J. Physiol. (1985), **365**, *pp.* 103–119
With 8 text-figures
Printed in Great Britain

PHARMACOLOGICAL EVIDENCE FOR L-ASPARTATE AS THE NEUROTRANSMITTER OF CEREBELLAR CLIMBING FIBRES IN THE GUINEA-PIG

BY H. KIMURA, K. OKAMOTO AND Y. SAKAI

From the Department of Pharmacology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama 359, Japan

(*Received 17 July 1984*)

SUMMARY

1. Climbing fibre responses (c.f.r.s) evoked by white matter stimulation and the depolarizations induced by iontophoretically applied L-glutamate and L-aspartate were recorded intracellularly from the proximal dendrites of Purkinje cells in *in vitro* slice preparations of the guinea-pig cerebellum.

2. Short pulses of L-glutamate and L-aspartate dose-dependently depolarized the Purkinje cell dendrite. Even small doses of these amino acids reduced the input resistance. The maximum decrease in input resistance induced by L-glutamate was 36% and that by L-aspartate was 38%.

3. Intracellular injection of Cs^+ allowed Purkinje cell dendrites to be depolarized to a range of -15 to $+30$ mV. The mean reversal potential for the c.f.r. (E_c) was found to be $+10{\cdot}2$ mV ($n = 4$). The mean reversal potentials obtained for L-glutamate (E_g) and for L-aspartate (E_a) were $+7{\cdot}3$ mV ($n = 7$) and $+5{\cdot}6$ mV ($n = 7$) respectively.

4. When external Na^+ concentration was reduced, E_c, E_a and E_g were linearly and similarly shifted in the negative direction, indicating that all these reversal potentials are determined primarily by a Na^+ conductance.

5. The effects of the glutamate antagonists 2-amino-5-phosphonovaleric acid (APV), γ-D-glutamylglycine (γ-DGG), *N*-methyl-DL-aspartic acid (NMDLA) and glutamic acid diethylester (GDEE) were compared as to the responses to L-glutamate and L-aspartate and Ca^{2+}-activated focal climbing fibre responses (c.f.c.f.r.s) in order to investigate the receptor type at the synapses formed by the climbing fibres with Purkinje cell dendrites.

6. The order of antagonistic potency to the c.f.c.f.r. was : APV (mean percentage blockade = 99%) > γ-DGG (87%) > NMDLA (71%) > GDEE (28%). The order of antagonistic potency to the response to L-aspartate was: γ-DGG (69%) > APV (66%) > NMDLA (60%) > GDEE (31%), and that to the response to L-glutamate was: GDEE (63%) > NMDLA (22%) > γ-GDD (15%) > APV (14%).

7. APV was found to be the most effective anatagonist of the c.f.c.f.r. Its action was reversible, selective for L-aspartate-induced depolarization and had no effect on the responses to L-glutamate. NMDLA, which has no activity as an agonist, was a greater suppressant of the responses to L-aspartate than those to L-glutamate.

8. These electrophysiological and pharmacological findings suggest that the receptor for the transmitter at the synapses formed by climbing fibres with Purkinje cell dendrites is of the L-aspartate-preferring type, and are thus consistent with the bio- and histochemical findings that L-aspartate may be the endogenous transmitter at this synapse.

INTRODUCTION

The climbing fibre (c.f.) which originates at the inferior olive is anatomically known to form excitatory synapses diffusively on the dendrite of cerebellar Purkinje cells (Palay & Chan-Palay, 1974). The electrophysiological properties of c.f. responses (c.f.r.s) were extensively investigated by Eccles (1965); Eccles, Llinás & Sasaki (1966*a*): and Eccles, Llinás, Sasaki & Voorhweve (1966*b*) by means of extra- and intracellular recordings from Purkinje cell somata in mammalian cerebella *in vivo*. The c.f.r. has also been investigated *in vitro* using cerebellar slice preparations and intrasomatic recording in the frog by Hackett, How & Cochran (1979), in the guinea-pig by Llinás & Sugimori (1980*a*) and in the rat by Crepel, Dhanjal & Garthwaite (1981) and Crepel, Dhanjal & Sears (1982). Although a possibility that an excitatory transmitter at c.f. synapses might be different from that at parallel fibre (p.f.) synapses has been suggested based on the observation that the equilibrium potential for the c.f.r. was shown to differ from that for the p.f. response in the frog (Hackett *et al.* 1979) and in the rat (Dupont, Crepel & Delhaye-Bouchaud, 1979; Crepel *et al.* 1981), no further electrophysiological attempt has been made to identify the transmitter(s) at the c.f. synapse. Although L-glutamate has been suggested to be a candidate for the transmitter at the p.f. synapse in the rat by Sandoval & Cotman (1978) and Stone (1979), the transmitter at c.f. synapses is more likely to be L-aspartate. For instance, it has been reported that the depolarization-induced release of L-aspartate (Wiklund, Toggenburger & Cuenod, 1982) and the aspartate level (Nadi, Kanter, McBride & Aprison, 1977) in the rat cerebellar tissue are decreased after degeneration of the c.f. system with 3-acetylpyridine. However, a detailed electrophysiological and pharmacological investigation of the transmitter mediating the climbing fibre response in cerebellar Purkinje cells has been hampered by the lack of effective antagonists capable of blocking both amino-acid-induced depolarization and synaptically evoked excitation (Davies & Watkins, 1979; Collingridge, Kehl & McLennan, 1983) and technical difficulties in obtaining a focal climbing fibre response elicited by synapses near to an impaled recording electrode.

The present *in vitro* study was undertaken to investigate the electrophysiological properties of the responses of Purkinje cell dendrites to the exogenous application of the neurotransmitter candidates, L-glutamate and L-aspartate and to c.f. stimulations, and to determine which one of the amino acids is likely to be the transmitter of the c.f. synapse. For these purposes, we have employed the technique of eliciting a Ca^{2+}-activated focal climbing fibre response (c.f.c.f.r.) (cf. Sawada, Takada & Yamamoto, 1983), and the reversal property and ionic dependency of the c.f.r. were compared with those of responses to L-glutamate and L-aspartate, and pharmacological investigation was made on the effects of various amino acid antagonists on this focal c.f.r. and on the depolarization of Purkinje cell dendrites induced by the application of L-glutamate and L-aspartate. The results further support the previous suggestion

based on bio- and histochemical observations that L-aspartate is more likely, than L-glutamate, to be the excitatory transmitter of c.f. synapses in the guinea-pig cerebellum.

METHODS

Male guinea-pigs, 300–400 g, were killed by stunning, and the cerebellum isolated. A block of the cerebellum was sectioned sagittally at 170 μm thickness, using a Vibratrom (Oxford Laboratories, CA, U.S.A.), parallel to the arborization of Purkinje cell dendrites (Okamoto, Kimura & Sakai, 1983). 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 a 1 h pre-incubation and throughout the experiment with the Krebs–Ringer bicarbonate medium (control medium) which was kept at 36·5 °C and contained (in mM): NaCl, 125; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; NaH_2PO_4, 1; $NaHCO_3$, 24; and glucose, 11; pH was adjusted to 7·4 by saturating with 95% O_2/5% CO_2 gas. A high Mg^{2+} perfusion medium was prepared by increasing the concentration of Mg^{2+} in the control media. Low-Na^+ media were prepared by iso-osmotically replacing Na^+ in control medium by $Tris^+$.

Agonists, antagonists and Ca^{2+} were applied iontophoretically from a three-barrel micropipette close to the recording site on the Purkinje cell dendrite. The individual barrels of the iontophoretic pipette contained the following solutions: L-glutamate (0·5 M, pH 8·5), L-aspartate (0·5 M, pH 8·5), L-glutamate 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-aminoadipic acid (DαAA) (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), DL-aspartic acid dimethylester (ADME) (0·5 M, pH 3·5), and $CaCl_2$ (1·1 M).

Responses to iontophoretically applied glutamate or aspartate and c.f. responses were recorded with the intracellular electrode in the dendrite (about 100 μm distant from the soma) of Purkinje cells in the slice under visual control (× 120). Recording electrodes filled with 3 M-KCl were routinely used, and those filled with 3 M-Cs acetate were utilized only to reverse the response to c.f. stimuli, L-glutamate and L-aspartate. Cs^+ were injected into an impaled Purkinje cell dendrite, prior to an experiment, by applying square pulses (1 nA, 800 ms duration, 1 Hz) for 25–30 min. Changes in the input resistance of the Purkinje cell dendrites were measured during iontophoretic applications of L-glutamate and L-aspartate by measuring the amplitude of the potential induced by a hyperpolarizing current pulse (100 ms, 1 nA) passed through the recording electrode.

C.f. responses were elicited by electrical stimulation of the cerebellar white matter of the slice with a bipolar metal electrode using square pulses (3–7 V, 20–200 μs duration, 0·5–1·0 Hz). For eliciting a c.f.c.f.r., the white matter of a slice under superfusion with control medium was stimulated to evoke a c.f.r., then the superfusing medium was changed to that containing low Ca^{2+} (1 mequiv/l) and high Mg^{2+} (10 mequiv/l) in order to suppress the c.f.r. Upon application of Ca^{2+} from the pipette, only those c.f.s having presynaptic terminals near the pipette tip could release a neurotransmitter and consequently evoked a c.f.c.f.r. Since the tip of the Ca pipette was found to block easily, probably by precipitation of insoluble salts, NaH_2PO_4 was omitted from the low-Ca^{2+}, high-Mg^{2+} medium.

In order to minimize the generation of obstructive Na^+ and Ca^{2+} spikes, the membrane potential of Purkinje cell dendrites was routinely shifted to −110 mV by direct current injection through an impaled recording electrode using a high input impedance (10^{10} Ω)bridge circuit (MEZ-8201, Nihon Kohden). Thus, all c.f.r.s illustrated in this paper are those recorded at a membrane potential of −110 mV, unless otherwise stated. Similarly, all records of responses to iontophoresed glutamate and aspartate are those at this hyperpolarized membrane potential.

All electrical signals observed were stored on magnetic tapes using a data recorder, and replayed on an ink-jet recorder (RIJ-3604, Nihon Kohden), on a pen-writing recorder or to a data processor (ATAC-350, Nihon Kohden) equipped with a *X*–*Y* recorder.

APV, APB, APP, cis-2,5-PDA and cis-2,4-PDA were purchased from Cambridge Research Biochemicals (G.B.). GDEE, NMDLA and ADME were from Sigma Chemical Co., U.S.A. All other

compounds, including Na L-glutamate and L-aspartic acid, were obtained from Wako Pure Chemical Industries (Toyko, Japan).

RESULTS

Properties of c.f.r. recorded intracellularly from Purkinje cell dendrites

Basic properties of Purkinje cell dendrites. Successful impalement with a recording electrode into Purkinje cell somata and dendrites always showed steady membrane potentials of about -65 mV ($-65{\cdot}3 \pm 6{\cdot}0$ (S.E. of mean) mV, $n = 48$), and -58 mV ($-58{\cdot}2 \pm 1{\cdot}2$ (S.E. of mean) mV, $n = 40$), respectively. The average resistance of seven somata impalements was $20{\cdot}0 \pm 3{\cdot}2$ (S.E. of mean) MΩ, and that from fifteen dendrites was $31{\cdot}5 \pm 1{\cdot}6$ (S.E. of mean) MΩ.

Intradendritic recording from the Purkinje cell dendrite was identified by the three criteria utilized by Llinás & Sugimori (1980*a*, *b*) and Okamoto *et al.* (1983). First, at the resting membrane potential, spontaneous Na^+ spikes generated at the soma propagating to the dendrites could consistently be observed. Secondly, Ca^{2+} spikes could be elicited by the depolarization of the membrane by the injection of direct or square-pulse currents through a recording electrode. Thirdly, electrical stimulation of the white matter could produce an all-or-none orthodromic potential characteristic of the c.f.r.

Reversal potential of the c.f.r. Attempts to reverse a c.f.r. by depolarizing the Purkinje cell dendrite by injecting direct current through a 3 M-KCl-filled recording electrode required anomalously large currents, probably because of the presence of depolarization-induced outward K^+ current (Langmoen & Hablitz, 1981). The intracellular injection of Cs^+ was used to block such outward currents (Johnston & Hablitz, 1980) by the use of a recording electrode filled with 3 M-Cs acetate. When Cs^+ was iontophoresed into a Purkinje cell dendrite by passing square pulses (1 nA, 800 ms duration, 1 Hz) for 25–30 min, the dendrite was usually depolarized to a final potential level of 20–30 mV. This depolarization was accompanied by the occurrence of Ca^{2+} and Na^+ spikes, but they were inactivated by a further depolarization to a potential level of about -15 mV. After this Cs^+ injection, the dendritic membrane potential could be shifted to any desired levels within the range -15 to 30 mV.

The typical reversal of the c.f.r. obtained with a Cs^+ electrode at an approximate potential of 10 mV is shown in Fig. 1*A* (third record). This linearity of the reversal of the c.f.r. from the same cell is graphically illustrated in Fig. 2*A*. The average reversal potential estimated from similar straight lines from a total of four dendrites was $10{\cdot}2 \pm 1{\cdot}5$ (S.E. of mean) mV.

Ionic dependency of the reversal potential of the c.f.r. When the Na^+ concentration was reduced from the control level of 150 mequiv/l to 108 and 75 mequiv/l and osmotic strength was maintained with $Tris^+$ in similar experiments, the reversal potential of the c.f.r. was shifted to more negative potential levels, as the Na^+ concentration was reduced (Fig. 3*C*). The relation between the logarithmic concentrations of external Na^+ and reversal potentials was linear with a slope of 36 mV for a 10-fold change in the Na^+ concentration.

Characterization of the excitatory response elicited by L-*glutamate and* L-*aspartate*

When the iontophoretic micropipette was carefully positioned as closely as possible to the recording site, the depolarizing actions of L-glutamate and L-aspartate were accompanied by a decrease in the membrane resistance which followed a time course similar to the concurrent depolarization and was observed even during low-dosed

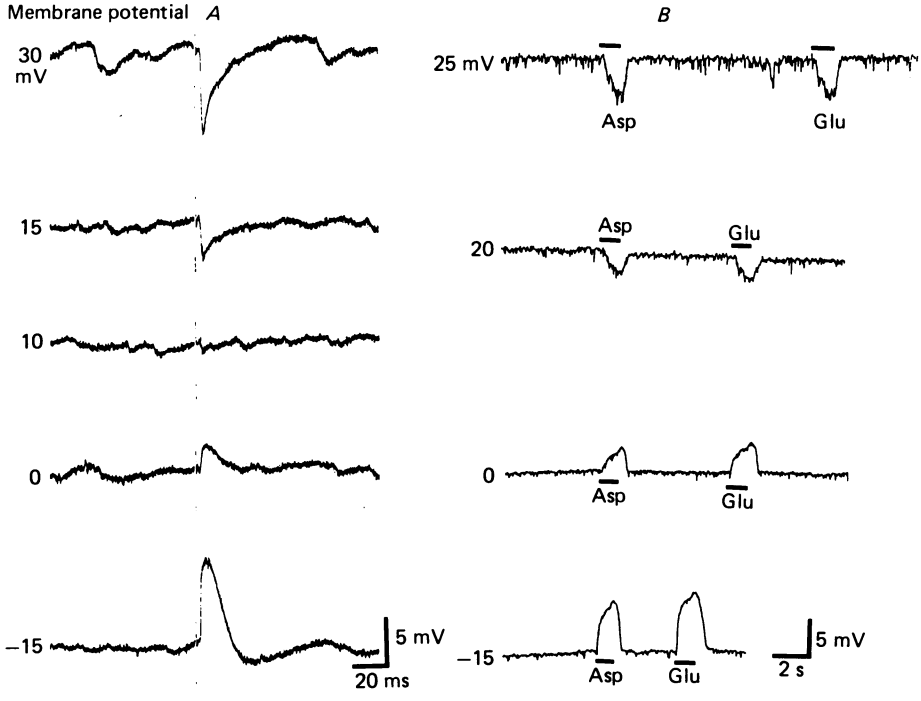

Fig. 1. Reversal of the c.f.r.s and glutamate- and aspartate-induced depolarizations in Cs^+-loaded Purkinje cell dendrites. *A*, c.f.r.s, and *B*, responses to L-glutamate (Glu) and L-aspartate (Asp) pulses (1 s duration) recorded from different dendrites at different membrane potentials. Cs^+ were injected through 3 M-Cs-acetate-filled recording electrodes by applying square pulses (1 nA, 800 ms, 1 Hz) for 30 min, and the membrane potential displaced by injecting direct currents.

iontophoretic applications of L-glutamate and L-aspartate (Fig. 4). During the peak depolarizations (5–40 mV) evoked by the application of L-glutamate (25–150 nA) the membrane resistance was decreased by $35 \pm 5\%$ ($n = 4$). The corresponding value for L-aspartate was by $38 \pm 5\%$ ($n = 4$). Similar depolarization (40 mV) induced by direct current injection through a recording electrode, on the other hand, decreased the membrane resistance by less than $8 \pm 6\%$ ($n = 4$).

Reversal potentials of responses to L-*glutamate and* L-*aspartate.* As shown in Fig. 1*B*, the depolarizing responses to L-glutamate and L-aspartate were clearly inverted by shifts of the membrane potential of the impaled Purkinje cell dendrite in the range −15 to 25 mV using a Cs-acetate-filled recording electrode. Their reversal potential estimated from six dendrites tested under linear conditions similar to those shown

in Fig. 2*B* was 7·3±1·1 (S.E. of mean) mV for glutamate and 5·6±1·0 mV for aspartate. Statistical comparisons using the Student's *t* test showed that the reversal potential of the c.f.r. (10·2±1·5 mV ($n = 4$); Fig. 2) was significantly ($P < 0.05$) different from that of the response to aspartate, while not significantly ($P < 0.2$) different from the response to glutamate. However, since responses to the amino acids

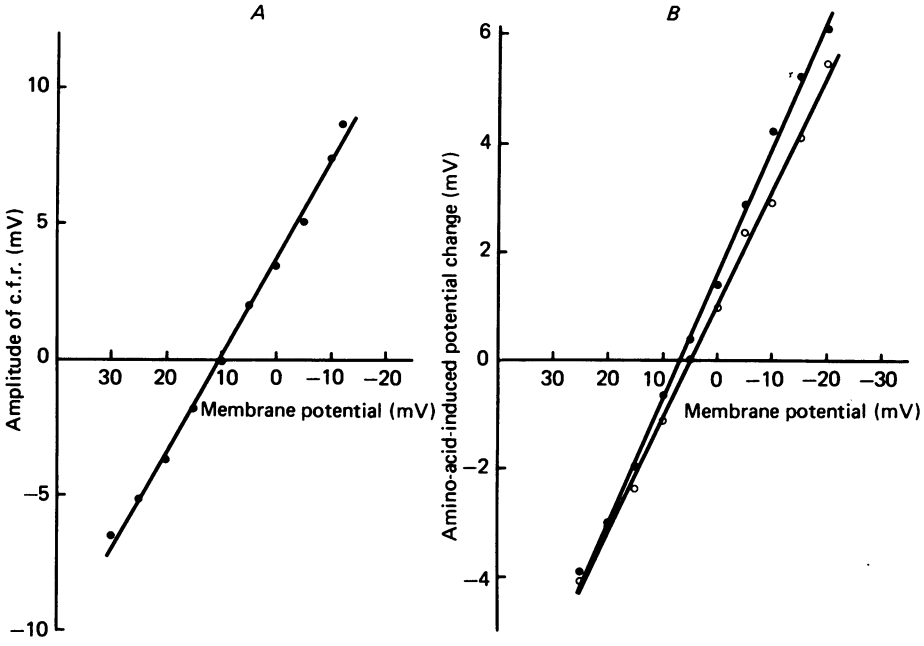

Fig. 2. Membrane potential–response amplitude plots drawn to show the reversal potentials for responses to L-glutamate and L-aspartate and for c.f.r.s in Cs^+-loaded Purkinje cell dendrites. *A*, relation between the mean peak amplitudes of c.f.r.s in four dendrites. *B*, relations between membrane potentials and the peak amplitudes of responses to L-glutamate (filled circles) and L-aspartate (open circles) in six dendrites. The regression lines (drawn by the method of least mean squares) indicate reversal potentials of 10·2±1·5 (S.E. of mean) mV ($n = 4$) for the c.f.r. (*A*), and of 7·3±1·1 ($n = 6$) for the response to L-glutamate and 5·6±1·0 mV ($n = 6$) for the response to L-aspartate (*B*).

were not significantly ($P < 0.4$) different from each other, it was not possible to assign either one of the amino acids to the transmitter for the c.f.r. based solely on the observed value of the mean reversal potential.

Ionic dependency of responses to L-glutamate and L-aspartate. As shown in Fig. 3*A* and *B*, the reversal potentials of hyperpolarizing responses to L-glutamate and L-aspartate were shifted in the hyperpolarizing direction by reducing the concentration of Na^+ in the media. The average slope of the change of the reversal potential for a 10-fold change of the concentration of the external Na^+ was 36 mV for glutamate and 37 mV for aspartate.

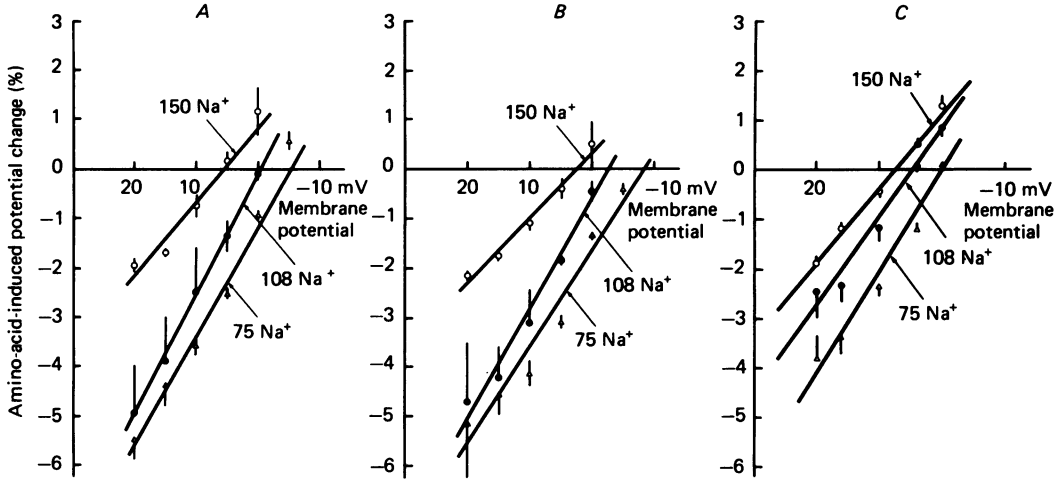

Fig. 3. Effect of varied external Na^+ concentrations on the reversal potential. *A*, *B* and *C*, relations between membrane potentials and the amplitudes of responses to L-glutamate (*A*) and L-aspartate (*B*) and the c.f.r.s (*C*) in the presence of 150 mequiv/l external Na^+ (open circles), 108 mequiv/l Na^+ (filled circles) and 75 mequiv/l Na^+ (triangles). $Tris^+$ were iso-osmotically substituted for Na^+. Values plotted are the means (±S.E. of mean) of three to five observations in the same Purkinje cell dendrites for *A* and *B*, while in the other dendrites for *C*. Regression lines were drawn by the method of least mean squares.

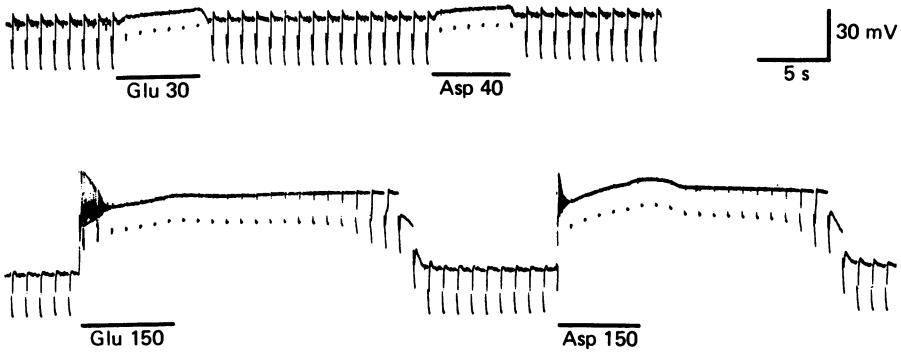

Fig. 4. Effects of L-glutamate and L-aspartate on the input resistance of Purkinje cell dendrites. The downward deflexions are membrane potential changes due to hyperpolarizing square pulses (0·8 nA, 100 ms, 1 Hz) applied through the recording electrode and indicate cell input resistance. L-Glutamate and L-aspartate were iontophoretically applied with the ejecting currents denoted in nA to the dendrite close to the recording site for the duration marked by horizontal bars. Upper and lower records were from different dendrites, and their membrane potentials were at the resting level of −58 mV. Depolarizations of 40 mV induced by direct current injection caused only 8% decrease of the membrane resistance, which was about one-fifth of the decrease induced by the amino acids.

C.f.c.f.r.

For the identification of the endogenous transmitter, it is necessary to demonstrate that not only the excitation induced by an exogenous transmitter candidate but also a synaptically evoked excitatory response is blocked to the same or similar extents by the same antagonist. As c.f. makes synaptic contacts diffusively with a Purkinje cell along its dendritic arborizations (Palay & Chan-Palay, 1974), an antagonist ejected from the iontophoretic pipette is thought to be effective only in the restricted region in the vicinity of the tip of the pipette, leaving synapses remote from the tip relatively unaffected. In fact, much higher (more than 10 times) doses of an antagonist were required to block an ordinary c.f.r. than to block a response to externally applied L-glutamate or L-aspartate. In order to solve this problem, c.f.r.s were elicited focally in this study. Typical examples of c.f.r.s thus evoked focally are shown in Fig. 5*A*, traces *a–d*. After a c.f.r. was evoked in control medium (Fig. 5*A*, trace *a*), the medium containing 1 mequiv/l Ca^{2+} and 10 mequiv/l Mg^{2+} was superfused for about 10 min. This led to the partial suppression of the c.f.r. to a steady amplitude (Fig. 5*A*, trace *b*). Within 5–10 s after the commencement of the ejection of Ca^{2+} from the iontophoretic pipette, the c.f.r. gradually increased in amplitude and reached a steady level (Fig. 5*A*, trace *c*). On termination of the ejection of Ca^{2+}, initial suppressed amplitude (Fig. 5*A*, trace *d*) was restored. The rate of falls of suppressed c.f.r.s. either by lowering the Ca^{2+} concentration or by antagonists (Figs. 5*A*, 7*A* and 8*A*) appeared to be rather small. It is probably due to the decrease of the Ca^{2+}-activated K^+ conductance (Meech & Standen, 1975; Heyer & Lux, 1976) induced by the generation of a Ca^{2+} component of the c.f.r., since even in 1 mequiv/l Ca^{2+}, 10 mequiv/l Mg^{2+} medium, the Ca^{2+} component still remained at the top of the c.f.r. (Kimura, Okamoto & Sakai, 1985*b*). These c.f.c.f.r.s were utilized to investigate the effects of several antagonists by applying them from another barrel of the same iontophoretic pipette.

Actions of individual antagonists

Weak antagonism by GDEE. GDEE is known to be a specific antagonist to quisqualate and L-glutamate at least in cat spinal neurones (McLennan & Lodge, 1979). In other C.N.S. neurones, however, rather inconsistent antagonistic actions have been reported (Collingridge *et al.* 1983). In the case of Purkinje cell dendrites in guinea-pig cerebellar slices, GDEE depressed excitatory responses to both L-glutamate and L-aspartate with preferential antagonism of L-glutamate (Fig. 5*B*). With the application current of 65 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.) %, $n = 6$) (Table 1). The Ca^{2+}-activated increment of the amplitude of a c.f.r., namely c.f.c.f.r., was also but much less potently suppressed by GDEE 28 % (27·6 + 6·6 (S.D.) %, $n = 5$) even when an iontophoretic current was increased to 100 nA (Fig. 5*A*, trace GDEE) (Table 1).

Rather weak antagonism by NMDLA. It has been reported that NMDLA is a very potent excitant of spinal neurones in the cat (Davies & Watkins, 1979) and of hippocampal neurones in the rat (Collingridge *et al.* 1982). However, in keeping with results of Crepel *et al.* (1982), application of NMDLA to Purkinje cell dendrites, by

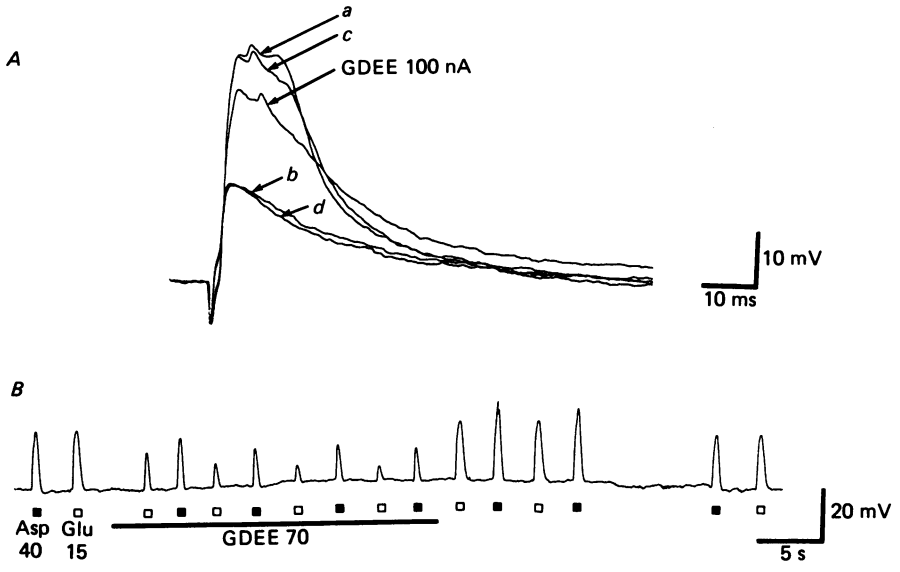

Fig. 5. Effects of GDEE on a c.f.c.f.r. and on L-glutamate- and L-aspartate-induced depolarizations in Purkinje cell dendrites in guinea-pig cerebellar slices. *A*, effect of GDEE on the c.f.c.f.r.: a c.f.r. (trace *a*), which was evoked by white matter stimulation and recorded intracellularly from the proximal dendrite of a Purkinje cell, was depressed to trace *b* 10 min after the change of a superfusion solution from control medium to the 1 mequiv/l Ca^{2+}, 10 mequiv/l Mg^{2+} medium. Then, Ca^{2+} was continuously applied from an adjacent barrel by iontophoresis (100 nA), this led to the augmentation of the c.f.r. (*b*) to the level of *c*. This increased component, namely from trace *b* to *c*, was tentatively termed as the c.f.c.f.r. Upon termination of the Ca^{2+} application, the c.f.c.f.r. rapidly diminished to leave trace *d*. GDEE applied from another adjacent barrel (100 nA) reduced the c.f.c.f.r. only by 28%, i.e. from trace *c* to that marked GDEE, this being practically the maximum inhibition by GDEE. The mean percentage blockade in five dendrites tested was $27{\cdot}6 \pm 6{\cdot}6$%. *B*, effects of GDEE on the depolarizations of a Purkinje cell dendrite induced by L-glutamate (open squares, 15 nA, 500 ms) and L-aspartate (filled squares, 40 nA, 500 ms): simultaneous application of GDEE (70 nA) from an adjacent barrel depressed the depolarizations induced by either amino acid, but responses to L-glutamate were preferentially antagonized. In six dendrites tested, the response to L-glutamate was blocked by $62{\cdot}8 \pm 19{\cdot}1$%, and that to L-aspartate was by $31{\cdot}1 \pm 18{\cdot}2$%. Records *A* and *B* were obtained from different dendrites, and their membrane potentials were maintained at -110 mV.

iontophoresis from a barrel adjacent to an amino-acid-containing barrel, showed no excitation. The protracted application of NMDLA up to 20 s never induced depolarization of the dendrite.

Alternatively, responses to iontophoresed L-glutamate and L-aspartate were consistently antagonized by NMDLA in a dose-dependent manner (in agreement with Crepel, Dupont & Gardette, 1983). Typical antagonistic effects of NMDLA on responses to iontophoresed L-glutamate and L-aspartate, recorded from a Purkinje cell dendrite, are shown in Fig. 6*B*, in which NMDLA exhibits preferential antagonism of the L-aspartate response. When NMDLA was applied with the ejecting current of 100 nA, 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.) %, $n = 5$) (Table 1).

Though not shown in Fig. 6*A*, NMDLA exhibited a dose-dependent antagonism of the c.f.c.f.r. When applied with 100 nA current, NMDLA was found to maximally and reversibly depress the amplitude of c.f.c.f.r., and an average decrease of the

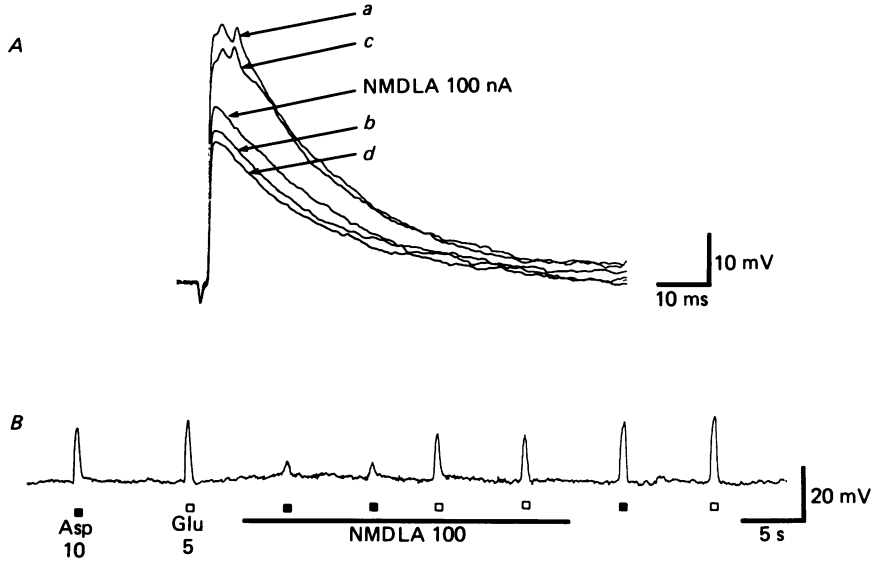

Fig. 6. Effects of NMDLA on a c.f.c.f.r. and L-glutamate- and L-aspartate-induced depolarizations. *A*, the iontophoretic application of NMDLA (100 nA) reduced the c.f.c.f.r. by about 70 %, namely from trace *c* to that marked NMDLA. The mean percentage blockade in five dendrites tested was 71·2 ± 17·0 %. Trace *a*, in control medium (c.f.r.). Traces *b–d* and NMDLA, in the 1 mequiv/l Ca^{2+}, 10 mequiv/l Mg^{2+} medium. Ca^{2+} was applied with 100 nA current to raise trace *b* to *c*. Trace *d*, recovery after the withdrawal of Ca^{2+}. (See Fig. 1 for the detail of c.f.c.f.r.) *B*, simultaneously applied NMDLA (100 nA) preferentially antagonized responses to L-aspartate (filled squares, 10 nA, 500 ms), while those to L-glutamate (open squares, 5 nA, 500 ms) were only slightly depressed by NMDLA. In five dendrites tested, the response to L-aspartate was suppressed by NMDLA by 60·0 ± 14·8 %, and that to L-glutamate was by 22·1 ± 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 maintained at −110 mV.

response was 71 % (71·2 + 17·0 (S.D.) %, $n = 5$) (Table 1). Thus, NMDLA (Fig. 6*A*) suppressed both response to c.f. stimuli and iontophoretic L-aspartate equally (Fig. 6*B*).

Selective antagonism by γ-DGG. γ-DGG which is known as the potent and selective antagonist to NMDLA (Crunelli, Forda & Kelly, 1983) was examined. In five out of seven Purkinje cell dendrites tested, the iontophoretic application of γ-DGG (30 nA) markedly and dose dependently reduced excitatory responses to L-aspartate without changing the membrane potential of the cell, whereas the response of the same neurone to L-glutamate was only slightly blocked. This preferential effect of γ-DGG on responses to L-aspartate is illustrated in Fig. 7*B*1. Although in the remaining two

cells responses to L-glutamate were depressed to certain extents as exemplified in Fig. 7*B*2, the preferential antagonism of L-aspartate response still remained. L-Aspartate responses were suppressed by 69 % (69·0 ± 12·4 (S.D.) %, $n = 7$) by γ-DGG, whereas L-glutamate-induced responses were suppressed by only 15 % (15·4 ± 10·0 (S.D.) %, $n = 7$) (Table 1).

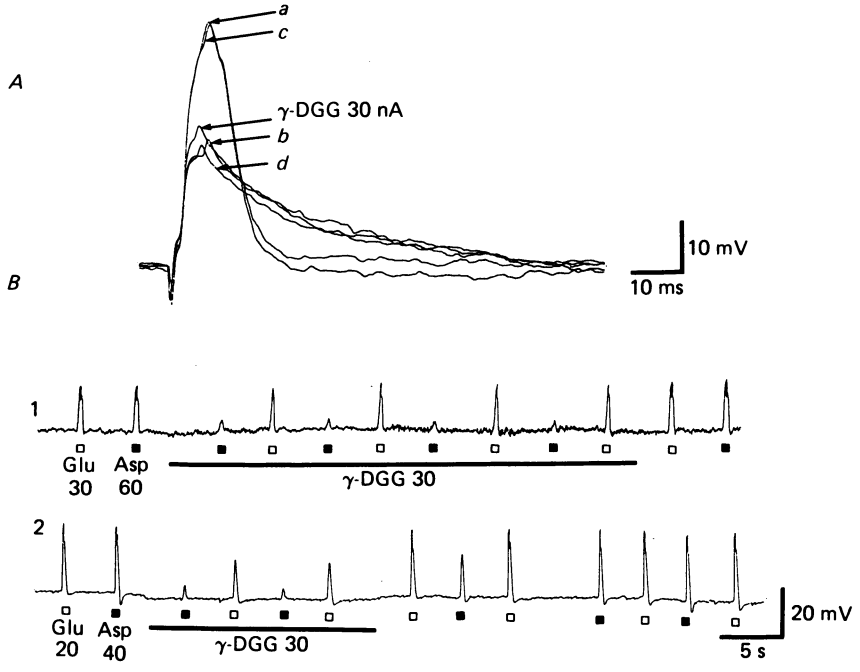

Fig. 7. Effects of γ-DGG on a c.f.c.f.r. and L-glutamate- and L-aspartate-induced depolarizations. *A*, upon application of γ-DGG (30 nA) the amplitude of the c.f.c.f.r. was suppressed by about 87 %. The mean percentage blockade in five dendrites tested was 87·2 ± 14·9 %. Trace *a*, in control medium. Traces *b*–*d* and γ-DGG: in the 1 mequiv/l Ca^{2+}, 10 mequiv/l Mg^{2+} medium. Ca^{2+} was applied with 100 nA current to raise trace *b* to *c*. Trace *d*, recovery after the withdrawal of Ca^{2+}. *B*1, simultaneous application of γ-DGG (30 nA) antagonized responses to L-aspartate (filled squares, 60 nA, 500 ms) with little or no effect on responses to L-glutamate (open squares, 30 nA, 500 ms). This type of antagonism was observed in five out of seven dendrites tested. *B*2, in the remaining two dendrites, responses to L-glutamate were depressed to some extent as shown here. Even in this case, however, L-aspartate responses were more greatly suppressed. In seven dendrites tested, the response to L-aspartate was depressed by γ-DGG by 69·0 ± 12·4 %, and that to L-glutamate was by 15·4 ± 10·0 %. Records *A*, *B*1 and *B*2 were obtained from different dendrites, and their membrane potentials were maintained at −110 mV.

γ-DGG suppressed the c.f.c.f.r. in a dose-dependent manner. In five cells tested γ-DGG (30 nA) markedly depressed the c.f.c.f.r. as shown in Fig. 7*A*, the suppression being about 87 % (87·2 ± 14·9 (S.D.) %, $n = 5$) (Table 1). With the iontophoretic pipette on the most sensitive spot the latency of onset of the γ-DGG-induced blockade was as short as 3–5 s. The maximum antagonism by γ-DGG was observed between 30 and 40 s after the commencement of ejection. Upon the termination of the iontophoretic current this antagonistic action of γ-DGG was rapidly reversible.

Selective antagonism by APV. APV, the most potent and selective antagonist to the *N*-methyl-D-aspartate (NMDA) receptor in the spinal cord (Watkins & Evans, 1981), 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 potent antagonist of the L-aspartate

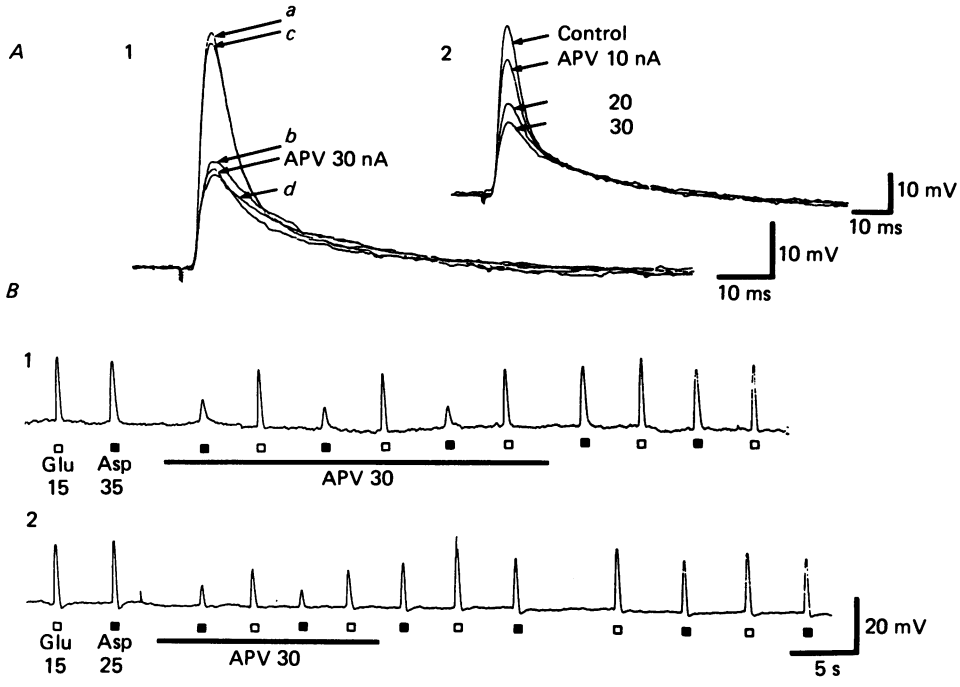

Fig. 8. Effects of APV on a c.f.c.f.r. and L-glutamate- and L-aspartate-induced depolarizations. *A*1, the iontophoretic application of APV (30 nA) almost abolished the c.f.c.f.r. The mean percentage blockade in five dendrites tested was 98·7 ± 4·9 %. Trace *a*, in control medium. Traces *b*–*d* and APV, in the 1 mequiv/l Ca^{2+}, 10 mequiv/l Mg^{2+} medium. Ca^{2+} was applied with 100 nA current to raise trace *b* to *c*. Trace *d*, recovery after the withdrawal of Ca^{2+}. *A*2, the dose dependency of the effect of APV on the c.f.c.f.r. APV was applied iontophoretically at doses as indicated in nA. *A*1 and 2 were recorded in the same dendrite. Similar dose dependency was observed in the antagonism by GDEE, NMDLA and γ-DGG. *B*1, the iontophoretic application of APV (30 nA) selectively reduced responses to L-aspartate (filled squares, 35 nA, 500 ms), while responses to L-glutamate (open squares, 15 nA, 500 ms) were only slightly depressed. This type of antagonism was observed in four out of five dendrites tested. *B*2, in only one remaining dendrite, responses to both L-glutamate and L-aspartate were suppressed as shown here. The preferential antagonism of responses to L-aspartate still remained, however. In five dendrites tested, the response to L-aspartate was blocked by APV by 65·7 ± 3·7 %, and that to L-glutamate was by 13·5 ± 13·2 %. Records *A*, *B*1 and 2 were obtained from different dendrites, and their membrane potentials were maintained at −110 mV.

responses than was γ-DGG. In each dendrite, the iontophoretic application (30 nA) of APV selectively and dose dependently suppressed L-aspartate-induced depolarization, and left the L-glutamate responses virtually unaffected (Fig. 8*B*1). In the remaining one cell both L-glutamate- and L-aspartate-induced depolarizations were reduced in amplitude, yet the L-aspartate responses were blocked to a greater degree

(Fig. 8*B*2). With an ejecting current of 30 nA for APV, responses induced by L-glutamate were depressed by about 14% (13·5 ± 13·2 (S.D.)%, $n = 5$), while those by L-aspartate were suppressed by about 66% (65·7 ± 3·7 (S.D.)%, $n = 5$) (Table 1).

APV antagonized the c.f.c.f.r. in a dose-dependent manner (Fig. 8*A*2) as in the cases of GDEE, NMDLA and γ-DGG. In five dendrites, the iontophoretic application of APV (30 nA) abolished the c.f.c.f.r. (98·7 ± 4·9 (S.D.)%, $n = 5$) (Table 1) without inducing any change in the membrane potential (Fig. 8*A*1). Thus, in addition to its

TABLE 1. Effects of various antagonists on the c.f.c.f.r. and responses to L-aspartate and L-glutamate

	Percentage blockade (mean ± S.D.) (*n*)		
Antagonists (nA)	C.f.c.f.r.	L-aspartate	L-glutamate
APV (30)	98·7 ± 4·9 (5)	65·7 ± 3·7* (5)	13·5 ± 13·2* (5)
γDGG (30)	87·2 ± 14·9 (5)	69·0 ± 12·4*** (7)	15·4 ± 10·0* (7)
NMDLA (100)	71·2 ± 17·0 (5)	60·0 ± 14·8† (5)	22·1 ± 13·3* (5)
GDEE (70 and 100)	27·6 ± 6·6 (5)	31·1 ± 18·2† (6)	62·8 ± 19·1** (6)
APB (100)	—	56·0 ± 4·5 (3)	42·2 ± 4·2 (3)
cis-2,5-PDA (200)	—	33·3 ± 3·5 (3)	35·7 ± 6·0 (3)
ADME (50)	—	32·8 ± 16·4 (5)	51·7 ± 18·0 (5)

All compounds tested were iontophoretically applied onto the dendritic region of Purkinje cells. The values indicate the percentage blockade of the depolarization induced by L-aspartate or L-glutamate alone at a hyperpolarized membrane potential of −110 mV. nA: ejecting current: same current intensity was used for c.f.c.f.r. and responses to the amino acids, except for GDEE for which 100 nA for c.f.c.f.r. and 70 nA for the amino acids. *n*: the number of cells tested. Statistically significantly different from percentage blockade of c.f.c.f.r. by each antagonist: $*P < 0{\cdot}001$, $**P < 0{\cdot}005$, $***P < 0{\cdot}05$, and † not significant.

preferential antagonism against L-aspartate responses, APV also blocked the c.f.c.f.r. even more effectively. The depressant action of APV on the c.f.c.f.r. became apparent within 10–15 s of commencing the ejection of this compound, and on terminating the ejection recovery was usually rapid (5–10 s).

General properties of antagonist and agonists

As shown in Table 1, the compounds which antagonized the depolarization induced by iontophoresed L-glutamate and/or L-aspartate were APV, γ-DGG, NMDLA, APB, cis-2,5-PDA, ADME and GDEE. On the other hand, APP, L-asparagine, L-glutamine and D-aspartic acid potentiated the depolarizations induced by L-glutamate and L-aspartate, probably in keeping with their agonistic properties or their ability to block reuptake (McLennan & Haldeman, 1973).

DαAA, cis-2,4-PDA, D-glutamic acid, *N*-acetyl-glutamic acid and DL-proline were virtually without effect on the responses to iontophoresed L-glutamate and L-aspartate.

DISCUSSION

The primary conclusion of the present study is that an L-aspartate-preferring receptor mediates excitatory synaptic transmission from c.f.s to Purkinje cell dendrites in the guinea-pig cerebellum. This is based on the following results: (1) the

reversal property and ionic dependency of the c.f.r. were almost the same as those of dendritic responses to iontophoresed L-aspartate. (2) NMDLA-selective antagonists, such as APV and γ-DGG, blocked the responses to L-aspartate, leaving those to L-glutamate relatively unaffected and potently antagonized c.f.c.f.r.s.

Reversal potentials

As shown in Figs. 1 and 2, the injection of Cs^+ into a Purkinje cell dendrite and direct intracellular recording from Purkinje cell dendrites enabled us to depolarize the cell beyond equilibrium potentials for L-glutamate, L-aspartate and c.f.r. (E_g, E_a and E_c, respectively), and the relation between amino-acid-induced potential changes and membrane potentials was nicely linear. Therefore, the reversal potentials obtained for the c.f.r. and for responses to L-glutamate and L-aspartate seemed to be fairly reliable.

Reversed hyperpolarizing c.f.r.s showed faster repolarizing phases compared with depolarizing c.f.r.s (Fig. 1*A*). Blockade by Cs^+ of the outward K^+ current seems to be primarily responsible for this slower recovery phase of depolarizing c.f.r.s.

If glutamate or aspartate is a neurotransmitter of c.f.s, E_c should be identical to E_g or E_a. Since c.f.s make multiple synapses on a Purkinje cell dendrite, those located near to the recording site are expected to be reversed first, while the reversal of c.f.r.s at remote synapses might occur at more depolarized potential levels. This may result in a positively shifted estimate of the value of E_c. Taking this possibility and the lack of statistically significant differences among observed E_g, E_a and E_c into account, it is not unreasonable to claim that either of these amino acids might be the neurotransmitter of the cerebellar c.f.s.

C.f.c.f.r.

Although c.f.r.s were not completely abolished in the low-Ca^{2+} (1 mequiv/l), high-Mg^{2+} (10 mequiv/l) medium used in this study, further lowering of the Ca^{2+} concentration and further raising of the Mg^{2+} concentration did not support stable and long-lasting intracellular recording from Purkinje cell dendrites. Upon iontophoretic application of Ca^{2+}, however, the suppressed c.f.r. in the low-Ca^{2+}, high-Mg^{2+} medium was augmented in amplitude to almost their initial value in control medium (Fig. 5*A*, traces *a–d*). Dingledine (1983) suggested that NMDA-mediated Ca^{2+} conductance may not only serve as a conventional charge carrier and an inducer of Ca^{2+}-activated K^+ conductance but also affect a number of cellular functions such as cytoplasmic transport, Ca^{2+}-dependent enzyme activities and local dendritic secretory processes. In the present study, however, the amplitude of glutamate- or aspartate-induced depolarization was not enhanced by simultaneous iontophoretic application of Ca^{2+} (data not shown). Therefore, it is unlikely that Ca^{2+} functions other than as a facilitator of presynaptic release of a transmitter and thus focally augment the c.f.r.

Under this condition, the same or similar dose of an antagonist equipotently depressed both c.f.c.f.r. and a response to L-aspartate (see Figs. 5–8 and Table 1). On the other hand, when c.f.r.s were evoked simply in control medium (2 mequiv/l Ca^{2+} and 1 mequiv/l Mg^{2+} present), much higher doses of an antagonist were required to block the c.f.r.s than required to block the responses to L-aspartate to a similar level.

Ionic mechanism for responses to L-glutamate and L-aspartate and the c.f.r.

As shown in Fig. 3, the reversal potentials for the c.f.r. and responses to L-glutamate and L-aspartate appear to be determined primarily by a Na^+ conductance. The slopes for the glutamate and aspartate responses and the c.f.r., however, were substantially smaller than would be predicted by the Nernst equation (Fig. 3*C*). A possible explanation for these small shifts may be the contribution of a K^+ conductance and this would also account for a reversal potential displaced from the Na^+ equilibrium potential in control medium. Another might be the greater contribution of Ca^{2+} in a low-Na^+ medium. In the case of the crayfish neuromuscular junction (Onodera & Takeuchi, 1976), a small but significant inward current is produced by L-glutamate even in a Na^+-free medium. In the presence of external Na^+, on the other hand, the glutamate current is reduced by increasing Ca^{2+} concentrations in the medium.

Changes in membrane conductance during depolarizations evoked by L-glutamate and L-aspartate

The depolarizations evoked by the application of L-glutamate to the dendritic regions of frog motoneurones was reported not to be associated with a change in the membrane resistance (Shapovalov, Shiriaev & Velumian, 1978). The application of small doses of L-glutamate was also reported to produce either no change or an apparent increase in input resistance of hippocampal CA1 pyramidal neurones of the guinea-pig (Hablitz & Langmoen, 1982). Moreover, Crepel *et al.* (1982) reported that the input resistance of the rat cerebellar Purkinje cells was unchanged by pulses of L-glutamate and L-aspartate, even when very large depolarizations were elicited.

In our experiments, on the contrary, the depolarizing actions of L-glutamate and L-aspartate on guinea-pig cerebellar Purkinje cell dendrites were accompanied by marked decreases in the membrane resistance (Fig. 4). This discrepancy may be due to the fact that Crepel *et al.* (1982) measured the input resistance at the soma while applying L-glutamate and L-aspartate in the dendrite.

Type of receptor in cerebellar Purkinje cell dendrites

It has been demonstrated recently that L-glutamate, L-aspartate and NMDA increase the input resistance of cultured central neurones of the mouse, and also that this increase was highly voltage dependent and Mg^{2+} sensitive (Mayer, Westbrook & Guthrie, 1984; Nowak, Bregestovski, Ascher, Jerbet & Prochiantz, 1984). Although we have not determined whether the response of guinea-pig cerebellar Purkinje cell dendrites to L-glutamate or L-aspartate also exhibits these properties, the type of the receptor on Purkinje cell dendrites seems to be different from those in cultured mouse central neurones. Thus, Nowak *et al.* (1984) suggested that the Mg^{2+}-sensitive glutamate-activated channels are of a NMDA type, because NMDA and L-aspartate elicited responses similar to those induced by L-glutamate, and that both kainate and quisqualate were ineffective. On the contrary, both kainate and quisqualate receptors appear to be present, while NMDA type receptors are absent in the cerebellar Purkinje cells of the rat (Crepel *et al.* 1982) and the guinea-pig (Kimura, Okamoto & Sakai, 1985*a*).

In agreement with Crepel *et al.* (1982), GDEE preferentially affected responses to L-glutamate rather than those to L-aspartate, and the c.f.c.f.r was only weakly

depressed in spite of larger application of this compound (Fig. 5). Moreover, NMDLA was more potent than GDEE in reducing the c.f.c.f.r. (Figs. 5 and 6, see also Crepel *et al.* 1982, 1983 for the rat cerebellum). Thus, it may be said that the c.f.c.f.r. is more susceptible to L-aspartate-preferring antagonists.

Based on the finding of the marked parallelism between the depressing actions of four antagonists, GDEE, NMDLA, γ-DGG and APV, on the c.f.c.f.r. and those on L-aspartate-induced depolarization (Table 1), we would like to give preference to L-aspartate rather than L-glutamate as being the endogenous neurotransmitter of the cerebellar c.f. at least in the guinea-pig. It should be noted, however, that the present result does not necessarily mean that L-aspartate is the endogenous neurotransmitter at c.f.s but may indicate that the post-synaptic receptor for c.f.s is a L-aspartate-preferring type. Other amino acids or related compounds, therefore, cannot be excluded from candidates for the transmitter of cerebellar c.f.s.

REFERENCES

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

Crepel, F., Dhanjal, S. S. & Garthwaite, J. (1981). Morphological and electrophysiological characterization of rat cerebellar slices maintained *in vitro*. *Journal of Physiology* **316**, 127–138.

Crepel, F., Dhanjal, S. S. & Sears, T. A. (1982). Effect of glutamate, aspartate and related derivatives on cerebellar Purkinje cell dendrites in the rat: an *in vitro* study. *Journal of Physiology* **329**, 297–317.

Crepel, F., Dupont, J. L. & Gardette, R. (1983). Voltage clamp analysis of the effect of excitatory amino acids and derivatives on Purkinje cell dendrites in rat cerebellar slices maintained in vitro. *Brain Research* **279**, 311–315.

Crunelli, V., Forda, S. & Kelly, J. S. (1983). Blockade of amino acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus. *Journal of Physiology*, **341**, 627–640.

Davies, J. & Watkins, J. C. (1979). Selective antagonism of amino acid-induced and synaptic excitation in the cat spinal cord. *Journal of Physiology* **297**, 621–635.

Dingledine, R. (1983). *N*-methyl aspartate activates voltage-dependent calcium conductance in rat hippocampal pyramidal cells. *Journal of Physiology* **343**, 385–405.

Dupont, J. L., Crepel, F. & Delhaye-Bouchaud, N. (1979). Influence of bicuculline and picrotoxin on reversal properties of excitatory synaptic potentials in cerebellar Purkinje cells of the rat. *Brain Research* **173**, 577–580.

Eccles, J. C. (1965). Functional meaning of the patterns of synaptic connection in the cerebellum. *Perspectives in Biology and Medicine* **8**, 289–310.

Eccles, J. C., Llinás, R. & Sasaki, K. (1966*a*). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *Journal of Physiology* **182**, 268–296.

Eccles, J. C., Llinás, R., Sasaki, K. & Voorhweve, P. E. (1966*b*). Interaction experiments on the responses evoked in Purkinje cells by climbing fibres. *Journal of Physiology* **182**, 297–315.

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

Hackett, J. T., Hou, S. M. & Cochran, S. L. (1979). Glutamate and synaptic depolarization of Purkinje cells evoked by parallel fibers and climbing fibers. *Brain Research* **170**, 377–380.

Heyer, C. B. & Lux, H. D. (1976). Control of the delayed outward potassium currents in bursting pace-maker neurones of the snail, *Helix pomatia*. *Journal of Physiology* **262**, 349–382.

Johnston, D. & Hablitz, J. J. (1980). Voltage clamp discloses slow inward current in hippocampal burst-firing neurones. *Nature* **286**, 391–393.

Kimura, H., Okamoto, K. & Sakai, Y. (1985*a*). Pharmacological characterization of postsynaptic receptors for excitatory amino acids in Purkinje cell dendrites in the guinea pig cerebellum. *Journal of Pharmacobio-Dynamics* **8**, 120–128.

KIMURA, H., OKAMOTO, K. & SAKAI, Y. (1985*b*). Climbing and parallel fiber responses recorded intracellularly from Prukinje cell dendrites in guinea pig cerebellar slices. *Brain Research* (in the Press).

LANGMOEN, I. A. & HABLITZ, J. J. (1981). Reversal potential for glutamate responses in hippocampal pyramidal cells. *Neuroscience Letters* **23**, 61–65.

LLINÁS, R. & SUGIMORI, M. (1980*a*). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *Journal of Physiology* **305**, 171–195.

LLINÁS, R. & SUGIMORI, M. (1980*b*). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**, 197–213.

MCLENNAN, H. & HALDEMAN, S. (1973). The actions of the dimethyl and diethyl esters of glutamic acid on glutamate uptake by brain tissue. *Journal of Neurochemistry* **20**, 629–631.

MCLENNAN, H. & LODGE, D. (1979). The antagonism of amino acid-induced excitation of spinal neurones in the cat. *Brain Research* **169**, 83–90.

MAYER, M. L., WESTBROOK, G. L. & GUTHRIE, P. B. (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**, 261–263.

MEECH, R. W. & STANDEN, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp. A component mediated by calcium influx. *Journal of Physiology* **249**, 211–239.

NADI, N. S., KANTER, D., MCBRIDE, W. J. & APRISON, M. H. (1977). Effects of 3-acetylpyridine on several putative neurotransmitter amino acids in the cerebellum and medulla of the rat. *Journal of Neurochemistry* **28**, 661–662.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., JERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462–465.

OKAMOTO, K., KIMURA, H. & SAKAI, Y. (1983). Ionic mechanisms of the action of taurine on cerebellar Purkinje cell dendrites *in vitro*: Intradendritic study. *Brain Research* **260**, 261–269.

ONODERA, K. & TAKEUCHI, A. (1976). Permeability changes produced by L-glutamate at the excitatory post-synaptic membrane of the crayfish muscle. *Journal of Physiology* **255**, 669–685.

PALAY, S. L. & CHAN-PALAY, V. (1974). *Cerebellar Cortex: Cytology and Organization*, pp. 242–287. Berlin, Heidelberg, New York: Springer Verlag.

SANDOVAL, M. E. & COTMAN, C. W. (1978). Evaluation of glutamate as a neurotransmitter of cerebellar parallel fibers. *Neurosciences* **3**, 199–206.

SAWADA, S., TAKADA, S. & YAMAMOTO, C. (1983). Selective activation of synapses near the tip of drug-ejecting microelectrode, and effects of antagonists of excitatory amino acids in the hippocampus. *Brain Research* **267**, 156–160.

SHAPOVALOV, A. I., SHIRIAEV, B. I. & VELUMIAN, A. A. (1978). Mechanisms of post-synaptic excitation in amphibian motoneurones. *Journal of Physiology* **279**, 437–455.

STONE, T. W. (1979). Glutamate as the neurotransmitter of cerebellar granule cells in the rat: electrophysiological evidence. *British Journal of Pharmacology* **66**, 291–296.

WATKINS, J. C. & EVANS, R. H. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology and Toxicology* **21**, 165–204.

WIKLUND, L., TOGGENBURGER, G. & CUÉNOD, M. (1982). Aspartate: Possible neurotransmitter in cerebellar climbing fibers. *Science* **216**, 78–80.