PHARMACOLOGICAL CHARACTERIZATION OF POSTSYNAPTIC RECEPTORS FOR EXCITATORY AMINO ACIDS IN PURKINJE CELL DENDRITES IN THE GUINEA PIG CEREBELLUM

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The responses of Purkinje cell dendrites to iontophoretically applied L-glutamate, L-aspartate, kainate and quisqualate were intradendritically recorded in the *in vitro* slice preparation of the guinea pig cerebellum. Kainate and quisqualate were found to be very potent excitants, while *N*-methyl-DL-aspartic acid (NMDLA) caused little or no effect on Purkinje cell dendrites. Thus, kainate and quisqualate receptors were certainly present, while there appeared to be no NMDA-preferring receptor in Purkinje cell dendrites in the guinea pig cerebellum. The effects of several antagonists such as 2-amino-5-phosphonovaleric acid (APV), γ -D-glutamylglycine (γ -DGG), NMDLA and glutamic acid diethylester (GDEE) *etc.* on responses to L-glutamate and L-aspartate were compared in order to search for potent and selective antagonists capable of differentiating the response to L-glutamate from that to L-aspartate. Among these compounds tested, APV and γ -DGG were found to be potent and selective antagonists to the L-aspartate-induced depolarization without affecting responses to L-glutamate. These two agents may be useful, at least in the *in vitro* study, to the identification of the excitatory amino acid which function as a neurotransmitter at the synapses formed by the climbing fiber or parallel fiber with Purkinje cell dendrites.

Keywords — excitatory amino acid; receptor; antagonist; intradendritic recording; cerebellar Purkinje cell; guinea pig

INTRODUCTION

Recent biochemical studies suggest that L-glutamate and L-aspartate may be the neurotransmitters respectively at the synapses formed by parallel fibers and climbing fibers with Purkinje cell dendrites in the mammalian cerebellum.¹⁻⁴⁾ However, the investigation of the possibility that these excitatory amino acids function as synaptic transmitters at these synapses has long been handicapped by the lack of suitable antagonists for either amino acid-induced or synaptic excitation. Therefore, further identifications on the transmitter role of L-glutamate and L-aspartate in Purkinje cell dendrites largely rely upon the discovery of potent and selective antagonists for either one of these excitatory amino acids.

On the basis of studies in the cat spinal cord,

Evans and Watkins (1981)⁵⁾ and McLennan (1981)⁶⁾ have proposed the existence of three pharmacologically distinguishable excitatory amino acid receptors. The first type is identified by its most potent and selective agonist *N*-methyl-D-aspartic acid (NMDA), and is antagonized by 2-amino-5-phosphonovaleric acid (APV). The second type is preferentially activated by kainate and is sensitive to antagonism by γ -D-glutamylglycine (γ -DGG), which also antagonizes NMDA actions. The third is affected by glutamate and quisqualate and is blocked by glutamic acid diethylester (GDEE).

In this study, a pharmacological analysis of the excitatory amino acid receptors on Purkinje cell dendrites and the evaluation of the potency and selectivity of several agonists and antagonists were carried out as the first step to further

investigations for identifying the neurotransmitters of the parallel fiber and climbing fiber in the guinea pig cerebellum.

MATERIALS AND METHODS

Male guinea pigs, 300–400 g, were killed by stunning, and the cerebellum was isolated. A block of the cerebellum was sectioned to be 170 μm thickness, using Vibratome (Oxford Lab., Calif, USA), in parallel to the arborization of Purkinje cell dendrites, *i.e.*, sagittally.⁷⁾ Two to three such slices were placed on the bottom of a superfusion chamber, and superfused (at a rate of about 1 ml/min) for 1 h as preincubation and throughout an experiment with the Krebs-Ringer bicarbonate medium (control medium) which was kept at 36.5 °C and containing (in mM) NaCl (125), KCl (5), CaCl_2 (2), MgCl_2 (1), NaH_2PO_4 (1), NaHCO_3 (24), and glucose (11), pH being adjusted to be 7.4 by saturating with 95%/5% O_2/CO_2 gas. When a high or low Mg^{2+} medium was required, the concentration of Mg^{2+} in control medium was simply changed.

Agonists and antagonists were applied iontophoretically from a three-barrel micropipette onto the Purkinje cell dendrite as closely as possible to the recording site. The following solutions were filled into individual barrels: L-glutamate (0.5 M, pH 8.5), L-aspartate (0.5 M, pH 8.5), kainate (0.05 M, pH 8.5), quisqualate (0.05 M, pH 8.5), L-glutamic acid diethylester (GDEE) (0.5 M, pH 3.5), *N*-methyl-DL-aspartic acid (NMDLA) (0.5 M, pH 8.0), γ -D-glutamylglycine (γ -DGG) (0.25 M, pH 8.0), 2-amino-5-phosphonovaleric acid (APV) (0.25 M, pH 8.0), 2-amino-4-phosphonobutyric acid (APB) (0.25 M, pH 8.0), *cis*-2,5-piperidine dicarboxylic acid (*cis*-2,5-PDA) (0.25 M, pH 8.0), 2-amino-3-phosphonopropionic acid (APP) (0.25 M, pH 8.0), L-asparagine (0.5 M, pH 8.0), L-glutamine (0.5 M, pH 8.0), D-aspartic acid (0.5 M, pH 8.0), D- α -amino adipic acid (D α AA) (0.5 M, pH 8.0), *cis*-2,4-piperidine dicarboxylic acid (*cis*-2,4-PDA) (0.25 M, pH 8.0), D-glutamic acid (0.5 M, pH 8.0), *N*-acetyl-DL-glutamic acid (0.5 M, pH 8.0), DL-proline (0.5 M, pH 8.0), and

DL-aspartic acid dimethylester (ADME) (0.5 M, pH 3.5).

Responses to iontophoretically applied glutamate, aspartate and other compounds were recorded with the microelectrode impaled into the dendrite (about 100 μm distant from the soma) of Purkinje cells in the slice under visual control ($\times 120$). Recording electrodes routinely used were filled with 3M KCl (70–90 Mohm).

In order to minimize disturbance by generation of Na- and Ca-spikes, the membrane potential of Purkinje cell dendrites was routinely shifted to -110 mV by direct current (DC) injection through an impaled recording electrode using a high input impedance (10^{10} ohm) bridge circuit (MEZ-8201, Nihon Kohden). Thus, all responses to iontophoresed agonists and antagonists shown are those at this hyperpolarized membrane potential. The pen-writing recorder (RJG-4004, Nihon Kohden) was used for recording responses to agonists and antagonists.

APV, APB, APP, *cis*-2,4-PDA and *cis*-2,5-PDA were purchased from Cambridge Res. Biochem. (G.B.). GDEE, ADME, quisqualate and NMDLA were from Sigma Chem. (USA). All other compounds used, including Na-L-glutamate and L-aspartic acid, were obtained from Wako Pure Chem. Ind. (Tokyo, Japan).

RESULTS

Intradendritic recordings were performed impaling a recording electrode by trial and error into a dendritic region of the Purkinje cell, which was about 100 μm from the Purkinje cell layer toward the pial surface. The mean membrane potential of the dendrites successfully impaled was about -58 mV (-58.2 ± 1.2 (s.e.m.) mV, $n=40$).

Three general criteria were utilized to identify intradendritic recording from a Purkinje cell dendrite. First, at the resting membrane potential, spontaneous Na-spikes generated at the soma propagating to the dendrite could consistently be observed. Secondly, Ca-spikes could be elicited by the depolarization of the membrane by injection of DC- or square pulse-currents

through a recording electrode. Thirdly, electrical stimulation of the white matter could produce an all or none orthodromic potential of the climbing fiber response.

Responses Elicited in Purkinje Cell Dendrites by L-Glutamate and L-Aspartate Agonists

The iontophoretic application of any one of excitatory amino acids at resting membrane potential induced a depolarization which was followed by the generation of Na-spikes, and finally generated Ca-spikes. In order to eliminate the interference of these regenerative spikes for the accurate estimation of effects of agonists and antagonists, the membrane was hyperpolarized to -110 mV by the application of DC-current through a recording electrode.

Figure 1 shows typical responses of Purkinje cell dendrites to iontophoretic pulse applications

FIG. 1. Depolarizations of Purkinje Cell Dendrites Induced by Iontophoresed L-Glutamate, L-Aspartate, Kainate and Quisqualate

Short pulse applications (700 ms duration for A, 500 ms for B) of L-glutamate and L-aspartate at a hyperpolarized membrane potential of -110 mV elicited abrupt depolarizations, which returned rapidly to the initial level on terminating the ejection. Depolarizations induced by the application of kainate (A) and quisqualate (B), at a membrane potential of -110 mV, outlasted the ejection of these substances and gradually returned to the initial membrane potential. All compounds were iontophoretically applied with the ejecting currents denoted in nA to the dendrite close to the recording site. Records A and B were from different dendrites.

(500–700 ms: duration) of L-aspartate, L-glutamate, kainate and quisqualate. Quisqualate and kainate were found to be very potent excitants of Purkinje cell dendrites (Fig. 1A, B). When quisqualate was applied iontophoretically as closely as possible to the recording site, the minimum current needed to depolarize the dendrite was usually less than 1 nA. On the basis of the ejecting currents used and the concentrations (1/10 of that of L-glutamate or L-aspartate) in the barrels of the iontophoretic pipette, these two agonists appeared to be about 10 times for kainate and about 30 times for quisqualate more

FIG. 2. Effects of GDEE (A) and NMDLA (B) on Responses to L-Glutamate and L-Aspartate

A. Simultaneous application of GDEE (70 nA) from an adjacent barrel depressed the depolarizations of a Purkinje cell dendrite induced by either L-glutamate (open squares, 20 nA, 300 ms) or L-aspartate (closed squares, 60 nA, 300 ms), but responses to L-glutamate were preferentially antagonised. In 6 dendrites tested, the response to L-glutamate was blocked by $62.8 \pm 19.1\%$, and that to L-aspartate was by $31.1 \pm 18.2\%$.

B. Simultaneously applied NMDLA (100 nA) preferentially antagonized responses to L-aspartate (closed squares, 30 nA, 400 ms), while those to L-glutamate (open squares, 10 nA, 400 ms) were only slightly depressed by NMDLA. In 5 dendrites tested, the response to L-aspartate was suppressed by NMDLA by $60.0 \pm 14.8\%$, and that to L-glutamate was by $22.1 \pm 13.3\%$. Note that NMDLA exhibited no excitatory action by itself, but it behaved as an antagonist to L-aspartate. Records A and B were obtained from different dendrites, and their membrane potentials were kept to be -110 mV.

active than L-aspartate or L-glutamate. As shown in Fig. 1, responses to kainate and quisqualate were always much more prolonged than that of L-aspartate and L-glutamate, resulting in very slow recovery.

In marked contrast with quisqualate and kainate, NMDLA, which has been reported to act as an agonist of L-aspartate, showed no excitatory effect in the case of Purkinje cell dendrites even with protracted application (data not shown), but partially antagonized responses to L-aspartate and L-glutamate (Fig. 4).

Effects of Amino Acid Antagonists on Responses to L-Aspartate and L-Glutamate

GDEE — Although GDEE has a demonstrated ability of antagonizing quisqualate and

glutamate responses selectively in the spinal cord *in vivo*,^{8,9} its antagonistic action has been reported to be rather inconsistent in other central nervous system (CNS) neurons.¹⁰ In the case of Purkinje cell dendrites in guinea pig cerebellar slices, low dosed applications of GDEE increased the amplitude and slightly prolonged the duration of depolarizations induced by L-glutamate and L-aspartate. On the contrary, GDEE applied in relatively high dosage antagonized responses to both L-glutamate and L-aspartate with stronger depressant effect on L-glutamate actions (Fig. 2A). With the application current of 70 nA for GDEE, the action of L-glutamate was blocked by about 63% (62.8 ± 19.1 (S.D.)%, $n=6$) and that of L-aspartate was by about 31% (31.1 ± 18.2 (S.D.)%, $n=6$) (Fig. 4).

NMDLA — NMDLA has been reported to be a very potent excitant of spinal neurons in the cat⁸) and of hippocampal neurons in the rat.¹⁰ In the case of cerebellar Purkinje cell dendrites in the guinea pig, however, even long-lasting iontophoretic application (20 s) of NMDLA failed to depolarize the membrane. Such negative results were also obtained even with the application current of up to 100 nA. Alternatively, NMDLA antagonized responses to L-glutamate and L-aspartate. With application current of 100 nA, NMDLA exhibited preferential antagonism to L-aspartate responses (Fig. 2B), the response to iontophoresed L-glutamate was depressed by about 22% (22.1 ± 13.3 (S.D.)%, $n=5$), while that of L-aspartate was blocked by about 60% (60.0 ± 14.8 (S.D.)%, $n=5$) (Fig. 4).

cis-2,5-PDA — It has been reported that *cis*-2,3-PDA antagonizes all three receptor types, *i.e.* kainate-, quisqualate-, and NMDA-preferring receptors,¹³ while about *cis*-2,5-PDA there seems to be very few information as to whether this compound is either agonistic or antagonistic to excitatory amino acids. The iontophoretic application of *cis*-2,5-PDA to cerebellar Purkinje cell dendrites antagonized responses to both L-glutamate and L-aspartate almost to the same extent, a large current of 200 nA being required to exhibit this antagonism. As

FIG. 3. Effects of γ -DGG (A) and APV (B) on Responses to L-Glutamate and L-Aspartate

A. Simultaneous application of γ -DGG (30 nA) antagonized responses to L-aspartate (closed squares, 40 nA, 300 ms) with little or no effect on responses to L-glutamate (open squares, 20 nA, 300 ms). In 7 dendrites tested, the response to L-aspartate was depressed by γ -DGG by $69.0 \pm 12.4\%$, and that to L-glutamate was by $15.4 \pm 10.0\%$.

B. The iontophoretic application of APV (30 nA) selectively reduced responses to L-aspartate (closed squares, 20 nA, 400 ms), while responses to L-glutamate (open squares, 10 nA, 400 ms) were only slightly depressed. In 5 dendrites tested, the response to L-aspartate was blocked by APV by $65.7 \pm 3.7\%$, and that to L-glutamate was by $13.5 \pm 13.2\%$. Records A and B were obtained from different dendrites, and their membrane potentials were kept to be -110 mV.

in the case of *cis*-2,3-PDA in other neurons, *cis*-2,5-PDA did not appear to distinguish the depolarization induced by L-glutamate from that induced by L-aspartate. With the application current of 200 nA, *cis*-2,5-PDA antagonized responses to L-glutamate by about 36% (35.7 ± 5.4 (S.D.)%, $n = 3$), and those to L-aspartate by about 33% (33.3 ± 4.5 (S.D.)%, $n = 3$) (Fig. 4).

APB — It has been reported that APB is a

relatively weak and less selective antagonist.¹¹⁾ Moreover, other studies^{10,12)} have shown that it not only enhanced the action of NMDA and quisqualate but showed weak agonistic actions on its own, though such actions were not observed in the present study. In three dendrites tested, with the application current of 100 nA, APB reduced responses to L-glutamate and L-aspartate. The excitation evoked by L-aspartate, compared with that by L-glutamate, was rather

FIG. 4. Effects of Various Compounds on Responses to L-Glutamate and L-Aspartate

All compounds tested were iontophoretically applied (5–30 s) as closely as possible to the recording site of dendritic region. The application currents and the number of observations were as follows: APV; 30 nA (5), γ -DGG; 30 nA (7), NMDLA; 100 nA (5), APB; 100 nA (3), *cis*-2,5-PDA; 200 nA (3), ADME; 50 nA (5), GDEE; 70 nA (6), APP; 100 nA (3), L-asparagine; 30 nA (3), D-aspartate; 40 nA (3), L-glutamine; 2 nA (3). The values shown are mean $\pm$ S.D., and indicate the % decrement (blockade) and % increment (potentiation) from the depolarization induced by L-glutamate or L-aspartate alone at the hyperpolarized membrane potential of -110 mV.

more susceptible to the antagonism by APB. APB (100 nA) suppressed L-aspartate responses by about 49% (49.0 ± 4.5 (S.D.)%, $n=3$), while L-glutamate responses by about 42% (42.2 ± 4.2 (S.D.)%, $n=3$) (Fig. 4).

ADME — Since little has been reported about the dimethyl derivatives of aspartate, we investigated the effect of ADME on responses to L-glutamate and L-aspartate. With low dosed application of ADME (2–10 nA), both L-glutamate- and L-aspartate-induced depolarizations were enhanced almost to the same degree (data not shown). Relatively large dosed application of ADME (20–50 nA) decreased the amplitude of depolarization induced by L-glutamate and L-aspartate with exhibiting preferential, but rather weaker than GDEE, antagonism to L-glutamate. The depolarization induced by iontophoretically applied L-aspartate was blocked by ADME (50 nA) by about 33% (32.8 ± 16.5 (S.D.)%, $n=5$), while that by L-glutamate was antagonized by about 52% (51.7 ± 18.0 (S.D.)%, $n=5$) (Fig. 4).

γ -DGG — In five out of seven Purkinje cell dendrites tested, the simultaneous application of γ -DGG (30 nA) markedly reduced excitatory responses to L-aspartate without being associated with any change in the membrane potential of the cell, whereas the response of the same neuron to L-glutamate was only slightly blocked. This preferential effect of γ -DGG on responses to L-aspartate is illustrated in Fig. 3A. Although in remaining two cells responses to L-glutamate were also depressed to certain extents (data not shown), the preferential antagonism of L-aspartate responses still remained. γ -DGG (30 nA) suppressed L-aspartate responses by about 69% (69.0 ± 12.4 (S.D.)%, $n=7$), while L-glutamate responses only by about 15% (15.4 ± 10.0 (S.D.)%, $n=7$) (Fig. 4).

APV — APV, the most potent and selective *N*-methyl-D-aspartate receptor antagonist in the spinal cord¹⁴⁾ and in rat dentate gyrus,¹⁵⁾ was also tested as to its selective action in Purkinje cell dendrites. In four out of five dendrites tested, APV was found to be a more selective an-

tagonist of L-aspartate responses than was γ -DGG. In each dendrite, the iontophoretic application (30 nA) of APV selectively suppressed L-aspartate-induced depolarization, leaving L-glutamate responses virtually unaffected (Fig. 3B). In the remaining one cell both L-glutamate- and L-aspartate-induced depolarizations were reduced in amplitude, yet still in this case the L-aspartate responses were more greatly blocked (data not shown). L-Glutamate-induced responses were depressed by about 14% (13.5 ± 13.2 (S.D.)%, $n=5$) by the iontophoretically applied APV (30 nA), while the corresponding value to L-aspartate was by about 66% (65.7 ± 3.7 (S.D.)%, $n=5$) (Fig. 4).

Mg Ion — Since Mg^{2+} is known to antagonize excitatory amino acids-mediated responses in central neurons,^{11,16)} some observations were made in Mg^{2+} -free, 0.5 and 20 meq/l- Mg^{2+} media. Even in these three media, depolarizations evoked in Purkinje cell dendrites by the iontophoretic application of L-glutamate and L-aspartate appeared to be unchanged.

The Compounds Facilitating Excitatory Amino Acid Responses — The effects of APP, L-asparagine, D-aspartate and L-glutamine on responses to L-glutamate and L-aspartate were studied. Large dosed application of each compound alone depolarized the membrane of Purkinje cell dendrites, while low doses of them did not exhibit the depolarization by themselves. With low doses, the application of any one of these compounds strongly potentiated responses to simultaneously applied L-glutamate and L-aspartate. These potentiating actions are summarized in Fig. 4.

Other Compounds Related to Excitatory Amino Acids — Other excitatory amino acid related compounds such as D α AA, *cis*-2,4-PDA, D-glutamate, *N*-acetyl-DL-glutamate and DL-proline were examined in order to evaluate their either depressing or enhancing effects on excitatory amino acids actions. Even large dosed applications of D α AA (60 nA), selective antagonist of NMDA,⁸⁾ and *cis*-2,4-PDA (100 nA) exhibited neither antagonistic nor potentiating

effect on responses to L-glutamate or L-aspartate. Remaining 3 compounds, D-glutamate, *N*-acetyl-DL-glutamate and DL-proline, also appeared to have no suppressing action on L-glutamate- or L-aspartate-induced depolarization. However, with large dosed application, these compounds tended to potentiate slightly the L-glutamate and L-aspartate actions.

DISCUSSION

Excitatory Amino Acid Receptors on Cerebellar Purkinje Cell Dendrites in the Guinea Pig

As mentioned in Introduction, three types of receptors, *i.e.*, *N*-methyl-D-aspartate, kainate, and quisqualate receptors have been suggested to exist in the cat spinal cord^{5,6)} on the basis of the orders of their agonistic potencies or of the selectivities of various antagonists. APV has been shown to be the most potent and selective NMDA receptor antagonist.¹⁷⁾ The kainate-preferring receptor is sensitive to antagonism by γ -DGG which also antagonizes NMDA-preferring receptor.¹⁵⁾ The quisqualate sensitive receptor is blocked by GDEE.⁹⁾

Quisqualate and kainate had marked depolarizing effect on Purkinje cell dendrites and their potency was much greater than that of L-glutamate or L-aspartate. Thus, both quisqualate and kainate receptors are certainly present on Purkinje cell dendrites. In contrast, NMDLA showed no excitatory effect on Purkinje cell dendrites, but had antagonistic actions to responses to L-glutamate and L-aspartate (in agreement with Crepel *et al.*, 1982 and 1983^{18,19)}), though in other CNS even in rat hippocampal CA1 neurons¹⁰⁾ and in cat caudate neurons²⁰⁾ NMDLA has been reported to be a potent excitant of those neurons. Moreover, a NMDA antagonist D α AA exhibited no antagonizing effect on responses to L-glutamate and L-aspartate in the present cerebellar preparation. These findings strongly suggest that there is no NMDA-preferring receptor in the Purkinje cell dendrites at least in the guinea pig cerebellum, like as in the rat cerebellum.¹⁸⁾

Effects of Antagonists on Depolarizations Induced

by L-Glutamate and L-Aspartate

Ester Derivatives of Excitatory Amino Acids

— With low doses application of GDEE and ADME, depolarizations induced by L-glutamate and L-aspartate were increased in amplitude with the duration being slightly prolonged. This potentiating action may be a consequence of competitive inhibition of the reuptake of excitatory amino acids by GDEE and ADME.²¹⁾ With rather high application current, GDEE and ADME blocked the actions of both L-glutamate and L-aspartate, exhibiting preferential antagonism to L-glutamate. Having been reported as to many NMDA specific antagonists, there are few reports concerning new compounds specific to L-glutamate actions. In spite of their rather low degree of specificity, both GDEE and ADME are thought to be still useful in investigating L-glutamate preferring receptor.

NMDLA — Although NMDLA was found to be a very potent excitant of spinal neurons in the cat⁸⁾ and of hippocampal neurons in the rat,¹⁰⁾ it had little or no excitatory effect on Purkinje cell dendrites. On the other hand, NMDLA appeared to behave as an antagonist rather than as an agonist. Ionophoretic application of L-aspartate was apparently more susceptible to antagonism by NMDLA than the excitation caused by L-glutamate. In cerebellar Purkinje cells, NMDLA can be useful to differentiate responses to L-aspartate from those to L-glutamate.

Selective Antagonism of γ -DGG and APV to L-Aspartate — APV and γ -DGG were found to be potent and selective antagonists of the NMDA-induced excitation in rat dentate gyrus¹⁵⁾ and rat hippocampal CA1 neurons.¹⁰⁾ It has also been reported that compounds which antagonize responses to NMDLA tend to reduce L-aspartate-induced excitation as well without blocking L-glutamate responses.¹⁴⁾ In agreement with this suggestion, upon applications of APV at a dose capable of reducing both L-glutamate- and L-aspartate-induced excitations, the latter found to be more sensitive to APV. Moreover, the input resistance being decreased by the appli-

cation of L-aspartate was recovered almost to the resting value by APV, while that by L-glutamate was only slightly affected (data not shown). An effect qualitatively similar to that observed with APV was also seen with γ -DGG in Purkinje cell dendrites. Thus, APV and γ -DGG may be useful for investigations of synapses at which L-aspartate may act as a neurotransmitter.

Mg Ions — Ault *et al.* (1980)¹⁶⁾ and Watkins *et al.* (1981)¹¹⁾ reported that both L-glutamate- and L-aspartate-induced excitations were suppressed in low Mg^{2+} -medium (0.5 meq/l- Mg^{2+}) and responses to L-aspartate was somewhat more sensitive to Mg^{2+} than was those to L-glutamate. In the case of Purkinje cell dendrites, however, even in the Mg^{2+} -free, low Mg^{2+} - (0.5 meq/l- Mg^{2+}) or high Mg^{2+} - (20 meq/l- Mg^{2+}) media, the depolarization evoked by the iontophoretic application of either L-glutamate or L-aspartate was not significantly altered. Watkins *et al.* (1981)¹¹⁾ suggested that Mg^{2+} may not act competitively with agonists such as L-glutamate and L-aspartate for the receptor site, but rather affects divalent cation ionophores. Moreover, in Purkinje cell dendrites, a primary involvement of a calcium current in responses to L-glutamate and L-aspartate could be excluded because of minor effect of Mg, Co or Cd ions on the reversal potential (data not shown). For these reasons, Mg^{2+} may not affect the responses to L-glutamate and L-aspartate in Purkinje cell dendrites.

Potentiating Effects of Several Compounds on Excitation Evoked by L-Glutamate and L-Aspartate — The enhancement of L-glutamate- and L-aspartate-induced depolarizations by APP may be due to the inhibition of the reuptake system for these excitatory amino acids.²¹⁾ L-Asparagine, D-aspartate and L-glutamine all strongly potentiated responses to L-glutamate and L-aspartate. Although potentiating effects of these three compounds might also be partly due to the inhibition of the reuptake as in the case of APP, other mechanisms seemed to be involved, because their potentiating actions were much greater than that of APP. The further investiga-

tion is required on this matter.

In conclusion, both quisqualate and kainate receptors are present in Purkinje cell dendrites of guinea pig cerebellum, whereas there seems to be no NMDA receptor. Among antagonists tested, γ -DGG, APV, NMDLA and GDEE may be useful for investigations of excitatory amino acid neurotransmitters at the synapses formed by the climbing fiber and parallel fiber with Purkinje cell dendrites, while others such as APB, *cis*-2,5-PDA, and ADME may be of lesser value because they are less potent and less selective.

REFERENCES

- 1) M. E. Sandoval and C. W. Cotman: Evaluation of glutamate as a neurotransmitter of cerebellar parallel fibers, *Neuroscience*, **3**, 199–206 (1978).
- 2) T. W. Stone: Glutamate as the neurotransmitter of cerebellar granule cells in the rat: electrophysiological evidence, *Br. J. Pharmacol.*, **66**, 291–296 (1979).
- 3) N. S. Nadi, D. Kanter, W. J. McBride and M. H. Aprison: Effects of 3-acetylpyridine on several putative neurotransmitter amino acids in the cerebellum and medulla of the rat, *J. Neurochem.*, **28**, 661–662 (1977).
- 4) L. Wiklund, G. Toggengruber and M. Cuénod: Aspartate: Possible neurotransmitter in cerebellar climbing fibers, *Science*, **216**, 78–80 (1982).
- 5) R. H. Evans and J. C. Watkins: Pharmacological antagonists of excitant amino acid action, *Life Sci.*, **28**, 1303–1308 (1981).
- 6) H. McLennan: On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system, "Glutamate as a Neurotransmitter," ed. by G. Di Chiara and G. L. Gessa, Raven Press, New York, 1981, pp. 253–262.
- 7) K. Okamoto, H. Kimura and Y. Sakai: Ionic mechanisms of the action of taurine on cerebellar Purkinje cell dendrites *in vitro*: Intradendritic study, *Brain Res.*, **260**, 261–269 (1983).
- 8) J. Davies and J. C. Watkins: Selective antagonism of amino acid-induced and synaptic excitation in the cat spinal cord, *J. Physiol. (London)*, **279**, 621–635 (1979).
- 9) H. McLennan and D. Lodge: The antagonism of amino acid-induced excitation of spinal neurones in the cat, *Brain Res.*, **169**, 83–90 (1979).
- 10) G. L. Collingridge, S. J. Kehl and H. McLennan: The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones *in vitro*, *J. Physiol. (London)*, **334**, 19–31 (1983).
- 11) J. C. Watkins, J. Davies, R. H. Evans, A. A. Francis and

- A. W. Jones: Pharmacology of Receptors for Excitatory amino acids, "Glutamate as a Neurotransmitter," ed. by G. Di Chiara and G. L. Gessa, Raven Press, New York, 1981, pp. 263–273.
- 12) R. H. Evans, A. A. Francis, A. W. Jones, D. A. S. Smith and J. C. Watkins: 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**, 65–76 (1982).
- 13) J. Davies, R. H. Evans, A. A. Francis, A. W. Jones and J. C. Watkins: Antagonism of excitatory amino acid-induced and synaptic excitation of spinal neurones by *cis*-2,3-piperidine dicarboxylate, *J. Neurochem.*, **36**, 1305–1307 (1981).
- 14) J. C. Watkins and R. H. Evans: Excitatory amino acid transmitters, *Annu. Rev. Pharmacol. Toxicol.*, **21**, 165–204 (1981).
- 15) V. Crunelli, S. Fonda and J. S. Kelly: Blockade of amino acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus, *J. Physiol. (London)*, **341**, 627–640 (1983).
- 16) B. Ault, R. H. Evans, A. A. Francis, D. J. Oakes and J. C. Watkins: Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations, *J. Physiol. (London)*, **307**, 413–428 (1980).
- 17) J. Davies, A. A. Francis, A. W. Jones and J. C. Watkins: 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation, *Neurosci. Lett.*, **21**, 77–81 (1981).
- 18) F. Crepel, S. S. Dhanjal and T. A. Sears: Effect of glutamate, aspartate and related derivatives on cerebellar Purkinje cell dendrites in the rat: an *in vitro* study, *J. Physiol. (London)*, **329**, 297–317 (1982).
- 19) F. Crepel, J. L. Dupont and R. Gardette: Voltage clamp analysis of the effect of excitatory amino acids and derivatives on Purkinje cell dendrites in rat cerebellar slices maintained *in vitro*, *Brain Res.*, **279**, 311–315 (1983).
- 20) P. L. Herrling, R. Morris and T. E. Salt: Effects of excitatory amino acids and their antagonists on membrane and action potentials of cat caudate neurones, *J. Physiol. (London)*, **339**, 207–222 (1983).
- 21) E. Puil: S-Glutamate: Its interactions with spinal neurones, *Brain Res. Rev.*, **3**, 229–322 (1981).