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High Concentrations of *N*-Acetylaspartylglutamate (NAAG) Selectively Activate NMDA Receptors on Mouse Spinal Cord Neurons in Cell Culture

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We examined the membrane action of the endogenous dipeptide and putative neurotransmitter *N*-acetylaspartylglutamate (NAAG) on the excitatory amino acid receptors of cultured mouse spinal cord neurons using electrophysiological methods. Responses to NAAG (1 μM-5 mM) were compared to those elicited by *N*-methyl-D-aspartate (1 μM-1 mM) and L-glutamate (0.5-500 μM). Under voltage clamp, concentration-response curves of agonist-evoked currents demonstrated that NAAG was much less potent than either L-glutamate or *N*-methyl-D-aspartate (NMDA), so that inward currents could be evoked only at NAAG concentrations above 300 μM. Analysis of the dipeptide by high-pressure liquid chromatography showed no evidence of contamination by excitatory amino acids, suggesting that NAAG has an intrinsic, although weak, neuroexcitatory action on spinal neurons.

Previous studies have shown that activation of NMDA receptors produces a voltage-dependent response. The current-voltage relationship of responses evoked by NAAG was also voltage-dependent. The peptide-activated conductance decreased with hyperpolarization in the presence of extracellular Mg^{2+}, such that little inward current could be evoked at a membrane potential of −80 mV. In addition, responses to NAAG were completely antagonized by 250 μM DL-2-amino-5-phosphonovaleric acid, a specific NMDA-receptor antagonist. Application of NAAG in Mg^{2+}-free medium resulted in an inward current with a large increase in membrane current noise. The spectral density function of this current noise could be fitted with a single Lorentzian with a decay time constant near 5 msec and a calculated single-channel conductance of 50-60 pS. Noise spectra of currents evoked by NMDA produced nearly identical values.

These results suggest that the neuroexcitatory action of NAAG on spinal cord neurons is due to selective activation of NMDA receptors; however, excitatory effects were only observed at high concentrations. In view of its low neuroexcitatory potency, the function of endogenous NAAG may be other than that of a neurotransmitter.

The evidence that one or several excitatory amino acid receptor(s) plays a role in synaptic transmission in the mammalian CNS is now quite convincing (Fagg and Foster, 1983; Watkins and Evans, 1981). Although L-glutamate or L-aspartate are potent activators of some or all of these receptors, their role as neurotransmitters has been challenged because of their high concentration in all neurons regardless of neurotransmitter phenotype, and their seemingly "nonspecific" excitatory effects on central neurons. As a result, several other endogenous neuroexcitatory compounds that might act on these receptors have come under consideration as transmitter candidates. These include the tryptophan metabolite, quinolinic acid (Perkins and Stone, 1982); the sulfur-containing amino acids L-cysteate and L-cysteine sulfinate (see Watkins and Evans, 1981); and a number of small peptides (Kanazawa et al., 1981; Reichelt and Edminson, 1977).

Of the later group, *N*-acetylaspartylglutamate (NAAG) is present primarily in nervous tissue (Curatolo et al., 1965; Miyamoto and Tsujio, 1967), binds to brain membranes with high affinity (Zaczek et al., 1983), and excites neurons both *in vivo* (Avoli et al., 1976; Zaczek et al., 1983) and in brain slices of prepyriform cortex and hippocampus (Bernstein et al., 1985; ffrench-Mullen et al., 1985). Since, with extracellular recording, the firing of units in response to stimulation of the lateral olfactory tract and to ionophoretic application of NAAG (but not of glutamate or aspartate) was equally blocked by DL-2-amino-4-phosphonobutyric acid (AP4), ffrench-Mullen et al. (1985) have suggested that NAAG could be the transmitter for lateral olfactory tract axons projecting onto prepyriform cortex neurons. However, Riveros and Orrego (1984) could find no effect of 1.25 mM NAAG on cortical slices of rat brain, using ^{45}Ca influx as an assay of excitatory action.

Spinal cord neurons have been extensively used in studies that have characterized the receptor types and conductance mechanisms activated by excitatory amino acids (for a review, see Mayer and Westbrook, 1986; Watkins and Evans, 1981). The spinal cord would appear to be an appropriate region in which to examine the membrane action of NAAG, since the spinal cord has the highest regional concentration of this dipeptide (Koller and Coyle, 1984a; Miyake et al., 1981); for example, regional NAAG levels are 10-fold higher in rat spinal cord than in piriform cortex or hippocampus (Meyerhoff et al., 1985). In addition, there are large numbers of NAAG binding sites in spinal cord (Koller and Coyle, 1985). Thus, we have used voltage-clamp techniques and fluctuation analysis to examine the membrane action of NAAG on cultured neurons dissociated from mouse spinal cord.

Materials and Methods

Spinal cords from 13-d mouse embryos (C57BL/6J) were dissociated and plated on collagen-coated tissue culture dishes, as previously described (Mayer and Westbrook, 1984). After 1-3 weeks in culture, electrophysiological experiments were performed at room temperature (25°C) on the stage of an inverted microscope. The recording solution contained (in mM): Na^+, 140, K^+, 2.5, Ca^{2+}, 2, Cl^-, 146.5, glucose, 10 and HEPES, 10; pH was adjusted to 7.3 with NaOH, and osmolarity to 325 mOsm with sucrose. In some experiments, Mg^{2+} (0.2–1.0 mM) was added to

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the recording solution. Tetrodotoxin (1 μM) and picrotoxin (50–100 μM) were usually added to the bath to reduce spontaneous synaptic activity. Agonists were diluted in the bath solution and applied by local perfusion from a 2–3 μM-tipped pipette similar to those used for patch recording. All compounds (except NAAG) were obtained from Sigma.

N-*Acetylaspartylglutamate*

One sample of NAAG was obtained from Peninsula Laboraories (Lot 005038). NAAG was also synthesized by the acetylation of α-aspartylglutamate (Bachem) using acetic anhydride at pH 9.0 (Miyamoto et al., 1966). The unreacted peptide and sodium ions were removed by passage of the reaction mixture over Dowex 50 cation exchange resin. NAAG was further purified using the reverse-phase, high-performance liquid-chromatographic (HPLC) procedure described below. This method was also used to characterize the purity of both the commercially available and newly synthesized NAAG before and after use in electrophysiological experiments.

HPLC

HPLC (Gilson Model 43) purification and characterization of NAAG were performed using isocratic elution on a reverse-phase C-18 column (Spherosorb ODS, 5 μm, 250 × 10 mm) in the presence of a mobile phase containing trifluoroacetic acid (0.1%) and acetonitrile (2.5%). To purify newly synthesized NAAG, 0.1–0.3 ml samples containing 1–5 mg NAAG were injected (flow rate = 4 ml/min), and the absorbance at 214 nm was monitored [Gilson 116 UV detector, 1.0 absorbance units full scale (AUFS)]. The fractions (1 ml) containing up to 20% of the peak absorbance (retention time, 6.7 min) were pooled and the solvent mixture was removed by evaporation (Speed-Vac). The purity of the newly synthesized NAAG was confirmed using ion-exchange HPLC, as described by Truckenmiller et al. (1985). To test the purity of both samples of NAAG that had been used in the electrophysiological experiments (see Fig. 2), the same reverse-phase HPLC system was used with a flow rate of 1 ml/min and a detector setting of 0.1 AUFS (retention time, 25–26 min).

Electrophysiology

Intracellular recordings from spinal cord neurons were obtained using patch electrodes (3–5 MΩ) in the whole-cell configuration (Hamill et al., 1981). Access resistance was usually 10 MΩ or less. The solution in the patch pipette contained (in mM): Cs^+, 140, Mg^{2+}, 2, Cl^-, 144, EGTA, 1.1, HEPES, 10; pH was adjusted to 7.2 with CsOH and osmolarity to 310 with sucrose. For voltage-clamp recordings, a discontinuous 1-electrode voltage clamp (Axoclamp-2, Axon Instruments) was used. Thus we could measure the actual membrane voltage and avoid the series resistance errors that can occur when patch-clamp amplifiers are used to clamp large currents. Membrane voltage and current were amplified, filtered at 1 kHz (8-pole Bessell), digitized, and stored on an LSI 11/73 computer for later analysis. Voltage commands were generated by the computer and monitored along with membrane current on an oscilloscope and a Brush chart recorder.

For fluctuation ("noise") analysis, we used a conventional patch-clamp amplifier (List EPC-7) with series-resistance compensation. Membrane current noise before ("control" record) and during a steady, agonist-evoked current was filtered at 0.15 Hz and 1 kHz (4- and 8-pole Butterworth, respectively), sampled at 2 kHz, and stored as continuous records of 2048 points. Records were inspected off-line and those with obvious spontaneous synaptic currents were deleted. Fast Fourier transforms of 8–12 records were averaged and plotted as spectral-density functions. Control noise spectra were subtracted from those obtained during application of NAAG and NMDA. The analytic methods developed for ACh-induced noise at the neuromuscular junction (Anderson and Stevens, 1973; Katz and Miledi, 1972) were used to interpret the power-density spectra. Specifically, spectra were fitted by eye to a single Lorentzian function of the form

$$S(f) = \frac{S(0)}{1 + (f/f_c)^2}$$

where $S(f)$ is the spectral density, $S(0)$ is the zero-frequency value, f is frequency, and f_c is the half-power frequency. Estimates of single-channel conductance (γ) were calculated as follows:

$$\gamma = \frac{S(0)\pi f_c}{2i(V_m - V_{eq})}$$

where i is the mean agonist-evoked current, V_{eq} is the reversal potential of the agonist-evoked current, and V_m is the membrane potential.

Results

The membrane effects of NAAG were tested on a total of 44 spinal cord neurons. The application of the dipeptide evoked a response in all neurons when sufficient concentrations of NAAG were used (see below).

Concentration–response relationship for NAAG

Previous studies of the excitatory action of NAAG have used either ionophoresis (ffrench-Mullen et al., 1985) or drop application of millimolar concentrations of peptide (Bernstein et al., 1985) in brain slices, making it difficult to assess the actual concentration reaching the neuronal membrane. In our initial attempts to characterize the action of NAAG, no excitatory effects were observed when spinal cord neurons, bathed in Mg^{2+}-free medium, were voltage-clamped at −50 mV, and brief applications of peptide at concentrations up to 250 μM were applied from a pipette near the soma. This contrasts with the brisk responses to much lower concentrations of L-glutamate, L-aspartate, kainate, NMDA, or quisqualate under the same conditions (G. L. Westbrook, unpublished observations). However, when the concentration of NAAG in the pipette was raised above 300 μM, an inward current could be observed in all spinal cord neurons tested.

To examine the concentration–response relationship, the inward current to sustained applications (5–30 sec) of several concentrations of NAAG was recorded. Neurons were bathed in Mg^{2+}-free recording solutions, since micromolar concentrations of Mg^{2+} block NMDA-receptor channels at membrane voltages near the rest potential (Mayer et al., 1984; Nowak et al., 1984), and this would tend to antagonize any action of NAAG on NMDA receptors. Each neuron was tested with 3 concentrations of NAAG to minimize interneuronal variability due to differences in cell size or sensitivity. Membrane currents evoked by 1 and 3 mM NAAG for 1 neuron at a holding potential of −50 mV are shown in Figure 1*A*. Responses showed "fading" or desensitization in the continued presence of higher concentrations of agonists. Also notable were increases in current after the end of NAAG application, similar to the "post-desensitization humps" described for cholinergic agonists at the frog end plate (Adams, 1975).

Since, as will be shown below, currents evoked by NAAG were due to activation of NMDA receptors, the dose–response relationship for NAAG was compared with that of NMDA for a series of spinal cord neurons. Nearly 30-fold less NMDA was sufficient to evoke similar amplitude currents (Fig. 1, *B, C*). Under the same conditions, L-glutamate evoked measureable inward currents due to activation of NMDA receptors beginning at concentrations above 0.5 μM (G. L. Westbrook and M. L. Mayer, unpublished observations).

Is the action of NAAG due to contamination by free amino acids?

Since the agonist potency of NAAG on spinal cord neurons was much lower than that of L-glutamate or L-aspartate, we were concerned that breakdown of the dipeptide to free amino acids could account for the excitatory action. Excitation by a contaminating amino acid would most likely be due to L-glutamate, since *N*-acetylaspartate has been reported to be inactive both on spinal neurons (Curtis and Watkins, 1960) and on neurons in prepyriform cortex (ffrench-Mullen et al., 1985). However, NAAG obtained from 2 independent sources (see Materials and Methods) gave identical results.

In addition, analysis by reverse-phase HPLC of the same solutions that had been used in physiological experiments showed no visible peak at the expected elution times for glutamate or

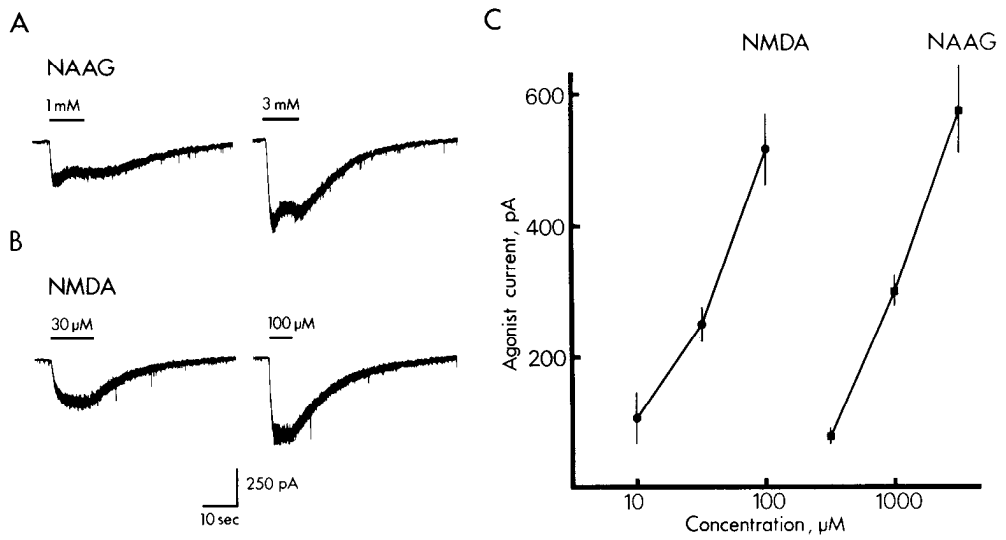

Figure 1. High concentrations of *N*-acetylaspartylglutamate (NAAG) are necessary to excite cultured spinal cord neurons. *A*, A spinal cord neuron was voltage-clamped at −50 mV in an Mg^{2+}-free solution containing 1 μM tetrodotoxin. Three pipettes containing 0.3, 1, and 3 mM *NAAG* were positioned within 50 μm of the soma. Constant pressure application of 1 mM NAAG (8 sec, 2 psi) and 3 mM NAAG (8.5 sec, 2 psi) evoked inward curents of 280 and 600 pA, respectively. *B*, Similar amplitude responses were obtained with 30 and 100 μM *NMDA* on another neuron under the same conditions. *C*, Concentration–response relationship for *NAAG* (n = 10) and *NMDA* (n = 5). Responses were taken as peak values before "fading" or desensitization (mean ± SEM); 3 concentrations were tested on each neuron.

aspartate. The elution profile for a sample of glutamate that was analyzed under these conditions is shown in Figure 2*A*, while the elution profile of the newly synthesized NAAG is shown in Figure 2*B*. Computer analysis (Gilson Data Master) of both NAAG samples did not show any peak corresponding to glutamate under these reverse-phase HPLC conditions. The smallest absorbance change detected in the computer analysis of the elution profiles corresponded to 0.004% of the NAAG absorbance in the sample. We estimate that the extinction coefficient of NAAG under the present HPLC conditions is 13-fold greater

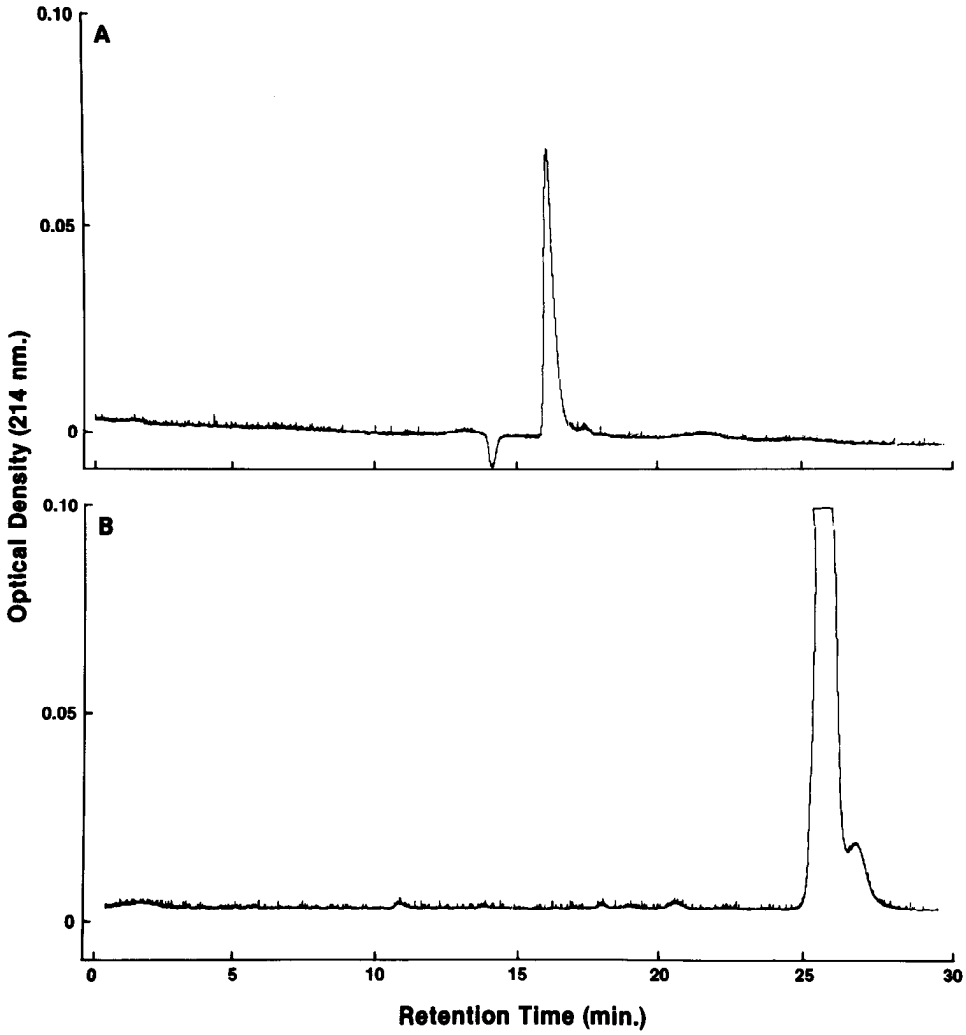

Figure 2. Samples of glutamate (40 μl of 10 mM) and newly synthesized NAAG (100 μl of 1 mM) were analyzed with the reverse-phase HPLC system described in Materials and Methods. The glutamate elution profile is shown in *A* and the NAAG profile in *B*. Significant absorbance was not detected in the region of glutamate elution at any concentration of NAAG tested (see text). The shoulder of absorbing material that eluted just after the NAAG profile may represent a small quantity of residual (*t*-butyloxy carbonyl)-aspartylglutamate present in the aspartylglutamate sample obtained from Bachem, which was used as the substrate for acetylation. This absorbance was not detected in the NAAG obtained commercially and could be eliminated by further chromatographic purification.

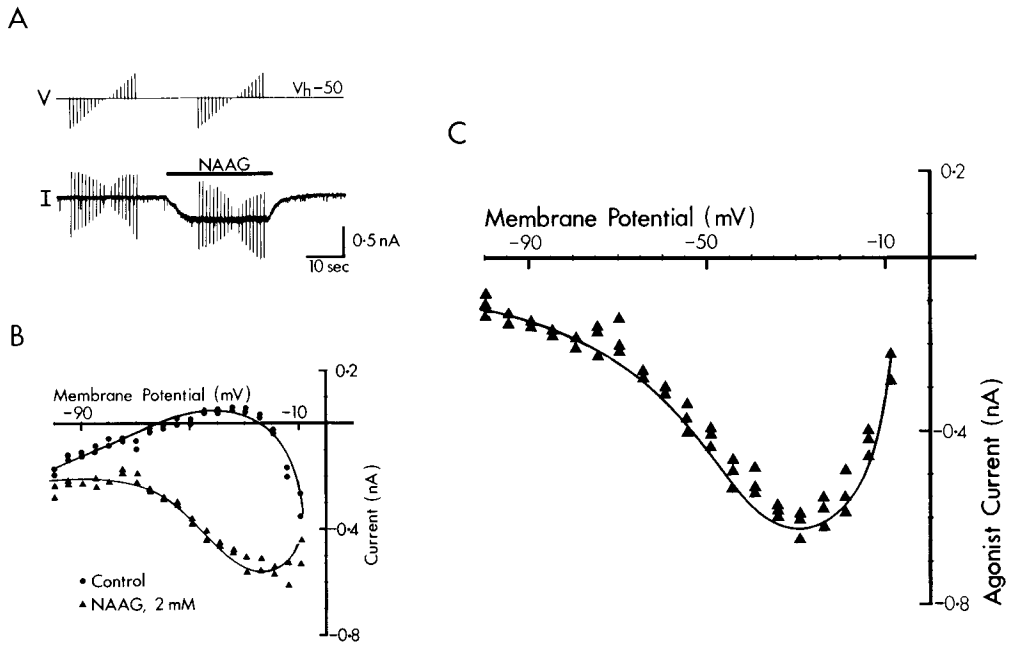

Figure 3. Responses to NAAG are voltage-dependent. *A*, A series of 30 msec voltage jumps was applied to a voltage-clamped spinal cord neuron from a holding potential of −50 mV. Voltage jumps to command voltages between −100 and −20 mV were made immediately before and during a 29 sec application of *NAAG*. The pressure pipette contained 2 mM NAAG, but, unlike Figure 1, the duration and frequency of a series of puffs (3 Hz, 100–250 msec, 2 psi) were adjusted to maintain a constant current of low amplitude; thus considerable dilution occurred. The current–voltage (*I–V*) relationship before (*circles*) and during (*triangles*) application of *NAAG* is shown in *B*. Note that the *control* curve shows evidence of a voltage-dependent inward current due to activation of voltage-dependent calcium channels, since outward currents are blocked by the Cs^+-containing solution in the patch electrode. *C*, Subtraction of the control record gives the *I–V* relationship of the conductance activated by NAAG. The *I–V* plot shows a negative slope conductance at membrane potentials negative to −30 mV, characteristic of activation of NMDA-receptor channels. The extracellular solution contained 200 μM Mg^{2+}.

than that of glutamate at 214 nm. Thus, we calculate that the maximum concentration of glutamate, if present at all, in a 1 mM solution of either NAAG sample was less than 0.5 μM. Similarly, we found no evidence of glutamate or aspartate in these samples when they were characterized by anion-exchange HPLC. These data support the conclusion that the activity of both NAAG samples on spinal cord neurons is due to NAAG itself, rather than to trace impurities of L-glutamate.

NAAG activates N-*methyl-D-aspartate receptors*

In the presence of physiological concentrations of extracellular Mg^{2+}, the conductance mechanism linked to NMDA receptors is voltage-dependent and, thus, current–voltage relationships can serve to distinguish activation of NMDA receptors from the relatively voltage-insensitive conductances linked to kainate/quisqualate receptors (MacDonald and Porietis, 1982; Mayer and Westbrook, 1984). A series of 30 msec voltage jumps from a membrane potential of −50 mV before and during application of NAAG (Fig. 3*A*) was used to construct current–voltage (*I–V*) relationships (Fig. 3*B*) in a recording solution containing 200 μM Mg^{2+}. The *I–V* plot in the absence of agonist was then subtracted from the plot in the presence of agonist to obtain the *I–V* plot for responses evoked by NAAG (Fig. 3*C*). At membrane potentials negative to −30 mV, the *I–V* relationship of responses to NAAG showed a negative slope and conductance that was characteristic of that evoked by NMDA-receptor activation (MacDonald et al., 1982; Mayer and Westbrook, 1985). Responses to NAAG in Mg^{2+}-free medium were essentially voltage-insensitive, as would be expected with NMDA-receptor activation (not shown).

Further indication of an action of NAAG on NMDA receptors was obtained using DL-2-amino-5-phosphonovalerate (AP5), a selective NMDA-receptor antagonist (Davies and Watkins, 1982). This antagonist completely and reversibly antagonized the inward current evoked by a brief pressure application of 2 mM NAAG (Fig. 4). This concentration of AP5 also completely antagonized responses to NMDA and L-aspartate while partially reducing responses to the mixed agonist, L-glutamate, on cultured spinal neurons (Mayer and Westbrook, 1984; Nelson et al., 1986).

Fluctuation analysis

Steady application of NAAG led to marked increases in the membrane current noise. In order to keep responses at steady submaximal (i.e., non-desensitizing) levels, trains of brief pressure applications were used, rather than continuous local perfusion. This method minimized the duration of agonist exposure and allowed us in some cases to test NMDA and NAAG on the same neurons. Figure 5*A* shows the membrane current immediately before and during application of NAAG. The spectral-density function was well fitted by a single Lorentzian function with a half-power frequency (f_c) of 28.6 Hz for the neuron shown in Figure 5*B*; this corresponds to a mean channel lifetime (τ) of 5.6 msec. Analysis of NMDA-induced current noise gave a nearly identical spectrum with τ = 5.5 msec (Fig. 5, *C, D*). For 6 neurons tested with NAAG, the estimated mean channel lifetime was 6.5 ± 0.2 msec (mean ± SEM, 13 observations); for NMDA, τ = 6.4 ± 0.7 msec (n = 4). The calculated single-channel conductance was 52 ± 3 pS for NAAG (mean ± SEM, n = 13) and 61 ± 4 pS for NMDA (n = 4).

The estimate of γ assumes that the agonist activates a single population of channels with a uniform size and shape; this is consistent with, but not proven by, the single Lorentzian spectrum obtained. We also did not attempt to demonstrate a proportional relationship between the mean induced current and variance; since the mean induced current was up to one-third

that of the maximal response, this is a potential source of error in our calculations (see, e.g., Anderson and Stevens, 1973). However, these values for the unitary properties of NMDA channels agree well with those reported for other cultured neurons (Nowak et al., 1984; Cull-Candy and Ogden, 1985) obtained using both fluctuation analysis and single-channel methods. This suggests that NAAG and NMDA activate the same population of channels.

Discussion

The primary finding of this study is that the neuroexcitatory effects of the endogenous dipeptide *N*-acetylaspartylglutamate on cultured spinal neurons result from the selective activation of NMDA receptors. The current–voltage relationship, antagonism by AP5, and single-channel properties derived from fluctuation analysis are all consistent with an action of NAAG on NMDA receptors. However, the NAAG concentration necessary to activate these receptors was much greater than for L-glutamate, L-aspartate, or NMDA.

The low potency of NAAG in our experiments could conceivably be due to differences in the properties of excitatory amino acid receptors acting on cultured spinal neurons from those in other brain regions or acting on other preparations. However, kainate, quisqualate, and NMDA—3 selective agonists used to characterize postsynaptic amino acid receptor subtypes on cultured spinal neurons *in vivo*—are also potent agonists on cultured spinal neurons and show the expected sensitivity to various antagonists (Mayer and Westbrook, 1984; Nelson et al., 1986). The order of agonist potency in displacing ^{3}H-AP5 binding to NMDA receptors on cortical membrane preparations (Olverman et al., 1984) also parallels the physiological activity of NMDA-receptor agonists on cultured spinal neurons, i.e., L-glutamate is the most potent activator of NMDA receptors in both assay systems. In addition, the conductance mechanism linked to NMDA receptors exhibits properties that are similar on pyramidal neurons in hippocampal slices to those seen on cultured neurons (Crunelli and Mayer, 1984). Thus it would seem that NAAG, at sufficient concentrations, could act on NMDA receptors in other brain regions, and that this might account for its excitatory properties. Although NMDA receptors are synaptically activated in the spinal cord (e.g., see Davies and Watkins, 1979), a neurotransmitter action of NAAG on NMDA receptors seems unlikely, owing to the low potency of NAAG and to the poor match between the regional distribution of NMDA-displaceable L-glutamate binding sites (Monaghan and Cotman, 1985) and regional variations in NAAG content (Koller and Coyle, 1984a).

The high-affinity binding of NAAG to brain membranes initially seemed to fulfill one of the postsynaptic criteria for a putative neurotransmitter (Zaczek et al., 1983). However, more recent studies have raised questions about the functional significance of the site to which NAAG binds. This binding site, which is distinct from the chloride-independent binding of NMDA, kainate, and AMPA/quisqualate (Foster and Fagg, 1984; Monaghan et al., 1983a) shows a high affinity for 2-amino-4-phosphonobutyrate (Butcher et al., 1983; Monaghan et al., 1983b) and is chloride-dependent (Koller and Coyle, 1984b). Recent evidence suggests that this site represents a chloride-dependent uptake site rather than a postsynaptic receptor (Fagg et al., 1986; Pin et al., 1984), raising doubts concerning the physiological role of both AP4-sensitive and NAAG-sensitive binding. For example, recent studies have not found a correlation between physiological activity of analogs of AP4 in the hippocampus and their ability to displace AP4 in a binding assay (Fagg and Lanthorn, 1985; Robinson et al., 1985).

The interpretation of some of the physiological evidence implicating NAAG as a neurotransmitter is also dependent on an understanding of the membrane action of AP4. ffrench-Mullen et al. (1985), using extracellular unit recordings of pyramidal neurons in the prepiriform cortex, reported that AP4 antagonized spike discharges induced either by NAAG or by electrical stimulation of the lateral olfactory tract. Since responses to L-glutamate or L-asparate were not affected by DL-AP4, it was suggested that NAAG was the endogenous transmitter acting on a novel postsynaptic receptor. This conclusion assumes that AP4 acts as a postsynaptic-receptor antagonist. However, the L-stereoisomer of AP4 at micromolar concentrations has been found to block synaptic transmission in the hippocampus by inhibiting transmitter release at a presynaptic site, rather than by blocking the postsynaptic receptors (Harris and Cotman, 1983). Furthermore, at high concentrations, AP4 has been shown to block NMDA receptors in the prepyriform cortex (Hori et al., 1982); it is of note that high concentrations of the D-stereoisomer of AP4 antagonize NMDA receptors in the spinal cord (Evans et al., 1982). AP4 may, in fact, have a unique postsynaptic action on some neurons (e.g., see Slaughter and Miller, 1981, for experiments on retinal neurons); however, further testing is required to determine if this is the case in the prepiriform cortex.

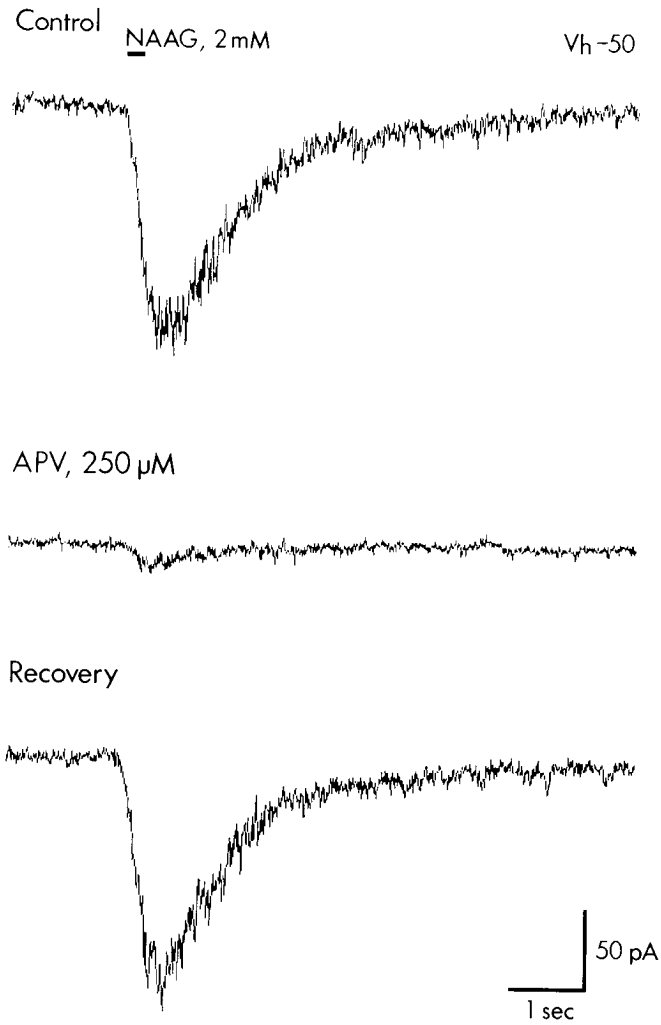

Figure 4. Responses to NAAG are blocked by DL-2-amino-5-phosphonovaleric acid (AP5). Brief pressure applications from a pipette containing 2 mM *NAAG* were applied at 15 sec intervals to the soma of a spinal cord neuron voltage-clamped at −50 mV. Immediately before the next application of NAAG, the neuron was locally perfused for 10 sec from another pipette containing 250 μM DL-AP5 (*APV*), which virtually eliminated the response to NAAG. The response to NAAG gradually returned to a full recovery (*bottom trace*) during the subsequent 60 sec.

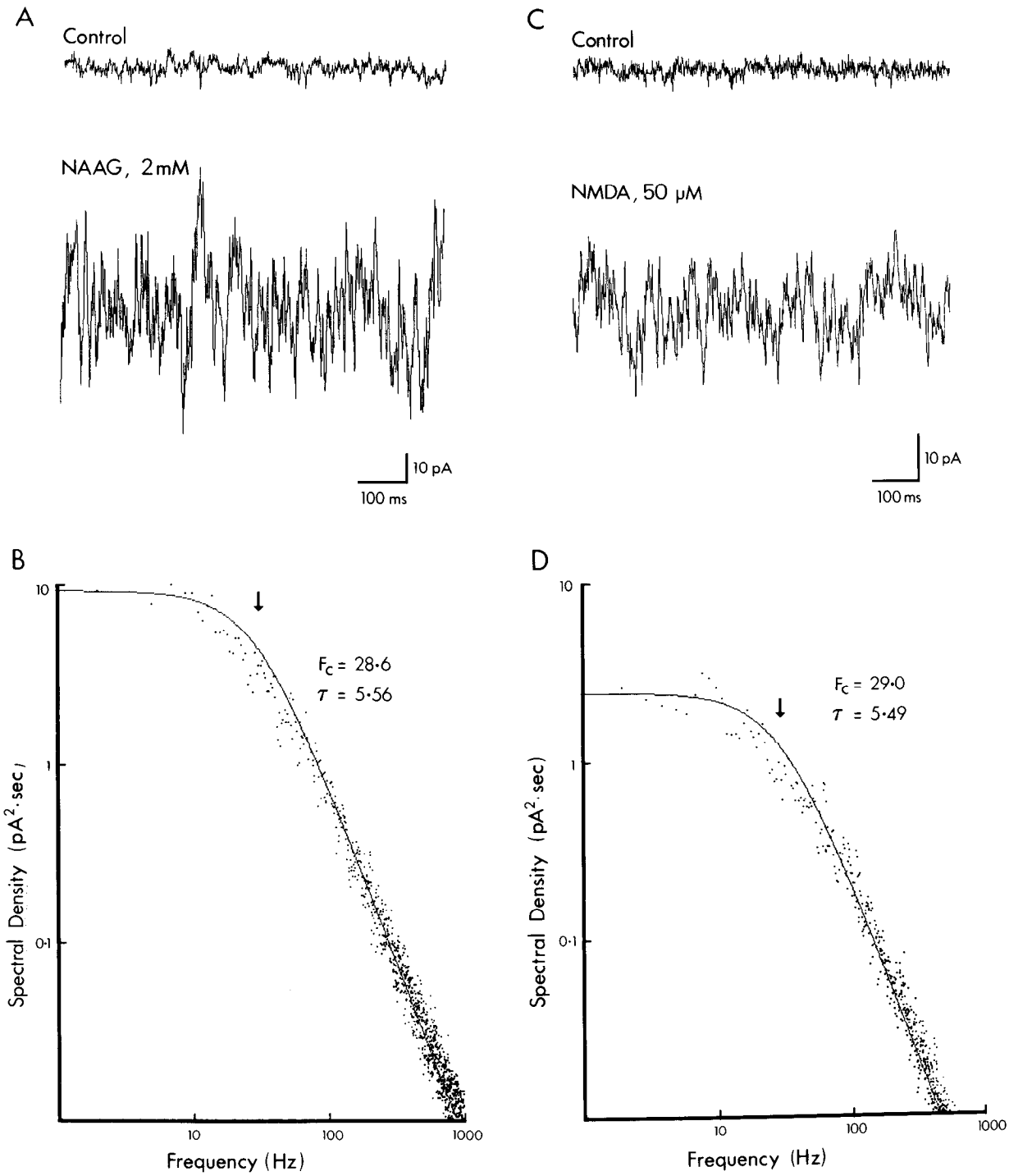

Figure 5. The conductance activated by NAAG shows unitary properties similar to NMDA-receptor channels. *A*, Membrane current noise before (*Control*) and during application of *NAAG* is shown for a spinal cord neuron at a membrane potential of −40 mV in Mg^{2+}-free medium. The spectral-density function of the membrane current noise evoked by NAAG is shown in *B*. The *arrow* indicates the location of the half-power frequency, F_c, of the single Lorentzian function fitted to the data points. The mean open time of the channels was estimated as $\tau = 1/2\pi F_c$. *C, D*, On another neuron, *NMDA* evoked an increase in noise with a nearly identical noise spectrum. Application methods were similar to those in Figure 3, and thus drug concentrations represent those in pipettes rather than at the cell surface. Mean inward current was 330 pA for NAAG and 80 pA for NMDA.

We conclude that *N*-acetylaspartylglutamate activates only the NMDA-receptor subtype on spinal cord neurons, when applied at concentrations not exceeding 3 mM. It is possible that as for L-glutamate (see Mayer and Westbrook, 1984) even higher concentrations of NAAG could have a mixed agonist action and activate kainate or quisqualate as well as NMDA receptors. Insufficient evidence is currently available for a decision as to whether this action contributes to the excitatory action of NAAG in other brain regions. However, the low neuroexcitatory potency of NAAG makes it reasonable to consider alternative roles for NAAG, as well as N-acetylaspartate and aspartate N-acetyltransferase (Truckenmiller et al., 1985). For example, their rel-

atively high concentration and differential distribution within the nervous system suggest a possible function in the metabolism of acidic amino acid-transmitter pools. In studies of spinal sensory ganglia, we have found NAAG-immunoreactivity within a subpopulation of neurons (Cangro et al., 1985); and *in vitro,* these ganglia readily use radiolabeled glutamine and glutamate as precursors for the apparent enzyme-mediated synthesis of NAAG (Cangro et al., 1986). The relative inability of these neurons to incorporate ^{3}H-aspartate directly into NAAG suggests a precursor role for the large intracellular pool of *N*-acetylaspartate in the biosynthesis of NAAG. Such metabolic interactions are consistent with the participation of NAAG and *N*-acetylaspartate in the regulation of the neurotransmitter pool of L-glutamate within subsets of neurons.

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