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EXCITATORY AMINO ACID-RECEPTOR-MEDIATED EPSPs IN RAT DORSOLATERAL SEPTAL NUCLEUS NEURONES *IN VITRO*

JOEL P. GALLAGHER AND HIROSHI HASUO*

From the Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550, USA

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SUMMARY

1. Intracellular recordings were made from rat dorsolateral septal nucleus (DLSN) neurones *in vitro*. We investigated depolarizations resulting from pressure application of excitatory amino acids and compared these to synaptically evoked excitatory postsynaptic potentials (EPSPs).

2. EPSPs evoked by focal fimbrial afferent stimulation in saline with 30–50 μM-bicuculline and 1·2 mM-Mg^{2+} yielded a linear amplitude–voltage relationship; their reversal potential was −3 mV. These EPSPs exhibited little sensitivity to 2-amino-5-phosphonopentanoate (APV), an *N*-methyl-D-aspartate(NMDA)-receptor-specific antagonist, but were markedly depressed by kynurenic acid, a broad-spectrum excitatory amino acid antagonist.

3. In Mg^{2+}-free solution, the amplitude and the duration of EPSPs were increased markedly masking the following inhibitory postsynaptic potential (IPSP) and the late hyperpolarizing potential (LHP). These facilitated and broadened EPSPs were sensitive to APV or Mg^{2+}. The APV or Mg^{2+}-sensitive component of the EPSP obtained by digital subtraction suggests a slower time course for the NMDA-receptor-mediated EPSP compared to the non-NMDA-receptor-mediated EPSP. On the other hand, in normal Mg^{2+} solution an EPSP evoked by either a single strong stimulus or by repetitive stimuli had APV-sensitive components.

4. The depolarizing potentials induced by pressure application of glutamate, kainate, α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), quisqualate or NMDA were compared. The amplitude–voltage relationship of depolarizations induced by NMDA obtained in a normal Mg^{2+} solution was non-linear, but approached linearity when the same responses were recorded in a Mg^{2+}-free solution. Depolarizations induced by kainate, AMPA and quisqualate were linear in their amplitude–voltage relationship in the presence or absence of Mg^{2+}. APV blocked NMDA-induced depolarizations specifically, while kynurenic acid blocked all the depolarizations induced by NMDA, quisqualate, or kainate.

5. Our data demonstrate the existence of NMDA-receptor-mediated synaptic potentials in the rat DLSN, the characteristics of which are similar to those in other central nervous system regions.

* Present address: Department of Physiology, Kurume University School of Medicine, 67-Asahi-machi, Kurume 830, Japan

MS 7332

INTRODUCTION

In the vertebrate central nervous system (CNS), receptors for the excitatory amino acid neurotransmitters are divided into three subtypes depending on their sensitivity to three specific agonists: kainate, quisqualate and NMDA (Watkins & Evans, 1981; Dale & Roberts, 1984; Mayer & Westbrook, 1987; Watkins & Olverman, 1987; Stone & Burton, 1988). Recently, characteristics of glutamate receptor subtypes coupled to ionic channels in CNS neurones have been investigated in detail (Cull-Candy & Usowicz, 1987; Jahr & Stevens, 1987; Ascher, Bregestovski & Nowak, 1988; Ascher & Nowak, 1988; Cull-Candy, Howe & Ogden, 1988; Shingai & Ebina, 1988). The NMDA receptor–channel complex, especially, has been demonstrated to have several unique characteristics (for reviews see Ascher & Nowak, 1987; Collingridge & Bliss, 1987).

Dorsolateral septal nucleus (DLSN) neurones receive excitatory input from the hippocampus (DeFrance, Shimono & Kitai, 1972; McLennan & Miller, 1974) and this input is mediated by an excitatory amino acid neurotransmitter (Storm-Mathisen, 1978). The majority of the hippocampal neurones which provide the dorsolateral septal input originate in hippocampal area CA3 and are branches of axons which form the well-known Schaffer collateral pathway (Lorente de Nó, 1934; Anderson, Blackstad & Lömo, 1966; Swanson, Sawchenko & Cowan, 1980). Three previous reports have concluded that this excitatory amino acid-mediated transmission is due to activation of a non-NMDA receptor (Joëls & Urban, 1984*a*, *b*; Stevens & Cotman, 1986). Since excitatory transmission in the Schaffer collateral–commissural pathway of rat hippocampus has been shown to involve activation of both non-NMDA and NMDA receptors (Blake, Brown & Collingridge, 1988), we initiated these studies to test the possibility that excitatory amino acid transmission onto DLSN neurones may also involve both non-NMDA and NMDA receptors. Indeed, high levels of NMDA-displaceable [^{3}H]L-glutamate binding sites have been demonstrated in the dorsolateral septum (Cotman, Monaghan, Ottersen & Storm-Mathisen, 1987), septal neurones respond to exogenously applied NMDA with an increase in firing rate (Joëls & Urban, 1984*b*), and NMDA-induced membrane current fluctuation has been recorded in cultured septal neurones (Shingai & Ebina, 1988). However, the involvement of NMDA receptors in synaptic potentials in the rat DLSN has not been reported and their possible role in synaptic transmission is still not clear. Here we demonstrate a role for NMDA receptors in synaptic potentials recorded intracellularly from DLSN neurones. We have also analysed the characteristics of NMDA receptors in the DLSN by comparing depolarizing potentials induced by various exogenously applied glutamate-receptor-specific agonists.

METHODS

Slices of rat brain containing the septal nuclei were obtained in a manner described previously (Stevens, Gallagher & Shinnick-Gallagher, 1984). Male Sprague–Dawley rats, 100–200 g, were killed by decapitation, their brains rapidly removed and immersed for 8–10 s in a cooled saline solution (4–6 °C) which was pre-bubbled with 95% O_2 and 5% CO_2. Transverse slices (500 μm thick) were cut with a Vibroslice (Camden Instruments) and left for 1 h in the oxygenated (95% O_2 and 5% CO_2) saline solution at room temperature (22–24 °C). The slice was later transferred to a recording chamber and submerged with superfusion of oxygenized saline solution at 32–33 °C. The volume

of our recording chamber was 1 ml and the superfusion rate was maintained at 2·5 ml/min. The composition of the normal saline solution was as follows (in mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$ 25; NaH_2PO_4, 1·2; D-glucose, 11, (pH 7·4). When the potassium concentration was changed an equimolar sodium concentration was substituted, and when the magnesium concentration was changed an equimolar calcium concentration was substituted. In some experiments, bicuculline (30–50 μM) or tetrodotoxin (TTX, 0·6 μM) was added to block inhibitory postsynaptic potentials (IPSPs). Intracellular recordings of membrane potential were made with glass microelectrodes filled with 4 M-potassium acetate (resistance, 70–150 MΩ) and were amplified with an Axoclamp 2A single-electrode voltage clamp amplifier (Axon Instruments). Resting membrane potential was $-59{\cdot}8 \pm 7{\cdot}1$ mV (mean ± S.D., $n = 75$) and input resistance was $105{\cdot}8 \pm 30{\cdot}3$ MΩ ($n = 53$). DC currents were injected through the recording electrode by means of a bridge-type circuit within the amplifier. Membrane potential and current signals were monitored continuously by a pen recorder (Gould, Model 220). These signals were also stored on magnetic tapes (A. R. Vetter Co., Redersburg, PA, USA) for future analysis. Synaptic responses were captured by a digital memory oscilloscope (DATA 6000, Data Precision) and later plotted by an *X–Y* plotter (7440A, Hewlett Packard). Digital subtraction of synaptic responses was conducted in a manner similar to that described by Forsythe & Westbrook (1988) and Collingridge, Herron & Lester (1988*a*). Bipolar concentric electrodes were used for focal stimulation of the fimbria/fornix (monophasic, 0·1 ms duration, 5–15 V for low-intensity and 30–50 V for high-intensity stimulation, respectively). Excitatory amino acids, dissolved in saline solution (30–100 μM), were applied from a large-bore pipette (diameter about 10 μm) placed close to the recording electrode (less than 300 μm away) by pressure pulses of 20 lbf/in^2 (138 kPa) for 5–200 ms. When drugs were bath-applied, effects were observed within 30 s and an apparent equilibrium was reached within 3–5 min. *N*-Methyl-D-aspartate (NMDA), L-glutamate, kainate, quisqualate, DL-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kynurenic acid, DL-2-amino-5-phosphonopentanoate (APV), tetrodotoxin (TTX) and (−)-bicuculline methiodide were obtained from Sigma, St Louis, MO, USA.

All data were represented by the number of neurones tested (n) with their mean ± standard deviation.

RESULTS

Characteristics of EPSPs recorded in normal (1·2 mM) Mg^{2+} solution

Fimbrial afferent stimulation produces excitatory postsynaptic potentials (EPSPs), fast inhibitory postsynaptic potentials (IPSPs) and late hyperpolarizing potentials (LHPs) in dorsolateral septal nucleus (DLSN) neurones when recorded in normal Mg^{2+} saline solution (for examples see Fig. 2*B b*; Gallagher, Stevens & Shinnick-Gallagher, 1984; Stevens, Gallagher & Shinnick-Gallagher, 1985, 1987; Stevens & Cotman, 1986). During analysis of orthodromically evoked EPSPs, we usually added the $GABA_A$ receptor antagonist bicuculline (30–50 μM) to the normal Mg^{2+} saline solution in order to minimize contamination of the EPSPs by fast IPSPs (Stevens *et al.* 1985). Afferent fimbrial stimulation produced short-latency (1·5–2 ms) EPSPs which declined with a half-decay time of 23 ms. Since the tail of the EPSP, even when the fast IPSP was blocked, seemed to be masked by a following LHP, an accurate assessment of the half-decay-time for the EPSP was not possible. When the membrane potential was hyperpolarized by DC current, the amplitude of the EPSP was increased. The relationship between amplitude of EPSP and membrane potential was almost linear over the potential range from −55 to −100 mV ($n = 7$). The amplitude of EPSPs recorded more negative than −90 mV tended to decline. However, this non-linearity was attenuated by 1 mM-Cs^{2+}, which blocks several potassium conductances (Rudy, 1988). The reversal potential of the EPSP suggested by extrapolation of a plot obtained from the EPSP amplitude *versus* membrane

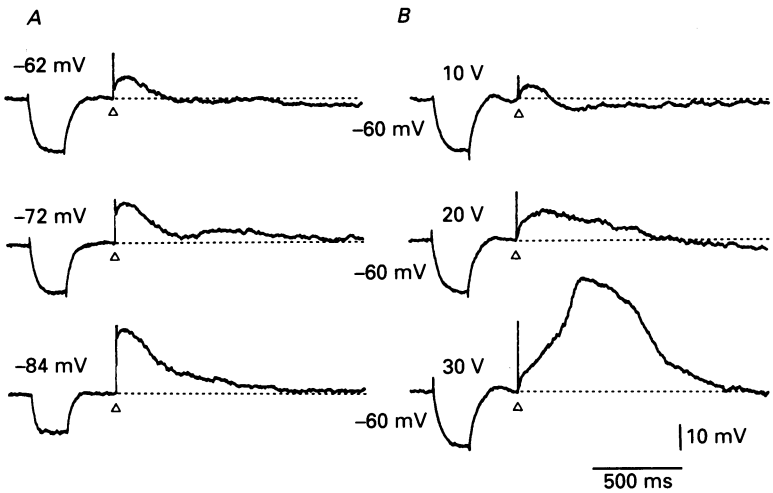

Fig. 1. Synaptic responses in Mg^{2+}-free solution. *A*, voltage dependence of synaptic potentials evoked by fimbrial stimulation (0·1 ms, 10 V at △). Input resistance was monitored by applying hyperpolarizing step pulses (0·1 nA, 200 ms). Dashed lines indicate the membrane potential levels. *B*, synaptic potentials evoked by different stimulus intensities. Upper, middle and lower traces were obtained by applying 10, 20 and 30 V stimulations (0·1 ms at △) at resting membrane potential of −60 mV. Note that synaptic potentials were obtained in absence of bicuculline.

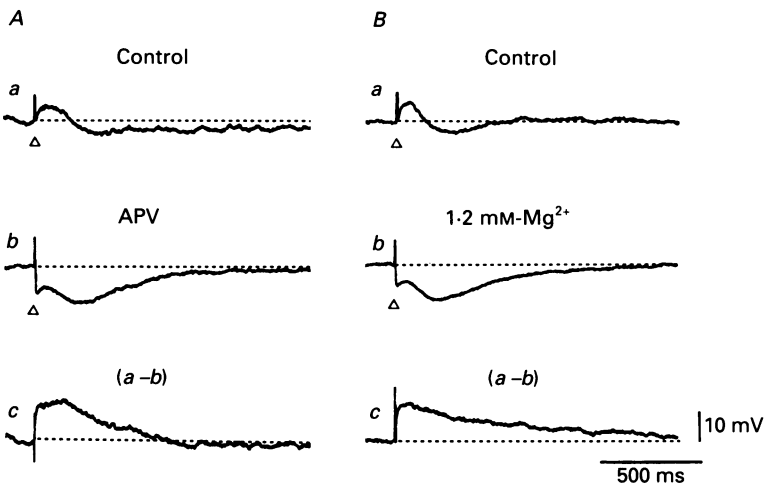

Fig. 2. Effects of APV *versus* Mg^{2+} and synaptic responses. *A*, effect of APV (50 μM) on synaptic responses recorded in Mg^{2+}-free solution: *a*, control synaptic potentials evoked by fimbrial stimulation (0·1 ms, 10 V at △); *b*, synaptic potential obtained in the presence of APV; *c*, difference between traces *a* and *b* obtained by digital subtraction. Dashed lines indicate the resting membrane potential level of −60 mV. *B*, effect of extracellular Mg^{2+} on synaptic potentials: *a*, synaptic potential evoked by fimbrial stimulation in Mg^{2+}-free solution; *b* synaptic potential in the presence of 1·2 mM-Mg^{2+}, *c*, difference between traces *a* and *b*. Traces shown in *A* and *B* were obtained from the same neurone. Bicuculline absent.

potential relationship recorded in a bicuculline and Cs^{2+}-containing solution was $-6{\cdot}0 \pm 6{\cdot}5$ mV ($n = 7$). This data was confirmed by measuring the actual reversal potential of EPSPs ($-3{\cdot}0 \pm 8{\cdot}5$ mV, $n = 3$) recorded with $CsCl_2$ (2 M)-filled microelectrodes. In eight neurones tested, the amplitudes of EPSPs evoked by low-

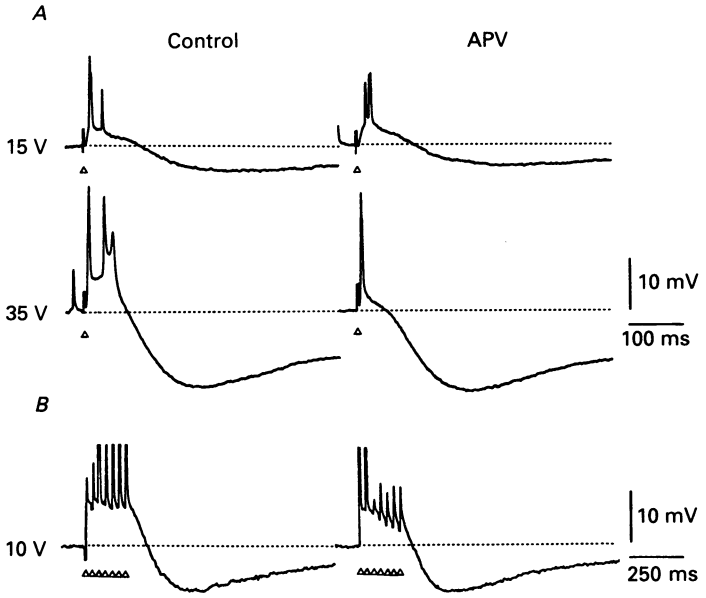

Fig. 3. APV-sensitive components of EPSPs evoked by a high-intensity stimulus or evoked by repetitive stimuli in normal Mg^{2+} solution. *A* left-hand traces depict control synaptic potentials evoked by lower intensity stimulus (upper, 15 V) and higher intensity stimulus (lower, 35 V). Right-hand traces depict synaptic potentials (upper, 15 V; lower, 35 V) obtained in the presence of APV (50 μM). Resting membrane potential was -58 mV. *B*, left-hand trace depicts the control synaptic responses evoked by repetitive fimbrial stimulation (10 V, 0·1 ms, seven times at 33 ms intervals). Right-hand trace was obtained in the presence of APV (50 μM). Resting membrane potential was -66 mV. Spikes of all action potentials were truncated. Bicuculline (50 μM) present.

frequency (0·1 Hz) and low-intensity stimuli (5–15 V) were not sensitive to APV (50 μM) which is a known NMDA-receptor-selective antagonist (Davies, Francis, Jones & Watkins, 1981). APV had no effect on membrane potential or input resistance, and did not affect either the IPSP or LHP recorded in normal Mg^{2+} saline solution. On the other hand, kynurenic acid (1 mM), a broad-spectrum excitatory amino acid antagonist (Ganong, Lanthorn & Cotman, 1983), depressed the amplitude of these low-frequency, low-stimulus-intensity-induced EPSPs by $90 \pm 8\%$ ($n = 7$). These data are consistent with the report of Stevens & Cotman (1986).

Characteristics of EPSPs evoked in Mg^{2+}-free solution

In Mg^{2+}-free solution, spontaneous activity was markedly increased and resulted in an increase in baseline noise, although no epileptiform burst-like activity became apparent. Application of bicuculline, kynurenic acid, APV or TTX reduced this spontaneous activity but did not eliminate it. The shape of both excitatory and inhibitory synaptic responses evoked by fimbrial afferent stimulation was altered

gradually by superfusion with Mg^{2+}-free saline solution, reaching equilibrium after 1 h of superfusion. The amplitude and duration of the depolarizing excitatory component were enhanced while the late hyperpolarizing component decreased in both amplitude and duration (Fig. 1). As the membrane potential was adjusted to a more hyperpolarized level, the amplitude of the depolarizing excitatory component increased (Fig. 1*A*), and, when the stimulus intensity was increased from 10 to 30 V, the depolarizing excitatory component was augmented in both amplitude and duration. At 30 V stimulation, a slow-time-course voltage-activated response was elicited (Fig. 1 *B*); its threshold was −39 mV.

We tested the sensitivity of the synaptic potentials, evoked in Mg^{2+}-free solution, to APV and Mg^{2+} in twenty-two neurones (Fig. 2). In the presence of APV (50 μM), synaptic potentials evoked by low-intensity fimbrial afferent stimulation (10 V) showed a clear IPSP–LHP sequence (Fig. 2*Ab*); this sequence was identical to that recorded in normal Mg^{2+} solution (Fig. 2*Bb*). The APV-sensitive component, which was estimated by digital subtraction of the synaptic potential obtained in the presence of APV from the EPSP recorded in the absence of APV (control), appeared as a slow depolarization (Fig. 2*Ac*). The Mg^{2+}-sensitive component, estimated in an identical manner, was also similar to the APV-sensitive component (Fig. 2*Bc*). This NMDA-receptor-mediated synaptic component obtained by digital subtraction may consist of membrane responses contaminated by active membrane properties, non-linear passive membrane properties, and other synaptic responses such as the fast IPSP and LHP. These contaminants could be minimized by recording EPSPs at a hyperpolarized membrane potential of −90 mV while blocking the fast IPSP with bicuculline. However, although only a few experiments reported here were carried out under these conditions, the results we obtained did not differ qualitatively from the majority of data collected at the resting membrane potential. The EPSP amplitude was enhanced after application of bicuculline (30–50 μM) to the Mg^{2+}-free solution. However, following addition of 1·2 mM-Mg^{2+} or APV (30–50 μM) the duration and amplitude of the bicuculline-enhanced EPSPs were depressed. The time course of the APV-sensitive component was prolonged with higher intensity stimulation. The minimum stimulus intensity to detect the APV-sensitive EPSP was about 5 V. At a low intensity of stimulation (less than 10 V), the half-decay-time of the APV-sensitive or Mg^{2+}-sensitive component varied from 30 to 200 ms. The time course of the APV-sensitive component appeared to be slower than that of the APV-resistant component.

APV-sensitive EPSP evoked in normal Mg^{2+} solution

The EPSP evoked at low-stimulus intensity in normal Mg^{2+} solution is not sensitive to APV. However, the EPSPs evoked by high-intensity stimuli or by repetitive stimuli were sensitive to APV (Fig. 3). As shown in Fig. 3, the EPSP evoked by a 15 V stimulus was not sensitive to APV, but the EPSP evoked by a 35 V stimulus was depressed by APV while the LHP was not affected. The absolute value of stimulus intensity needed to evoke an APV-sensitive EPSP varied among preparations; however, larger intensity stimuli (more than 30 V) evoked EPSPs which were usually sensitive to APV. Since Herron, Lester, Coan & Collingridge (1986) reported that NMDA receptors contribute to postsynaptic activation during

high-frequency synaptic transmission in the hippocampus, we tested whether NMDA receptors contributed to the synaptic responses evoked by repetitive stimuli in the DLSN. Repetitive stimuli (seven pulses at 33 ms intervals) of relatively lower intensity (10 V, 0·1 ms) were applied in the presence of bicuculline. The depolarizing component of the resultant synaptic potential was depressed by APV, especially in its later phase (Fig. 3*B*).

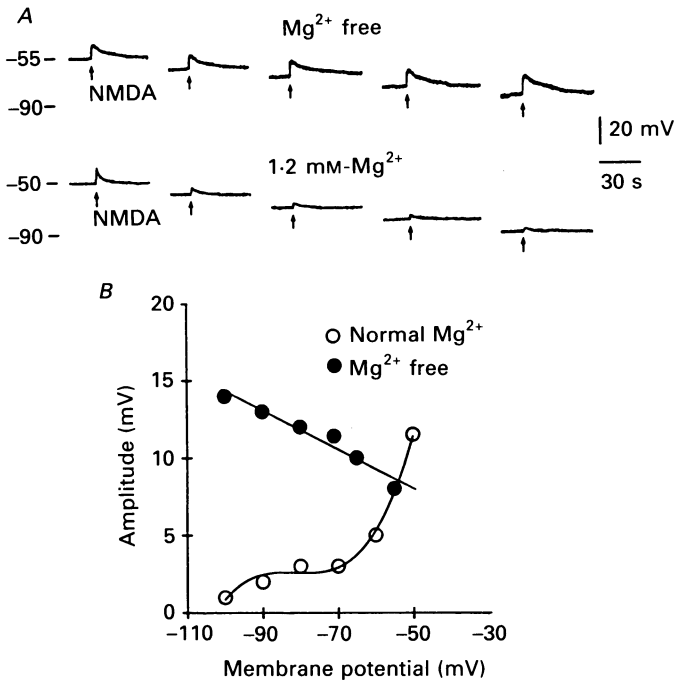

Fig. 4. Voltage dependence of NMDA-induced potentials in Mg^{2+}-free solution and in normal Mg^{2+} solution. *A*, actual recordings of NMDA-induced potentials are shown. Membrane potentials shown to left (mV). Upper traces were obtained in Mg^{2+}-free solution. NMDA was applied by pressure ejection from a puff pipette containing 100 μM-NMDA (20 lbf/in^2(= 138 kPa) 10 ms) at the time indicated by arrows. Lower traces were obtained in normal Mg^{2+} solution. NMDA was applied by pressure ejection (20 lbf/in^2, 20 ms). *B*, relationship between the amplitude of NMDA-induced potentials and membrane potential. The data used for this plot are depicted in *A*.

Depolarizations induced by excitatory amino acids applied exogenously

We applied glutamate and glutamate-receptor-subtype-specific agonists, such as kainate, AMPA and NMDA (Watkins & Evans, 1981; Mayer & Westbrook, 1987; Watkins & Olverman, 1987; Stone & Burton, 1988) to the DLSN neurones in the presence of TTX (0·6 μM). Excitatory amino acids were pressure ejected from a large-bore pipette located close to the recording electrode. The concentration of each excitatory amino acid in the puff pipette was 100 μM except for AMPA which was 30 μM. TTX was added to the saline solution to reduce possible contamination of the response resulting from a neurotransmitter released by a local interneurone

subsequent to application of an excitatory amino acid (Gallagher & Hasuo, 1989). Almost all neurones tested ($n = 26$) responded to glutamate ($n = 5$), quisqualate ($n = 4$), kainate ($n = 7$) and NMDA ($n = 20$) with depolarizations when recorded at the resting membrane potential level. AMPA or quisqualate appeared to be more potent

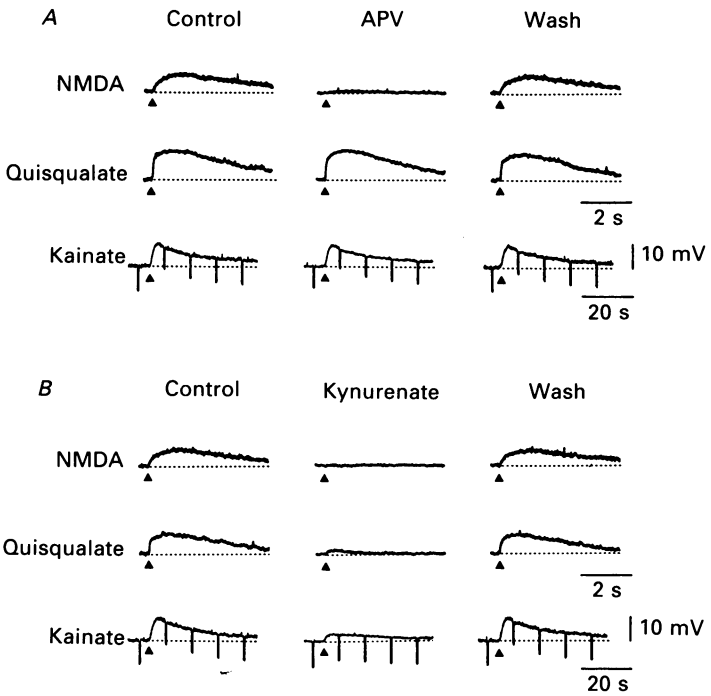

Fig. 5. Effects of antagonists on excitatory amino acids applied exogenously. *A*, effect of APV (50 μM) on excitatory amino acid-induced potentials. NMDA, quisqualate or kainate (50 μM in each puff pipette) were applied (30, 30 or 80 ms, respectively) at the time indicated by ▲. Resting membrane potential was −73 mV. Middle-column traces were obtained 6–8 min after superfusion of 50 μM-APV. Right-column traces were obtained 10–15 min after wash-out. Downward deflections in the lower traces (kainate responses) are tonic potentials (500 ms, 0·2 nA). *B*, effect of kynurenic acid (1 mM) on excitatory amino acid-induced potentials. Excitatory amino acids were applied by the same methods as in *A*. Middle-column traces were obtained after 6–8 min superfusion of 1 mM-kynurenic acid. Right-column traces were obtained 10–15 min after wash-out. Traces in *A* and *B* were obtained from the same neurone. TTX was added to the bathing solution.

than kainate or NMDA. The decay time course of glutamate-induced depolarizations was significantly faster than those of any of the other agonists. The amplitude and time course of an NMDA-induced depolarization obtained in Mg^{2+}-free solution ($n = 8$) were similar to those of kainate or AMPA-induced depolarizations in a Mg^{2+}-containing solution. We tested the voltage dependence of these excitatory amino acid-induced depolarizations in normal Mg^{2+} solution. The glutamate ($n = 2$), kainate ($n = 4$) or quisqualate ($n = 2$) -induced depolarizations were linear, but the NMDA ($n = 3$) -induced depolarization became smaller with hyperpolarization of the membrane potential between −50 and −100 mV (Fig. 4). However, an NMDA-induced depolarization obtained in Mg^{2+}-free solution ($n = 6$) showed a similar linear voltage dependence as was observed with non-NMDA-receptor-agonist-induced

depolarizations obtained in normal Mg^{2+} (Fig. 4). There was no significant difference in reversal potentials estimated by extrapolation between an NMDA-induced depolarization obtained in Mg^{2+}-free solution and non-NMDA-induced depolarizations obtained in normal Mg^{2+} solution. The reversal potentials of kainate, AMPA and NMDA-induced depolarizations were (in mV) $2{\cdot}3 \pm 10$ ($n = 4$), $-4{\cdot}0 \pm 5{\cdot}3$ ($n = 4$) and $-3{\cdot}4 \pm 12$ ($n = 6$), respectively.

Excitatory amino acid antagonists

Effects of the excitatory amino acid antagonists, APV and kynurenic acid, were tested in Mg^{2+}-free solution. NMDA, quisqualate or kainate (50 μM) were applied by a brief puff (30, 30 and 80 ms, respectively) to the same DLSN neurone (Fig. 5). The NMDA-induced depolarization was depressed markedly by 50 μM-APV, but quisqualate and kainate-induced depolarizations were not affected. On the other hand, 1 mM-kynurenate depressed not only NMDA but also quisqualate and kainate-induced depolarizations (Fig. 5*B*). Among them, the NMDA-induced depolarization was most sensitive to kynurenic acid.

Effect of glycine on an NMDA-induced depolarization

Johnson & Ascher (1987) reported that glycine enhances NMDA-induced currents in mouse-cultured brain neurones using the patch clamp technique. We tested whether glycine would augment an NMDA-induced potential in rat DLSN. NMDA-induced potentials were obtained by pressure application of NMDA every 2 min in a Mg^{2+}-free solution containing TTX. After application of glycine (1–10 μM), the NMDA-induced potential was not changed in amplitude or duration ($n = 3$, data not shown). The most plausible reason for our negative results, other than differences in the NMDA receptor–channel complex, is that the NMDA receptor–channel complex in our slice preparation was already potentiated by the endogenous glycine present in the tissue. Indeed, an essential requirement for glycine in agonist activation of NMDA receptors has been recently suggested (Kleckner & Dingledine, 1988).

DISCUSSION

Our results demonstrate: (1) the presence of an NMDA-receptor-mediated EPSP and (2) the sensitivity of depolarizations due to exogenously applied glutamate receptor subtype agonists on rat DLSN neurones.

Involvement of NMDA receptor activation during synaptic transmission in DLSN

Since a large component of the enhanced synaptic response recorded from DLSN neurones in Mg^{2+}-free solution is blocked by APV, these EPSPs may involve NMDA receptor activation. On the other hand, in normal Mg^{2+} solution, fast EPSPs evoked by low-frequency and low-intensity fimbrial stimulation were not sensitive to APV in rat DLSN (Joëls & Urban, 1984*a*, *b*; Stevens & Cotman, 1986). Similar results were reported to occur during synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus (Collingridge, Kehl & McLennan, 1983). It should be noted, however, that an NMDA-receptor-mediated component of EPSPs evoked by the Schaffer collateral–commissural pathway at 0·2 Hz in 2 mM-

Mg^{2+}-containing medium has been suggested in the rat hippocampus by using new non-NMDA antagonists (Andreasen, Lambert & Jensen, 1988). On the other hand, high-frequency repetitive stimuli or a high-intensity stimulus could evoke an APV-sensitive component in EPSPs recorded from the rat DLSN. These latter results may be similar to those reported in the rat hippocampus (Herron *et al.* 1986; Coan & Collingridge, 1987; Collingridge, Herron & Lester, 1988*b*). All these different observations can be explained by the voltage-dependent blockade of NMDA channels by Mg^{2+} (Fig. 4; Mayer, Westbrook & Guthrie, 1984; Nowak, Bregestovski, Ascher, Herbert & Prochiantz, 1984). Higher intensity stimuli or high-frequency stimuli liberate more excitatory amino acid transmitter which depolarizes membranes to a sufficient level and duration to reduce the Mg^{2+} block of NMDA channels. Indeed, in Mg^{2+}-free solution, an APV-sensitive component of EPSPs was detected by a single low-intensity stimulus, as low as 5 V. This indicates that the threshold for neurotransmitter release in NMDA-receptor-mediated and non-NMDA-receptor-mediated synapses is essentially the same. Furthermore, the short latency of the APV-sensitive component is consistent with a monosynaptic rather than polysynaptic activation. Possibly a single neurotransmitter substance, such as L-glutamate, normally activates two types of receptor on DLSN neurones. These findings are consistent with speculations from studies in the rat hippocampus (Herron, Lester, Coan & Collingridge, 1985; Collingridge *et al.* 1988*a*), *Xenopus* embryo spinal cord (Dale & Roberts, 1985) and lamprey spinal cord (Dale & Grillner, 1986). However, at present, we cannot totally eliminate the possibility that another neurotransmitter may participate in and modulate excitatory transmission in the DLSN.

Slow time course of the APV-sensitive component of the EPSP

In the present experiments, the NMDA-receptor-mediated component of the orthodromically induced EPSP was estimated by digital subtraction of the synaptic potential recorded in the presence of APV from that recorded in its absence (control) (cf. Collingridge *et al.* 1988*a*; Forsythe & Westbrook, 1988). The half-decay-time of the APV-sensitive component of the EPSP (about 70 ms) was considerably longer than that of the non-NMDA-receptor-mediated EPSP (about 20–30 ms, see also Stevens & Cotman, 1986). One hypothesis we have considered to explain this slow decay time may be the involvement of a second messenger system(s) in the NMDA-induced response. Thus far, NMDA responses do not appear to be affected by pertussis toxin pre-treatment (J. P. Gallagher & H. Hasuo, unpublished observations).

Comparison of glutamate and glutamate-receptor-subtype-specific agonist-induced depolarizations

Glutamate-induced depolarizations in rat DLSN had a shorter decay time course than those of the other excitatory amino acids tested. Possible explanations for this difference include variable diffusion characteristics of the respective agonists and/or substrate specificity by the normally active uptake processes for the excitatory amino acids (Balcar & Johnston, 1972; Garthwaite, 1985). The difference in rise time among these excitatory amino acid-induced depolarizations was not significant; however, this may be due to differences in positioning of the puff pipettes.

According to binding studies, quisqualate-induced responses may not be mediated by a single receptor subtype; these studies suggested a cross-over with kainate and possibly NMDA receptors (Forster & Fagg, 1984). AMPA and related substances may be purer agonists for a 'quisqualate' receptor than is quisqualate itself (Krogsgaard-Larsen & Honoré, 1983). However, in the present experiments, we could not detect any difference between AMPA and quisqualate responses. Also, we could not detect any interaction of kainate with NMDA receptors. These results indicate that a cross-over action of quisqualate or kainate with NMDA and non-NMDA receptors is minor on DLSN neurones. As almost all neurones tested responded to quisqualate, kainate and NMDA, co-existence of NMDA and non-NMDA receptors appears to be common in the DLSN.

IPSP and late hyperpolarizing potential (LHP) in Mg^{2+}-free solution

In Mg^{2+}-free solution, an IPSP and an LHP evoked by fimbrial afferent stimulation are not clearly seen. Usually, biphasic synaptic responses are recorded at the resting membrane potential level (Fig. 1). Since application of APV unmasks the IPSP–LHP sequence as observed in normal Mg^{2+} solution, it is unlikely that the IPSP–LHP sequence is altered by an unknown transmitter released in the Mg^{2+}-free solution. Rather, the IPSP–LHP responses are probably shunted due to the NMDA-receptor-mediated conductance increase. The IPSP, which is mediated by $GABA_A$ receptors, may be masked by the synaptic depolarizing potential in Mg^{2+}-free solution, because the initial depolarizing potential in a Mg^{2+}-free solution was enhanced by bath application of bicuculline to this preparation. In addition, in the hippocampal preparation, recurrent inhibition which is mediated by $GABA_A$ receptor activation was not affected by a Mg^{2+}-free solution (Collingridge *et al.* 1988*a*). Nevertheless, a possible NMDA receptor-mediated presynaptic inhibition of GABA release or an interaction of $GABA_B$ receptors and NMDA receptors at the postsynaptic membrane cannot be excluded.

In summary, our results suggest that the fimbrial afferent excitatory transmitter(s) can activate *both* non-NMDA and NMDA receptors, but the NMDA component is most clearly revealed at more depolarized membrane potentials or in a Mg^{2+}-free solution. It is not yet clear whether this receptor is of the quisqualate, kainate or some other type.

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