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EXCITATORY SYNAPTIC POTENTIALS IN NEURONS OF THE DEEP NUCLEI IN OLIVO-CEREBELLAR SLICE CULTURES

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Abstract—Excitatory postsynaptic potentials evoked in neurons of the deep cerebellar nuclei, either by electrical stimulation within the nuclei in cerebellar slice cultures or by electrical stimulation of olivary explants in olivo-cerebellar co-cultures, were investigated in the rat by means of intracellular recordings. In neurons of the deep cerebellar nuclei, stimulation of the nuclear tissue, as well as stimulation of the olivary tissue, induced a fast rising excitatory postsynaptic potential, followed by an inhibitory postsynaptic potential and a long-lasting excitation. The fast rising excitatory postsynaptic potential and the following inhibitory postsynaptic potential were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione. The remaining depolarization was abolished by D-(−)-2-amino-5-phosphonovalerate, suggesting that this potential was mediated by *N*-methyl-D-aspartate receptors. With only D-(−)-2-amino-5-phosphonovalerate added to the bath, the slow excitation was depressed, whereas the fast excitatory and inhibitory postsynaptic potentials were not affected. In the presence of bicuculline, the 6-cyano-7-nitroquinoxaline-2,3-dione- and the D-(−)-2-amino-5-phosphonovalerate-sensitive excitatory postsynaptic potentials elicited by stimulation of the olivary tissue had the same latency, and were both graded with stimulation strength. The time-to-peak and the duration of the D-(−)-2-amino-5-phosphonovalerate-sensitive excitatory postsynaptic potentials were considerably longer than those of the 6-cyano-7-nitroquinoxaline-2,3-dione-sensitive excitatory postsynaptic potentials. In magnesium-containing bathing solution, the amplitude of the 6-cyano-7-nitroquinoxaline-2,3-dione-sensitive excitatory postsynaptic potentials increased, while the amplitude of the D-(−)-2-amino-5-phosphonovalerate-sensitive excitatory postsynaptic potentials decreased with membrane hyperpolarization. Removal of magnesium from the bathing solution resulted in an increase in *N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic potentials whose amplitude increased with membrane hyperpolarization.

Recordings from Purkinje cells within the same slice cultures revealed that 6-cyano-7-nitroquinoxaline-2,3-dione reversibly abolished graded excitatory postsynaptic potentials induced by stimulation within the cerebellar cortex as well as all-or-none climbing fibre responses induced by stimulation of the olivary tissue. Furthermore, D-(−)-2-amino-5-phosphonovalerate did not affect these synaptic responses, even in magnesium-free bathing solution.

Many excitatory synaptic pathways within the central nervous system of vertebrates are thought to use excitatory amino acids as neurotransmitters. In the cerebellum, this includes the two main extrinsic afferent systems, the climbing fibres and the mossy fibres, as well as an intracerebellar system, the parallel fibres. The climbing fibres originate within the inferior olive and most likely release glutamate as their neurotransmitter.[7,36] In Purkinje cells, activation of this pathway induces a powerful all-or-none excitatory postsynaptic potential (EPSP), whose electrophysiological properties have been extensively studied.[4,6,9,15,21] Climbing fibres also give rise to collaterals which terminate in the deep cerebellar nuclei (DCN),[35] where they form excitatory connections.[28] A detailed analysis of the excitatory synapses of the neurons of the DCN, including the characterization of receptor subtypes, is still missing, most probably because it is difficult to activate identified afferent pathways to the DCN selectively in acute cerebellar slice preparations. The connectivity of these neurons places them in a key position within the cerebellar network: neurons of the DCN are the main target of Purkinje cells, and their own axons form the major output of the cerebellum.

We have recently reported that in cerebellar slice cultures, neurons of the DCN bear two classes of ionotropic excitatory amino acid receptors, i.e. *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, while Purkinje cells express only non-NMDA receptors.[3,27] Furthermore, we have shown that in olivo-cerebellar co-cultures, olivary fibres grow into cerebellar tissue and form synaptic contacts with Purkinje cells, and that activation of these fibres induces typical climbing fibre responses in these Purkinje cells.[21] In the present work, we took advantage of the unique opportunity provided by

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Abbreviations: D-APV, D-(−)-2-amino-5-phosphonovalerate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DCN, deep cerebellar nucleus; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; NMDA, *N*-methyl-D-aspartate.

these olivo-cerebellar co-cultures to characterize the receptors activated in neurons of DCN by olivary afferents.

EXPERIMENTAL PROCEDURES

Preparation of slice cultures

Parasagittal slices, 400 μm thick, of the cerebellum were prepared from newborn Wistar rat pups killed by decapitation. Transverse slices, 400 μm thick, of the lower medulla containing the inferior olive were obtained from three- to four-day-old rats. The ventral medial portion of the anterior medulla containing the inferior olives was separated by means of a razor blade cut. Slices of cerebellum alone, or together with slices of inferior olive, were cultured using the roller-tube technique as previously described.[11,12,21] Cultures and co-cultures were fed once and twice weekly, respectively, with a medium consisting of horse serum (25%), basal medium (Eagle's, 50%) and Hanks' or Earle's balanced salt solution (25%) supplemented with glucose to a final concentration of 6.5 g/l. Cultures were treated for 24 h with a combination of anti-mitotic drugs (5-fluorodeoxyuridine, cytosine-β-D-arabino-furanoside and uridine, all 1 μM) by day 4 and exposed to ionizing radiation (X-rays, 600–800 rad) at the time of explantation in order to reduce glial proliferation.

Electrophysiology

After 10–40 days *in vitro*, cultures were transferred to a temperature-controlled (32°C) recording chamber mounted on the stage of an inverted microscope and superfused at 0.5–1 ml/min with Tyrode's balanced salt solution containing (in mM): Na^+, 148.9; K^+, 2.7; Cl^-, 148.2; Ca^{2+}, 3.8; Mg^{2+}, 0.5; HCO_3^-, 11.6; $H_2PO_4^-$, 0.4; D-glucose, 5.6. Visually identified Purkinje cells and neurons of the CDN were impaled with thin-walled microelectrodes filled with 2 M $KMeSO_4$ (pH 7.4; resistance about 50 MΩ). To study synaptic potentials at depolarized membrane potentials, most of these experiments were performed with microelectrodes additionally containing QX222 (0.2 M), a derivative of the local anaesthetic lidocaine which blocks sodium channels. Recordings were obtained in either the bridge mode or the discontinuous current-clamp mode of a high-input impedance amplifier (Axoclamp-2, Axon Instruments Inc., Foster City, CA, U.S.A.) and fed into a data acquisition system (Axolab-1, Axon Instruments Inc., interfaced with an MS-DOS computer). Synaptic potentials in neurons of the DCN or Purkinje cells were evoked with anodal current pulses (0.1–0.5 ms, 10–100 μA) delivered through micropipettes filled with Tyrode's solution (resistance about 1–3 MΩ). The stimulation electrode was placed at one of the three following locations: within or at the border of the deep cerebellar nuclei ("nuclear stimulation", indicated by ● in all figures), in the olivary tissue or the fibre tracts from olivary to cerebellar tissue ("olivary stimulation", indicated by ▲ in all figures) or within the cerebellar cortex ("cortical stimulation", indicated by ■ in all figures). Figure 1 shows a schematic drawing of stimulation sites and connections studied in olivo-cerebellar co-cultures. Cerebellar cultures contained the same elements except the olivary tissue and olivary fibres.

D-(−)-2-Amino-5-phosphonovalerate (D-APV) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were obtained from Tocris Neuramin. QX222 was a gift from Astra (Sweden). All other drugs were purchased from Sigma.

RESULTS

The pharmacological properties of EPSPs of neurons of the DCN and of Purkinje cells were investigated in cerebellar cultures and in olivo-cerebellar co-cultures after 10–40 days *in vitro*. Neurons of the DCN and Purkinje cells were identified on the basis of their localization within cerebellar cultures and their morphology as observed with contrast-enhancing microscopy.[3]

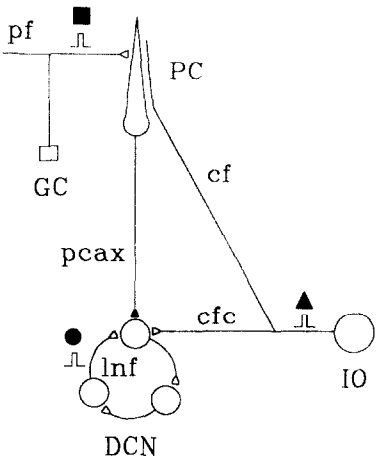

Fig. 1. Schematic drawing of stimulation sites and connections studied in olivo-cerebellar co-cultures. Cell types: neurons of the DCN, Purkinje cells (PC), granule cells (GC), neurons of the inferior olive (IO). Synaptic connections: climbing fibres (cf), climbing fibre collaterals (cfc), parallel fibres (pf), axons of Purkinje cells (pcax), local nuclear fibres (lnf). Stimulation sites: within or at the border of the deep cerebellar nuclei ("nuclear stimulation", indicated by ● in all figures), in the olivary tissue or the fibre tracts from olivary to cerebellar tissue ("olivary stimulation", indicated by ▲ in all figures) or within the cerebellar cortex ("cortical stimulation", indicated by ■ in all figures). Cerebellar cultures contained all these elements except olivary cells and their fibres.

Pharmacology of postsynaptic potentials

In response to electrical stimulation within or at the border of the DCN ("nuclear stimulation") as well as to stimulation within the olivary tissue ("olivary stimulation"), a sequence of EPSPs and inhibitory postsynaptic potentials (IPSPs) was recorded in neurons of the DCN ($n = 26$). Typically, this sequence consisted of a fast rising depolarization, followed by a hyperpolarization and a long-lasting depolarization of the membrane. The amplitude of each of these three components increased with stimulation intensity (Fig. 2A, B).

Except for the longer latency of the synaptic potentials evoked by olivary stimulation, no major difference has been observed between the responses induced by nuclear stimulation in cultures and those produced by olivary stimulation in co-cultures. In some olivo-cerebellar co-cultures, however, olivary stimulation elicited a second EPSP–IPSP sequence (Fig. 2C), which resulted from oscillatory firing of olivary neurons, an observation which resembles those made in other olivo-cerebellar preparations.[28]

Bath application of CNQX (2.5–10 μM) reversibly abolished the fast rising EPSPs and IPSPs induced in neurons of the DCN by nuclear or olivary stimulation (Fig. 3A, B). The remaining slow EPSPs were blocked by addition of D-APV (10–20 μM) to the superfusate. With only D-APV added to the bath, the slow EPSPs were blocked, whereas the fast EPSPs and the IPSPs were not affected (Fig. 3A, B). The data, therefore, indicate that fast and slow EPSPs were mediated through activation of non-NMDA and NMDA receptors, respectively. The blockade of the IPSPs by CNQX suggests that these IPSPs are at least di-synaptic and a consequence of the activation of excitatory synapses which involve non-NMDA receptors. After all EPSPs had been abolished by application of CNQX and D-APV, nuclear stimulation (but not olivary stimulation) could, however, still induce IPSPs of short latency, due to direct activation of axons of Purkinje cells or of inhibitory nuclear neurons (cf. Fig. 3A).

Time-course and voltage dependence of excitatory postsynaptic potentials

With 20 μM D-APV and 20 μM bicuculline present in the bathing solution, only fast rising EPSPs could be evoked by nuclear (Fig. 4A) or olivary (Fig. 4E) stimulation. The amplitude of these EPSPs was graded with the stimulation intensity (Fig. 4A); their time-to-peak and half-decay time were 5.2 ± 3.7 ms (mean $\pm$ S.D.; $n = 7$) and 9.9 ± 5.2 ms ($n = 7$), respectively. These fast EPSPs were mediated by non-NMDA receptors since they were abolished by CNQX (2.5–10 μM) (Fig. 4B). In bicuculline- and CNQX-containing solutions, slow graded EPSPs were elicited in response to nuclear (Fig. 4C) or olivary (Fig. 4E) stimulation. Their time-to-peak and half-decay time were 16.7 ± 6.2 ms ($n = 11$) and 30.5 ± 18.5 ms ($n = 11$), respectively. These slow EPSPs were mediated by NMDA receptors since they were abolished by 10 μM D-APV (Fig. 4D). The fast CNQX- and the slow D-APV-sensitive EPSPs had the same latency (Fig. 4E).

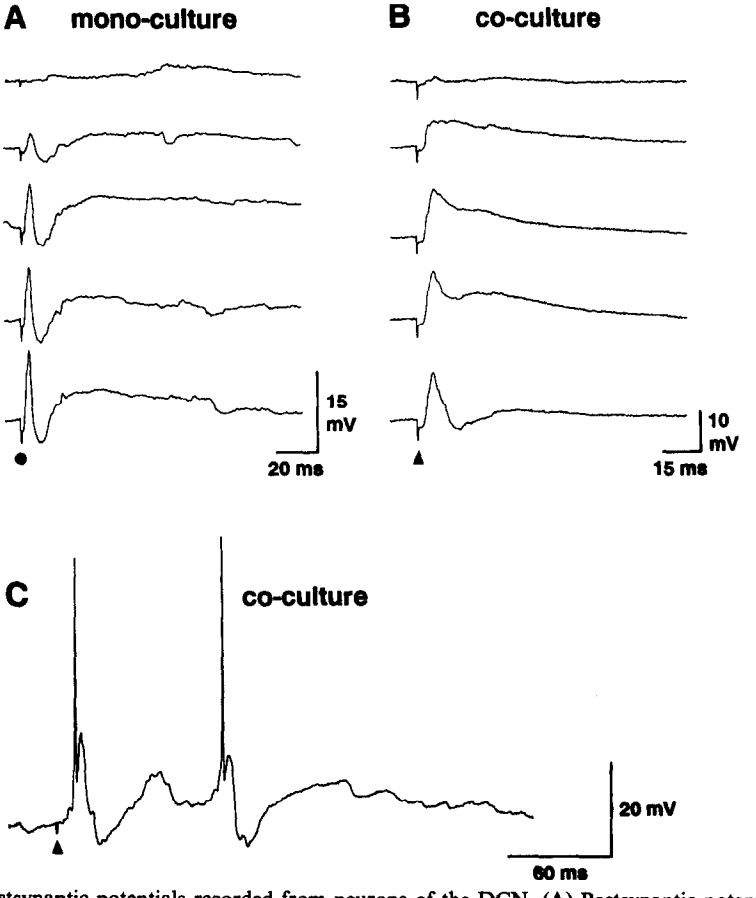

Fig. 2. Postsynaptic potentials recorded from neurons of the DCN. (A) Postsynaptic potentials evoked by nuclear stimulation at increasing intensities (top to bottom rows) in a cerebellar culture after 36 days *in vitro* (resting membrane potential −70 mV). (B) Postsynaptic potentials evoked by the stimulation of the olivary explant with increasing intensities (top to bottom rows) in a co-culture after 19 days *in vitro* (resting membrane potential −63 mV). (C) Complex sequence of EPSPs and IPSPs induced by electrical stimulation of the olivary explant in another co-culture after 26 days *in vitro* (resting membrane potential −60 mV). Symbols as in Fig. 1.

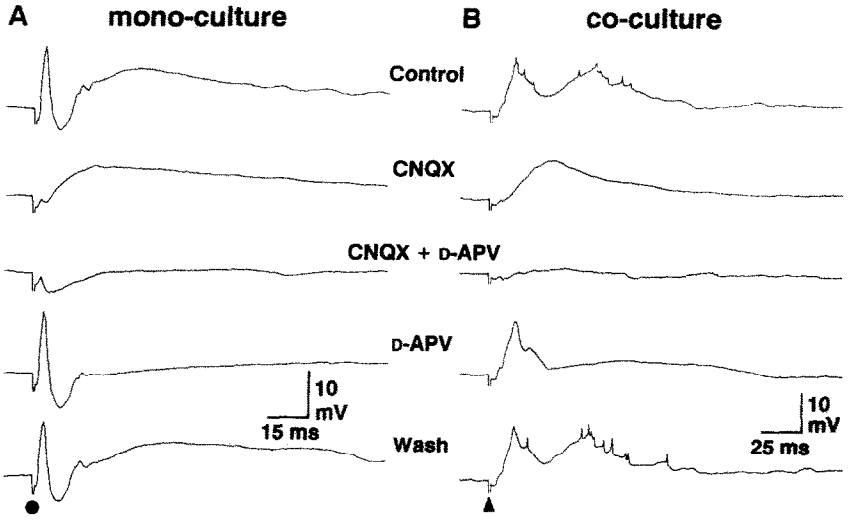

Fig. 3. Effects of CNQX (5 μM) and D-APV (10 μM) on postsynaptic potentials recorded in neurons of the DCN. (A) Synaptic responses evoked by nuclear stimulation. (B) Synaptic responses evoked by the stimulation of the olivary explant in a co-culture after 23 days *in vitro* (resting membrane potential −61 mV). Each trace represents the average of 10 sweeps. Note that in both neurons, CNQX reversibly abolished the fast rising EPSP and the following IPSP, while D-APV blocked only the slow EPSP. Symbols as in Fig. 1.

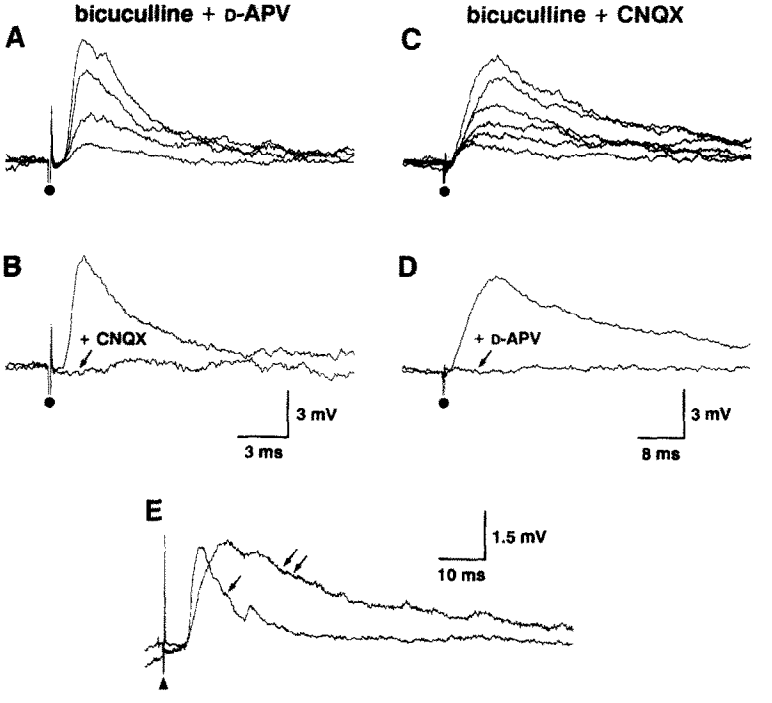

Fig. 4. NMDA- and non-NMDA-mediated EPSPs recorded from a neuron of the DCN in a co-culture after 22 days *in vitro*. Bicuculline (20 μM) was added to all external solutions. (A) In the presence of D-APV (20 μM), nuclear stimulation of increasing intensities induced fast rising, graded EPSPs of short duration. (B) These D-APV-resistant EPSPs were abolished by CNQX (5 μM). (C) After wash-out of D-APV, the same neuron responded to nuclear stimulation with slowly rising, graded EPSPs of longer duration. (D) CNQX-resistant EPSPs were abolished by D-APV (20 μM). (E) Superimposed traces of NMDA- (double arrows) and non-NMDA (single arrow) receptor-mediated EPSPs evoked by stimulation of the olivary explant in the presence of CNQX or D-APV (same neuron as in A–D). Note identical latency and different time-course of the two EPSPs. Each trace represents the average of three sweeps. In A–D, the cell was kept at its resting membrane potential, −65 mV; in E, the cell was depolarized to −55 mV by intracellular current injection. Symbols as in Fig. 1.

The voltage dependence of NMDA- and non-NMDA-mediated EPSPs induced in neurons of the DCN was studied with microelectrodes containing QX222 in order to block voltage-gated sodium channels. In bicuculline-, D-APV- and magnesium-containing solutions, the amplitude of the non-NMDA receptor-mediated EPSPs increased when the membrane was hyperpolarized, and decreased with depolarization (Fig. 5A, $n = 6$). In contrast, the amplitude of the EPSPs mediated by NMDA receptors (recorded in bicuculline-, CNQX-, and magnesium-containing solutions) gradually decreased with membrane hyperpolarization and increased with depolarization (Fig. 5B, $n = 13$). At membrane potentials more depolarized than -45 mV, these EPSPs often induced regenerative potentials, most probably due to the occurrence of dendritic calcium spikes (data not shown). To assess the effect of external magnesium on the voltage dependence of these NMDA receptor-mediated EPSPs, cultures were bathed in magnesium-free saline containing CNQX (10 μM) and bicuculline (20 μM). The intensity of the stimulation was then adjusted to a level that would elicit a synaptic potential without triggering the large and long-lasting suprathreshold depolarizations described below. Under these conditions, the amplitude of the NMDA receptor-mediated EPSPs increased when the membrane was hyperpolarized from -45 mV to -90 mV (Fig. 5C; $n = 6$). After return to a solution containing 0.5 mM magnesium the NMDA receptor-mediated EPSPs again displayed their characteristic voltage dependence (Fig. 5D).

Wash-out of external magnesium increased the frequency of spontaneous EPSPs, induced spontaneous depolarizing shifts which elicited fast action

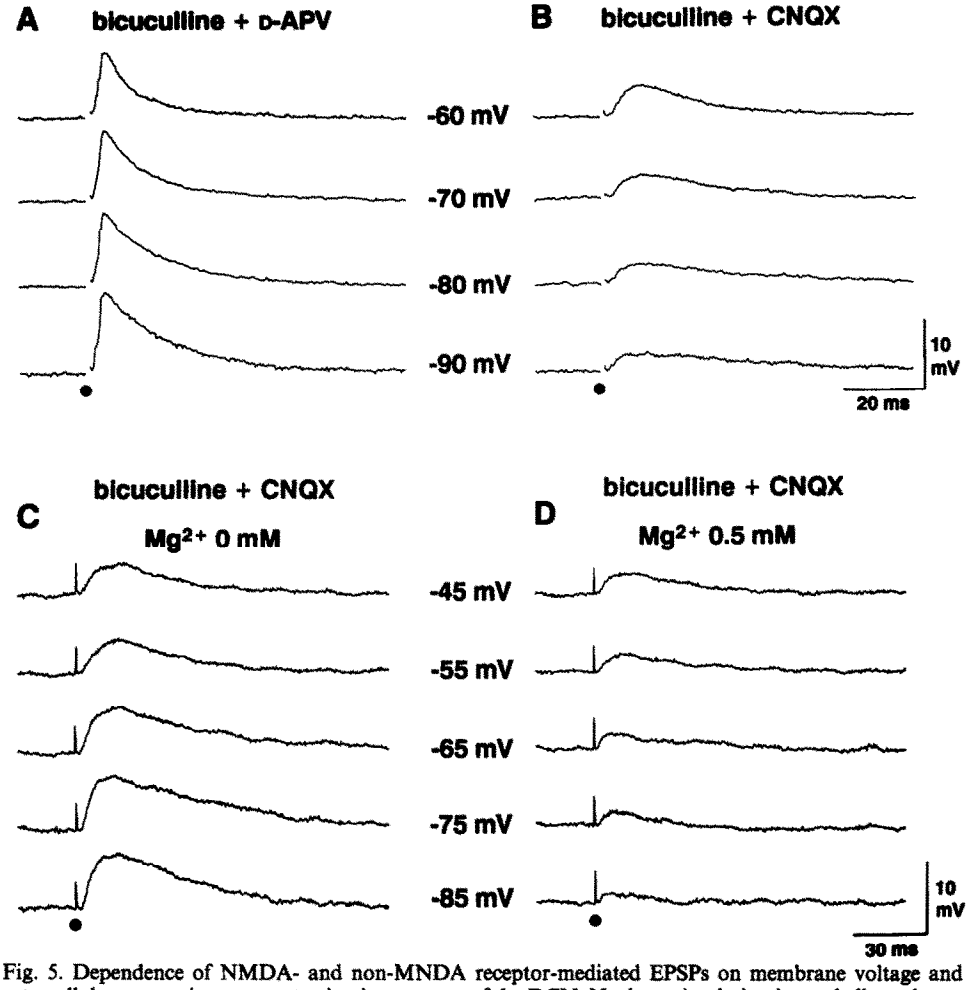

Fig. 5. Dependence of NMDA- and non-MNDA receptor-mediated EPSPs on membrane voltage and extracellular magnesium concentration in a neuron of the DCN. Nuclear stimulation in cerebellar cultures after 21 days (A, B) and 25 days (C, D) *in vitro*. Bicuculline (20 μM) was added to all external solutions. (A) EPSPs induced in the presence D-APV (20 μM) at different membrane potentials, as indicated at the right of each trace. (B) EPSPs induced in the presence of CNQX (5 μM) at the same membrane potentials as in A. (C) EPSPs recorded in the presence of CNQX (5 μM) induced by nuclear stimulation at five different membrane potentials in magnesium-free bathing solution. (D) The same EPSPs in C following wash of external magnesium (0.5 mM). Recordings were obtained in discontinuous current-clamp mode and with a microelectrode containing QX222 (0.2 M). Each trace represents the average of three sweeps. Symbols as in Fig. 1.

potentials and greatly enhanced NMDA receptor-mediated EPSPs. As illustrated in Fig. 6, nuclear stimulation (cerebellar culture) evoked a small depolarizing synaptic potential in magnesium-containing solution. In magnesium-free bathing solution, the same stimulation elicited a large and long-lasting depolarization which closely resembled the spontaneously occurring depolarizing shifts. All these evoked and spontaneous synaptic depolarizations recorded in the presence of CNQX were reversibly abolished by 20 μM D-APV. The observation of these evoked or spontaneously occurring depolarizing responses most likely represents population responses and therefore suggests strong excitatory connection between neurons of the DCN.

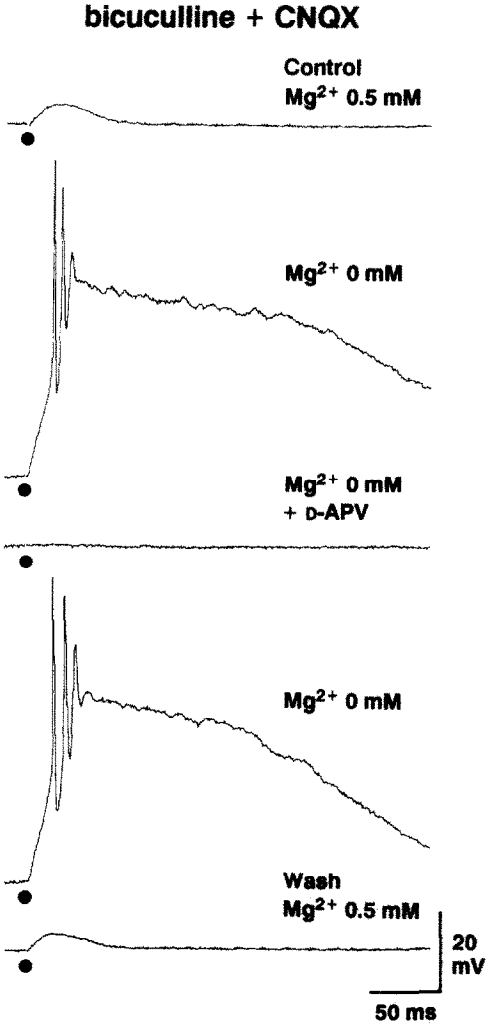

Fig. 6. Enhancement of NMDA receptor-mediated EPSPs following wash-out of external magnesium in a neuron of the DCN. Nuclear stimulation in a cerebellar culture after 25 days *in vitro*. Bicuculline (20 μM) and CNQX (5 μM) were added to all external solutions. Upper to lower traces: CNQX-resistant EPSPs recorded in control, after wash-out of external magnesium, in the presence of D-APV (20 μM), after wash-out of D-APV and after return to the initial solution. Resting membrane potential was −70 mV. Recordings were obtained in discontinuous current-clamp mode. Symbols in Fig. 1.

Pharmacology of excitatory postsynaptic potentials in Purkinje cells

In view of the remarkably strong NMDA receptor-mediated EPSPs in neurons of the DCN, culture conditions might result in an atypical expression of excitatory amino acid receptors. We have, therefore, compared the synaptic responses of neurons of the DCN to those of Purkinje cells within the same cultures. In cerebellar cultures, cortical stimulation elicited depolarizing postsynaptic potentials in Purkinje cells, the amplitudes of which were graded with stimulation strength (Fig. 7A). In olivo-cerebellar co-cultures, olivary stimulation elicited an all-or-nothing complex response in Purkinje cells (Fig. 7C). These two responses are reminiscent of parallel and climbing fibre inputs of Purkinje cells. Washing out the external magnesium led to an increase in the frequency of spontaneous IPSPs and EPSPs (not shown), but failed to reveal an additional component of the evoked synaptic responses (Fig. 7B, C). CNQX (2.5–10 μM) blocked the evoked EPSPs, while D-APV (20 μM) had no effect (Fig. 7B, C; $n = 6$).

DISCUSSION

Synaptic potentials in neurons of the deep cerebellar nuclei

In slice cultures, selective activation of the inferior olive or local intranuclear stimulation induced both fast and slow EPSPs, interrupted by an IPSP in neurons of the DCN. Our observation that the amplitude of EPSPs evoked by activation of climbing fibres could be graded by the stimulation strength indicates that neurons of the DCN are contacted by several olivary fibres, in contrast to the all-or-none climbing fibre response of Purkinje cells in the same preparation.[21]

EPSP–IPSP sequences recorded from neurons of the DCN have also been observed in an isolated brainstem–cerebellar preparation following activation of olivary neurons with harmaline.[28] Moreover, similar synaptic sequences were evoked *in situ* by stimulation of the climbing fibre system in Deiter's neurons[1,33] which, in many respects, are comparable to neurons of the DCN. In these studies, EPSPs were attributed to the direct effect of climbing fibres on the recorded neurons, while IPSPs were thought to be elicited indirectly through climbing fibre activation of Purkinje cells, the axons of which strongly inhibit nuclear cerebellar and vestibular neurons. The present observations in olivo-cerebellar co-cultures substantiate this view since the IPSPs induced in neurons of othe DCN by olivary stimulation were blocked by CNQX, demonstating that they were generated through a synaptic loop which includes an excitatory synapse. Furthermore, the inhibitory neurons activated by the climbing fibres were most likely Purkinje cells, since the IPSPs were not affected by D-APV.

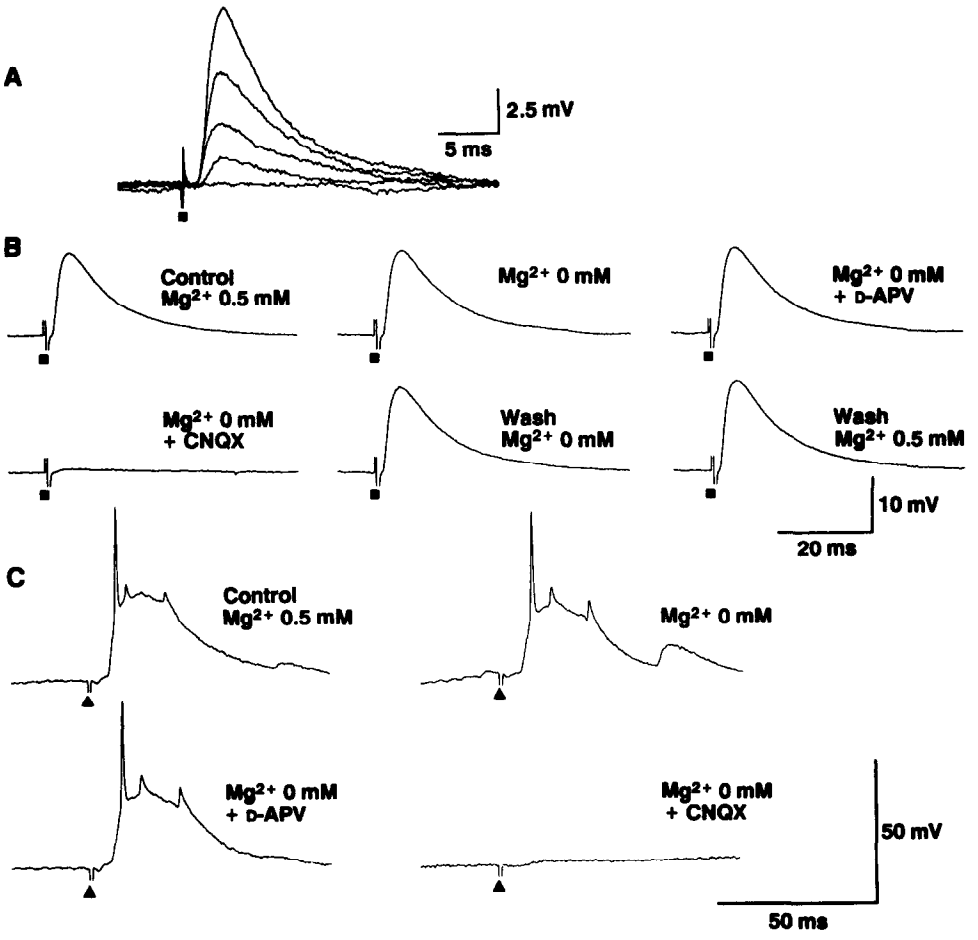

Fig. 7. EPSPs evoked in a Purkinje cell by cortical stimulation in a cerebellar culture (A, B) and by olivary stimulation in an olivo-cerebellar co-culture (C). (A) Superimposed sweeps showing the graded nature of the EPSPs at various intensities of stimulation. (B) Effects of magnesium-free solution, D-APV (20 μM) and CNQX (5 μM) on the graded EPSPs. ■ indicates time of intracortical stimulation. (C) Effects of magnesium-free solution, D-APV (20 μM) and CNQX (5 μM) on the synaptic response evoked in a Purkinje cell by olivary stimulation (A). Bicuculline (20 μM) was added to all external solutions in order to block inhibitory synaptic potentials. Note that the responses were completely and reversibly blocked by CNQX, but not by D-APV. Resting membrane potential was −71 mV (A) and −65 mV (B, C). Other symbols in Fig. 1.

N-Methyl-D-aspartate and non-N-methyl-D-aspartate receptor-mediated excitatory synaptic potentials in neurons of the deep cerebellar nuclei

In contrast to EPSPs recorded in Purkinje cells, our results clearly show that in the DCN, EPSPs induced by local electrical stimulation, as well as by selective activation of climbing fibres, were mediated by NMDA and non-NMDA receptors. This is consistent with previous pharmacological studies showing that exogenous application of excitatory amino acids, such as glutamate, aspartate and homocysteate, produced mixed NMDA and non-NMDA responses in neurons of the DCN.[3,13] In addition to their different pharmacological profiles, the NMDA and non-NMDA receptor-mediated EPSPs exhibited different time courses and voltage dependencies: the NMDA component of the EPSPs had a slower rise time and a slower decay time when compared to the non-NMDA component. Recently, it has been demonstrated in hippocampal neurons that the activation kinetics of NMDA receptors and a prolonged occupation (slow unbinding) of these receptors by glutamate are sufficient to explain the slow time course of NMDA receptor-mediated EPSPs.[14,25] As expected from the characteristic voltage-dependent block of the NMDA channel by magnesium,[29,30] the NMDA receptor-mediated EPSPs evoked in neurons of the DCN displayed an unconventional voltage dependence in the presence of external magnesium. The properties of the two components of the EPSPs are similar to those found in other neurons of the central nervous system, such as hippocampal CA1 pyramidal cells and interneurons,[2,5,14,32] dentate granule cells[22,24] and neocortical pyramidal cells.[17,18,34]

A surprising finding of our study was the large contribution of NMDA receptors to the overall synaptic activity in neurons of the DCN. Indeed,

when these cells were recorded at their resting membrane potential in magnesium-containing solutions (and in the presence of robust IPSPs), D-APV clearly abolished a substantial part of the EPSPs. Interestingly, a similarly large NMDA receptor-mediated EPSP component has been described in vestibular neurons.[20]

Non-N-methyl-D-aspartate receptor-mediated excitatory synaptic potentials in Purkinje cells

Experimental data from extracellular recordings *in vivo*,[19] whole-cell recordings in acute slices[10,23,26,31] and in co-cultures of granule cells and Purkinje cells[16] support the view that the postsynaptic receptors involved at the synapses between parallel fibres and Purkinje cells, as well as at those between climbing fibres and Purkinje cells, are of the non-NMDA type. Our present observations concerning the EPSPs evoked in cultured Purkinje cells are in full agreement with these studies. Moreover, our observations indicate a physiological pattern of expression of excitatory amino acid receptors in tissue-cultured cerebellar neurons.

Physiological significance of N-methyl-D-aspartate receptors in neurons of the deep cerebellar nuclei

The physiological significance of NMDA receptors being in control of resting discharge of vestibular neurons (which are also the targets of Purkinje cells and climbing fibres) has been emphasized by the demonstration that infusion of D-APV into guinea-pig vestibular nuclei induced a severe postural and occulomotor syndrome, similar to that observed following acute vestibular deafferentation.[8] These results suggest the presence of a tonic activation of NMDA receptors of the nuclear neurons under normal physiological conditions. Among the different excitatory afferents that might activate these NMDA receptors, the climbing fibre system should be considered a candidate. The functional consequences of such a tonic activation of NMDA receptors for the functional properties of neurons of the DCN remain to be elucidated. An attractive hypothesis is that these receptors modulate the coupling between the dendritic inputs and the output of the nuclear neurons, as has been suggested for CA1 hippocampal pyramidal cells[32] and also for vestibular neurons.[20]

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