SYNAPSE 31:203–209 (1999)

Heparin Modulates the Single Channel Kinetics of Reconstituted AMPA Receptors from Rat Brain

SRIKUMAR SINNARAJAH,[1] VISHNU SUPPIRAMANIAM,[2] KOLAPPA PREM KUMAR,[2] RANDY A. HALL,[3] BEN A. BAHR,[4] AND VITALY VODYANOY[1]*

[1]*Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, Alabama 36849*
[2]*Department of Biology, Tuskegee University, Alabama 36088*
[3]*Center for the Neurobiology of Learning and Memory, University of California, Irvine, California 92697*
[4]*Department of Pharmaceuticals Sciences, University of Connecticut, Storrs, Connecticut 06269*

KEY WORDS glutamate receptor; bilayer; ion channel; proteoglycan

ABSTRACT Glutamate receptors specifically activated by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) have been reported to interact with the highly sulfated glycosaminoglycan, heparin, and to subsequently express lower binding affinity for [^{3}H]AMPA. The present study examined whether heparin also modifies the kinetic properties of single channel activity expressed by isolated AMPA receptors from rat forebrain. Upon application of 280 nM AMPA, the partially purified receptors reconstituted in lipid bilayers expressed bursting channel activity that was inhibited by dinitroquinoxaline-2-3,-dione (DNQX). Treating the receptors with heparin (10 μg/ml) produced no change in conductance but the mean burst length for 280 nM AMPA was nearly doubled. Heparin also prolonged the lifetime of open states of the individual ion channels 3–5-fold, perhaps by causing a decrease in the closing rate constant for channel gating. Heparin had no effect on the lifetime of the closed state or on the amplitude of currents. The single channel open time was voltage-dependent and an increase of applied voltage caused a decrease in the heparin effect on channel open times. While the lifetime of the open channel was increased 3–4 times by heparin at 20 mV, there was no significant change induced at 43 mV. The equivalent electric charge of the channel gate was increased by 40%. The heparin effects were specific as another polysaccharide, dextran, and a monomeric constituent of heparin, glucosamine 2,3-disulfate, failed to have any effect on the receptors. These findings suggest that heparin-containing extracellular matrix components can interact with AMPA receptors and influence their functional properties. **Synapse 31:203–209, 1999.**

INTRODUCTION

Insight into the structure and function of AMPA-type glutamate receptors has been greatly advanced as details concerning their molecular properties have become available (see Boulter et al., 1990; Bahr et al., 1992b, 1996; Wenthold et al., 1992; Martin et al., 1993) and an array of modulatory agents such as aniracetam (Ito et al., 1990), ampakines (Arai et al., 1994; Staubli et al., 1994), cyclothiazide (Patneau et al., 1993; Yamada and Tang, 1993), and GYKI 52466 (Zorumski et al., 1993) have been discovered. One aspect, however, that has received only scant attention concerns the interaction of these receptors with their synaptic surroundings and the forces that hold them there. The relevance of such questions extends beyond purely structural considerations, since the synaptic environment may possibly alter the properties of AMPA receptors (Hall et al., 1993, 1996a) and changes in the channel kinetics of these receptors have been proposed to account at least in part for long-lasting changes in synaptic efficacy (Ambros-Ingerson et al., 1991; Staubli et al., 1992; Ambros-Ingerson and Lynch, 1993). While processes acting from within the cell may be most critical in this regard (see Greengard et al., 1991; Wang et al., 1991; Rosenmund et al., 1994; Yakel et al., 1995), the possibility exists that elements of the extracellular

Contract grant sponsor: FAA; Contract grant number: 93-G-058; Contract grant sponsor: NINDS; Contract grant number: 1K01NS02018-01; Contract grant sponsor: AFOSR; Contract grant number: 89-0383.

Current address for R.A. Hall: Howard Hughes Medical Institute, Duke University, Durham, NC 27710.

*Correspondence to: Vitaly Vodyanoy, Department of Anatomy, Physiology and Pharmacology, 207 Greene Hall, Auburn University, Auburn, AL 36849.
E-mail: vodyavi@vetmed.auburn.edu.

Received 6 April 1998; accepted 23 June 1998.

matrix that connects pre- and postsynaptic membranes also impart an influence on AMPA receptors.

Indeed, matrix receptors and other types of adhesion molecules appear to be critical for the changes in synaptic current associated with long-term potentiation (Staubli et al., 1990; Xiao et al., 1991; Lüthi et al., 1994; Rønn et al., 1995). In addition, the influence of matrix recognition may extend further and probably includes a role as well in the stabilization of the potentiation (Bahr et al., 1997). Connection with matrix elements often involves interaction with the glycosidic moiety of proteoglycans (see Culp et al., 1986; Ruoslahti, 1988, 1989) and it was therefore of interest when AMPA receptors were found to interact with heparin-agarose during the course of purifying the native receptors from rat brain (Bahr et al., 1992b; Hall et al., 1996b). Since heparin alters the biological activity of many proteins to which it binds (see Jackson et al., 1991), it was not entirely surprising that its interaction with AMPA receptors produced a change in the ligand binding properties of the receptors (Hall et al., 1996b). The present study tested whether the heparin-induced changes on AMPA binding are associated with changes in channel properties. To address this issue, the experiments described used AMPA receptors isolated from adult rat forebrain and reconstituted in phospholipid bilayers.

MATERIALS AND METHODS

Receptor purification was accomplished by using techniques described elsewhere (Bahr et al., 1992b). Briefly, brains from adult rats were rapidly removed and forebrain membranes prepared using conventional centrifugation techniques. The membranes were solubilized in 1% (wt/vol) Triton X-100 and then subjected to a sequence of three chromatographic steps in 1% n-octylglucoside: 1) DEAE anion exchange, 2) wheat-germ lectin affinity, and 3) polyethyleneimine anion-exchange. These steps produced a 30 to 60-fold increase in the number of AMPA binding sites (assayed with [^{3}H]AMPA) per unit protein. A band of 105 kDa corresponding to the expected molecular weight of AMPA receptor subunits (Hollmann et al., 1989; Keinanen et al., 1990) was evident in polyacrylamide gels of purified material and this band reacted intensely with antibodies against the GluR-1 subunit in immunoblots (Bahr et al., 1992b). These biochemical results indicate that the starting material for the reconstitution experiments contained high concentrations of partially purified AMPA receptors. Preparation of brain membranes, solubilization, and [^{3}H]AMPA binding assays were as described previously (Hall et al., 1996b).

Receptors were reconstituted as previously reported in small "tip-dip" bilayers (Vodyanoy et al., 1993; Hall et al., 1996b). The electrical measurement system, assay procedures, and data processing were as described previously (Vodyanoy, 1989). Bilayers are typically formed from 1,2-diphytanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc., Alabaster, AL) in a buffer composed of (in mM) 125 NaCl, 5 KCl, 1.25 NaH_2PO_4, and 5 Tris-HCl, pH 7.4. Patch-bilayers were typically formed in asymmetric saline conditions ("outside out" configuration) where 110 KCl, 4 NaCl, 2 $NaHCO_3$, 0.1 $CaCl_2$, 1 $MgCl_2$, 2 MOPS are the mM concentrations inside, and 125 NaCl, 5 KCl, 1.25 NaH_2PO_4, 5 Tris (pH 7.4) are those outside. The bilayer was formed by the successful transfer of two phospholipid monolayers upon the tip of a patch pipette, after which receptors were added to the bulk solution bathing the cis side of the preformed bare membrane. After receptor incorporation, AMPA (Tocris Neuramin, St. Louis, MO) and heparin, dextran, or glucosamine 2.3-disulfate (Sigma, St. Louis, MO) were applied to the same side of the bilayer with continuous electrical measurements. All patch-clamp data recorded by a VCR system were analyzed off-line with the pCLAMP 6 software (Axon Instruments, Inc., Foster City, CA). Recorded single-channel events were subjected to computer analysis of amplitude and time distributions. Recorded signals were filtered at 1–5 kHz and sampled at 0.2–0.5 msec intervals, after which they were reduced to series of datasets each containing 2,000–110,000 data points. The minimum detectable dwell time (0.2 msec) was calibrated by detection of brief events.

RESULTS

AMPA receptors reconstituted by the tip-dipping method were activated by the addition of AMPA to the pseudo-extracellular solution, resulting in current fluctuations usually with multiple conductance levels with bursts at principal conductance level of about 50 pS. Figure 1A shows traces of a recording from a patch bilayer containing AMPA receptors exposed to 280 nM AMPA with the voltage clamped at −35.5 mV. Analysis of the amplitude histogram revealed that the composite current was an integral multiple of single channel currents of low and high conductance states as previously shown (Vodyanoy et al., 1993). An addition of 10 μg/ml heparin (approximately 0.5 μM) resulted in profound changes of the burst dwell times (Fig. 1B). Heparin did not activate the receptors in the absence of AMPA. Table I illustrates the effect of heparin on AMPA receptor single-channel currents elicited by 280 nM AMPA at −35.5 mV. Addition of heparin resulted in about a twofold increase ($P < 0.05$) of the mean burst duration and a fivefold decrease ($P < 0.001$) of the interburst interval. The mean number of short closings per duration of an apparent open state of burst was not influenced by the heparin, but the duration of openings (Fig. 1a,b) and the mean probability of open time within bursts were significantly increased ($P < 0.001$). The principal channel conductance was not affected by heparin (Fig. 1a,b), but the population of open states (relative areas) showed more than a twofold increase.

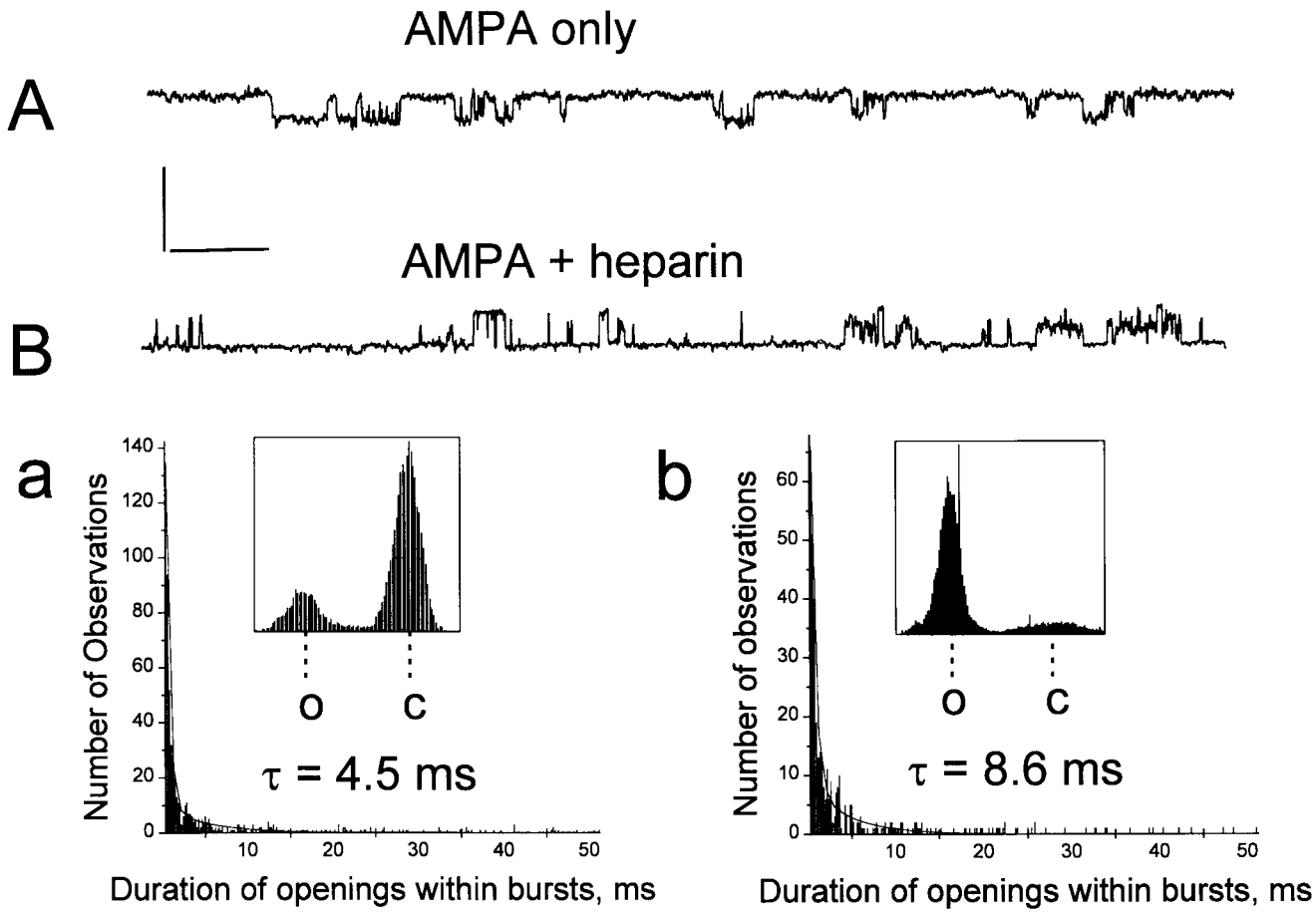


Fig. 1. Effects of heparin on channel activity elicited by AMPA. **A:** The portion of the trace of channel fluctuations elicited by 280 nM AMPA. Openings are downwards. Ten µl of a suspension of partially purified AMPA receptor (1 pmol AMPA binding/ml) were added to the cis-compartment (1 ml) with stirring. AMPA was then added to the same compartment and membrane current was registered with the voltage clamped at −35.5 mV. The vertical and horizontal bars are 4 pA and 860 ms, respectively. The trace shown in the figure consisted of 25,600 data points and lasted 10240 ms. **B:** The trace of channel current fluctuations initially elicited by 280 nM AMPA and then modified by addition of heparin (0.01 mg/ml) recorded at −35.5 mV. **a** and **b:** Single-channel records in the presence of 280 nM AMPA (**a**) and after addition of heparin (**b**) were analyzed for openings of 56 pS conductance within bursts. Openings of lower conductance were also present in the records, but were excluded from analysis. The current traces of 20 sec were segmented with a bin width of 0.2 ms. Histograms of duration of openings within bursts analyzed by Marquardt least squares method (PSTAT module of Axon pCLAMP 6.0 software) with two-exponential functions with time constants as follows: **a,** 280 nM AMPA, 0.37 ± 0.27, 4.47 ± 0.04 (SE) ms; **b,** 0.01 mg/ml heparin, 0.52 ± 0.07, 8.60 ± 0.02 (SE) ms. Inserts **a** and **b** show amplitude distributions of channel current traces **A** and **B,** respectively. The single-channel currents of the traces consisting of 25,600 points sampled at intervals of 0.2 ms, segmented with a bin width of 0.2 pA. The amplitude histograms of the traces exhibits two maxima at the channel closed current level, **c,** and the channel-open current level, **o,** with channel conductance and relative area of open states as follows: insert **a,** 280 nm AMPA, 56.0 ± 5.2 (S.D.) pS, 26%; insert **b,** 0.01 mg/ml heparin, 59.2 ± 5.6 (S.D.) pS, 60%.

Overall, the addition of heparin doubled the probability of the open state within the total time recorded (Table I). Channel currents under the influence of heparin were blocked by 10 µM DNQX (data not shown), thus heparin has no effect on the antagonism of the ligand binding site.

Single channel currents were determined from the experimental current amplitude distributions of all the digitized points in current traces at different voltages. The currents were plotted as a function of membrane voltage for currents induced by AMPA in the absence or presence of heparin (Fig. 2). Using the Hodgkin-Huxley model (Hille, 1984) for the channel conductance ($g = I/(V - V_0)$ where I is a current, V is voltage, and V_0 is a reversal potential obtained from I-V graphs), V_0 and g were respectively determined to be 3.0 ± 0.3 mV (SD) and 44.0 ± 4.0 pS for control channels, and 4.90 ± 0.7 mV and 50.0 ± 8.0 pS for those treated with heparin. The unitary channel conductance, thus, was not affected by heparin. The small reversal potentials found in this analysis indicated the typical cationic nature of the AMPA channel (Choay, 1989).

The most peculiar effect of heparin on the AMPA-elicited channels was the voltage-dependent modulation of single channel open time. As shown in Figure 3, the heparin-induced increase in open time at 20 mV gradually became smaller at higher voltage levels. Interestingly, channel open time of AMPA current mea-

TABLE I. Effect of heparin on AMPA receptor single-channel currents elicited by 280 nM AMPA at −35.5 mV

Record information	AMPA only	AMPA + heparin
Number of events	1,405	920
Total record time, sec	20.2	20.4
Number of conductance levels	1	1
Number of bursts	38	25
Interburst interval, ms*	124 ± 30	25 ± 11
Mean burst duration, ms (±SD)**	254 ± 395	483 ± 1141
Duration of openings within bursts, ms (±SD)	4.45 ± 0.03	8.60 ± 0.02
Channel conductance within bursts, pS (±SD)	56.0 ± 5.2	59.2 ± 5.6
Mean closings per burst (±SD)[a]	17 ± 24	17 ± 28
Mean P (open) within bursts (±SD)[b]	0.56 ± 0.38	0.71 ± 0.34
P (open) for total record	0.320	0.608

The experimental conditions are as in Figure 1, A and B.
[a]Mean number of short closings per duration of an apparent open state of burst.
[b]Mean probability of the channel being open found from a histogram of the open dwells within bursts.
The difference in values after addition of heparin is statistically significant by the *t*-test: $*P < 0.05$; $**P < 0.001$.

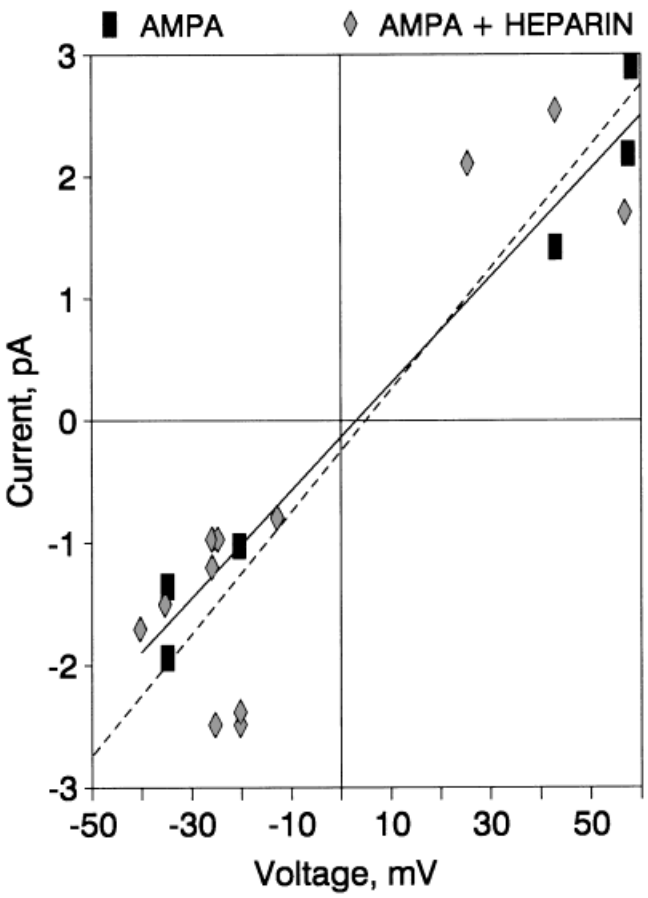


Fig. 2. The current–voltage relationships for the AMPA receptor channels modulated by heparin. The currents for AMPA elicited single channels and for channels modulated by heparin were plotted as functions of membrane voltage using linear regression. Squares and a solid line ($r^2 = 0.97$) represent the lowest defined current transition obtained from the amplitude analysis of current records of about 10.2 sec, consisting of 51,200 points measured after addition of AMPA only (280 nM). Diamonds and a dashed line ($r^2 = 0.79$) represent channel currents after addition of heparin (0.01 mg/ml). The figure summarizes the results of five individual bilayer experiments.

sured in the absence of heparin also exhibited voltage dependency to a significant degree ($P < 0.05$; one-sample *t*-test comparing the slope to zero). In contrast, closed channel time was not modulated by heparin or voltage. The voltage dependence of the apparent rate constant (k) of the channel transition to the open state can be expressed by the equation $k = k_0 \exp(zF\Delta V/RT)$, where k_0 is the rate constant in the absence of membrane voltage ($\Delta V = 0$) depending on the nonelectrical conformational energy increase upon opening the channel, z is the equivalent valence of the channel gate, T is absolute temperature, and R and F are thermodynamic constants (Hille, 1984). From this equation and the definitions of the channel open times ($\tau_{open} = 1/k$ and $\tau_0 = 1/k_0$) we can write that $\log(\tau_{open}) = -(zF/2.303RT)\Delta V + \log(\tau_0)$. The plot of the logarithm of the channel open time as a function of membrane voltage should be a straight line with a negative slope. The slope should be equal to $-(zF/2.303RT)$, and V-intercept equal to $\log(\tau_0)$. Determined from the slopes of plots for open times in Figure 3, the equivalent valence of the channel gate opened by 280 nM AMPA was significantly increased from 1.02 ± 0.29 to 1.43 ± 0.05 by heparin ($P < 0.05$).

In control experiments, AMPA-elicited channels were measured in the absence and presence of 10 μg/ml heparin, 10 μg/ml of another glycosaminoglycan (dextran), and 520 μM glucosamine 2,3-disulfate, a monomeric constituent of heparin. Note that 10 μg/ml heparin is roughly equivalent to 26 μM of monomeric residues. In Figure 4, it can be seen that modulation of channel open time was only associated with heparin treatment. To test if such specificity applies to the heparin effects on AMPA binding, different concentrations of the glycosaminoglycans and the monomeric sugar residue were incubated with solubilized AMPA receptors (Table II). Binding of 10 nM [^{3}H]AMPA was decreased by only heparin, the loss of binding presumably due to a lower affinity state induced by the sulfated glycosaminoglycan. The correlated specificity of the changes on binding and channel properties further suggests that heparin constrains AMPA receptors in a low affinity/high conductance state, a state which has been used to describe AMPA receptors residing in their native postsynaptic density environment (see Hall and Bahr, 1994).

DISCUSSION

The properties of the reconstituted receptors in the present report correspond well with those described for AMPA receptors found in binding and physiological studies (Jahr and Stevens, 1987; Tang et al., 1989, 1991; Vodyanoy et al., 1993; Wyllie and Cull-Candy, 1994). Hall et al. (1996b) reported that heparin associates with AMPA-type glutamate receptors and that this interaction alters the binding affinity for agonist but not antagonist molecules. The data presented here demonstrate that the heparin-induced changes to AMPA

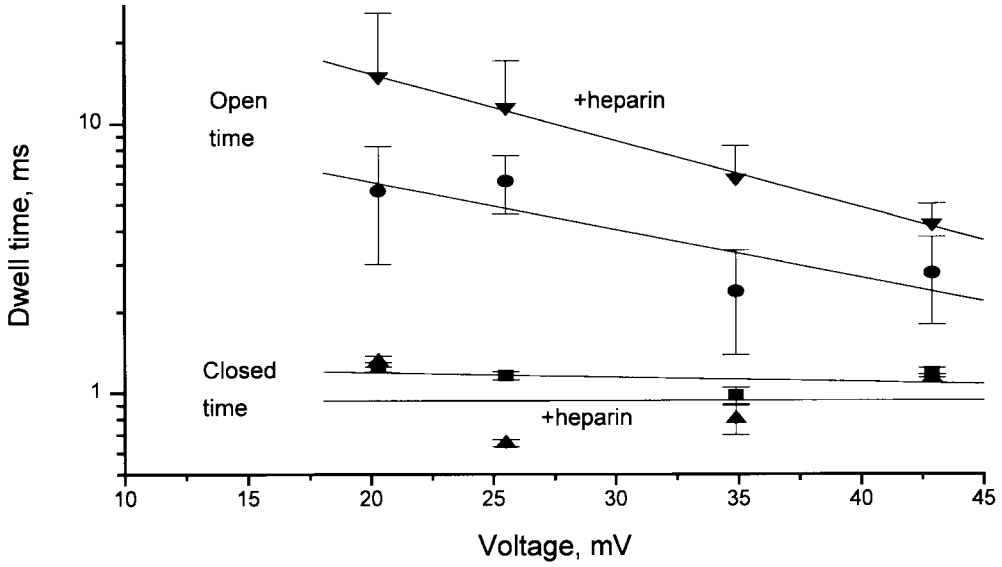


Fig. 3. Voltage dependence of dwell times of AMPA receptor channels. Receptors were reconstituted in small bilayer patches. Channel currents were initially elicited by the addition of 280 nM AMPA to the cis-compartment and then heparin was added to the same compartment and currents were subsequently recorded and digitized. Best fits to dwell time histograms usually required two-exponential functions. Typical analyzed records were of 2–20 sec of total record time and contained 2,000–100,000 events. Each point represent the summary of 3–4 individual experiments. Values of open times before and after heparin was added at 20.3, 25.5, and 34.9 mV were significantly different at the levels of $P < 0.001$, 0.025, and 0.05, respectively. Values of open times before and after heparin was added at 43 mV were not significantly different. The open times AMPA elicited single channels ($r^2 = 0.72$) and for channels modulated by heparin ($r^2 = 0.96$) were plotted as functions of membrane voltage using linear regression.

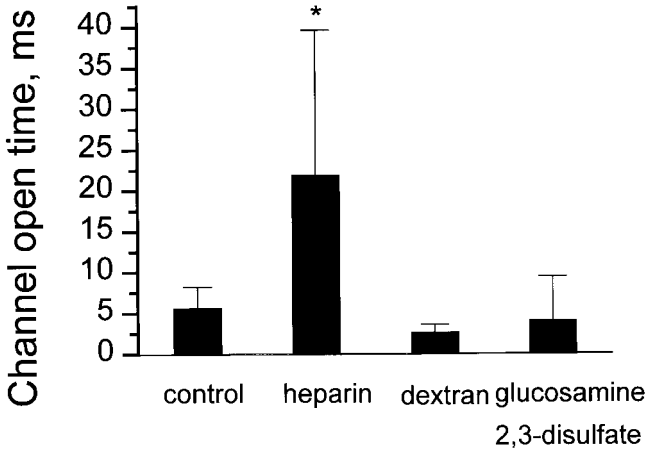


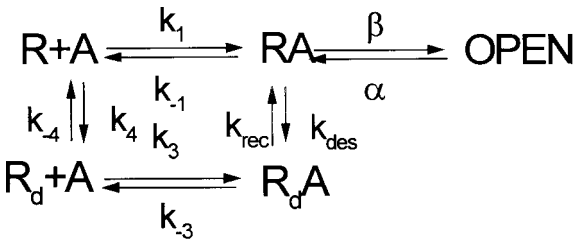
Fig. 4. Specific effects of heparin on AMPA elicited single channel open time. In bilayer experiments, channel fluctuations were elicited by 280 nM AMPA (control) and then heparin (0.01 mg/ml), dextran (0.01 mg/ml), or glucosamine 2,3-disulfate (520 nM) at 20 mV. Open times before and after heparin was added were significantly different at the level of $P < 0.001$.

TABLE II. Change in [^{3}H]AMPA binding in the presence of heparin, dextran, and glucosamine 2,3-disulfate

Treatment, concentration	Change in [^{3}H]AMPA binding[a]
Control	0 ± 1 (16)
Heparin (mg/ml)	
0.1	−40 ± 7 (5)*
1.0	−67 ± 4 (10)*
Dextran (mg/ml)	
0.1	−3 ± 3 (9)
1.0	−6 ± 4 (8)
10	−7 ± 5 (8)
Glucosamine 2,3 Disfulate (mM)	
0.3	+2 ± 3 (8)
5	+1 ± 2 (8)
10	−2 ± 5 (9)

[a]Means of the percent change ± SE are shown with the number of experiments in parentheses. In binding experiments percentage of control [^{3}H]AMPA (10 nM) binding remaining in the presence of heparin, dextran, or glucosamine 2,3-disulfate.
*The difference is statistically significant, *t*-test, $P < 0.001$.

receptors also include the modulation of channel kinetics. Heparin does not appear to cause a generalized degradation of the binding domain or of receptor conformation in general, but rather acts on specific aspects of receptor kinetics. Heparin produced no change in the single channel conductance, but the mean burst duration, the duration of openings within bursts, and the probability of all open states increased almost twofold. The five-state cyclic model successfully applied for AMPA receptors (Vyklicky et al., 1991; Ambros-Ingerson and Lynch, 1993) can be used for analysis of our kinetic data. The change in two parameters of the model can be responsible for an increase of the duration of bursts: a decrease in α, the closing rate constant for



channel gating, and a reduction of k_{des}, the rate constant for entry into the desensitized state (Vyklicky et al., 1991). The reduction of k_{des}, however, affects only the duration of bursts and does not change the open times inside bursts. In contrast, the decrease in α provides the increase of duration of both bursts and channel open times. According to the present experiments, heparin effectively prolongs both duration of bursts and open channel times. This leaves the suggestion that parameter α, the closing rate constant for channel gating α, is influenced by the interaction between heparin and the AMPA receptor.

There were some reports about heparin affecting properties of receptor-associated ion channels. It has been shown that heparin blocked Ca^{2+} channels associated with IP_3 receptors purified from smooth muscle and reconstituted in bilayers (Mayrleitner et al., 1991). Using the whole-cell patch clamp method, heparin was also found to suppress Ca^{2+}-dependent K^+ currents in rabbit jejunum smooth muscle (Komori et al., 1990) and L-type Ca^{2+} channels in mammalian cardiac myocytes (Lacinova et al., 1993). In contrast to these inhibitory effects, it has been shown in bilayer experiments that heparin opened the calcium release channel (ryanodine receptor) obtained from rabbit skeletal muscle (Bezprozvanny et al., 1993). Due to the polyanionic nature of heparin (Choay, 1989), it can bind relatively large amounts of cations and the observed effects of heparin on different channels might be mediated by the depletion of Na^+, K^+, Ca^{2+}, and/or Mg^{2+}. There are two arguments against such an interpretation regarding the results reported here. First, concentrations of cations used were much higher than the concentration of heparin to produce noticeable change in the ions activities. It has been shown that 100 μg/ml heparin at most could reduce free [Ca^{2+}] by 4% (Lacinova et al., 1993). In the present work, we used ten times lower concentrations of heparin. Second, the reduction of cation concentrations in the vicinity of the open channel would produce a decrease in the amplitude of the channel current, yet we found no significant change in the amplitude of the single channel current when heparin was added to the system.

Considering the large negative charge of heparin, it may be suggested that the effects of heparin are mediated by nonspecific binding and alterations in membrane surface potential. It would be manifested by alterations and shift of I-V characteristics of channel currents. However, no significant changes were observed after addition of heparin in both slope and reversal potentials of our I-V characteristics. Thus, our results do not support the nonspecific effects of membrane surface charge in heparin effects.

Since heparin prolongs open channel times and does not affect closing times, the most plausible explanation of heparin effects would be that the polyanionic oligosaccharide binds to a molecular moiety near the open mouth of the AMPA channel, where cationic sites are thought to be located (Burnashev et al., 1992). Because the nonpolar glycosaminoglycan dextran produced no effect, interactions between negative charges of heparin and certain positively charged residues of AMPA channels might be important. This idea is consistent with the present data showing that heparin caused a 40% increase of the equivalent charge of the AMPA channel gate. Thus, effects of heparin can be explained by the fact that this relatively large rigid polyanion requires an open channel to bind; the presence of the molecule keeps the channel open and deactivation cannot close the channel until the molecule leaves. An increase in voltage can help to remove heparin and allow the channel to close. The molecules of glucosamine 2,3-disulfate are small enough to remain bound to the AMPA channel when the gate is closed. The interaction of heparin with AMPA receptors is similar to the known interaction of polycyclic cations with Na^+ channels, where open gates are required for the molecule to bind and remain open until the molecule leaves (Cahalan and Almers, 1979).

With the overall finding that heparin binds to and alters the properties of AMPA receptors, it is of interest that glycosaminoglycan-containing proteoglycans are abundant in the brain (Herndon and Lander, 1990) and are known to have effects on a large number of physiological processes (see Ruoslahti, 1989). Most pertinent are specific proteoglycans that have been shown to be involved in the mechanisms of neurotransmission (Bahr et al., 1992a) as well as in the formation and maintenance of neuromuscular junctions (Nastuk and Fallon, 1993). This broad spectrum of action may complicate the interpretations concerning the effects of heparin on AMPA receptors in intact preparations. Nonetheless, the data in the present report raise the interesting possibility that native AMPA receptors associate with endogenous proteoglycans such as agrin (Tsen et al., 1995) or its functional equivalent at central synapses and that these associations play a role in synaptic function.

ACKNOWLEDGMENTS

The authors thank Dr. Gary Lynch for supporting this research, Dr. Markus Kessler for useful discussions, and Mr. Alex Quan and Mr. Randy O. Boddie for technical assistance.

REFERENCES

Ambros-Ingerson J, Lynch G. 1993. Channel gating kinetics and synaptic efficacy: A hypothesis for the expression of long-term potentiation. Proc Natl Acad Sci USA 90:7903–7907.

Ambros-Ingerson J, Larson J, Xiao P, Lynch G. 1991. LTP changes the waveform of synaptic responses. Synapse 9:314–316.

Arai A, Kessler M, Xiao P, Ambros-Ingerson J, Rogers G, Lynch G. 1994. A centrally active drug that modulates AMPA receptor gated currents. Brain Res 638:343–346.

Bahr BA, Noremberg K, Rogers GA, Hicks BW, Parsons SM. 1992a. Linkage of the acetylcholine transporter-vesamicol receptor to proteoglycan in synaptic vesicles. Biochemistry 31:5778–5784.

Bahr BA, Vodyanoy V, Hall RA, Suppiramaniam V, Kessler M, Sumikawa K, Lynch G. 1992b. Functional reconstitution of alpha-

amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors from rat brain. J Neurochem 59:1979–1982.
Bahr BA, Hoffman KB, Kessler M, Hennegriff M, Park GY, Yamamoto R, Kawasaki BT, Vanderklish PW, Hall RA, Lynch G. 1996. Distinct distributions of AMPA receptor subunits and a related 53,000 M_R antigen in brain tissue. Neuroscience 74(3):707–721.
Bahr BA, Staubli U, Xiao P, Chun D, Ji ZX, Esteban ET, Lynch G. 1997. Arg-Gly-Asp-Ser-selective adhesion and the stabilization of long-term potentiation: Pharmacological studies and the characterization of a candidate matrix receptor. J Neurosci 17(4):1320–1329.
Bezprozvanny I, Ondrias K, Kaftan E, Stoyanovsky DA, Ehrlich BE. 1993. Activation of the calcium release channel (ryanidine receptor) by heparin and other polyanions is calcium dependent. Mol Biol Cell 4:347–352.
Boulter J, Hollmann M, O'Shea-Greenfield A, Hartley M, Deneris E, Maron C, Heinemann S. 1990. Molecular cloning and functional expression of glutamate receptor subunit genes. Science 249:1033–1037.
Burnashev N, Monyer N, Seeburg PH, Sakmann B. 1992. Divalent ion permeability of AMPA receptor channel is dominated by the edited form of a single subunit. Neuron 8:189–198.
Cahalan MD, Almers W. 1979. Block of sodium conductance and gating current in squid giant axons poisoned with quaternary strychnine. Biophys J 27(1):57–73.
Choay J. 1989. Structure and activity of heparin and its fragments: An overview. Semin Thromb Hemost 15:359–364.
Culp LA, Laterra J, Lark MW, Beyth RJ, Tobey SL. 1986. Heparin sulphate proteoglycan as mediator of some adhesive responses and cytoskeletal reorganization of cells on fibronectin matrices: Independent versus cooperative functions. In: Functions of the proteoglycans. Chichester, UK: Wiley (Ciba Foundation Symposium 124), pp 158–173.
Greengard P, Jen J, Nairn AC, Stevens CF. 1991. Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. Science 253:1135–1138.
Hall RA, Bahr BA. 1994. AMPA receptor development in rat telencephalon: [^{3}H]AMPA binding and Western blot studies. J Neurochem 63:1658–1665.
Hall RA, Massicotte G, Kessler M, Baudry M, Lynch G. 1993. Thiocyanate equally increases affinity for two DL-alpha-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor states. Mol Pharmacol 43(3):459–464.
Hall RA, Quan A, Kessler M, Lynch G. 1996a. Ultraviolet radiation, thiol reagents, and solubilization enhance AMPA receptor binding affinity via a common mechanism. Neurochem Res 21(8):963–968.
Hall RA, Vodyanoy V, Quan A, Sinnarajah S, Suppiramaniam V, Kessler M, Bahr BA. 1996b. Effects of heparin on the properties of solubilized and reconstituted rat brain AMPA receptors. Neurosci Lett 217:179–183.
Herndon ME, Lander AD. 1990. A diverse set of developmentally regulated proteoglycans is expressed in the rat central nervous system. Neuron 4:949–961.
Hille B. 1984. Ionic channels of excitable membranes. Sunderland: Sinauer Assoc., pp 23–57.
Hollmann M, O'Shea-Greenfield A, Rogers S, Heinemann S. 1989. Cloning by functional expression of a member of the glutamate receptor family. Nature 342:643–648.
Ito I, Tanabe S, Konda A, Sugiyama H. 1990. Allosteric potentiation of quisqualate receptors by a nootropic drug aniracetam. J Physiol 424:533–543.
Jackson RL, Busch SJ, Cardin AD. 1991. Glycosaminoglycans: Molecular properties, protein interactions, and role in physiological processes. Physiol Rev 71(2):481–539.
Jahr CE, Stevens CF. 1987. Glutamate activates multiple single channel conductances in hippocampal neurons. Nature 325:522–525.
Keinanen K, Wisden W, Sommer B, Werner P, Herb A, Verdoorn TA, Sakmann B, Seeburg PH. 1990. A family of AMPA-selective glutamate receptors. Science 249:556–560.
Kirsch J, Wolters I, Triller A, Betz H. 1993. Gephyrin antisense oligonucleotides prevent glycine receptor clustering in spinal neurons. Nature 366:745–748.
Komori S, Bolton TB, Roles AG. 1990. Proteins in muscarinic receptors inward current and outward currents in rabbit jejunum smooth muscle. J Physiol (London) 427:395–419.
Lacinova L, Cleemann L, Morad M. 1993. Ca^{2+} channel modulating effects of heparin in mammalian cardiac myocytes. J Physiol 465:181–201.
Lüthi A, Parent J-P, Figurov D, Muller D, Schachner M. 1994. Hippocampal long-term potentiation and neural cell adhesion molecules L1 and NCAM. Nature 372:777–779.
Martin LJ, Blackstone CD, Levey AI, Huganir RL, Price DL. 1993. AMPA glutamate receptor subunits are differentially distributed in rat brain. Neuroscience 53:327–358.
Mayrleitner M, Chadwick CC, Timerman AP, Fleischer S, Schindler H. 1991. Purified IP_3 receptor from smooth muscle forms an IP_3 gated and heparin sensitive Ca^{2+} channel in planar bilayers. Cell Calcium 12:505–514.
Nastuk MA, Fallon JR. 1993. Agrin and the molecular choreography of synapse formation. Trends Neurosci 16(2):72–76.
Patneau DK, Vyklicky L Jr, Mayer ML. 1993. Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. J Neurosci 13(8):3496–3509.
Rønn LCB, Bock E, Linnemann D, Jahnsen H. 1995. NCAM-antibodies modulate induction of long-term potentiation in rat hippocampal CA1. Brain Res 677:145–151.
Rosenmund C, Carr DW, Bergeson SE, Nilaver G, Scott JD, Westbrook GL. 1994. Anchoring of protein kinase A is required for modulation of AMPA/kainate receptors on hippocampal neurons. Nature 368: 853–856.
Ruoslahti E. 1988. Structure and biology of proteoglycans. Annu Rev Cell Biol 4:229–255, 1988.
Ruoslahti E. 1989. Proteoglycans in cell regulation. J Biol Chem 264:13369–13372.
Staubli U, Vanderklish P, Lynch G. 1990. An inhibitor of integrin receptors blocks long-term potentiation. Behav Neural Biol 53:1–5.
Staubli U, Ambros-Ingerson J, Lynch G. 1992. Receptor changes and LTP: An analysis using aniracetam, a drug that reversibly modifies glutamate (AMPA) receptors. Hippocampus 2:49–58.
Staubli U, Rogers G, Lynch G. 1994. Facilitation of glutamate receptors enhances memory. Proc Natl Acad Sci USA 91:777–781.
Tang CM, Dichter M, Morad M. 1989. Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. Science 243(4897):1474–1477.
Tang CM, Shi QY, Katchman A, Lynch G. 1991. Modulation of the time course of fast EPSCs and glutamate channel kinetics by aniracetam. Science 254(5029):288–290.
Tsen G, Halfter W, Kroger S, Cole GJ. 1995. Agrin is a heparan sulfate proteoglycan. J Biol Chem 270(7):3392–3399.
Vodyanoy V. 1989. Cyclic nucleotide-gated electrical activity in olfactory receptors. In: Brand JG, Teeter JH, editors. Receptor events and transduction mechanisms in taste and olfaction. New York: Marcel Dekker, pp 319–345.
Vodyanoy V, Bahr BA, Suppiramaniam V, Hall RA, Baudry M, Lynch H. 1993. Single channel recordings of reconstituted AMPA receptors reveal low and high conductance states. Neurosci Lett 150:80–84.
Vyklicky L Jr, Patneau DK, Mayer ML. 1991. Modulation of excitatory synaptic transmission by drugs that reduce desensitization at AMPA/kainate receptors. Neuron 7(6):971–984.
Wang L-Y, Salter MW, MacDonald JF. 1991. Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. Science 253:1132–1135.
Wenthold RJ, Yokotani N, Doi K, Wada K. 1992. Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies. J Biol Chem 267:501–507.
Wyllie DJ, Cull-Candy SG. 1994. A comparison of non-NMDA receptor channels in type-2 astrocytes and granule cells from rat cerebellum. J Physiol (London) 475(1):95–114.
Xiao P, Bahr BA, Staubli U, Vanderklish PW, Lynch G. 1991. Evidence that matrix recognition contributes to the stabilization but not induction of LTP. Neuroreport 2:461–464.
Yakel JL, Vissavajjhala P, Derkach VA, Brickey DA, Soderling TR. 1995. Identification of a Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in non-N-methyl-D-aspartate glutamate receptors. Proc Natl Acad Sci USA 92:1376–1380.
Yamada KA, Tang CM. 1993. Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. J Neurosci 13(9):3904–3911.
Zorumski CF, Yamada KA, Price MT, Olney JW. 1993. A benzodiazepine recognition site associated with the non-NMDA glutamate receptor. Neuron 10:61–67.