The Journal of Neuroscience
January 1986, 6(1): 102-106

N-Methyl-D-Aspartate Receptor Antagonists Reduce Synaptic Excitation in the Hippocampus

John J. Hablitz and Iver A. Langmoen[1]

Section of Neurophysiology, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030

The hypothesis that synaptic excitation in the CA1 region of the hippocampus is mediated in part by N-methyl-D-aspartate (NMDA) receptors was tested using intra- and extracellular recording techniques. Synaptic potentials elicited by stratum radiatum stimulation were examined in individual neurons before and after bath application of the NMDA receptor antagonist, DL-2-amino-5-phosphonovalerate (APV). This antagonist reduced both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). When IPSPs were suppressed by the addition of picrotoxin, EPSPs were seen in isolation. APV reduced these EPSPs but did not block synaptic transmission. This antagonist demonstrated anticonvulsant actions when tested against picrotoxin-induced epileptiform activity.

These results suggest that, as in the spinal cord and neocortex, synaptic excitation in the CA1 region of the hippocampus is partially mediated by APV-sensitive NMDA receptors. The fact that synaptic activity is not blocked by NMDA antagonists indicates that EPSPs in CA1 neurons are not mediated solely by this receptor.

Synaptic excitation in the hippocampal CA1 region is thought to be mediated by an excitatory amino acid neurotransmitter (Cotman and Nadler, 1981). Electrophysiological (Collingridge et al., 1983a; Hablitz, 1982) and anatomical (Monaghan et al., 1983) studies have indicated that multiple types of receptors, including one for N-methyl-D-aspartate (NMDA), are present on CA1 pyramidal neurons. Although NMDA receptors have been implicated in the regulation of certain types of synaptic plasticity (Collingridge et al., 1983b; Harris et al., 1984; Wigström and Gustafsson, 1984), a role for them in normal synaptic transmission in the hippocampus has not been established.

NMDA receptors have been implicated in synaptic transmission in the spinal cord and neocortex. The specific NMDA antagonist, DL-2-amino-5-phosphonovalerate (APV), at doses that selectively depress responses to iontophoretically applied NMDA, preferentially reduces polysynaptic excitation of feline spinal neurons (Davies and Watkins, 1983; Davies et al., 1981). In the isolated frog spinal cord, NMDA receptor antagonists depress electrically evoked ventral root potentials (Evans et al., 1982) and eliminate spinal seizures induced by convulsant drugs (Ryan et al., 1984). An excitatory postsynaptic potential (EPSP) evoked in pyramidal cells of rat sensorimotor cortex has also been reported to be APV-sensitive, and is apparently an NMDA receptor-mediated synapse (Thomson et al., 1985).

Received March 25, 1985; revised June 24, 1985; accepted July 17, 1985.

This work was supported by NIH Grants NS18145 and AA06077. Dr. Langmoen was a recipient of a travel grant from the G. E. Strangelands Fund.

Correspondence should be addressed to John J. Hablitz, Ph.D., Section of Neurophysiology, Department of Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

[1] Dr. Langmoen's permanent address is Department of Neurosurgery, Ulleval Hospital, Oslo, Norway.

Here we report that bath application of APV, which has previously been shown to specifically antagonize NMDA-induced excitation of hippocampal neurons (Collingridge et al., 1983a; Hablitz and Langmoen, 1984), reduces intracellularly recorded EPSPs in CA1 neurons *in vitro*. Furthermore, we show that this antagonist has anticonvulsant actions when tested against picrotoxin-induced epileptiform activity. These results demonstrate that, as in the spinal cord (Davies and Watkins, 1983) and neocortex (Thomson et al., 1985), synaptic excitation in the CA1 region of the hippocampus is mediated in part by APV-sensitive NMDA receptors.

Materials and Methods

Experiments were carried out on hippocampal slices from rats weighing 100–150 gm. Transverse slices, nominally 400 μm thick, were taken from the middle third of the hippocampus and maintained *in vitro*, as described previously (Hablitz and Langmoen, 1982). The initial perfusing solution consisted of (in mM) NaCl, 125; KCl, 3.5; NaH_2PO_4, 1.25; CaCl, 2; $MgSO_4$, 2; $NaHCO_3$, 26; and glucose, 10.

Microelectrodes were prepared from thin-walled capillaries and filled with 4M KC_2H_3O (40–70 MΩ). Recordings were made using a single-electrode current-clamp circuit (Hablitz, 1984), and digitized using a microcomputer. Afferent fibers in stratum radiatum were activated via bipolar platinum iridium electrodes. These electrodes were placed in the dendritic layer approximately 75–125 μm from the cell body layer.

Extracellular recordings of the presynaptic fiber volley and field EPSP were made at distances of 0.5–1 mm from the stimulating electrode, as described by Andersen et al. (1978). A graded series of orthodromic stimuli were delivered at 0.2 Hz before and after bath application of APV. In intracellular studies, microelectrodes were advanced through the pyramidal cell layer. After a stable intracellular impalement had been obtained, control recordings were made of the response to a graded series of orthodromic stimuli, as well as to a family of hyperpolarizing and depolarizing current pulses. The perfusate was then changed to one containing APV and responses reassessed after a waiting period of 15 min. In the studies of epileptiform activity, picrotoxin (50 μM), a potent convulsant agent (Hablitz, 1984), was present in both the control and APV-containing saline.

Results

Extracellular studies

In order to establish that APV was not acting to reduce the excitability of afferent fibers in stratum radiatum, a series of experiments using extracellular recordings of the presynaptic fiber volley and field EPSP were performed. An example of the dendritic response to stratum radiatum stimulation is shown in the inset to Figure 1*A*. The initial diphasic negative deflection represents impulse propagation in the presynaptic fibers and is a measure of the number of afferent fibers activated (Andersen et al., 1978). The following negative wave represents an extracellular counterpart of the EPSP, recordable intracellularly. Initial experiments were designed to determine if APV affected fiber excitability. When the amplitude of the presynaptic fiber volley is plotted as a function of stimulus strength (Fig. 1*A*), it

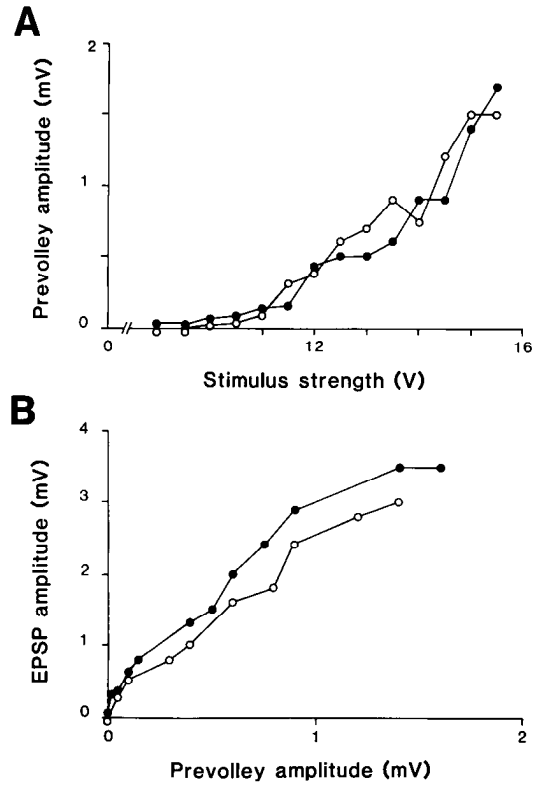

Figure 1. Alterations in input–output curves produced by APV. *A*, Plot of presynaptic fiber volley amplitude as a function of stimulus strength. Measurements were made from an average of 10 traces obtained at each stimulus strength. APV had no effect on fiber excitability. *B*, Field EPSP amplitude as a function of prevolley amplitude. Data are from the same experiment as *A*. APV caused a reduction in the field EPSP, consistent with an antagonist action. Control, ●. 200 μM DL-APV, ○.

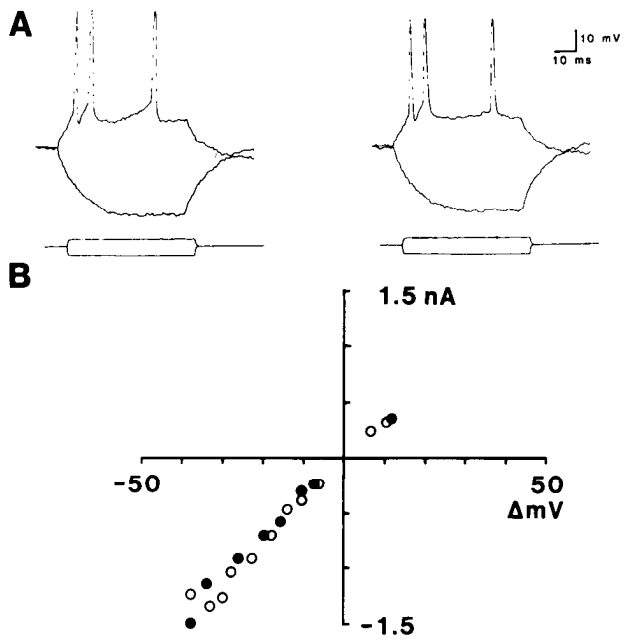

Figure 2. Effect of DL-APV on hippocampal CA1 neurons. *A*, Specimen records showing the response to depolarizing and hyperpolarizing current pulses before (*left*) and after (*right*) addition of 100 μM APV to the bathing medium. *B*, Current–voltage relation for the same neuron, showing that antagonist application did not affect passive membrane properties. Input resistance was 31 and 28 MΩ in the control (●) and drug (○, 100 μM APV) periods, respectively.

can be seen that this relationship is not affected by APV application. Similar results were obtained in six experiments, including two where an APV concentration of 500 μM was used. In contrast to the lack of effect on the fiber volley, the field EPSP was reduced by APV, as shown in Figure 1*B*. This antagonist, at a concentration of 200 μM ($N = 4$), produced a 27 ± 15% reduction in the field EPSP.

Passive membrane properties

The results of a typical experiment are shown in Figure 2. Specimen records of the response to hyperpolarizing and depolarizing current pulses are shown in Figure 2*A* and indicate that APV did not affect the firing properties or passive input resistance of this cell. A more complete assessment of the current–voltage relationship was made by delivering a family of depolarizing and hyperpolarizing current pulses and measuring the resultant voltage deflection. Addition of APV to the bathing medium did not affect the current–voltage relationship (Fig. 2*B*).

In nine neurons, the average input resistance was 28.9 ± 3 MΩ in the control period and 29 ± 6 MΩ after antagonist perfusion. There was no consistent change in membrane potential associated with APV perfusion. When small changes (2–4 mV) did occur, steady depolarizing currents were used to adjust the membrane potential to its control value.

Effect on intracellularly recorded EPSPs

The response to orthodromic stimulation before and after 100 μM APV is shown in Figure 3. Subthreshold stimulation (upper trace) elicited the EPSP–IPSP sequence typically seen in CA1 neurons, while stronger stimulation (lower trace) evoked an action potential at the peak of the EPSP. Following wash-in of APV, the subthreshold EPSP was reduced by 20% and the IPSP was barely discernible. After antagonist perfusion, the stronger stimulus no longer evoked an action potential. Although this stronger stimulus resulted in an EPSP comparable in size to that evoked by the weak stimulus in control saline, a smaller IPSP was observed in the presence of APV. In four neurons tested, APV, at a concentration of 100 μM, produced a 23 ± 8% decrease in the EPSP.

Although these results indicate that NMDA receptor antagonists can reduce EPSPs in CA1 neurons, quantitative analysis of changes in the EPSP was hindered by the ubiquitous occurrence of IPSPs. Since the difference between the latency of onset of excitation and of inhibition is approximately 2 msec (Andersen et al., 1964), there is considerable temporal overlap of the two potentials. To examine the effect of EPSPs uncontaminated by IPSPs, slices were bathed in a saline containing 50 μM picrotoxin (to block GABA-mediated inhibition; Hablitz, 1984) and 4 mM calcium plus 4 mM magnesium (to reduce the occurrence of epileptiform activity; Wigström and Gustafsson, 1984). As shown in the upper trace of Figure 4*A*, under these

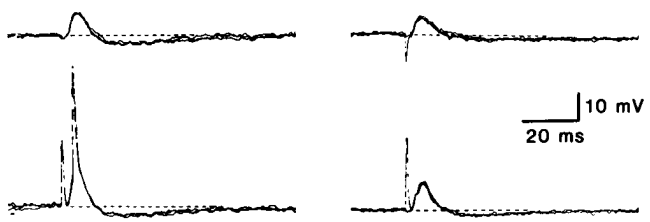

Figure 3. Depression of synaptic responses by an NMDA antagonist. Responses to stimulation of afferent fibers in stratum radiatum at two stimulus strengths are shown. Control, left traces. 100 μM DL-APV, right traces. Each trace consists of three superimposed sweeps. *Dotted lines*, the resting membrane potential, which was −60 mV.

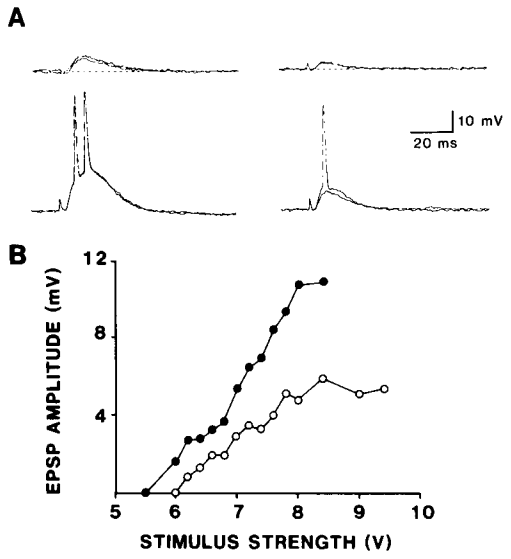

Figure 4. Reductions of EPSPs by APV in picrotoxin-treated slices. *A*, The strength of orthodromic stimulation was varied to produce either a subthreshold EPSP (*upper record*) or repetitive firing (*lower record*). Control, *left*. 100 μM DL-APV, *right*. APV reduced the amplitude of the EPSP and the number of action potentials produced by the suprathreshold stimulus. Resting potential was −65 mV. *B*, Plot of EPSP amplitude as a function of stimulus strength showing a depression of EPSP amplitude in the presence of APV. Control, ●. 100 μM APV, ○.

conditions, subthreshold EPSPs without associated IPSPs could be recorded in response to afferent stimulation. Suprathreshold activation (lower trace) produced repetitive firing. The effect of APV was to decrease the amplitude of the EPSPs and reduce the number of action potentials generated by stronger stimulation. Synaptic input–output curves relating stimulus strength to EPSP amplitude were constructed. One can see that APV increased the threshold for eliciting a detectable response and decreased the maximum EPSP amplitude (Fig. 4*B*). A reduction of 39 ± 11% ($N = 7$) was observed with 100 μM APV. Action-potential generation in response to orthodromic stimulation was

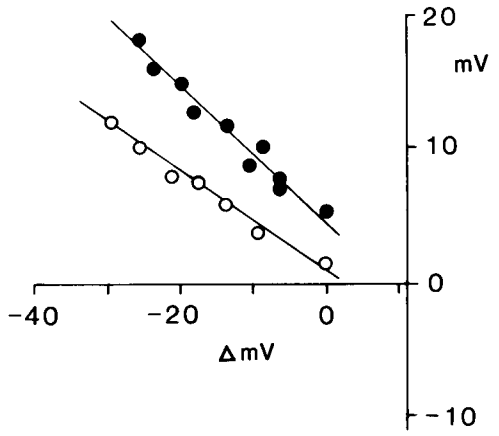

Figure 5. Effect of DL-APV on the voltage dependence of the EPSP. Synaptic responses were obtained at varying membrane potentials by superimposing the EPSP on long-duration (300 msec) hyperpolarizing current pulses of varying amplitude. EPSP amplitude was calculated as the difference between the steady state value of the membrane potential during the current pulse and the peak of the synaptic response. The *regression lines* fitted to the data had correlation coefficients of 0.98 and 0.99 for the control (●) and APV (○) conditions, respectively. The EPSP conductance increase was decreased by APV.

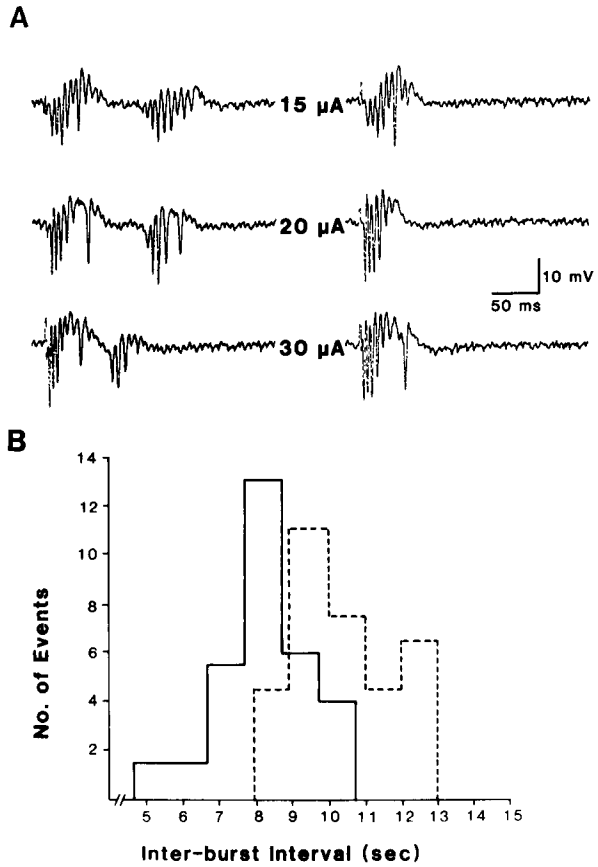

Figure 6. Anticonvulsant action of APV on picrotoxin-induced epileptiform activity. *A*, Extracellular recordings were made of epileptiform burst discharges in the CA1 region in response to stratum radiatum stimulation. Control (*left half*), an initial burst discharge is followed by a secondary epileptiform event. The latency of this late event decreases as the stimulus strength is increased. 200 μM DL-APV (*right half*), the secondary epileptiform discharges, which probably represent a reactivation of CA1 following an epileptiform event in CA3, are abolished by APV. *B*, Effect of APV on the frequency of occurrence of spontaneous epileptiform discharges. Histograms of interburst intervals for spontaneous epileptiform discharges recorded in the CA1 region are shown (control, —). Bath application of the NMDA antagonist APV produces a lengthening of the mean interburst interval (100 μM DL-APV, – – –).

never completely suppressed by antagonist application, although the strength needed for excitation was increased.

If the reduction in the EPSP was due to a postsynaptic blockade of the synaptic conductance mechanism, there should be a decrease in the conductance increase associated with synaptic activation. Since the input resistance of the neurons was not affected by APV, relative changes in synaptic conductance can be determined from plots of EPSP amplitude versus membrane potential. Figure 5 shows the relationship between membrane potential and EPSP amplitude before and after APV was added to the bathing medium. The slope of this relation, which is an indicator of synaptic conductance, was reduced by APV. Similar results were obtained in two other neurons; the slope was decreased by an average of 31% ($N = 3$).

Epileptiform activity and APV

In slices bathed in a saline containing the normal concentration of divalent cations plus picrotoxin, stimulation of afferent fibers in stratum radiatum produced the response pattern shown in Figure 6*A*. A single shock typically triggered an initial epileptiform burst response, which was followed by a late secondary discharge (upper record). As the stimulus strength was increased,

the latency of the late discharge decreased (middle record), and the two responses eventually merged (lower record). We have shown previously that this late secondary discharge is due to a reverberation of activity between the CA1 and CA3 regions (Gjerstad et al., 1981; Hablitz, 1984). Following bath application of 200 μM APV, late discharges were either abolished (upper and middle records) or reduced (lower record). The initial epileptiform events were relatively unaffected.

To further examine possible anticonvulsant properties of APV, we monitored the frequency of occurrence of spontaneous epileptiform discharges before and after antagonist perfusion. As shown in Figure 6*B*, APV decreased the spontaneous discharge rate. In a total of seven such experiments, the mean interval between events before and after APV application was 5.57 ± 1.9 and 7.7 ± 1.8 sec, respectively. This difference was statistically significant (Mann-Whitney *U* test; $p < 0.05$). This effect was reversible, and upon washing, the rate returned to one every 5.04 ± 0.86 sec.

Discussion

The present observations indicate that APV is effective in reducing orthodromically evoked EPSPs in CA1 neurons. At the concentrations employed, we have previously shown APV to antagonize responses of CA1 cells to iontophoretically applied NMDA, while not affecting responses to quisqualate (Hablitz and Langmoen, 1984). Extracellular studies (Collingridge et al., 1983a) have also demonstrated a selective action of APV on NMDA-mediated responses. This suggests that an NMDA receptor is involved in synaptic transmission in this region of the hippocampus. This is in keeping with the finding that a high density of NMDA-sensitive ^{3}H-L-glutamate binding sites is found in the terminal fields of afferent fibers in stratum radiatum (Monaghan et al., 1983).

Antagonist application reduced the amplitude of the EPSPs, while having little effect on passive membrane properties. This, and the observed decrease in EPSP conductance, suggests that APV was acting directly on postsynaptic receptors, decreasing their sensitivity to the synaptically released transmitter. The apparent lack of antagonism of synaptic responses reported by others (Collingridge et al., 1983b; Koerner and Cotman, 1982) may reflect an insensitivity of extracellular recording techniques in detecting the subthreshold effects observed here. The persistence of evoked synaptic activity that is APV-insensitive indicates that both NMDA and non-NMDA receptors are involved in excitation of hippocampal neurons.

The idea that synaptic responses in the CA1 region are mediated via multiple receptors would explain the different voltage sensitivities of the EPSP and NMDA responses. We have shown that both the response to glutamate and the EPSP in CA1 neurons decrease in amplitude as the membrane potential is made less negative, and reverses near 0 mV (Hablitz and Langmoen, 1982). In contrast, NMDA responses are maximal around the cell's resting potential and decrease with both hyperpolarization and depolarization (Hablitz, 1982; Hablitz and Langmoen, 1984). These results suggest that non-NMDA receptors are the predominant contributors to the EPSP. If the NMDA receptor-mediated response could be isolated, response amplitude would be expected to be a nonlinear function of membrane potential, decreasing with both hyperpolarization and depolarization. Such NMDA receptor-mediated responses have been observed in the neocortex (Thomson et al., 1985).

Hippocampal pyramidal neurons typically respond to orthodromic activation with a mixed EPSP–IPSP. The inhibitory component of this response is often so strong that the EPSP is barely discernible. Blockade of GABA-mediated inhibition with penicillin (Dingledine and Gjerstad, 1979) or picrotoxin (Hablitz, 1984) results in an unmasking of EPSPs. The disinhibition observed in the present studies could counteract the expected antagonist effect of APV on excitatory synapses, with a decrease of EPSP amplitude being masked by a concomitant decrease in the shunting effect of the IPSP. Such a mechanism could account for previous failures to observe an effect of APV on synaptic responses. This hypothesis is supported by the observation that, when EPSPs were studied in relative isolation following picrotoxin exposure, APV was more effective than under normal recording conditions.

IPSPs in the hippocampus are thought to be generated by both recurrent (Andersen et al., 1964) and feedforward (Alger and Nicoll, 1982; Dingledine and Gjerstad, 1980) inhibitory pathways. Our experiments have shown that the fiber volley recorded in stratum radiatum, a measure of the excitability of presynaptic fibers (Andersen et al., 1978), was not affected by APV. Thus, the observed reduction in IPSPs can be attributed to a loss of excitatory input to interneurons. This could result from a postsynaptic action of APV on inhibitory interneurons receiving excitatory inputs from afferent fibers in stratum radiatum, i.e., an effect on feedforward inhibition or via a decrease in recurrent inhibition due to a decreased probability of pyramidal cell discharge. These alternative mechanisms cannot be distinguished on the basis of the present results and are not mutually exclusive.

The alterations in spontaneous and evoked epileptiform activity induced by APV are in keeping with previous reports of anticonvulsant actions of excitatory amino acid antagonists (Croucher et al., 1982; Meldrum et al., 1983). The finding that these agents can reduce the frequency of occurrence of epileptiform activity and its spread between hippocampal subregions without suppressing evoked synaptic activity indicates that they are potentially useful anticonvulsant agents. APV's predominant anticonvulsant effect was to reduce or abolish late, secondary epileptiform discharges in the CA1 region. This activity is due to reactivation of the CA1 area following a paroxysmal discharge in CA3 (Gjerstad et al., 1981; Hablitz, 1984). The greater susceptibility of the late discharge to APV antagonism could result from an action of APV on some specific group of fibers mediating transmission of this discharge. It is more likely, however, that it is due to a cumulative effect of APV at the several synapses in the multisynaptic pathway underlying this type of activity. Since spontaneous discharges, which were reduced in frequency by APV, are known to originate in the CA3 region of the hippocampus, the observation of alterations in discharge frequency indicates that APV also affects synaptic interactions in that region, implying a widespread distribution of physiologically relevant NMDA receptors.

References

Alger, B. E., and R. Nicoll (1982) Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied in vitro. J. Physiol. (Lond.) *328:* 105–123.

Andersen, P., J. C. Eccles, and Y. Løyning (1964) Pathway of postsynaptic inhibition in the hippocampus. J. Neurophysiol. *27:* 608–619.

Andersen, P., H. Silfvenius, S. H. Sundberg, O. Sveen, and H. Wigström (1978) Functional characteristics of unmyelinated fibres in the hippocampal cortex. Brain Res. *144:* 11–18.

Collingridge, G. L., S. J. Kehl, and H. McLennan (1983a) The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. J. Physiol. (Lond.) *334:* 19–31.

Collingridge, G. L., S. J., Kehl, and H. McLennan (1983b) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J. Physiol. (Lond.) *334:* 33–46.

Cotman, C. W., and J. V. Nadler (1981) Glutamate and aspartate as hippocampal transmitters: Biochemical and pharmacological evidence. In *Glutamate: Transmitter in the Central Nervous System,* P. J. Roberts, J. Storm-Mathisen, and G. A. R. Johnston, eds., pp. 117–154, Wiley, New York.

Croucher, M. J., J. F. Collins, and B. S. Meldrum (1982) Anticon-

vulsant action of excitatory amino acid antagonists. Science *216:* 899–901.

Davies, J., and J. C. Watkins (1983) Role of excitatory amino acid receptors in mono- and polysynaptic excitation in the cat spinal cord. Exp. Brain Res. *49:* 280–290.

Davies, J., A. A. Francis, A. W. Jones, and J. C. Watkins (1981) 2-amino-5 phosphonovalerate (2-APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. Neurosci. Lett. *21:* 77–81.

Dingledine, R., and L. Gjerstad (1979) Penicillin blocks hippocampal IPSPs, unmasking prolonged EPSPs. Brain Res. *168:* 205–209.

Dingledine, R., and L. Gjerstad (1980) Reduced inhibition during epileptiform activity in the in vitro hippocampal slice. J. Physiol. (Lond.) *305:* 297–313.

Evans, R. H., A. A. Francis, A. W. Jones, D. A. S. Smith, and J. C. Watkins (1982) The effects of a series of ω-phosphonic α-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. Br. J. Pharmacol. *75:* 65–75.

Gjerstad, L., P. Andersen, I. A. Langmoen, A. Lundervold, and J. J. Hablitz (1981) Synaptic triggering of epileptiform discharges in CA1 pyramidal cells in vitro. Acta Physiol. Scand. *113:* 245–252.

Hablitz, J. J. (1982) Conductance changes induced by DL-homocysteic acid and N-methyl-DL-aspartic acid in hippocampal neurons. Brain Res. *247:* 149–153.

Hablitz, J. J. (1984) Picrotoxin-induced epileptiform activity in hippocampus: Role of endogenous versus synaptic factors. J. Neurophysiol. *51:* 1011–1027.

Hablitz, J. J., and I. A. Langmoen (1982) Excitation of hippocampal pyramidal cells by glutamate in the guinea-pig and rat. J. Physiol. (Lond.) *325:* 317–331.

Hablitz, J. J., and I. A. Langmoen (1984) Possible NMDA receptor mediation of synaptic transmission in the hippocampal CA1 region. Soc. Neurosci. Abstr. *10:* 415.

Harris, E. W., A. H. Ganong, and C. W. Cotman (1984) Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res. *323:* 132–137.

Koerner, J. F., and C. W. Cotman (1982) Response of Schaffer collateral-CA1 pyramidal cell synapses of the hippocampus to analogues of acidic amino acids. Brain Res. *251:* 105–115.

Meldrum, B. S., M. J. Croucher, G. Badman, and J. F. Collins (1983) Antiepileptic action of excitatory amino acid antagonists in the photosensitive baboon, *Papio papio.* Neurosci. Lett. *39:* 101–104.

Monaghan, D. T., V. R. Holets, D. W. Toy, and C. W. Cotman (1983) Anatomical distributions of four pharmacologically distinct ^{3}H-L-glutamate binding sites. Nature *306:* 176–179.

Ryan, G. P., J. C. Hackman, and R. A. Davidoff (1984) Spinal seizures and excitatory amino acid-mediated synaptic transmission. Neurosci. Lett. *44:* 161–166.

Thomson, A. M., D. C. West, and D. Lodge (1985) An N-methylaspartate receptor-mediated synapse in rat cerebral cortex: A site of action of ketamine? Nature *313:* 479–481.

Wigström, H., and B. Gustafsson (1984) A possible correlate of the postsynaptic condition for long-lasting potentiation in the guinea pig hippocampus in vitro. Neurosci. Lett. *44:* 327–332.