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Contribution of NMDA receptors to postsynaptic potentials and paired-pulse facilitation in identified neurons of the rat nucleus accumbens in vitro

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Summary. The principal aim of this study was to characterize the transmitter mechanisms mediating fast postsynaptic potentials in identified neurons of the rat nucleus accumbens. Using the biocytin-avidin labeling technique, impaled neurons were identified as medium spiny neurons. The basic membrane characteristics of these neurons were determined. Local electrical stimulation or stimulation of the corpus callosum elicited a depolarizing postsynaptic potential consisting of an EPSP often followed by an IPSP. The quisqualate/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (4 μM) abolished most of the depolarizing postsynaptic potential. The N-methyl-D-aspartate receptor antagonist D(−)-2-amino-5-phosphonopentanoic acid depressed a small part of the decay phase of the depolarizing postsynaptic potential. Paired-pulse facilitation of postsynaptic potentials was found using interstimulus-intervals between 10 and 150 ms. N-methyl-D-aspartate receptors were found to contribute only slightly to the facilitation of the decay phase of the depolarizing postsynaptic potential, but not to its rising phase. This contribution was particularly clear under conditions of reduced $GABA_A$ receptor mediated inhibition. The present study indicates that postsynaptic responses of medium spiny neurons in the nucleus accumbens to local stimulation or stimulation of neocortical afferents are primarily mediated by quisqualate/kainate receptors. The contribution of NMDA receptors is normally limited to a portion of the decay phase of these responses, but is enlarged in the absence of GABAergic inhibition and following paired-pulse stimulation.

Key words: NMDA receptors – Nucleus accumbens – Quisqualate/kainate receptors – Paired-pulse facilitation – Postsynaptic potential – Rat

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Introduction

The nucleus accumbens (Acb), which is the main constituent of the ventral striatum (Heimer and Wilson 1975; Heimer et al. 1985), plays an important role in converting limbic inputs into motor behaviour and in mediating responses to rewarding stimuli (Mogenson et al. 1980; Fibiger and Phillips 1988). It has been suggested that dysfunctioning of this forebrain structure is involved in several forms of mental illness, such as schizophrenia, and in drug addiction (Seeman 1987; Swerdlow and Koob 1987; Koob and Bloom 1988). Despite the recently acquired insights into the intrinsic organization of the Acb (Mogenson et al. 1980; Heimer et al. 1985; Chang and Kitai 1986), little is known about the transmitter mechanisms by which limbic inputs affect the medium spiny neurons that project to the ventral pallidum and mesencephalon (Chang and Kitai 1985; Nauta et al. 1978). Glutamate, or related excitatory amino acids, are probably utilized by afferent fibers originating in the hippocampal formation, amygdala, prefrontal cortex and midline thalamic nuclei (Walaas and Fonnum 1980; Christie et al. 1987; Fuller et al. 1987). In this study the contribution of glutamatergic synapses to fast synaptic responses evoked by afferent or local stimulation in the Acb was investigated using an in vitro slice preparation.

In characterizing the glutamatergic transmitter system, the distinction between quisqualate/kainate (Q/K) and N-methyl-D-asparate (NMDA) receptors should be taken into account (Crunelli et al. 1985; Watkins and Olverman 1987). While in many CNS regions Q/K receptors mediate the major part of EPSPs elicited by activation of glutamatergic fibers, the NMDA receptor subtype contributes only little to these EPSPs when the membrane potential of the postsynaptic neuron is close to resting level (Crunelli et al. 1985; Herron et al. 1986; Honoré et al. 1988). Exceptions to this generalization, however, have been reported (Jones 1987; Nakanishi et al. 1988; D'Angelo et al. 1990). One of the main reasons

for the recent interest in the NMDA receptor is its central role in induction of long-term potentiation, a form of synaptic plasticity that has been associated with memory formation (Harris et al. 1984; Herron et al. 1986; Melchers et al. 1988). Behavioral experiments have shown that injection of the specific NMDA receptor antagonist D-amino-7-phosphonoheptanoic acid into the Acb impairs spatial navigation learning in rats without causing motor disturbances (Scheel-Krüger and Willner 1991).

It has been proposed that a major part of the EPSP evoked in the Acb is mediated by NMDA receptors (Uchimura et al. 1989b). Our previous extracellular recordings, however, suggested that Q/K instead of NMDA receptors mediate most of the EPSP (Pennartz et al. 1990). A major aim of the present investigations was to elucidate this controversy using intracellular recording techniques. The recorded neurons were labeled by injection of biocytin to identify the morphological cell types generating the fast synaptic response studied here.

In a previous in vivo study, a pronounced paired-pulse facilitation of synaptic responses in the subiculum-accumbens pathway was demonstrated (Boeijinga et al. 1990). This phenomenon is also encountered in locally-evoked potentials in the nucleus accumbens in vitro, although less pronounced than in vivo (Pennartz et al. 1990). In addition to the aim outlined above, we investigated whether NMDA receptors contribute to paired-pulse facilitation.

Material and methods

Preparation

Thirty-five slices were prepared from male Wistar albino rats (100–180 g) that had been anesthetized with ether or ketamine (50–100 mg/kg, i.m.). After decapitation, the brain was removed from the skull and cooled in Ringer solution at 3–7° C for 1 min. Slices of 400 µM thickness were cut frontally by a vibroslice (Campden, UK) and transferred to the recording chamber. Slices were fully submerged except for labeling experiments, which were usually carried out using an interface chamber. The slice preparation was continuously superfused (1–2 ml/min) with oxygenated (95% O_2, 5% CO_2) Ringer solution (33–35° C, pH 7.3) of the following composition (in mM): NaCl 132, KCl 3.5, $MgSO_4$ 1.3, $CaCl_2$ 2.5, NaH_2PO_4 1.0, $NaHCO_3$ 26.2, D-glucose 10.0. The slices were allowed 1 hour rest prior to recording.

Recording and stimulation

Synaptic responses were elicited by applying bipolar, biphasic rectangular current pulses (0.2 ms duration, 0.1–0.2 Hz) through two 60 µm thick stainless steel electrodes, insulated except at the tip and separated by 100–200 µm. The stimulation electrodes were placed just under the slice surface, either within the Acb or in the rostral corpus callosum overlying the Acb. The distance between the stimulation and recording site varied between 0.3 and 1.0 mm. Most intracellular recordings were made in the core region of the Acb (Paxinos and Watson 1986). The recording electrodes were glass micropipettes filled with 4 M KAc (80–120 MΩ). Intracellular potentials were recorded using the bridge mode of an Axoclamp 2A amplifier and displayed on a digital Nicolet 3091 oscilloscope. Responses to synaptic stimulation were averaged ($N=4$) on line using a Motorola Exorset microcomputer, unless noted otherwise, and stored on disk for further analysis. The DC membrane potential was continuously monitored on a chart recorder.

Drugs

Stock solutions of D(−)-2-amino-5-phosphonopentanoic acid (D-AP5; Tocris), glycine, bicuculline methiodide and picrotoxin (all from Sigma) were dissolved in distilled water. 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris) was dissolved in dimethylsulphoxide and diluted 10^4 times for preparing test solutions. In a final concentration of 0.01%, dimethylsulphoxide itself had no effect on locally-evoked synaptic potentials (4 slices tested).

Statistical evaluation of paired-pulse facilitation and pharmacological effects was done using Wilcoxon's matched-pairs signed rank test. Numerical values are expressed as mean ± SEM.

Histology

Biocytin (Sigma; cf. Horikawa and Armstrong 1988) was injected intracellularly by passing negative current (0.2 to 0.4 nA) through the recording electrode for at least 5 min. Usually only 1 or 2 cells were labeled per slice. Following termination of the experiment, the slice was stored in fixative (4% paraformaldehyde, 0.5% glutaraldehyde, 15% picric acid in 0.1 M sodium phosphate buffer, pH 7.4). The slice was sectioned at a thickness of 40 µM on a freezing microtome, collected into phosphate buffered saline and rinsed several times. Sections were incubated overnight in phosphate buffered saline containing avidin-Texas Red™ (Vector; dilution 1:100), 0.3% Triton-X100 and 0.1% NaN_3 and examined by fluorescent microscopy. Extensively labeled cells that were first visualized by avidin-Texas Red, were permanently stained using a peroxidase reaction to allow drawing and reconstruction of the cells by light microscopy. Briefly, sections were incubated for 1 h in buffer containing 1:100 diluted biotin-HRP solution (Vector). Subsequently, avidin solution was added to this medium and incubated for 2 h. Following repetitive rinsing, sections were immersed in buffer containing 0.05% diaminobenzidine and 0.03% H_2O_2. Cells were drawn under oil immersion (1000×) using a drawing tube.

Methodological controls

In general we found that slices obtained from ketamine-anesthetized rats were of better quality than those obtained from ether-anesthetized rats. This may be due to the anti-excitotoxic action of ketamine (Rothman et al. 1987). However, if ketamine would not be readily washed out of the tissue after slice preparation, it might influence the experimental results. Therefore, we checked in 4 cells whether the effect of ketamine was reversible.

At the relatively high concentration of 2 mM, which may suppress both NMDA and non-NMDA receptor-mediated potentials (Duchen et al. 1985; Thomson et al. 1985; Ashford et al. 1989), ketamine reduced the peak amplitude of the postsynaptic response from 20 ± 6 mV to 10 ± 2 mV (cf. Table 1). The suppressive effect of ketamine could be reversed by washing out for approximately 15 min (washout value: 21 ± 4 mV), in agreement with other studies (Thomson et al. 1985; Duchen et al. 1985). This was also the case in cells exposed to picrotoxin or bicuculline, in which large NMDA receptor-mediated components were evoked (cf. Fig. 4). In addition we found that neurons obtained from ether-anesthetized ($N=9$) rats exhibited the same sensitivity to CNQX and D-AP5 as neurons obtained from ketamine-anesthetized rats ($N=26$).

As pointed out below, the effect of CNQX can be quantified by measuring the change in peak amplitude of the postsynaptic response. In ether-anesthetized rats, control responses of 33 ± 7 mV amplitude were reduced to 5 ± 1 mV during application of 4 µM CNQX ($N=3$). In ketamine-anesthetized rats, very similar values

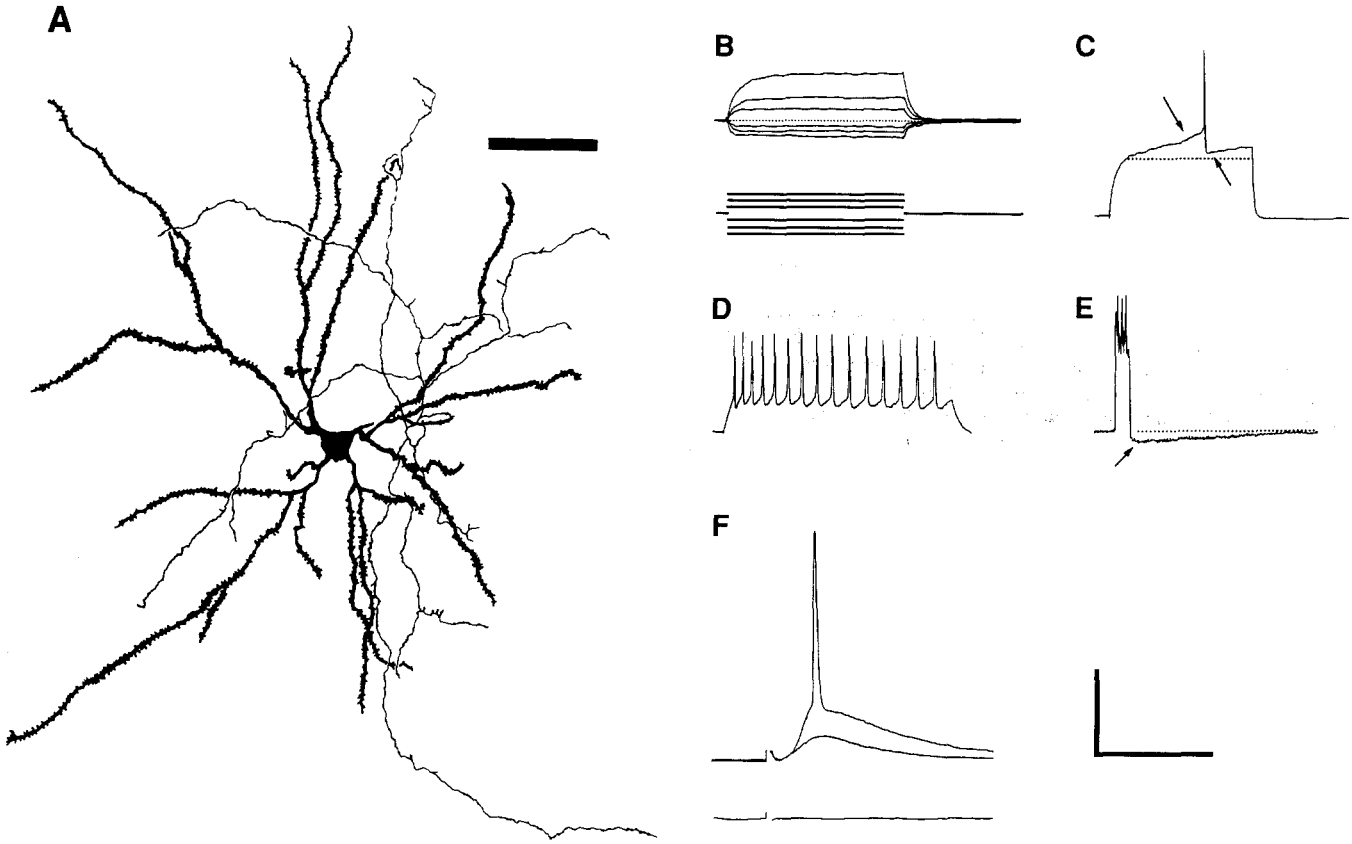

Fig. 1A–F. Morphology, membrane properties and locally-evoked synaptic potentials of recorded neurons in the nucleus accumbens. All traces in this figure represent single sweeps. (**A**), medium spiny neuron reconstructed from serial sections using a drawing tube. Calibration bar: 50 μM. (**B**), membrane potential changes (upper part) were elicited by injection of currents (lower part). A clear inward rectification in the hyperpolarizing direction is observed. (**C**), in a different medium spiny neuron (resting membrane potential: −82 mV), depolarizing current (1.1 nA, 380 msec) successively elicited a slow depolarizing ramp potential, an action potential and a short-lasting afterhyperpolarization (indicated by arrows). The arbitrarily drawn dashed line serves to highlight the slow depolarizing ramp and the short-lasting AHP. (**D**), In this cell (resting membrane potential: −78 mV) depolarizing current (1.5 nA) elicited a spike train exhibiting moderate frequency adaptation. Due to sampling at 1.8 kHz, spikes have been partially truncated. (**E**), a membrane depolarization (elicited by 0.75 nA) is followed by a long-lasting afterhyperpolarization (indicated by arrow; spikes are truncated due to sampling at 125 Hz). The resting membrane potential of this medium spiny neuron was −83 mV. (**F**, upper traces), depolarizing postsynaptic potentials evoked at subthreshold and suprathreshold stimulation intensity. (**F**, lower trace), extracellular control trace recorded at the same location as the upper trace. (**B**) and (**F**) were recorded from the neuron shown in (**A**); resting membrane potential: −78 mV. Calibration bars (**B**): 40 mV (voltage trace), 2.6 nA (current trace) and 100 ms; (**C**): 45 mV and 320 ms; (**D**): 90 mV and 70 ms; (**E**): 30 mV and 1.6 sec; (**F**): 40 mV and 20 ms

were obtained (control: 34±3 mV; CNQX: 5±1 mV; $N=6$). Since D–AP5 affected the decay phase of the postsynaptic potential but not its rising phase and peak amplitude, the half-decay time of the postsynaptic potential was quantified. In ether-anesthetized rats, the half-decay time was 14±3 ms in control conditions and 11±2 ms following D–AP5 application ($N=6$). The values found for ketamine-anesthetized rats (12±1 and 10±1 ms respectively; $N=14$) were not significantly different from ether-anesthetized rats. These results indicate that significant lingering effects of ketamine can be ruled out in the experiments presented below. Consequently, the results from both groups have been pooled.

Results

Morphology and membrane properties of labeled neurons

Thirty-six cells recorded in the ventral striatum were sufficiently labeled with biocytin to allow morphological identification. An example of a neuron reconstructed from avidin-HRP stained sections is presented in Fig. 1A. All labeled neurons were characterized by the presence of spines on their distal dendrites. Their somata were round, polygonal or oval in shape; both somata and primary dendrites were devoid of spines. The average maximal soma diameter amounted to 17±1 μm (range 9–20 μm). The mean number of primary dendrites per cell was 4.2±0.2. Axons originated from the soma or from one of the primary dendrites and branched profusely in the dendritic domain of the parent cell. Occasionally one of the axon collaterals could be traced into the ventral pallidum or subpallidal area.

The cells ($N=36$) had a mean resting membrane potential (RMP) of −72±2 mV and never fired spontaneously. The spike threshold, as estimated by injection of depolarizing current pulses, was −44±3 mV. Action potential amplitude and duration were 86±4 mV (quan-

tified from RMP level) and 1.8 ± 0.1 ms, respectively. The input resistance and time constant, as estimated by small (0.2 to 0.4 nA, 50–150 ms duration) depolarizing current pulses, amounted to 39 ± 4 MΩ and 7.7 ± 0.6 ms. Further characteristics of these neurons included a marked inward rectification in the hyperpolarizing direction (Fig. 1B; cf. Uchimura et al. 1989a), a slow depolarizing ramp potential preceding a spike (Fig. 1C), a short-lasting afterhyperpolarization following a spike (Fig. 1C) and, in response to a strong depolarizing current pulse, a regular spike train exhibiting little or moderate frequency adaptation (Fig. 1D). After termination of a depolarizing current pulse, an afterhyperpolarization was usually observed that could last up to 3 sec (Fig. 1E).

Postsynaptic potentials

Responses evoked by either local stimulation or stimulation of the rostral corpus callosum were recorded in 45 neurons. Eleven of these cells were labeled by biocytin and identified as medium spiny neurons. It may be assumed that most, if not all, of the 45 neurons belong to the same morphological phenotype, since their membrane characteristics were systematically monitored and appeared to be rather homogeneous. Local and callosal stimulation yielded very similar responses; consequently the results were pooled.

In 3 cells, local stimulation evoked spikes which were considered antidromically activated since their latency was constant in spite of varying stimulation intensity. Furthermore, they followed stimulation trains consisting of 6–8 pulses spaced by 7 ms (cf. Lemon and Prochazka 1984). In 42 neurons depolarizing postsynaptic potentials (DPSPs) were elicited. When the stimulation intensity was adjusted just below the spike threshold, the onset latency and rise time of the DPSP were 3.3 ± 0.1 and 4.5 ± 0.3 ms, respectively. The peak amplitude of the DPSP was 30 ± 2 mV. The onset latency of the DPSP remained nearly constant in spite of varying stimulation intensity. In all cells tested, suprathreshold stimuli evoked only 1 spike in the postsynaptic response. At 1.1 to 1.7 times spike threshold intensity, the spike latency was 5.8 ± 0.3 ms. The peak-to-half decay time in these neurons was 11 ± 0.6 ms. Examples of locally-evoked DPSPs recorded from a medium spiny neuron are shown in Fig. 1F.

In order to determine the nature of the postsynaptic potential, the effects of DC current injection on its shape and amplitude were studied. At slightly hyperpolarized levels, up to -90 mV, an increase in the amplitude of the DPSP was noted. Upon further hyperpolarization, this increase was attenuated. Injection of depolarizing current always reduced the amplitude of the DPSP.

In about half of the neurons tested, depolarization to -65 to -45 mV revealed a biphasic response pattern, consisting of a shortened DPSP followed by a hyperpolarizing postsynaptic potential (HPSP) that lasted 80 to 150 ms (not illustrated, but see Chang and Kitai 1986 and Uchimura et al. 1989b). The reversal potential of the HPSP could not be accurately estimated since the tail of the preceding DPSP component partially overlapped the HPSP. In the presence of the $GABA_A$ antagonists picrotoxin (50 μM) or bicuculline (25 μM), the HPSP was abolished and the remaining DPSP was prolonged. The RMP and input resistance were not notably altered during application of these drugs.

In the remaining half of the cells, spikes followed by afterhyperpolarizations were generated at strongly depolarized levels. Since these interfered with the postsynaptic potential, a clear reversal could not be demonstrated in these neurons.

Role of quisqualate/kainate and NMDA receptors in mediating postsynaptic potentials

The involvement of various glutamate receptor subtypes in mediating the DPSP was tested using the Q/K receptor antagonist CNQX and the NMDA receptor antagonist D–AP5. Before application of these antagonists to the slice, the peak amplitude of the DPSP was measured during a control period of 10 min. In these experiments, the stimulation intensity was adjusted just below spike threshold. The results of this part of the study are summarized in Table 1.

The DPSP amplitude recorded in the control situation averaged 34 ± 3 mV and was reduced to 5 ± 1 mV in the presence of 4 μM CNQX ($N=9$; $P<0.01$; Fig. 2A–B; washout value: 28 ± 6 mV; Fig. 2D). In addition to the strong blocking effects of CNQX on Q/K receptors, this drug has been reported to be a non-competitive antagonist at the strychnine-insensitive glycine binding site of the NMDA receptor complex (Harris and Miller 1989; Thomson 1989). Since the endogenous concentration of glycine was not determined in our slice preparation, the possible contribution of this additional antagonistic action to the suppression of the DPSP was examined. Addition of 30 μM glycine to the bathing medium has

Table 1. Quantified effects of glutamate receptor antagonists on postsynaptic potentials in the nucleus accumbens. In the upper part of the table, peak amplitudes of depolarizing postsynaptic potentials and SEMs (mV) are given for control, drug application and washout conditions. In the lower part of the table, half-decay times of the postsynaptic potential and SEMs are shown for the same conditions. Statistical comparisons were made with respect to the control period preceding drug application using Wilcoxon's matched-pairs signed rank test. n.s., not significant; *, $P<0.01$; for $N=4$ Wilcoxon's test cannot be applied

Drug(s) applied	Number of cells	Control	Drug application	Washout
Peak amplitude (mV)				
Ketamine	4	20 ± 6	10 ± 2	21 ± 4
CNQX	9	34 ± 3	5 ± 1*	28 ± 6 n.s.
CNQX/glycine	4	32 ± 5	4 ± 1	27 ± 4
CNQX/D–AP5	4	32 ± 5	5 ± 1	26 ± 6
D–AP5	19	30 ± 2	30 ± 1 n.s.	29 ± 2 n.s.
Half-decay time (ms)				
D–AP5	19	13 ± 1	11 ± 1*	13 ± 2 n.s.

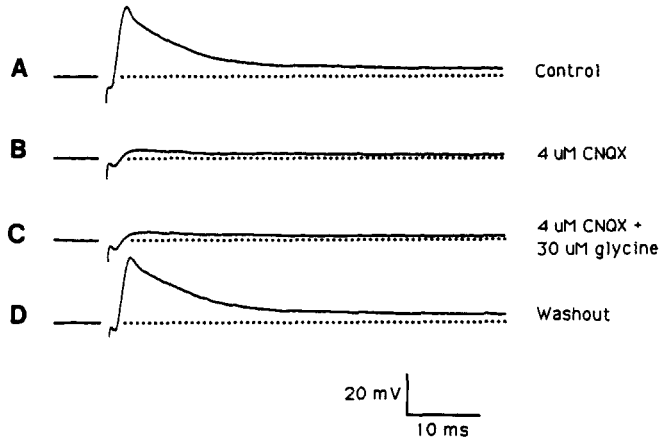

Fig. 2A–D. Effect of a quisqualate/kainate receptor antagonist on DPSPs evoked by local stimulation. (**A, B**), 4 μM CNQX almost completely abolished the DPSP. (**C**), addition of 30 μM glycine to medium already containing 4 μM CNQX did not restore the DPSP. (**D**), after washout of CNQX and glycine the DPSP amplitude recovered to control level. The resting membrane potential of this cell was −84 mV

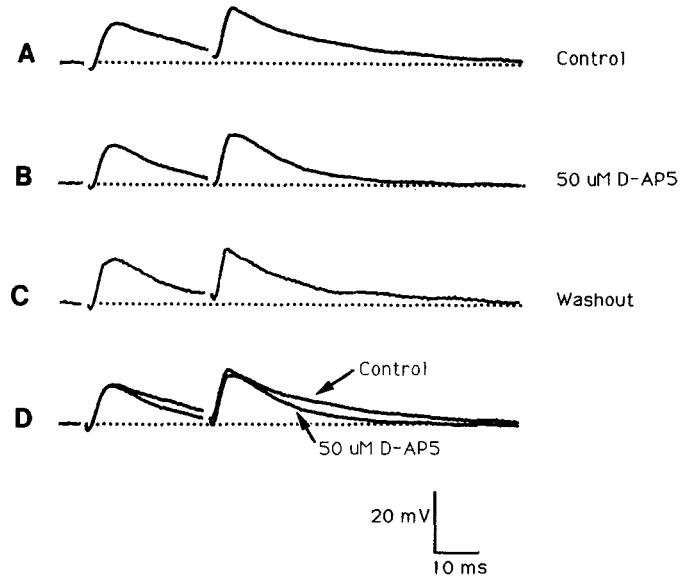

Fig. 3A–D. Effects of a NMDA receptor antagonist on conditioning and test responses in a paired-pulse stimulation paradigm (30 msec interval). (**A**), in the control situation the DPSP of the test response was facilitated as compared to the DPSP of the conditioning response. (**B, C**), 50 μM D–AP5 reversibly reduced the late part of the conditioning and test response. Note that facilitation of the DPSP peak amplitude was still present during D–AP5 application. (**D**), paired-pulse responses evoked in the control (**A**) and D–AP5 (**B**) situation are superimposed to show the effects of D–AP5 more clearly. The resting membrane potential of this cell was −69 mV

been shown to counteract the antagonizing effect of CNQX at the NMDA receptor complex (Harris and Miller 1989). In 4 cells, 4 μM CNQX reduced the DPSP amplitude from 32 ± 5 mV to 4 ± 1 mV. The addition of 30 μM glycine did not restore the DPSP to any extent (mean value: 4 ± 1 mV; Fig. 2B–C). Glycine was also ineffective when applied simultaneously with CNQX. No changes in RMP and input resistance were observed during glycine and CNQX application. Thus we conclude that the blocking effect of CNQX is not due to an action on the glycine binding site of the NMDA receptor complex.

In order to determine whether NMDA receptors mediate the DPSP that remained during CNQX application, D–AP5 was added to the bathing medium already containing CNQX. In 4 cells tested, 4 μM CNQX reduced the peak amplitude of the DPSP from 32 ± 5 mV to 5 ± 1 mV; for application of both 4 μM CNQX and 50 μM D–AP5 this value was also 5 ± 1 mV. Thus, D–AP5 did not suppress the part of the DPSP that remained during superfusion of CNQX.

To assess the contribution of NMDA receptors to the DPSP in the presence of functionally active Q/K receptors, synaptic responses were obtained during a control period and during superfusion of D–AP5. Again, stimulation intensity was adjusted just below spike threshold. In 19 neurons, a DPSP peak amplitude of 30 ± 2 mV was measured in the control period. Following addition of 50 μM D–AP5 to the bathing medium, the DPSP amplitude was not significantly changed (30 ± 1 mV; washout value: 29 ± 2 mV). However, a reduction was noted in the late phase of the DPSP (Fig. 3). This change was quantified by measuring the peak-to-half decay time of the synaptic response. The half-decay time in the control situation was 13 ± 1 msec and during D–AP5 it was significantly reduced to 11 ± 1 ms ($P < 0.01$; washout value: 13 ± 2 ms). A similar, albeit smaller, reduction of the decay phase by D–AP5 was observed at stimulation intensities 30–60% of the spike threshold intensity ($N = 7$). D–AP5 had no significant effects on the RMP or input resistance.

To examine whether the above mentioned GABAergic component of the postsynaptic potential may influence the NMDA receptor mediated component of the DPSP, picrotoxin (50 μM) or bicuculline (25 μM) was added to the perfusate in combination with D–AP5 (50 μM). In 7 of 8 cells tested, both $GABA_A$ antagonists were observed to lower the spike threshold intensity and to enhance and prolong the DPSP evoked by a single stimulus. The enhancement and prolongation were particularly clear at stimulation intensities above spike threshold (Fig. 4A–B, conditioning response). Occasionally secondary spikes occurred during the plateau phase of the DPSP. D–AP5 reversibly abolished this prolongation of the DPSP ($N = 6$; Fig. 4B–E).

Contribution of NMDA receptors to paired-pulse facilitation

Postsynaptic responses to paired-pulse stimulation exhibited a facilitation of the DPSP in 26 of 28 neurons studied (Fig. 3A). This facilitation was found for interstimulus intervals ranging from 10 to 150 ms. The effects of 50 μM D–AP5 on paired-pulse facilitation were studied by evaluating three response parameters, using a standard interval of 30 ms: the peak amplitudes of the conditioning and test DPSP (quantified at stimuli below spike threshold), the half-decay times of the DPSPs, and

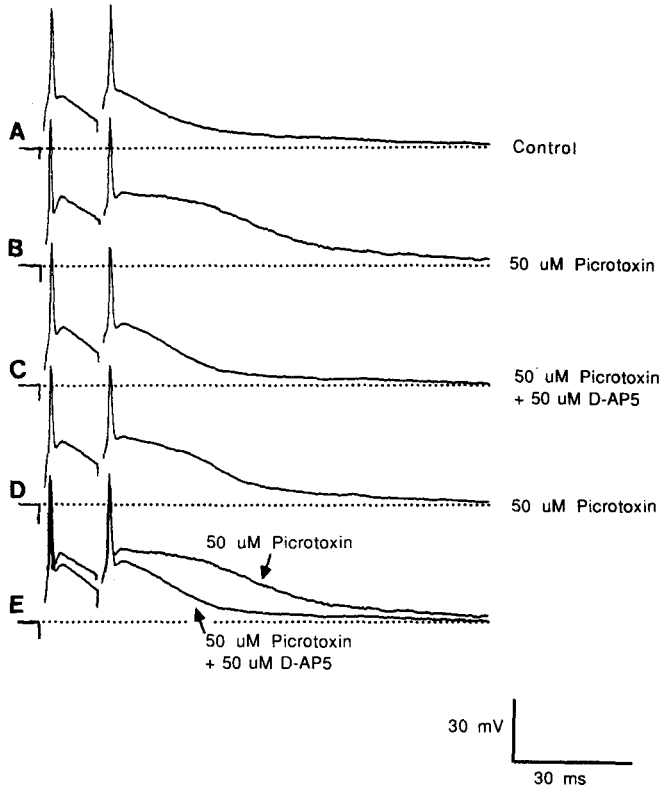

Fig. 4A–E. Effects of a NMDA receptor antagonist on DPSPs that were first modified by a $GABA_A$ antagonist. In this experiment, suprathreshold, paired-pulse stimulation in the rostral corpus callosum was used. Spikes are partially truncated due to digital sampling at 3 kHz. All traces in this figure represent single sweeps. (**A, B**), application of 50 μM picrotoxin enhanced and prolonged the late part of both the conditioning and test response. This prolongation was more prominent in the test than in the conditioning response. (**B, C, D**), 50 μM D–AP5 reversibly suppressed the prolongation in both responses. (**E**) responses evoked in the presence of picrotoxin (B) and in the presence of picrotoxin plus D–AP5 (**C**) are superimposed to illustrate the more severe suppression of the test response by D–AP5 as compared to the conditioning response. The resting membrane potential of this neuron was −76 mV

the occurrence of a spike in the test response at a stimulation intensity just below spike threshold for the conditioning response.

In the control period preceding D–AP5 administration, all three of these parameters exhibited paired-pulse facilitation: the peak amplitude of the test DPSP was larger than that of the conditioning DPSP (29 ± 3 and 25 ± 2 mV respectively; $N = 11$; $P < 0.02$). Using a stimulation intensity just below spike threshold for the conditioning response, an action potential was generated on top of the test DPSP in 11 of 12 neurons studied (in the remaining neuron, the spike threshold intensity of the conditioning and test response were equal). Furthermore, the half-decay time of the test response was significantly longer than that of the conditioning response (19 ± 3 and 15 ± 1 ms respectively; $N = 10$, $P < 0.01$).

As noted above, D–AP5 did not significantly affect the peak amplitude of the conditioning DPSP; neither did it reduce the peak amplitude of the test DPSP (control: 29 ± 3 mV; D–AP5: 28 ± 2 mV; washout value: 29 ± 2 mV; $N = 11$). Furthermore, D–AP5 reversibly abolished facilitation of the action potential in 4 of 12 neurons studied. In the remaining 8 neurons, no consistent and reversible effects of D–AP5 on this response parameter were observed.

Facilitation of the late part of the DPSP was only slightly but consistently affected by D–AP5. This was more clearly seen at suprathreshold stimuli than at subthreshold stimuli. In 14 cells that were tested for paired-pulse effects, the half-decay time of the conditioning DPSP was reduced from 15 ± 1 to 12 ± 1 ms ($P < 0.01$; washout value: 15 ± 1 ms). The half-decay time of the test response was reduced from 19 ± 3 to 14 ± 2 ms by D–AP5, and this reduction was significantly larger than that found for the conditioning response ($P < 0.01$; washout value: 18 ± 2 ms). During D–AP5 application, a slight facilitation was still left in the late part of the DPSP since the half-decay time of the test response (14 ± 2 ms) remained larger than that of the conditioning response (12 ± 1 ms) during D–AP5 ($P < 0.05$).

The contribution of NMDA receptors to paired-pulse facilitation was especially clear under conditions of reduced GABAergic inhibition (50 μM picrotoxin or 25 μM bicuculline). Using suprathreshold stimulation for the conditioning response, the prolongation of the test DPSP was found to exceed the prolongation of the conditioning DPSP (Fig. 4B). This particular facilitation effect was seen at interstimulus intervals lasting up to 150 ms. As in single-pulse experiments, 50 μM D–AP5 abolished most of the prolonged DPSP component in both responses (Fig. 4B–D). In the superimposed sweeps shown in Fig. 4E, the difference between the two double-pulse responses (obtained under picrotoxin and picrotoxin plus D–AP5 conditions) can be interpreted as being mediated by NMDA receptors. This difference was found to be larger in the test response than in the conditioning response ($N = 4$).

Discussion

Morphology and membrane properties of labeled neurons

All neurons labeled in this study exhibited the morphological characteristics of medium spiny neurons. Previously, these neurons were observed to project outside the striatal complex (Chang and Kitai 1985; Smith and Bolam 1990), as was confirmed by the present findings.

In comparing the membrane properties of medium spiny neurons in the core region of the Acb to those recorded in the caudate-putamen, many similarities can be noted. Both Acb and caudate-putamen medium spiny neurons exhibit a strong inward rectification in the hyperpolarizing direction, a slow ramp-like depolarization visible just below spike threshold and a relative lack of frequency adaptation in spike trains (cf. Kita et al. 1984, 1985; Kawaguchi et al. 1989). Moreover, their time constants, input resistances as well as the shape and time

course of their postsynaptic responses are similar. Altogether, these electrophysiological results support the notion that the Acb and caudate-putamen are part of one large striatal complex, underscoring the anatomical findings and concepts introduced by Heimer and Wilson (1975).

Postsynaptic potentials

Local or callosal stimulation elicited DPSPs in almost all neurons tested. In at least a large portion of the neurons, depolarizing current injection revealed a biphasic synaptic response consisting of an early DPSP followed by a late, bicuculline- and picrotoxin-sensitive HPSP. In agreement with other studies (Chang and Kitai 1986; Uchimura et al. 1989b) the HPSP can be identified as a $GABA_A$ receptor-mediated IPSP and the DPSP as a glutamate receptor-mediated EPSP. The EPSP component is likely to be due to monosynaptic activation considering its constant onset latency in spite of varying stimulation intensity. Most probably, these activated glutamatergic fibers originate in limbic structures, such as the hippocampal formation, prefrontal cortex and amygdala (Walaas and Fonnum 1980; Christie et al. 1987; Fuller et al. 1987). The origin of the IPSP, however, is less clear and cannot be deduced from the present experiments.

Injection of small hyperpolarizing currents led to an increase in the amplitude of the DPSP but further hyperpolarization attenuated this increase. This effect may be explained by the strong inward rectification observed at hyperpolarized levels.

At RMP level, the EPSP component of the DPSP appeared to be mediated almost completely by Q/K receptors. The first piece of evidence supporting this conclusion was that 4 μM CNQX – even in the presence of 30 μM glycine – strongly suppressed the DPSP. The small synaptic component that remained during application of CNQX was not blocked by D–AP5 and may reflect a fraction of Q/K receptors not occupied by CNQX (cf. Yamada et al. 1989). Secondly, in the presence of active Q/K receptors, D–AP5 only suppressed part of the decay phase of the DPSP. This observation is in agreement with the slow rise time of NMDA receptor-mediated activity recorded in cultured neurons from the hippocampus and the spinal cord (Forsythe and Westbrook 1988). Since low-affinity glutamate receptor antagonists were used in previous studies on synaptic responses in the ventral and dorsal striatum (Cordingley and Weight 1986; Cherubini et al. 1988; Uchimura et al. 1989b; Walsh et al. 1989), the present investigations are the first to suggest that fast EPSPs evoked by local or callosal stimulation are almost completely mediated by Q/K receptors.

The contribution of NMDA receptors to EPSPs may be enhanced under several conditions. First, a reduction of GABAergic inhibition allows expression of a considerable NMDA receptor-mediated component. Secondly, this component is moderately increased by paired-pulse stimulation (Fig. 4). These different effects may originate from two properties of the NMDA receptor-channel complex. First, its slow half decay time (Forsythe and Westbrook 1988; D'Angelo et al. 1990) may, in paired-pulse experiments, give rise to temporal summation in the test response and thus to facilitation. Second, the voltage-dependent relief of magnesium block of the NMDA receptor-coupled channel at depolarized membrane potentials (Nowak et al. 1984; Forsythe and Westbrook 1988; D'Angelo et al. 1990) may account for our observations. In paired-pulse experiments, a larger amount of glutamate or aspartate may be hypothesized to be released following the test pulse as compared to the conditioning pulse. This assumption is supported by the residual calcium hypothesis (reviewed by Zucker 1989). A larger amount of excitatory amino acid would give rise to a larger depolarization and thus a larger NMDA receptor mediated component. Likewise, the enlargement of the NMDA receptor mediated component in the presence of $GABA_A$ antagonists may be explained by a decreased shunting inhibition and thus a stronger depolarization at the subcellular sites of afferent input.

Uchimura et al. (1989b) suggested that a major portion of the postsynaptic potential, evoked under normal conditions in the Acb, is mediated by NMDA receptors. The difference between their results and the findings reported here may be ascribed to (1) a difference in the Acb subregion that was investigated (dorsomedial part vs. core of the Acb); (2) a smaller influence of IPSPs in their study, or (3) aspecific effects of high concentrations of DL–APV (250 μM) and D-α-aminoadipic acid (1 mM) used in their study. A concentration of 50 μM D–AP5, as used here, has been shown to be sufficient in antagonizing responses to NMDA in other slice preparations (Crunelli et al. 1985; Dingledine et al. 1986; Melchers et al. 1988; cf. Watkins and Olverman 1987).

Paired-pulse facilitation

Evidence for paired-pulse facilitation was found when comparing the spike threshold intensity, peak amplitude and half-decay time of the DPSP in the conditioning and test response. The contribution of NMDA receptors to this form of short-term plasticity was investigated using 50 μM D–AP5. Whereas the facilitation of the early part of the DPSP was not significantly affected by D–AP5, it slightly reduced the facilitation in the late part of the DPSP. The NMDA receptor-mediated component of paired-pulse facilitation of this part was enhanced by reducing GABAergic inhibition. The hypothesized mechanism of these effects was outlined above.

Previous reports on short-term plasticity stress the predominant role of presynaptic terminals in mediating paired-pulse facilitation (Zucker 1989). While our results do not rule out a presynaptic mechanism (paired-pulse facilitation is still observed during application of D–AP5; cf. Fig. 3), our findings suggest the additional involvement of postsynaptic sites. Almost all striatal NMDA receptors have been shown to reside on postsynaptic elements (Greenamyre and Young 1989).

Previously, functions of the NMDA receptor have

been primarily associated with long-term synaptic plasticity (Harris et al. 1984; Herron et al. 1986; Melchers et al. 1988). In the present study it was shown that this receptor contributes, albeit modestly, to paired-pulse facilitation in the Acb. Similar findings have recently been reported in the rat hippocampal slice preparation, where paired-pulse facilitation was studied under conditions of reduced GABAergic inhibition and in medium containing only 10–20 μM Mg^{2+} (Muller and Lynch 1988). By means of this property the responses to glutamatergic inputs may be amplified as these inputs successively reach the spines and dendrites of their target neurons.

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