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EFFECTS OF EXCITATORY AMINO ACIDS AND THEIR ANTAGONISTS ON MEMBRANE AND ACTION POTENTIALS OF CAT CAUDATE NEURONES

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SUMMARY

1. The electrical activity of caudate neurones was recorded with intracellular electrodes in halothane anaesthetized cats. Agonists and antagonists of excitatory amino acid receptors were applied by micro-ionophoresis and their effects on membrane- and action potentials and on cortically evoked synaptic potentials evaluated.

2. The agonists, L-aspartate (asp), L-glutamate (glu), *N*-methyl-DL-aspartate (NMA), quinolinate and quisqualate all depolarized the membrane, caused repetitive firing, reduced the apparent amplitude of the cortically evoked excitatory post-synaptic potentials (e.p.s.p.s) and increased the amplitude of the associated inhibitory post-synaptic potential. Two of the agonists, NMA and quinolinate, additionally caused the appearance of up to 500 ms long depolarizations (plateaus) on the falling phase of action potentials. These plateaus were seen in about two-thirds of the cells in this sample while in the other third the excitatory effects of NMA and quinolinate were indistinguishable from those of glu and quisqualate.

3. The *N*-methyl-D-aspartate (NMDA) receptor antagonist D-α-aminoadipate (DAA) reversibly inhibited the effects of NMA and quinolinate but only on those cells where these two agents evoked action potential plateaus while on the same cells the effects of asp, glu and quisqualate were either only weakly antagonized or not affected. On cells not displaying plateaus to NMA or quinolinate none of the effects of the agonists could be antagonized by DAA. DAA applications that completely antagonized the effects of NMA never reduced the amplitudes of cortically evoked e.p.s.p.s.

4. *Cis*-2,3-piperidine dicarboxylate also blocked the effects of NMA and asp at low application currents while at higher currents it enhanced the effects of glu or asp although still retaining its NMA antagonistic activity.

5. High-frequency stimulation of the cortico-caudate pathway resulted in long-lasting depolarizations and repetitive firing, but plateaus of the type caused by NMA or quinolinate were not seen.

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6. It is concluded that micro-ionophoretic application of excitatory amino acids can excite some caudate cells by at least two different mechanisms, one of which is pharmacologically similar to the one proposed for the NMDA receptor. Furthermore, the present as yet indirect evidence suggests that the receptor responsible for the cortically evoked e.p.s.p. is not of the NMDA type.

INTRODUCTION

The monosynaptic cortico-caudate projection (Kitai, Kocsis, Preston & Sugimori, 1976; Vandermaelen & Kitai, 1980; Webster, 1965) is a neuronal pathway which may use an excitatory amino acid as a transmitter (Divac, Fonnum & Storm-Mathisen, 1977; Fonnum, Storm-Mathisen & Divac, 1981; Kim, Hassler, Haug & Paik, 1977; McGeer, McGeer, Scherer & Singh, 1977; Roberts, McBean, Sharif & Thomas, 1982). Although there have been a number of electrophysiological studies using extracellular recording where excitatory amino acids have been applied by micro-ionophoresis to neurones in the caudate nucleus, most of these agents were used in order to create a background of firing against which inhibitory drugs could be tested (Ben-Ari & Kelly, 1976; Davies & Tongroach, 1978, 1979; Fry, Zieglgaensberger & Herz, 1980; Jones, 1981; McLennan & York, 1966; Norcross & Spehlmann, 1981; Woodruff, McCarthy & Walker, 1976). Only a few authors directly addressed the question of the specificity of the effects of excitatory amino acids (Spencer, 1976; Stone, 1979). There are only two reports using intracellular techniques in conjunction with micro-ionophoretic application of excitatory amino acids in the caudate but both only examined the effects of L-glutamate and no antagonists were used (Bernardi, Floris, Marciani, Morocutti & Stanzione, 1976; Herrling & Hull, 1980).

Largely due to the efforts of Watkins and his colleagues, a number of compounds have become available that have led to the postulation of three receptors for excitatory amino acids, quisqualate, kainate and *N*-methyl-D-aspartate receptors (Watkins, 1981). Recent studies using biochemical methods have indicated that some of these receptors might also be found in the striatum (Scatton & Lehman, 1982; Teichberg, Goldberg & Luini, 1981).

The present report is an attempt to evaluate some of these new excitatory amino acid agonists and antagonists in the cat caudate nucleus using intracellular recording and micro-ionophoretic techniques and to determine the pharmacological characteristics of the receptors responsible for the generation of the cortically evoked excitatory post-synaptic potential (e.p.s.p.) (Buchwald, Price, Vernon & Hull, 1973; Hull, Bernardi, Price & Buchwald, 1973; Kitai *et al.* 1976).

Parts of the present results have been previously published as abstracts (Herrling, Misbach-Lesenne & Salt, 1982; Herrling & Salt, 1982).

METHODS

Surgery

Forty-five mongrel cats of either sex and weighing from 2·7 to 4·0 kg were used. The saphenous vein and femoral artery were cannulated for I.V. drug injections and monitoring of the blood pressure, respectively. The skull was exposed and the frontal sinuses were opened bilaterally to allow insertion of two pairs of stainless-steel stimulation electrodes into the precruciate cortex where the

cell bodies of the neurones projecting to the head of the caudate nucleus are located in the cat (Oka, 1980; Royce, 1982; Webster, 1965).

Two square bone-plates (1 × 1 cm) were removed above the heads of the caudate nuclei, their centres were located at AP +17, L/R 4 (Snider & Niemer, 1961), the dura excised and the cortical tissue overlying the caudate was removed by suction until the lateral ventricles were reached. The walls of the finished wells (i.d. 6 mm) were supported by insertion of a tube of Gelfilm (Upjohn). Subsequently, a 10 mm long silicon rubber tube cut at one end to fit the dorsal surface of the caudate nucleus was inserted and gentle pressure applied to reduce cardiovascular and respiratory pulsations. A thin strip of gauze was introduced between the Gelfilm and the silicon tube to drain blood and cerebrospinal fluid from the well. The caudate surface was covered with a thin layer of liquid paraffin to prevent drying. Further measures to reduce pulsations were a bilateral pneumothorax and drainage of the cisterna magna. Tracheotomy permitted artificial ventilation: 1·0–1·6 l/min, 20 cycles/min.

Body temperature was maintained at 37·8 °C, the level of expired CO_2 was measured with a Gould-Goddard MKII Capnograph and kept between 3·6 and 4·2%. Cell recordings were only included if the blood pressure was above 80 mmHg.

Anaesthesia

Surgical manipulations were performed under an ultra-short acting barbiturate (sodium methohexital, Brietal, Lilly) which was injected periodically. At the end of surgery, the barbiturate was discontinued, 1 ml of gallamine triethiodide (Flaxedil, Abbott) was given I.V. and artificial respiration was commenced with 0·5–1·0% halothane (Fluothane, ICI) in pure oxygen. The effect of the paralysing agent usually wore off after one hour as judged by the paw withdrawal reflex. No paralysing agent was re-injected except in the rare cases where strong respiratory movements interfered with the stability of the recording. The absence of paralysing agent in most experiments allowed an evaluation of the depth of anaesthesia as judged by reflexes, blood pressure and expired CO_2 levels. All stereotaxic pressure points were treated with Xylocaine-Gel (Astra).

Electrodes

The intracellular electrodes were pulled on a vertical puller (Narishige) from Hilgenberg borosilicate glass (o.d. 3 mm, i.d. 1·63 mm) with inner filament to a taper of 20 mm. They were filled with 1·6 M-K citrate adjusted to a pH of 7·2 with citric acid and their tips were briefly dipped in a 10% (v/v) solution of silicon column coat (Miles) in acetone. Such electrodes had resistances of 40–200 MΩ (90 ± 28, mean ± S.D., $n = 33$) measured in the c.s.f. just before touching the brain. In the tissue, electrode resistances could exceed 300 MΩ.

As ionophoretic electrodes, multibarrelled pipettes made from seven lengths of glass (Hilgenberg borosilicate, o.d. 1·5, i.d. 1·02 mm with inner filament) were used. The central barrel was left straight, but the six others were bent at one end to avoid electrical interactions between the drug barrels. They were first twisted and then pulled to a taper of 18–20 mm and their common tips broken to 7·9 ± 1·2 μm ($n = 30$) to give, when filled, suitable resistances. The tapering part was then bent and glued alongside the intracellular electrodes with a drop of cyanoacrylic glue (Cyanolit 202, 3 M) without lateral displacement and with intertip distances of about 50 μm. The assemblies were prepared one day before the experiments and were used for only one penetration of 4–5 mm into the head of the caudate nucleus.

Drugs

Each barrel of the ionophoretic electrodes contained one of the following drug solutions: D-α-aminoadipic acid (Sigma), 0·2 M; L-aspartic acid (Sigma), 0·5 M; L-glutamic acid (Sigma), 1·0 M; *N*-methyl-DL-aspartic acid (Sigma), 0·2 M; *N*-methyl-D-aspartic acid (Sandoz), 0·2 M; *cis*-2,3-piperidine dicarboxylic acid (kindly donated by Dr J. C. Watkins, Bristol), 0·1 M; quinolinic acid (Sigma), 0·2 M; quisqualic acid (Sigma), 0·1 M. All drugs were adjusted to a pH of 9–9·5 with NaOH with the exception of L-glutamate (glu) which was adjusted to pH 8·0. One barrel was filled with a NaCl solution (165 mM, pH 9·8) and was used for current controls. The central barrel of the ionophoretic arrays contained a NaCl solution (165 mM) at neutral pH that was used for current balancing (see below). The drugs were either freshly prepared for each experiments or kept frozen between experiments. Electrodes were filled just prior to use and all drugs were ejected as anions.

All drugs were applied at currents ranging from −5 to −150 nA from a device with six constant current sources and an automatic balancing unit that could neutralize electric fields at the electrode tips and was built in our workshop by W. Bauer. Usually, +10 nA retaining current was applied to the drug barrels. The balance channel was only used occasionally with each drug because it became apparent that it sometimes accumulated some of the ejected drugs as previously described (Engberg, Flatman & Lambert, 1979). All drug effects were qualitatively similar with and without balancing.

Intracellular potentials were recorded with commercially available equipment, displayed on an oscilloscope and stored on tape for further analysis. From tape, the potentials could be either printed on paper with a c.r.o. recorder (Fiberstatic M, Toennies, Freiburg, F.R.G.) or digitalized by a computer (PDP 11/10, programs written by P. Linscheid).

Because of the usually very high resistances of the electrodes which resulted in poor current passing ability, the pronounced depolarizations and intense firing induced by the agonists, no attempt was made to measure changes in input resistance during the experiments.

At the end of each successful experiment the animals were deeply anaesthetized (3·5 % halothane) and the brain perfused through the carotid artery with 10 % formaldehyde in physiological saline for subsequent histological localization of the electrode tracks.

RESULTS

Identity and characteristics of recorded cells

Results were obtained from fifty-eight cells impaled within the caudate nucleus (A: +16 to +18, Snider & Niemer, 1970) at depths ranging from 400–4700 μm below its dorsal surface. Although no intracellular staining of the recorded neurones was attempted in this study, it is very probable that most impaled cells were medium spiny neurones because other authors (Bishop, Chang & Kitai, 1982; Kitai *et al.* 1976; Kocsis, Sugimori & Kitai, 1977), using horseradish peroxidase filled intracellular electrodes, reported that the great majority of impaled cells in the caudate nucleus were of this type. These cells were held for 10–70 min and most of them were silent unless stimulated electrically, synaptically or by drugs.

The resting potentials were estimated from the potential drop that occurred at the end of the recordings when the cells were lost and as in a previous study (Herrling & Hull, 1980) had a value of about −50 mV (50 ± 9 mV, range 40–65 mV, $n = 26$). No correction was applied for junction potentials caused by the potassium citrate in the electrodes (Kandel, Spencer & Brinley, 1961). The resting potential was near the equilibrium potential of the cortically evoked inhibitory post-synaptic potential (i.p.s.p.) as indicated by the fact that it was usually only visible when the cells were depolarized by the application of an excitatory amino acid.

The mean amplitude of action potentials was 40 ± 9 mV, range from 30–70 mV with durations of 0·5 to 1 ms at the half maximal amplitude. The above is probably a low estimate of their amplitude as it seemed that some of it was lost through stray capacitance that could not be compensated (Cornwall & Thomas, 1981). This was probably due to the high capacitance of the recording electrode, the depth at which cells were found below the caudate surface, usually more than 1 mm, and the fact that the ionophoretic barrels were glued directly alongside the recording electrode.

Pharmacology

Effects of the agonists on the membrane and action potential. The agonists, L-aspartate (asp), L-glutamate (glu), *N*-methyl-DL-aspartate (NMA), *N*-methyl-D-aspartate

(NMDA), quinolinate and quisqualate all depolarized caudate neurones by 5–20 mV and induced repetitive firing of the usually silent caudate neurones.

The agonists could be divided into two groups with respect to the firing pattern they induced: the first group included glu and quisqualate. These agents depolarized the membrane and increased the firing rate of all cells they were tested on (ten and twenty cells, respectively, Figs. 1, 2) until total inactivation occurred. These excitations were termed type 1.

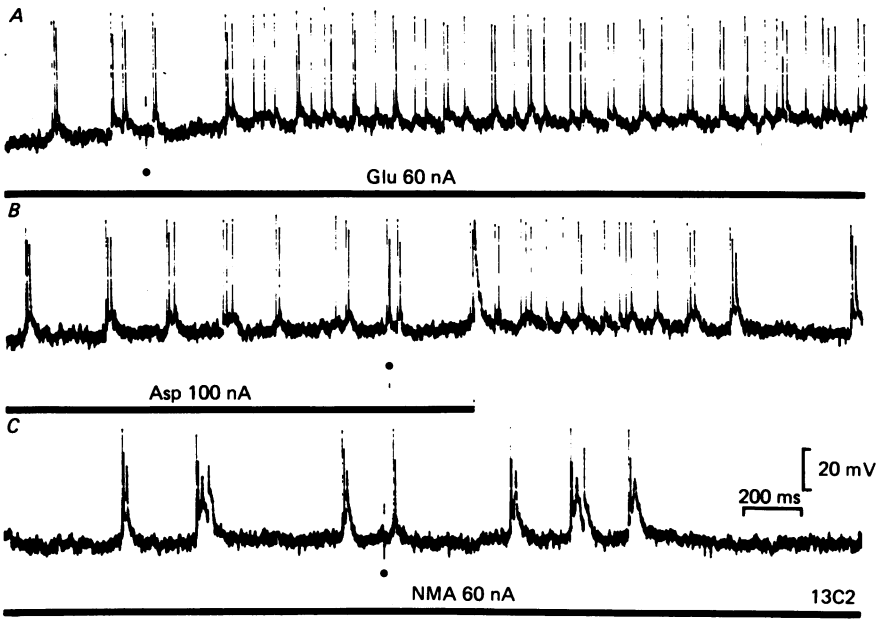

Fig. 1. Effects of glu, asp and NMA on the firing pattern and membrane potential of a caudate neurone. *A*, glu was applied at the indicated current for the whole duration of the trace. The depolarization of the membrane was associated with a relatively regular increase in firing. *B*, asp also depolarized the membrane and elicited similar firing but there were also some burst-like depolarizations. *C*, the depolarization of the membrane induced by NMA was accompanied exclusively by bursts which in this instance were shorter than the plateaus described in the text. In this and all other traces time is on the abscissa and the membrane potential on the ordinate, positive upwards. ●: stimulus artifact from the cortical stimulation for this and subsequent illustrations. The number at the lower right corner is the cell identification. In some illustrations action potentials were retouched for clarity.

The second group included NMA, NMDA and quinolinate. On thirteen of forty-one cells, NMA or NMDA (both are hereafter referred to as NMA) displayed the same effects on membrane and action potentials as the agonists of the first group (Fig. 2). On the other twenty-eight cells however, NMA elicited a characteristic and different firing pattern. At low application currents a large proportion of the action potentials occurred superimposed on large depolarizations with a fast onset and offset that lasted up to 500 ms (Figs. 1, 3). These excitations were termed type 2. As the mechanism causing these depolarization is at present unknown we have called them 'plateau potentials' or 'plateaus' for short. On most of these plateaus partially inactivated

action potentials could be seen (Figs. 1, 3) and the firing rate could reach up to 100 Hz. Quinolinate was applied to fourteen cells on which NMA and quisqualate had also been tested. Four of these cells belonged to the sample where NMA caused only regular firing without plateaus and on these quinolinate displayed the same effect (Fig. 2). On the remaining ten cells NMA and quinolinate both elicited plateaus while quisqualate did not, even using application currents that caused inactivation of the action potentials (Fig. 3).

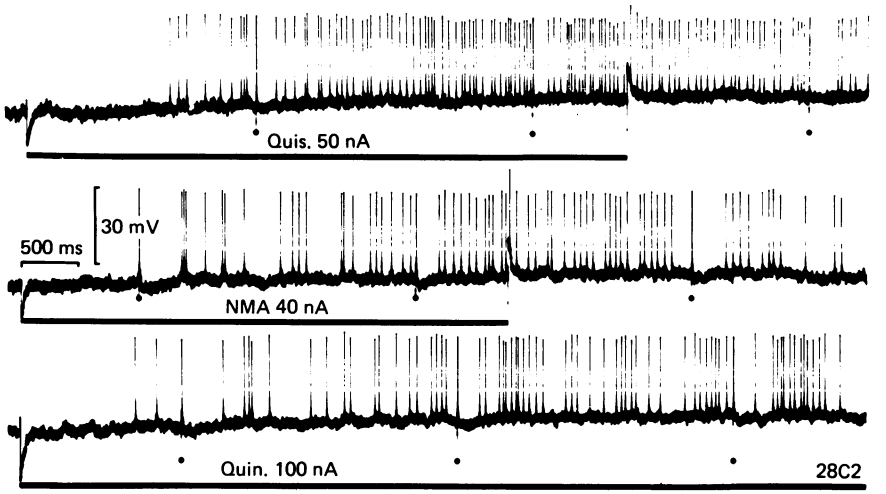

Fig. 2. The effects of quisqualate (Quis.), NMA and quinolinate (Quin.) on membrane potential and firing pattern of a neurone not displaying plateaus.

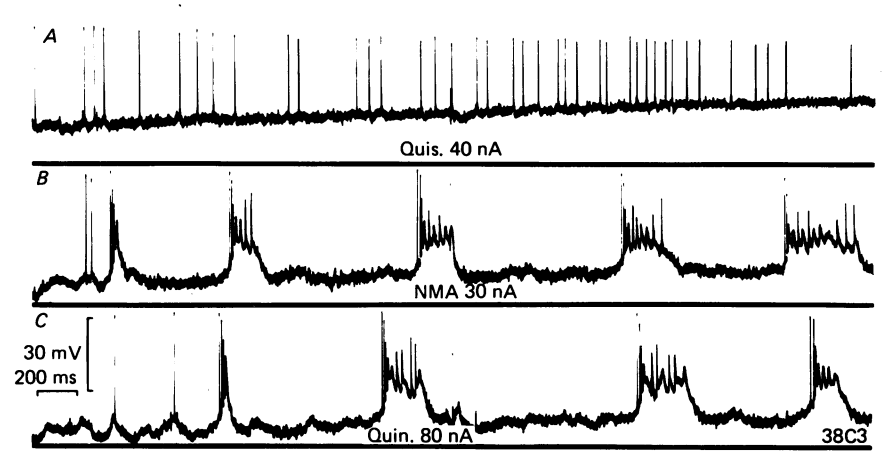

Fig. 3. The effects of quisqualate, NMA and quinolinate on the membrane potential and firing pattern of a neurone displaying pronounced plateaus. *A*, quisqualate depolarized the membrane and elicited repetitive firing but no plateaus up to a level where the action potentials were largely inactivated. *B* and *C* both NMA and quinolinate provoked very distinct plateaus. With quinolinate it was usually necessary to apply more current to reach excitation levels equal to those of NMA on the same cell.

Aspartic acid, usually thought to be more NMDA-like than quisqualate-like (Watkins, 1981), behaved in an intermediate fashion in the caudate. On cells displaying plateaus during NMA applications asp elicited both regular, quisqualate-like firing and short plateaus that however never reached the length achieved during NMA applications (Fig. 1).

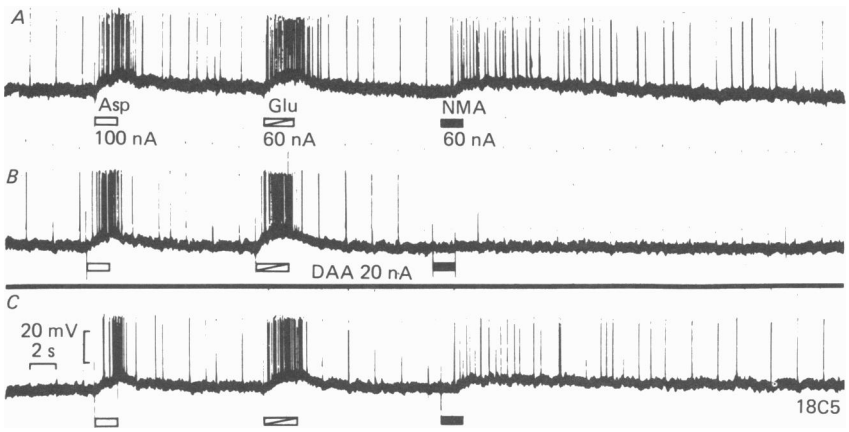

Fig. 4. Selective inhibition of NMA by DAA. *A*, control where asp, glu and NMA were applied. *B*, the same compounds were applied at the same currents in the presence of DAA. The excitations induced by NMA were totally abolished while those due to asp and glu were only slightly affected. *C*, recovery after the end of the DAA application. The inhibitory effects of DAA on NMA were usually maximal after about one minute of application and recovery had a similar time course.

Antagonists. The selective NMDA receptor antagonist D-α-aminoadipic acid (DAA; Biscoe, Davies, Dray, Evans, Francis, Martin & Watkins, 1977; for review see Watkins, 1981) was tested on eight cells to which NMA and glu were applied alternately in regular pulses, and on nine further cells which were alternately excited by NMA and quisqualate. NMA caused depolarizations and plateaus on thirteen of these cells, and these effects were selectively antagonized by DAA while the excitatory effects of glu seemed almost unaffected. In the remaining four cells NMA elicited depolarizations accompanied by regular firing and these were not sensitive to DAA. If asp was also included as an agonist, DAA inhibited its excitatory effects only to a similar extent as those of glu at currents which had completely abolished the effects of NMA (Fig. 4). The ionophoretic currents needed to expel DAA at doses sufficient to inhibit the effects of NMA selectively were usually equal to or less than those used with NMA on the same cell to achieve a distinct but submaximal effect with this agonist. If DAA was applied at much higher currents than those used to antagonize NMA, selectivity was occasionally reduced, i.e. glu or asp were then also more distinctly affected. Nevertheless, even in those cases the antagonist effects of DAA were much more potent against NMA responses than against glu or asp responses. Excitations induced by quisqualate appeared unaffected by DAA (Fig. 5). DAA was tested on four cells against NMA, quinolinate and quisqualate. Both NMA and quinolinate caused plateaus on two cells and only simple excitations on the remaining two. DAA blocked the effects of both NMA and quinolinate, but not

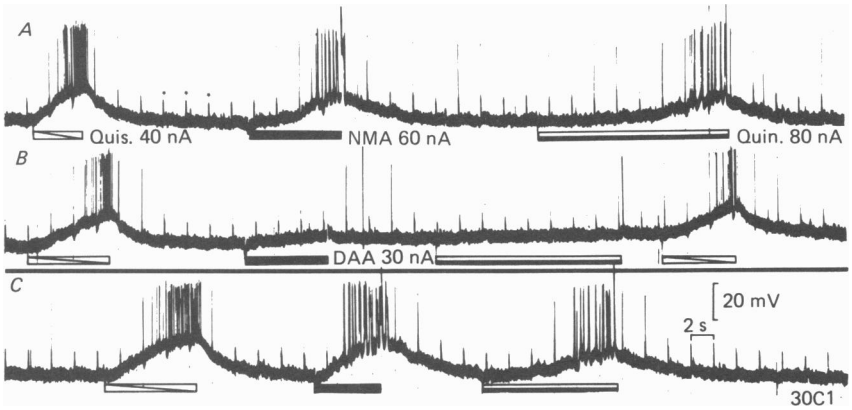

Fig. 5. Selective inhibition of NMA and quinolinate by DAA. *A*, control where quisqualate, NMA and quinolinate were applied. Note that the different firing pattern can also be seen at this slow sweep speed. Cortically evoked e.p.s.p.s are indicated by *. *B*, the same compounds applied with the same current intensity in the presence of DAA. Both effects of NMA and quinolinate were abolished while quisqualate induced excitations were still very pronounced. The cortically evoked e.p.s.p.s were not visibly diminished by DAA. *C*, recovery.

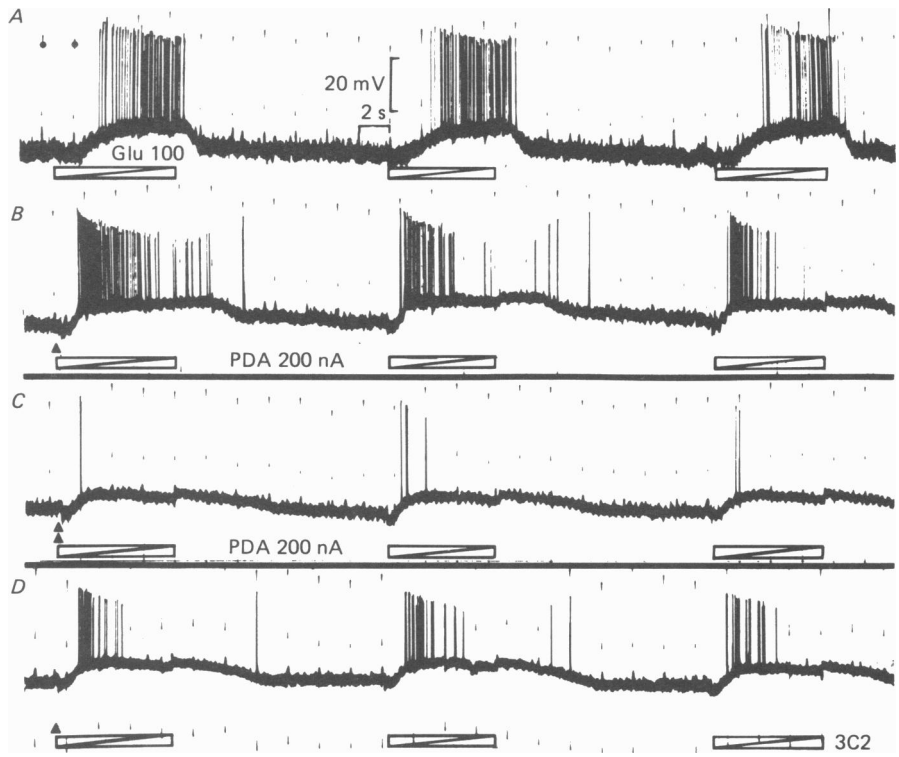

Fig. 6. The effect of PDA on excitations evoked by glu. *A*, control, where regular pulses of glu were applied. *B*, the effects of glu during PDA. Glu depolarized the membrane much faster and longer than during control and there was a distinct progressive inactivation of the action potentials. The arrow indicates 240 s after the beginning of the application of PDA. *C*, after a prolonged application of PDA glu depolarized the membrane for even longer periods and the accompanying action potentials were nearly completely inactivated. The arrows indicate 480 s after the beginning of the PDA application. *D*, partial recovery, the arrow indicates 460 s after the end of the PDA application.

quisqualate, on the cells showing plateaus (Fig. 5). On the cells which did not show plateaus the actions of none of the agonists were affected.

The antagonist *cis*-2,3-piperidine dicarboxylic acid (PDA), thought to discriminate less between the receptors for excitatory amino acids (Davies, Evans, Francis, Jones & Watkins, 1981; Salt & Hill, 1982), was tested on ten cells. PDA caused an

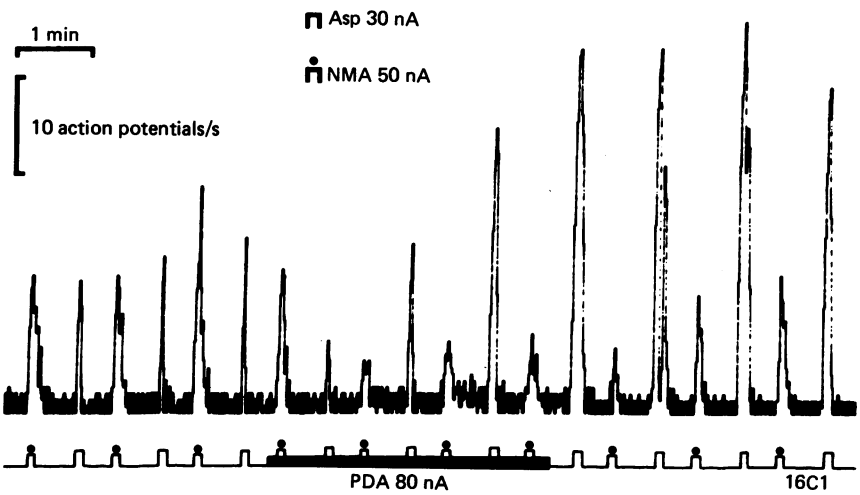

Fig. 7. Rate-meter record of a cell where PDA at low doses inhibited both asp and NMA. At high doses NMA remained inhibited while the excitations induced by asp were distinctly stronger.

augmentation of the effects of glu on three cells. It caused a slow depolarization of the membrane that however did not usually reach threshold for firing. The depolarizations induced by glu had a shorter rise time during the application of PDA so that the first action potentials induced by regular glu pulses occurred with a shorter latency after the pulse onset. If PDA was applied for sufficient durations it could provoke an inactivation of action potentials induced by glu (Fig. 6). If PDA was tested against NMA, asp or glu (five cells, all displaying plateaus to NMA) complicated interactions were seen: NMA was always inhibited and recovered slowly after the end of the application of PDA. Asp was blocked in three cells during the initial part of the PDA application, but was then strongly potentiated if the antagonist was applied for longer periods while the effects of NMA remained inhibited (Fig. 7).

Effects of agonists and antagonists on cortically evoked synaptic potentials. The cortico-caudate pathway was stimulated every 2 s which resulted in e.p.s.p.–i.p.s.p. sequences (Buchwald *et al.* 1973; Hull *et al.* 1973). Often however the i.p.s.p.s were not visible if cells were not depolarized by excitatory amino acids, presumably because their membrane potential at rest was near the equilibrium potential of the ion responsible for this i.p.s.p. (Herrling & Hull, 1980).

As expected, all agonists decreased the amplitude of the e.p.s.p.s and increased the amplitudes of the i.p.s.p.s. In this respect no difference was found between the effects of type 1 or 2 agonists (Fig. 8).

The NMDA antagonist DAA had no inhibitory effect on cortically evoked e.p.s.p.s even at currents of up to ten times those needed to totally antagonize the effects of NMA on the same cell and applied for several minutes (Fig. 9). These negative results

with DAA could however be due to the inability of ionophoretically released DAA to reach enough of the synapses responsible for this e.p.s.p. Therefore a further series of experiments was performed to determine if the cortically evoked e.p.s.p. is mediated by NMDA receptors. If this were the case then stimulation of the cortico-caudate pathway might result in plateau potentials if it is assumed that such synaptic receptors are of the same kind as those stimulated by ionophoretically

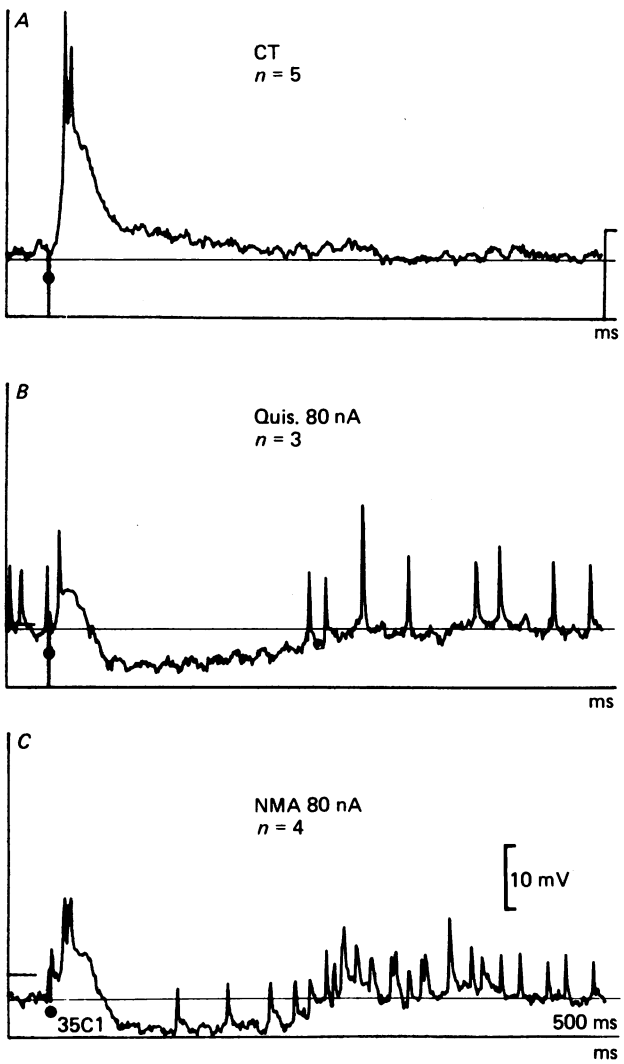

Fig. 8. The effects of quisqualate and NMA on computer averaged cortically evoked e.p.s.p.–i.p.s.p. sequences. *A*, control (CT), the membrane potential (*ca.* −60 mV) was slightly more negative than the equilibrium potential of the ion responsible for the i.p.s.p. *B*, during a quisqualate-induced depolarization (21 mV) the e.p.s.p. amplitude was distinctly reduced while the i.p.s.p. was now clearly visible as a hyperpolarization following the e.p.s.p. *C*, the e.p.s.p.–i.p.s.p. sequence was similarly affected by NMA induced depolarizations (26 mV).

applied NMA on the cells displaying plateaus. At stimulation frequencies of 0·5 Hz plateaus were never observed even at the maximal available current of 5 mA and pulse duration of 300 μs. If the precruciate cortex was stimulated with higher frequency trains (50 Hz, 0·5–5 mA, pulse length 100–300 μs, train duration 2–3 s), the e.p.s.p.s summated and this led to long-lasting depolarizations accompanied by repetitive firing, both of which could outlast the train by several seconds. This was done on six cells where the effects of NMA were not determined and on six other cells that were found to display plateaus upon application of NMA. In all cases no plateaus were seen during the synaptically evoked excitation (Fig. 10).

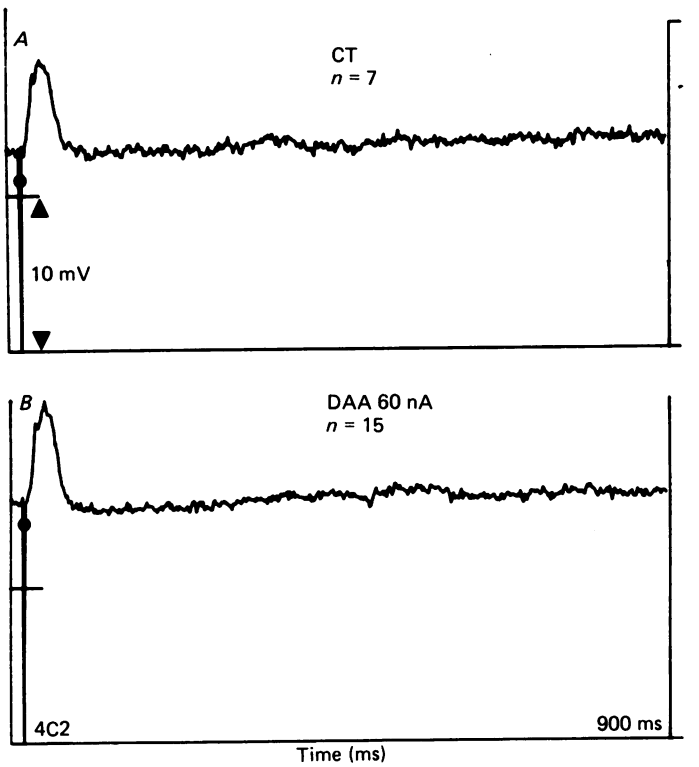

Fig. 9. The effect of DAA on cortically evoked e.p.s.p.– i.p.s.p. sequences averaged by computer. *A*, control (CT), here the membrane potential was near the equilibrium potential of the i.p.s.p. *B*, the e.p.s.p.–i.p.s.p. sequence during the application of DAA at a time when the effects of ionophoretically applied NMA were completely abolished. There was no evidence of an inhibition of the e.p.s.p. by DAA.

DISCUSSION

The plateau effects reported here have not been previously described on caudate cells. Other workers have applied NMA to different mammalian central neurones during intracellular recordings: In mouse spinal neurones grown in dissociated cultures excitatory amino acids produced depolarizations some of which looked similar to the type 2 effects described here (MacDonald & Wojtowicz, 1980, 1982).

However the pharmacology of these effects in dissociated spinal neurones was different from the one described here and the brain structure from which these cultured neurones originated could not be determined. In lumbar motoneurones recorded *in vivo* (Engberg, Flatman & Lambert, 1978; Lambert, Flatman & Engberg, 1981) the agonist effects of asp, glu, quisqualate and NMDA, amongst others, were also investigated and our results are in agreement concerning asp, which was attributed to the group that included glu and quisqualate as opposed to NMDA. In cerebellar Purkinje cells NMA seemed to be a much weaker agonist than quisqualate and no plateaus were reported (Crepel & Dhanjal, 1981).

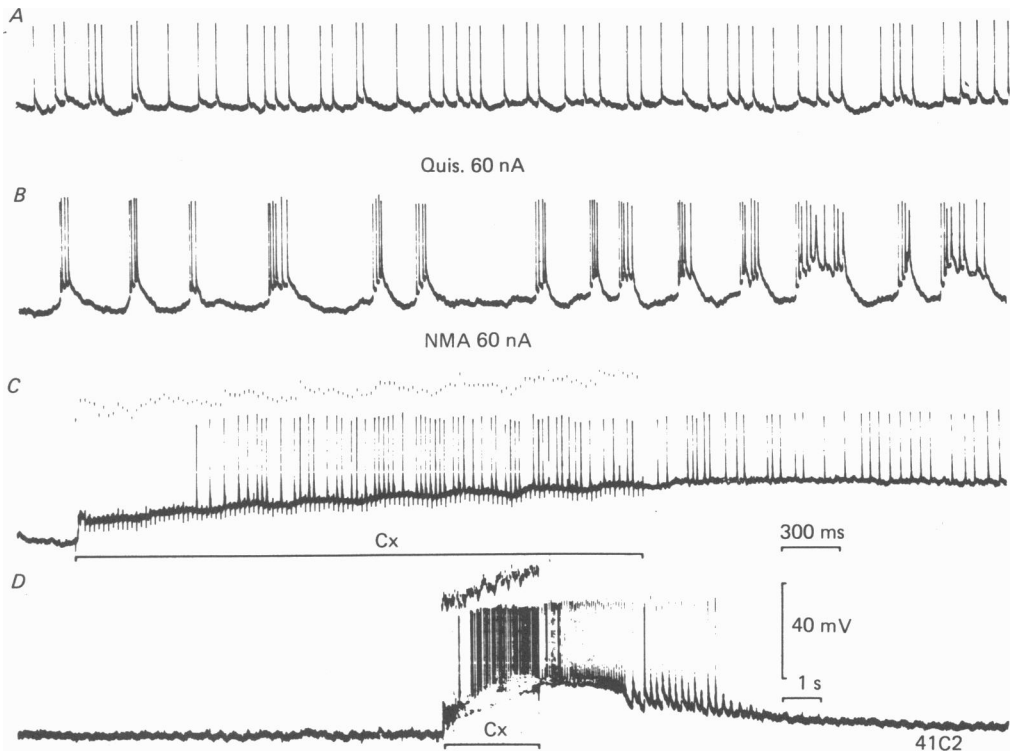

Fig. 10. Comparison of the effects of quisqualate, NMA and high frequency cortical stimulation on the membrane potential and firing pattern of a caudate neurone. *A*, regular firing induced by quisqualate. *B*, plateaus induced by NMA. *C*, regular firing and depolarization induced by a stimulation train at 50 Hz, 4 mA, 300 μs. The time calibration is the same for *A*, *B* and *C*. *D*, the effect of the same train shown in *C* at a faster recording speed. The depolarizing waves seen at the end of the repolarization phase are not plateaus but probably potentiated e.p.s.p.s as it can be seen that some of them do not have action potentials.

The only brain structure where plateau-like depolarizations have been seen is the hippocampus (unpublished observations of B. Gaehwiler in cultured hippocampal tissue and of B. Misbach & E. VanDeusen in hippocampal slices). In another study of hippocampal slices 'complex depolarizations' were seen upon the application of NMDA (Dingledine, 1981).

Selective antagonism by DAA of NMDA induced excitations was first demonstrated by Biscoe *et al.* (1977) on spinal neurones and has been repeatedly confirmed by other workers as reviewed by Watkins (1981).

The present results show that this selective inhibition of at least one type of NMA effect can also be found on neurones of the cat caudate nucleus. As quinolinate was equally effected by DAA in the caudate, our results support Stone & Perkins' (1981) conclusion from experiments in the cortex that quinolinate acts at NMDA receptors.

The observation that in about a third of the cells NMA and quinolinate elicited type 1 excitations that could not be blocked by DAA suggests that these two compounds can interact with a receptor different from the above described NMDA receptor. It is also possible that NMA and quinolinate are acting indirectly to release a transmitter stimulating type 1 receptors.

In earlier reports using extracellular recording techniques PDA was termed a broad spectrum antagonist of excitatory amino acids because it was shown to inhibit excitations elicited by NMDA, quisqualate and kainate in the spinal cord (Davies *et al.* 1981) and those of asp, glu, kainate, NMDA and quisqualate but not ACh on neurones of the caudal trigeminal nucleus (Salt & Hill, 1981, 1982). In the present study, PDA predominantly antagonized NMA, sometimes asp and never glu. Higher doses of PDA still blocked NMA but slightly depolarized the membrane potential and strongly augmented the effects of asp and glu. The reason for this discrepancy might be due to differences in receptor specificities of different types of neurones. However, because of the relatively small sample of cells that were tested with PDA in the caudate a more definite judgement on this point must await further experiments.

One stated aim of this study was to determine the nature of the receptors responsible for the cortically evoked e.p.s.p. The results presented above unfortunately provide only negative evidence. In our hands even large amounts of DAA over extended periods of time did not inhibit this e.p.s.p. while they clearly antagonized the effects of NMA on the same cells. This could be taken as an argument against the involvement of plateau-mediating NMDA receptors at this synapse, and this argument is further supported by our observation that high frequency stimulation of the cortico-caudate pathway generated depolarizations with repetitive firing but never plateaus.

There is one report where the effects of DAA were studied in the rat striatum (Stone, 1979). In agreement with our findings, this antagonist showed no selectivity towards asp and glu in 70% of the cells while it inhibited asp more than glu in the remaining ones and at high doses both asp and glu were blocked.

In contrast to our observations, Stone (1979) reported that cortically evoked action potentials could be inhibited by doses of DAA that also affected excitations induced by glu. The reason for this discrepancy might be that Stone used electrode assemblies where the distance between the tip of the recording electrode and the tips of the ionophoretic electrodes was from 2 to 10 times shorter than the intertip distance used in our experiments, resulting in higher DAA concentrations around the recorded neurones. Such higher concentrations of DAA might also result in a reduction of the specificity of the antagonist.

In conclusion, the results presented here provide evidence that excitatory amino acids can activate cat caudate neurones by several mechanisms. The first, as yet

poorly characterized mechanism, consists of depolarizations associated with regular firing elicited by NMA and quinolinate and not antagonized by DAA. The second, depolarizations associated with regular firing, elicited by glu and quisqualate might involve quisqualate receptors similar to those described by Watkins (1981) because of the matching agonist specificity. The third, and best characterized mechanism, which could be blocked by DAA and PDA, is the one where NMA elicited depolarizations associated with plateaus. This type of excitation was probably due to the stimulation of NMDA receptors similar to those described by Watkins (1981) in view of the similar pharmacology with respect to agonists and antagonists. It is also noteworthy that this receptor can also be stimulated by an endogenous substance, quinolinic acid and that it is probably not the receptor responsible for the cortically evoked monosynaptic e.p.s.p. in cat caudate neurones.

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