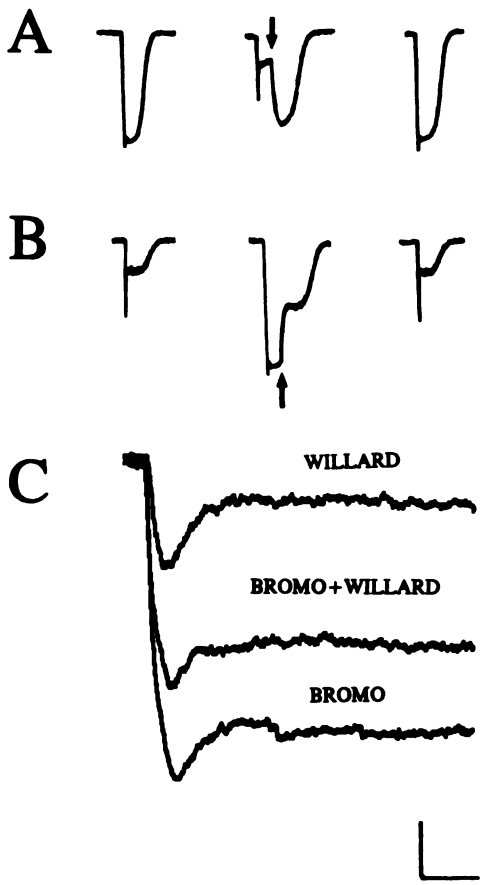


Fig. 5. Bromowillardiine and willardiine exhibit cross-desensitization and interact competitively. Neurons were voltage-clamped at −50 mV and exposed to agonist for 500 msec. A, *Left trace*, control response to 1 mM bromowillardiine. *Middle trace*, after an application of 1 mM willardiine, 1 mM bromowillardiine (*arrow*) produces a significantly smaller inward current. *Right trace*, recovery of the response to bromowillardiine 30 sec later. B, The converse experiment to that shown in A. *Left trace*, control response to 1 mM willardiine; *arrow*, response subsequent to 1 mM bromowillardiine. Note the decrease in current produced by willardiine. *Right trace*, recovery of the willardiine response. C, Coapplications of agonists reveal a competitive interaction between bromowillardiine and willardiine. The response of a single neuron to 500-msec applications of 1 mM bromowillardiine (*BROMO*), 1 mM (*WILLARD*), and the combination of 1 mM each (*BROMO + WILLARD*) is shown. The combination always gave a response that was intermediate between the responses to the agonists alone. *Calibration bar*, A and B, 200 pA, 10 sec; C, 200 pA, 100 msec.

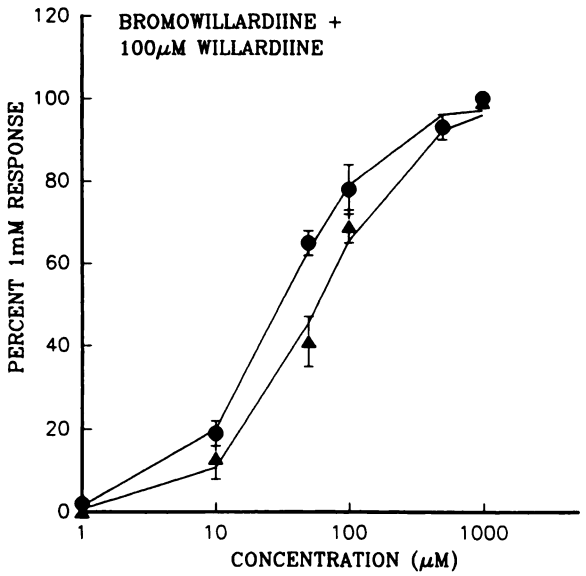
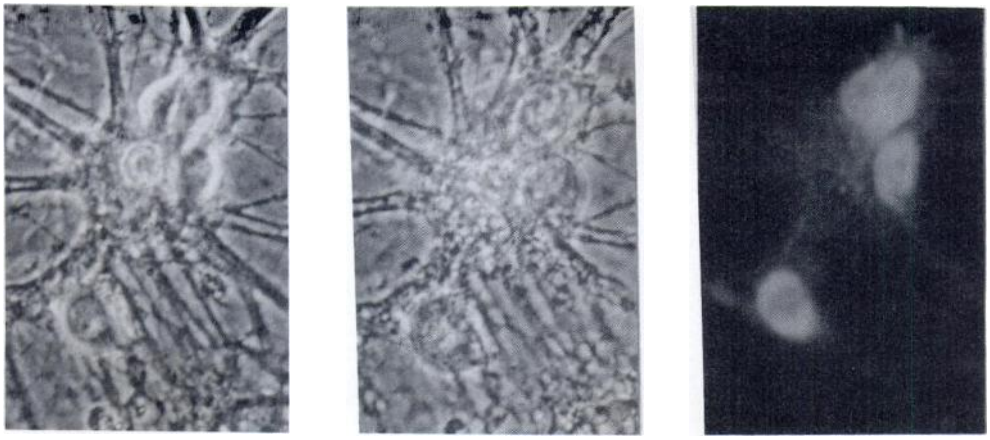


Fig. 6. Coapplications of bromowillardiine and willardiine demonstrate a competitive interaction. Using a coapplication paradigm similar to that described in Fig. 5C, the effect of 100 μM willardiine on the response to various concentrations of bromowillardiine was examined. The displayed data depict the control bromowillardiine dose-response curve (●) and the shift in the dose-response curve produced by willardiine (▲). *Points* represent mean ± standard error. *Solid lines* were fit using the equations in the legends to Figs. 2 and 4. The IC_{50} for willardiine was 94 ± 15 μM.

channel complex in cultured postnatal rat hippocampal neurons. The substitution of bromine for hydrogen at the 5-position of the willardiine ring enhances the steady state efficacy of the agonist by about 5-fold and decreases the ability of the molecule to promote desensitization. At comparable concentrations, bromowillardiine gates responses that are about 80% of those produced by the nondesensitizing non-NMDA agonist kainate. These results support previous observations that only one ionotropic non-NMDA receptor exists on cultured central nervous system neurons (2, 5, 6) and that desensitization contributes to differences in steady state efficacy among these agonists. However, we cannot exclude the possibility that bromowillardiine elicits a desensitizing current that declines with a time constant of <5 msec, which would not be detected in whole-cell recordings.

Prior studies have suggested that willardiine is a "quisqualate-like" agonist, whereas bromowillardiine is more "kainate-like" (13). Our results support this contention, in that willardiine, like quisqualate, gates responses that exhibit prominent desensitization, whereas bromowillardiine produces considerably larger currents that exhibit little use-dependent decline. However, unlike kainate, a rapidly desensitizing phase of response does occur with bromowillardiine. In postnatal hippocampal neurons, quisqualate activates peak currents with an EC_{50} of 36 μM (23), making this agent about 5 times as potent as willardiine. Evans *et al.* (14) have previously reported that quisqualate is about 5 times more potent than willardiine in a rat spinal cord preparation. Kainate is about 6 times less potent than bromowillardiine based on our dose-response data in cultured hippocampal neurons (EC_{50} values of 176 μM versus 31 μM), consistent with its lower potency in rat dorsal root C fibers (13). Yet, kainate is more effective than bromowillardiine based on steady state currents induced by comparable concentrations.

These data further support the notion that non-NMDA agonists can be divided into two classes, based on their actions in cultured neurons. One class includes glutamate, quisqualate, AMPA, 4-methylhomoibotenate, β-*N*-oxalylamino-L-alanine, and willardiine, agonists that induce rapidly desensitizing responses. The second group consists of kainate and domoate, agonists that fail to induce desensitization (1, 2, 4, 22). Based on competition and cross-desensitization experiments, all of these agonists appear to act at a common receptor-channel complex in cultured hippocampal neurons, suggesting that desensitization is agonist specific. Bromowillardiine is an intermediate agent, producing less desensitization and larger steady state currents than quisqualate-like agonists. This suggests that relatively simple changes in agonist structure may produce agonists more like one group or the other and that agonist structure may be important in allowing the conformational changes necessary for receptor desensitization. The addition of bromine to the willardiine structure increases the bulk of the



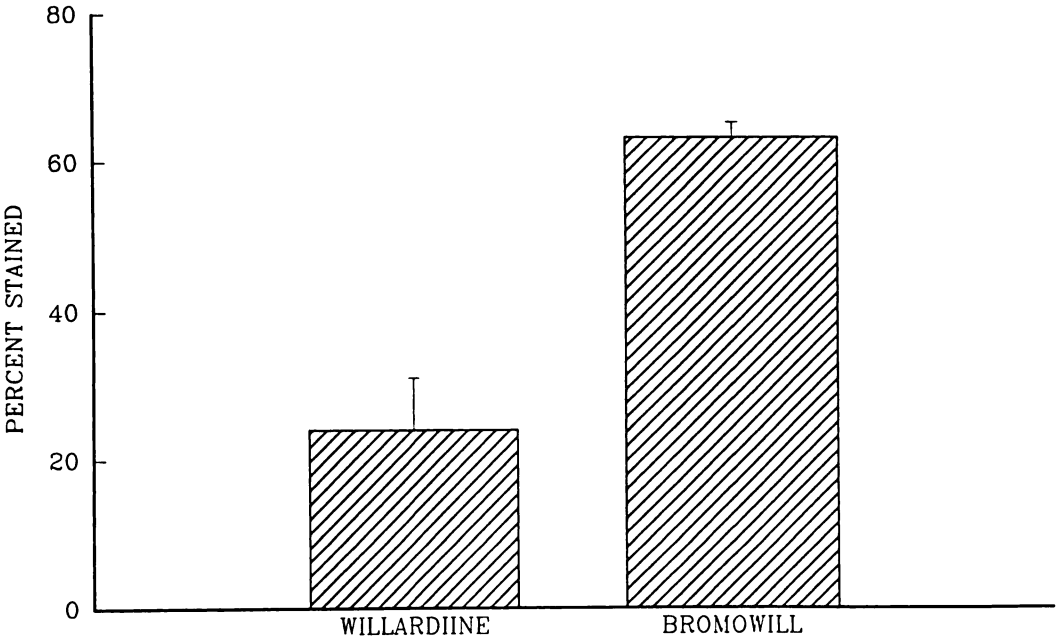


Fig. 7. Bromowillardiine is a more effective acute excitotoxin than willardiine. *Upper*, a culture was exposed to 1 mM bromowillardiine for 20 min. The photographs depict the control condition (*left*), the culture immediately after bromowillardiine had been applied for 20 min (*middle*), and the culture after staining with 5 μM propidium iodide for 5 min (*right*). Magnification, 400×. *Lower*, in experiments similar to those described above, cultures were exposed to 1 mM willardiine or 1 mM bromowillardiine for 20 min. Cells stained by propidium iodide were considered damaged by the treatment. The plot displays the percentage of neurons (mean ± standard error) from 10 fields/culture stained with propidium iodide. Results are pooled from three separate experiments.

molecule, although how this changes the action of the agent is uncertain (24, 25). In both invertebrate and vertebrate preparations, structural changes in non-NMDA agonists that alter the three-dimensional molecular configuration have profound effects on efficacy, suggesting that agonist structure is important for binding and channel gating (26, 27).

Although in vertebrate central neurons it appears that agonists with a "kainoid" C-4 unsaturated side chain exhibit little desensitization (25), this structural distinction is not absolute. In acutely dissociated rat dorsal root ganglion neurons, both kainate and domoate induce desensitizing currents (28), making it unlikely that agonist structure is the determining factor for desensitization at all non-NMDA receptors. Additionally, an important feature of rapid non-NMDA desensitization in postnatal hippocampal neurons is that the process is rarely 100% complete. In almost all neurons, there is a residual current that flows through the channel during prolonged agonist administration. The mechanisms responsible for this residual current are at present unclear. It is also uncertain whether the same channels that produce rapid desensitization are also responsible for the steady state current (3, 29). Recent studies using cloned AMPA receptors have identified a segment of 38 amino acids, preceding the predicted fourth transmembrane-spanning region, that greatly affects the kinetics of currents produced by desensitizing non-NMDA agonists and that may be responsible for differences in peak and steady state currents (30). Given the differences in peak and steady state responses produced by willardiine and bromowillardiine, it is possible that these agents have preferential effects on different structural forms of the receptor.

Our results also suggest that physiological differences between desensitizing and nondesensitizing non-NMDA agonists extend to differences in acute excitotoxicity. Nondesensitizing agonists, including bromowillardiine, gate large steady state currents and damage about 60% of postnatal hippocampal neurons during a 20–30-min exposure. In contrast, desensitizing agonists such as willardiine damage only about 25% of neurons during a similar exposure (22). Thus, prominent desensitization appears to limit the degree of acute neurodegeneration that occurs during prolonged exposures to certain agonists in vertebrate neurons.

Acknowledgments

The authors thank Dr. William Klunk for many helpful discussions.

References

1. Kiskin, N. I., O. A. Krishtal, and A. Y. Tsyndrenko. Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neurosci. Lett.* **63**:225–230 (1986).
2. Trussell, L. O., L. L. Thio, C. F. Zorumski, and G. D. Fischbach. Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc. Natl. Acad. Sci. USA* **85**:2834–2838 (1988).
3. Tang, C. M., M. Dichter, and M. Morad. Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. *Science (Washington D. C.)* **243**:1474–1477 (1989).
4. Patneau, D. K., and M. L. Mayer. Structure-activity relationship for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *J. Neurosci.* **10**:2385–2399 (1990).
5. O'Brien, R. J., and G. D. Fischbach. Characterization of excitatory amino acid receptors expressed by embryonic chick motoneurons *in vitro*. *J. Neurosci.* **6**:3275–3283 (1986).
6. Zorumski, C. F., and J. Yang. AMPA, kainate and quisqualate activate a common receptor-channel complex on embryonic chick motoneurons. *J. Neurosci.* **8**:4277–4286 (1988).
7. Lerma, J., L. Kushner, R. S. Zukin, and M. V. L. Bennett. *N*-Methyl-D-aspartate activates different channels than do kainate and quisqualate. *Proc. Natl. Acad. Sci. USA* **86**:2083–2087 (1989).
8. Rassendren, F.-A., P. Lory, J.-P. Pin, J. Bockaert, and J. Nargeot. A specific quisqualate agonist inhibits kainate responses induced in *Xenopus* oocytes injected with rat brain RNA. *Neurosci. Lett.* **99**:333–339 (1989).
9. Keinanen, K., W. Wisden, B. Sommer, P. Werner, A. Herb, T. A. Verdoorn, B. Sakmann, and P. H. Seeburg. A family of AMPA-selective glutamate receptors. *Science (Washington D.C.)* **249**:556–560 (1990).
10. Boulter, J., M. Hollman, A. O'Shea-Greenfield, M. Hartley, E. Deneris, C. Maron, and S. Heinemann. Molecular cloning and functional expression of glutamate receptor subunit genes. *Science (Washington D. C.)* **249**:1033–1037 (1990).
11. Foster, A. C., and G. E. Fagg. Acidic amino acid binding sites in mammalian neuronal membranes: their characteristics and relationship to synaptic receptors. Brain Res. Rev. **7**:103–164 (1984).
12. Monaghan, D. T., R. J. Bridges, and C. W. Cotman. The excitatory amino acid receptors: their classes, pharmacology and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* **29**:365–402 (1989).
13. Davies, J., R. H. Evans, A. W. Jones, D. A. S. Smith, and J. C. Watkins. Differential activation and blockade of excitatory amino acid receptors in the mammalian and amphibian central nervous systems. *Comp. Biochem. Physiol. C Comp. Pharmacol.* **72**:211–224 (1982).
14. Evans, R. H., A. W. Jones, and J. C. Watkins. Willardiine: a potent quisqualate like excitant. *J. Physiol. (Lond.)* **308**:71P–72P (1980).
15. Agrawal, S. G., and R. H. Evans. The primary afferent depolarizing action of kainate in the rat. *Br. J. Pharmacol.* **87**:345–355 (1986).
16. Huettner, J. E., and R. W. Baughman. Primary culture of identified neurons from the visual cortex of postnatal rats. *J. Neurosci.* **6**:3044–3060 (1986).
17. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. Improved patch clamp techniques for high resolution current recordings from cells and cell-free membrane patches. *Pfluegers Arch.* **391**:85–100 (1981).
18. Favaron, M., H. Manev, H. Alho, M. Bertolino, B. Ferret, A. Guidotti, and E. Costa. Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. *Proc. Natl. Acad. Sci. USA* **85**:7351–7355 (1988).
19. Macklis, J. D., and R. D. Madison. Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. *J. Neurosci. Methods* **31**:43–46 (1989).
20. Mayer, M. L., and G. L. Westbrook. Mixed agonist actions of excitatory amino acids on mouse spinal cord neurones under voltage clamp. *J. Physiol. (Lond.)* **354**:29–53 (1984).
21. Honore, T., S. N. Davies, J. Drejer, E. J. Fletcher, P. Jacobsen, D. Lodge, and F. E. Nielsen. Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science (Washington D. C.)* **241**:701–703 (1988).
22. Zorumski, C. F., L. L. Thio, G. D. Clark, and D. B. Clifford. Blockade of desensitization augments quisqualate excitotoxicity in cultured postnatal rat hippocampal neurons. *Neuron* **5**:61–66 (1990).
23. Thio, L. L., D. B. Clifford, and C. F. Zorumski. Characterization of a rapidly desensitizing glutamate current in cultured postnatal hippocampal pyramidal neurons. *Soc. Neurosci. Abstr.* **14**:1195 (1988).
24. Watkins, J. C., and H. J. Olverman. Agonists and antagonists for excitatory amino acid receptors. *Trends Neurosci.* **10**:265–272 (1987).
25. Watkins, J. C., P. Krogsgaard-Larsen, and T. Honore. Structure activity relationships in the development of excitatory amino receptor agonist and competitive antagonists. *Trends Pharmacol. Sci.* **11**:25–33 (1990).
26. Boden, J., B. W. Bycroft, S. R. Chhabra, J. Chiplin, P. J. Crowley, R. J. Grout, T. J. King, E. McDonald, P. Raferty, and P. N. R. Usherwood. The action of natural and synthetic isomers of quisqualic acid at a well defined glutamatergic synapse. *Brain Res.* **385**:205–211 (1986).
27. Brehm, L., F. S. Jorgensen, J. J. Hansen, and P. Krogsgaard-Larsen. Agonists and antagonists for central glutamate receptors. *Drug News Perspect.* **1**:138–144 (1988).
28. Huettner, J. E. glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron* **5**:255–266 (1990).
29. Baev, K. V., K. I. Rusin, and B. V. Safronov. Development of L-glutamate- and glycine-activated currents in spinal cord neurones during early chick embryogenesis. *J. Physiol. (Lond.)* **423**:381–395 (1990).
30. Sommer, B., K. Keinanen, T. A. Verdoorn, W. Wisden, N. Burnashev, A. Herb, M. Kohler, T. Takagi, B. Sakmann, and P. H. Seeburg. Flip and Flop: a cell-specific functional switch in glutamate-operated channels in the CNS. *Science (Washington, D. C.)* **249**:1580–1585 (1990).

Send reprint requests to: Dr. Charles F. Zorumski, Department of Psychiatry, Washington University Medical School, 4940 Audubon Ave, St. Louis MO 63110.