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Effects of glutamate antagonists on the activity of aromatic L-amino acid decarboxylase

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Summary. This study examines the hypothesis that glutamate tonically suppresses the activity of the enzyme aromatic L-amino acid decarboxylase (AADC), and hence the biosynthesis of dopamine, to explain how antagonists of glutamate receptors might potentiale the motor actions of L-DOPA in animal models of Parkinson's disease. A variety of glutamate antagonists were therefore administered acutely to normal rats, which were sacrificed 30–60 min later and AADC activity assayed in the substantia nigra pars reticulata (SNr) and corpus striatum (CS). The NMDA receptor-ion channel antagonists MK 801, budipine, amantadine, memantine and dextromethorphan all caused a pronounced in creased in AADC activity, more especially in the SNr than CS. The NMDA glycine site antagonist (R)-HA 966 produced a modest increase in AADC activity in the CS but not SNr, whilst the NMDA polyamine site antagonist eliprodil, the NMDA competitive antagonist CGP 40116 and the AMPA antagonist NBQX were without effect. The results suggest that an increase in dopamine synthesis might contribute to the L-DOPA-facilitating actions of some glutamate antagonists.

Keywords: L-DOPA – Aromatic L-amino acid decarboxylase – Glutamate antagonists – Substantia nigra – Corpus striatum

The discovery of hyperactive glutamatergic neurones in the basal ganglia, secondary to dopamine loss from the nigrostriatal pathway, has profound implications for sufferers of Parkinson's disease. Not least because it offers hope for the first time in over 30 years that alternative drug treatments of Parkinson's disease may be found, which do not involve dopamine replacement therapy. Early excitement concerning the antiparkinsonian potential of glutamate antagonists, however, has been short-lived. Selective antagonists of the NMDA and AMPA subtypes of the glutamate receptor, have a variable and limited capacity to restore motor activity in rodent and primate models of parkinsonism (see Starr, 1995a; b for reviews). Severe motor and other side effects are common, reflecting the importance of glutamate as an excitatory neurotransmitter throughout the brain.

This does not mean that we will have to abandon any idea of using glutamate antagonists to treat Parkinson's disease. Monotherapy with these drugs may still be possible when the exact stoichiometry of the subunits that make up the various subpopulations of NMDA receptor has been worked out. Corticostriatal neurones appear to mediate excitation of striataopallidal output cells via NR2B-containing NMDA receptors, so that NR2B antagonists could prove to be highly selective antiparkinsonian agents with fewer side effects. Alternatively, since the neurodegenerative process that results in the demise of nigrostriatal neurones is believed to involve glutamate toxicity, compounds which block glutamate might prove useful in preventing or retarding the death of dopamine neurones (see Sonsalla et al., this issue).

A completely different approach might be to use glutamate antagonists in combination with dopamine agonists. It is evident that competitive and non-competitive NMDA receptor blockers interact with dopamine D_1 and D_2 agonists in complex and highly unpredictable ways (Starr, 1995a), whereas many investigators have demonstrated unequivocally that glutamate antagonists can potentiate the antiparkinsonian efficacy of L-DOPA (reviewed in Starr, 1995b). How they do this is still not clear, although we recently found that the extracellular concentration of dopamine formed from a threshold dose of L-DOPA, in the substantia nigra pars reticulata (SNr) of reserpine-treated rats, was markedly increased in the presence of MK 801, CGP 40116 or HA 966, at doses of the antagonists which did not release dopamine by themselves (Biggs et al., 1996). We have therefore considered the possibility that blocking the excitatory action of glutamate in the nigra, results in an increased bioconversion of L-DOPA to dopamine, by activating the enzyme aromatic L-amino acid decarboxylase (AADC) (Hadjiconstantinou et al., 1995). The present work tests out this hypothesis by assaying the activity of AADC in the corpus striatum (CS) and SNr of normal rats, following pretreatment with an assortment of NMDA and AMPA antagonists.

Methodology

Male Wistar albino rats weighing 240–360 g were injected with glutamate antagonists i.p. and sacrificed 0.5 h (NBQX) or 1 h later (other drugs). Control animals received injections of vehicle (1 ml/kg i.p.). The brains were rapidly removed and placed into ice-cold saline and the CS and SNr dissected out. The tissues were homogenised in 0.25 M ice-chilled sucrose and centrifuged for 10 min. Aliquots (20 µl) of the supernatant were added to an incubation mixture comprising: 50 mM sodium phosphate buffer (pH 7.2), 0.5 mM L-DOPA, 10 µM pyridoxal-5'-phosphate, 0.1 mM EDTA, 0.17 mM ascorbic acid, 1 mM pargyline and 1 mM mercaptoethanol (total volume 400 µl), and incubated for 20 min at 37°C. The reaction was stopped by the addition of 80 µl ice-cold 0.5 M perchloric acid, containing isoprenaline as internal standard. The concentration of dopamine in the mixture was quantified by HPLC with electrochemical detection and protein by the method of Lowry et al. (1951). AADC activity was expressed as nmol dopamine/mg protein/20 min, after correcting for tissue levels at zero time and recovery. Data were analysed by one way ANOVA and group differences were evaluated by Newman Keuls test.

Results

Basal enzyme activity was found to be 19.7 ± 1.0 nmol dopamine/mg protein/20 min for CS, and 22.0 ± 1.9 for SNr (means ± SEM of 6 determinations). MK 801 at 0.01 mg/kg, was without effect on behaviour and AADC activity. However, at 0.1 mg/kg, MK 801 stimulated hyperlocomotion, and this was accompanied by 97.5% and 230.5% increases in AADC activity in the CS and SNr respectively at 1 h posttreatment (Fig.1). A higher dose of MK 801 (1 mg/kg) induced severe ataxia and hypoactivity, together with lesser increases in corresponding AADC activity (56.8% in CS, 113.6% in SNr; Fig. 1).

Since MK 801 has a high affinity for the cation channel associated with the NMDA receptor, we next tested a number of antiparkinsonian agents purported to have a low affinity for this site. These were all tested at the fixed dose of 40 mg/kg i.p. (12.5 mg/kg for budipine), which is normally sufficient to induce a modest increase in locomotion in normal rats. Figure 2 indicates that amantadine, memantine, dextromethorphan and budipine all evoked a pronounced increase in AADC activity in the CS and SNr. Save for memantine, these increases were considerably larger in magnitude in the SNr than in the CS, as occurred with MK 801. The response to dextromethorphan suggested the existence of responders and non-responders (data only for the responders are shown).

To determine whether other NMDA and AMPA antagonists were similarly effective in this regard, we also tested the NMDA glycine site antagonist (*R*)-HA 966 (5 mg/kg), the NMDA polyamine site antagonist eliprodil (10 mg/kg), the competitive NMDA antagonist CGP 40116 (5 mg/kg), and the AMPA antagonist NBQX (10 mg/kg). Apart from a modest rise in AADC activity in the CS of 45.7% detected after (*R*)-HA 966, no other changes in AADC activity were observed with these drug treatments.

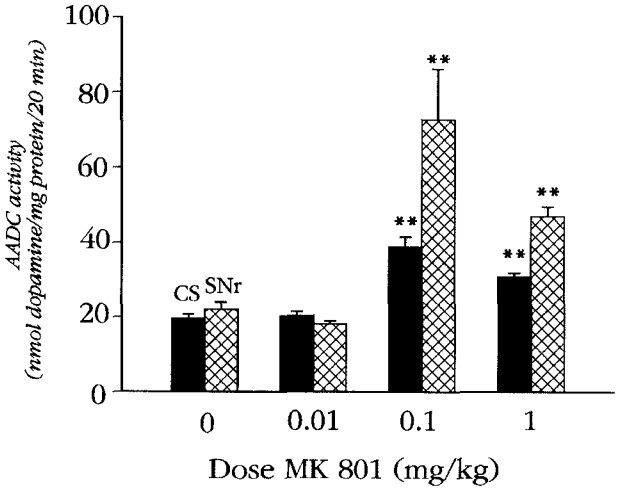

Fig. 1. Effects of MK 801 on levels of AADC in the corpus striatum (*CS*) and substantia nigra pars reticulata (*SNr*) of rats. Treatments were administered i.p. and the animals sacrificed 1 h later. CS, solid bars; SNr, hatched bars. Results are means + S.E.M. of at least 6 experiments. ** $p < 0.001$ versus controls

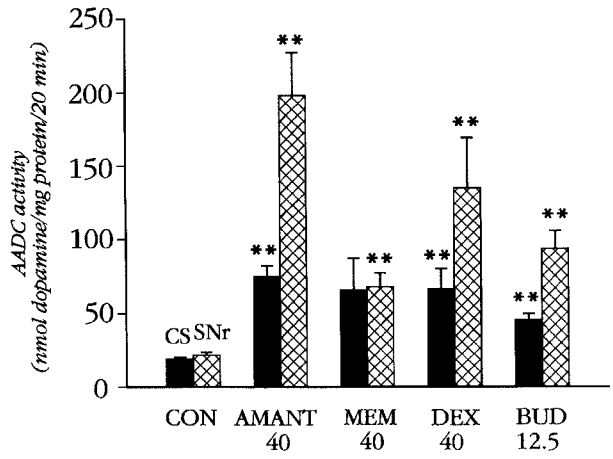

Fig. 2. Effects of low affinity NMDA-channel blockers on AADC activity in corpus striatum (*CS*) and substantia nigra pars reticulata (*SNr*) of rats. All drugs were injected i.p. 1 h before sacrifice. *CON* Controls; *AMANT* amantadine; *MEM* memantine; *DEX* dextromethorphan; *BUD* budipine; CS solid bars; SNr hatched bars. Results are means + S.E.M. of at least 6 experiments. ** $p < 0.001$ versus controls

Discussion

The results of this study demonstrate quite clearly that antagonists of the phencyclidine binding site located within the ion channel associated with the NMDA receptor, exert a remarkable acute facilitatory effect on the activity of the enzyme AADC. This effect is almost invariably more pronounced in the SNr than in the CS, and provides further evidence that the decarboxylation step in the biosynthesis of dopamine may also be subject to important physiological control. Earlier work has revealed that AADC activity is suppressed by dopamine agonists and increased by dopamine antagonists, with similar effects being mediated by dopamine D_1 and D_2 receptors (Hadjiconstantinou et al., 1993). These authors argued in favour of an autoregulatory control of AADC by dopamine released into the synapse. Interestingly, the maximum increases they saw in the CS were no greater than 41% (with SCH 23390 treatment), which is comparable to the 46.2% (their study) and the 56.8% increase (our study) obtained in the CS after 1 mg/kg MK 801. These modest changes consequently make the 800% increase in AADC activity we saw in the SNr after amantadine all the more remarkable. Our data suggest that AADC is considerably more susceptible to modulation by glutamate antagonists than by dopamine antagonists, and that this modulatory influence is more keenly felt in the somatodendritic (nigral) than in the axon terminal (striatal) region of the dopamine neurone.

In recent years there has been a re-evaluation of the role of AADC in dopamine synthesis and the factors that control it (for reviews see Opacka-Juffry and Brooks, 1995; Zhu and Juorio, 1995). Zhu et al. (1992) have reported two mechanisms for increasing AADC activity via dopamine autoreceptors – an early (30 min) and a late (2 h) induction. The former represents

an increase in activity of the existing enzyme (phosphorylation induction?), whereas the latter corresponds to a synthesis of new enzyme protein, just like the mechanisms that determine the activity of tyrosine hydroxylase. The fact that antipsychotic treatment increased the brain content of AADC mRNA, but not that of tyrosine hydroxylase mRNA, suggested to Buckland et al. (1992) that AADC may be more important than tyrosine hydroxylase for the long term regulation of dopamine synthesis.

These speculations only apply to the normal brain, where the starting point for dopamine synthesis requires the sequential hydroxylation and decarboxylation of tyrosine. In Parkinson's disease the situation is different, since the administration of large quantities of L-DOPA depends solely upon AADC for its bioconversion to dopamine. Here, the activity of AADC is paramount in determining the rate and amount of dopamine that can be formed. Of course, the loss of dopamine neurones will mean that AADC activity is reduced in this compartment, but this loss is more than compensated by the presence of AADC in tryptaminergic neurones and glia, which may also act as a source of dopamine in the parkinsonian brain (Opacka-Juffry and Brooks, 1995). Nevertheless, the continued administration of L-DOPA to experimental animals, to simulate the chronic treatment of Parkinson's disease with this drug, results in a down-regulation of AADC in the brain (Hadjiconstantinou et al., 1993). It is possible, therefore, that the combination of AADC loss in dopamine neurones and reduced activity of the remaining enzyme, contribute to the gradual loss of efficacy of L-DOPA as an antiparkinsonian treatment.

We have yet to show that glutamate receptor blockade affects the amount as well as the activity of AADC. If it does, then one potential use of glutamate antagonists in the therapy of Parkinson's disease may be as an adjunct to L-DOPA, to prevent the down-regulation of AADC by L-DOPA. In fact, clinicians may already unwittingly be doing just this when they administer amantadine to boost the efficacy of L-DOPA. The aminoadamantane class of drugs has provided us with two important antiparkinsonian agents, amantadine and memantine, whose mechanism of action has eluded us. The fact that they are both weak NMDA channel blockers (Kornhuber et al., 1991), and both strongly potentiate AADC activity, could be how they work in the clinic. The same applies to the newly licensed drug budipine, which is also a weak glutamate antagonist (Jackisch et al., 1994), and to dextromethorphan, which is antiparkinsonian in some patients and not others (Bonuccelli et al., 1992; Montastruc et al., 1994). The latter finding is particularly interesting, given that dextromethorphan stimulates both motor activity (Kaur and Starr, 1995) and AADC activity (see Fig. 2 above) in only a fraction of animals. Thus it would appear that there are two populations of animals and patients – responders and non-responders to dextromethorphan.

As already indicated above, the precise site(s) of decarboxylation of L-DOPA to dopamine in the parkinsonian brain is unknown. Our finding that NMDA antagonists exerted a greater effect over AADC activity in the SNr than in the CS, is congruent with the growing belief that dopamine replacement with L-DOPA in parkinsonism, is possibly more important in the nigra than in the striatum. This is because the time course of the motor stimulation

afforded by L-DOPA in 6-hydroxydopamine-hemilesioned rats, more closely matches the appearance of dopamine in nigral dialysates than it does in striatal dialysates (Orosz and Bennett, 1992). Furthermore, the present data may indicate a greater hyperactivity of glutamatergic inputs to the SNr (e.g. from the subthalamic nucleus) than to the striatum (e.g. from the cortex), both of which contribute to the neuropathology of Parkinson's disease.

Finally, it is interesting to note that not all of the glutamate antagonists we tested were able to modulate AADC activity. There was a small rise in striatal AADC after (*R*)-HA 966, but otherwise only the NMDA channel blockers were active in these experiments. This could mean that a subpopulation of NMDA receptors is coupled to AADC, which are insensitive to competitive blockade, or to non-competitive blockade via the glycine or polyamine regulatory sites. This finding means that the ability of HA 966 and CGP 40166 to facilitate the release of dopamine newly formed from L-DOPA in dialysis experiments, may be unrelated to an increase in the rate of decarboxylation, and that we have to look for an alternative mechanism of action (Biggs et al., 1996). The inactivity of NBQX further suggests that AMPA receptors are not linked to AADC.

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