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NMDA receptor antagonists increase the release of dopamine in the substantia nigra of reserpine-treated rats

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Abstract

Microdialysis of the substantia nigra pars reticulata in freely moving rats disclosed a steady release of dopamine and its metabolites which was greatly reduced after reserpine (4 mg/kg s.c.) and α-methyl-*p*-tyrosine (200 mg/kg i.p.) pretreatments. Local infusion of high K^+ (100 mM) or L-3,4-dihydroxyphenylalanine (L-DOPA, 10 μM) significantly increased dialysate levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), but not homovanillic acid (HVA) in this model. Intranigral application of the non-competitive NMDA receptor antagonist dizocilpine (150 nM), or the competitive NMDA receptor antagonist *R*-DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate (CGP 40116, 10 μM), via the dialysis probe, did not affect the release of dopamine or its metabolites in intact rats, but further suppressed these releases in reserpine plus α-methyl-*p*-tyrosine-treated animals. When the same amounts of dizocilpine or CGP 40116 were coinfused with L-DOPA, however, they potentiated the recovery of dopamine 12–24 times, and of DOPAC 5–10 times (but not HVA), as well as producing detectable behavioural arousal. The facilitation of dopamine formation from L-DOPA by NMDA receptor antagonists in the substantia nigra pars reticulata could explain the enhancement of L-DOPA's antiparkinsonian activity by these compounds in behavioural experiments.

Keywords: Glutamate receptor antagonist; Dopamine; Reserpine; Parkinsonism; Microdialysis

1. Introduction

The observation that glutamate receptor antagonists can potentiate the antiakinetic action of L-3,4-dihydroxyphenylalanine (L-DOPA) in dopamine-depleted rodents and primates has important implications for our appreciation of the aetiology and treatment of Parkinson's disease (for review see Starr, 1995). Not only does it lend weight to the notion that glutamatergic hyperactivity is a neuropathological feature of parkinsonism (Bergman et al., 1990), but it also supports the hypothesis that glutamate receptor blockers might be used to improve the efficacy of dopamine replacement therapy (Klockgether and Turski, 1989). The mechanism of this motor synergism, however, has not yet been satisfactorily explained.

It is assumed that L-DOPA increases motility through its enzymatic conversion to dopamine, which is then liberated to elicit an interactive motor response by the combined stimulation of postsynaptic dopamine D_1 and D_2 receptors (for review see Clark and White, 1987). Behavioural experiments with selective agonists of these receptors reveal that impairment of glutamate transmission at NMDA receptors facilitates motor responding to dopamine D_1 receptor stimulation, but strongly inhibits motor responding to dopamine D_2 receptor stimulation (Goodwin et al., 1992; Morelli et al., 1992). Thus while an increase in the dopamine D_1 receptor-dependent component of locomotion by NMDA receptor antagonists could conceivably contribute to their potentiation of L-DOPA, it is unlikely that this is the only mechanism.

Further support for this argument is provided by behavioural data obtained with the non-competitive NMDA receptor-channel blocker dizocilpine. In reserpine-treated rats and mice, dizocilpine has been observed to increase the locomotor response to L-DOPA, at doses which are considerably smaller than those required to enhance motor responding to the dopamine D_1 receptor-selective agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine hydrochloride (SKF 38393; i.e. 0.00625 mg/kg versus 0.4 mg/kg; Kaur et al., 1994; Klockgether and Turski, 1990). To explain this dose discrepancy, we postulated that very low doses of dizocilpine might enhance the functional cooperativity that normally exists between dopamine D_1

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and D_2 receptors, since this will clearly be an important factor as far as the animal's responsiveness to L-DOPA is concerned. If anything, however, we found the reverse was true, since dizocilpine strongly suppressed the combined effects of dopamine D_1 and D_2 receptor stimulation, when this was applied in the form of the mixed dopamine D_1/D_2 receptor agonist apomorphine, or as a mixture of selective dopamine D_1 and D_2 receptor agonists (Starr and Starr, 1993a, b). On the basis of current evidence, therefore, it is not possible to reconcile these behavioural data in terms of a simple interaction between glutamate receptor antagonists and dopamine newly formed from L-DOPA, at postsynaptic receptor sites.

The present study consequently considers an alternative possibility, which is that dizocilpine and other such compounds act presynaptically, to enhance the biotransformation of L-DOPA and/or release of dopamine. This process was monitored by including the relevant drugs in the solution perfusing microdialysis probes implanted in the reticular segment of the substantia nigra. The nigra was chosen as an investigational site for several reasons. Firstly, it receives a putative glutamatergic input from the subthalamic nucleus, which becomes overactive under parkinsonian conditions (Bergman et al., 1990). Secondly, dizocilpine evokes a robust locomotor response from the dopamine-depleted nigra (St-Pierre and Bédard, 1994). Thirdly, the nigra has been shown to be a major site of L-DOPA decarboxylation and motor stimulation in the parkinsonian brain (Orosz and Bennett, 1992; Robertson and Robertson, 1989; Robertson et al., 1991). And lastly, recent evidence suggests the substantia nigra pars reticulata may play a role in the positive behavioural and biochemical interactions that occur between glutamate receptor antagonists and dopamine D_1 receptor agonists in the 6-hydroxydopamine model of parkinsonism (Fenu et al., 1995). We shall present evidence to show that the release of dopamine newly synthesised from L-DOPA in the substantia nigra pars reticulata is greatly exaggerated by behaviourally relevant concentrations of competitive and non-competitive antagonists of the NMDA receptor.

2. Materials and methods

2.1. Drugs

Chloral hydrate, monoamine standards, L-3,4-dihydroxyphenylalanine (L-DOPA), reserpine and DL-α-methyl-*p*-tyrosine methyl ester (α-MTP) were all obtained from Sigma, UK. Dizocilpine maleate was purchased from Research Biochemicals International, UK and *R*-DL-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoate (CGP 40116) was a gift from Ciba Geigy, Basle. α-MPT was dissolved in 0.9% physiological saline with gentle warming. Reserpine was first dissolved in a minimum quantity of warmed glacial acetic acid and made up to volume with saline. Both drugs were injected in a volume of 0.1 ml/100 g body weight. L-DOPA, dizocilpine and CGP 40116 were all dissolved to the required concentration in artificial cerebrospinal fluid (ACSF) prior to infusion (see below).

2.2. Animals and surgery

All surgical and experimental procedures were conducted in accordance with the Animals (Scientific Procedures) U.K. Act, 1986. Male Wistar rats (A.R. Tuck), weighing 220–260 g, were initially group housed at 22 ± 1°C, under fluorescent lighting from 07.00–17.00 h, and allowed free access to rat diet and water. Rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and fixed in a stereotaxic frame (Kopf). Concentric microdialysis probes, 2 mm active membrane length and 0.2 mm external diameter, constructed in our laboratory as described previously (Whitton et al., 1992a), were implanted bilaterally into the substantia nigra pars reticulata (stereotaxic coordinates in mm: A −5.2 from bregma, L 2.2 from midline and V 8.3 below dura – see König and Klippel, 1963). Dialysis probes were secured to the skull with dental acrylic cement (Duralay). Animals were then placed individually in Perspex experimental cages. Following recovery from anaesthesia, rats were injected either with reserpine (4 mg/kg s.c.) to deplete brain monoamines, or with vehicle (monoamine-intact controls). Groups of 8 rats were used for each experimental protocol.

2.3. Microdialysis

Microdialysis was performed 18 h after probe implantation and reserpine injection. Probes were perfused for a 30 min equilibration period, at a rate of 0.5 μl/min, with ACSF of the following composition (mM): NaCl 125, KCl 2.5, $MgCl_2$ 1.18 and $CaCl_2$ 1.26 (pH 7.0). Thirty-minute dialysates were then collected for 90 min, after which time α-methyl-*p*-tyrosine (200 mg/kg i.p.) was administered and a further two samples collected. At this point, the perfusion fluid was substituted for one containing L-DOPA (10 μM) and/or dizocilpine (150 nM) or CGP 40116 (10 μM) for 60 min, or high K^+ (100 mM) for 30 min, before returning to drug-free ACSF for the remainder of the experiment. High K^+ ACSF was made by substituting KCl for NaCl. Doses of drugs were calculated on the basis of their systemic efficacy in earlier behavioural experiments (Kaur et al., 1994; Starr and Starr, 1993a).

A similar protocol was observed in a separate set of experiments, in which monoamine-intact rats received saline instead of α-methyl-*p*-tyrosine, followed by dizocilpine (150 nM) or CGP 40116 (10 μM) alone. In all experiments changes in the animals' behaviour were noted, but were not quantified.

2.4. Histology

Dialysis probe placements within the substantia nigra pars reticulata were verified histologically. At the termina-

tion of each experiment, animals were injected with sodium pentobarbitone (200 mg/kg i.p.; Expiral, Sanofi) and perfused transcardially with phosphate-buffered formol saline (pH 7.2). After 120 min, the brains were carefully removed, sectioned coronally and probe tracks determined by low-power binocular microscopy.

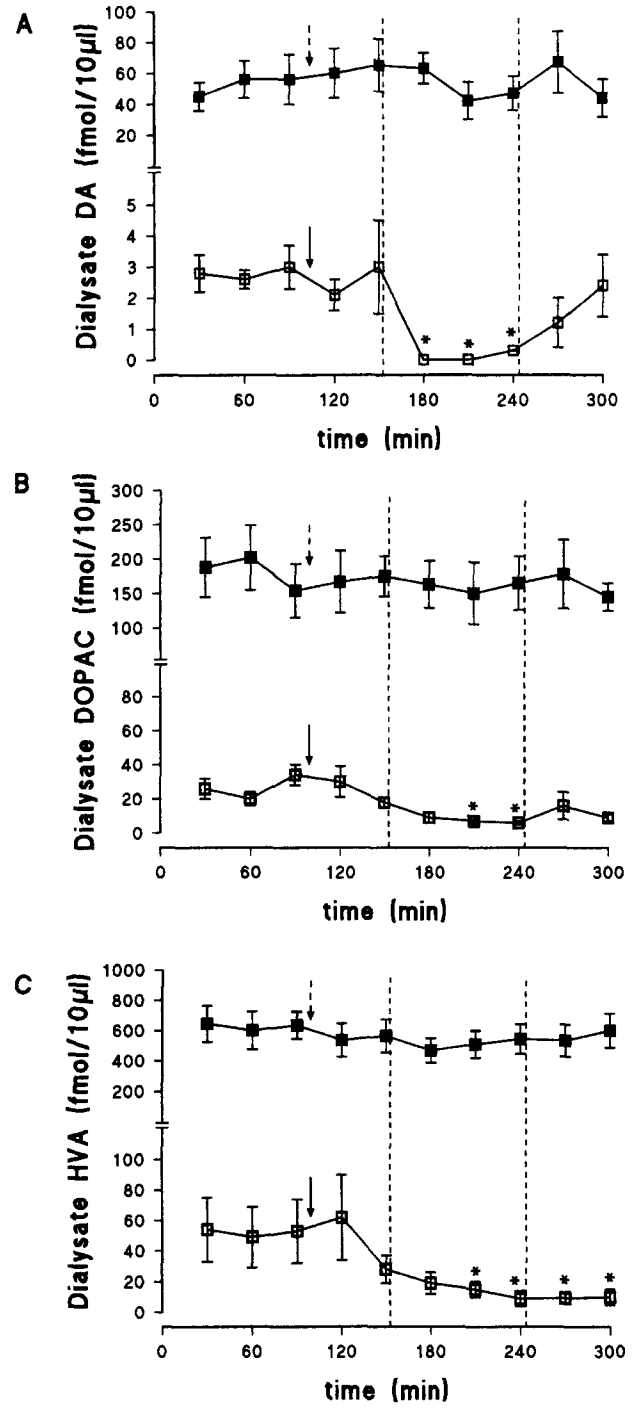

Fig. 1. Effect of dizocilpine on basal nigral dialysate levels of dopamine and metabolites. After implanting the dialysis probes bilaterally in the substantia nigra pars reticulata, rats were injected with either reserpine (4 mg/kg s.c., open squares) or saline (filled squares) and dialysis performed 18 h later (0.5 µl/min). Levels of dopamine, DOPAC and HVA are the means ± S.E.M. of 8 determinations. Saline (0.1 ml/100 g i.p.) was injected at the broken arrow, whilst α-methyl-*p*-tyrosine (200 mg/kg i.p.) was injected at the solid arrow. Vertical broken lines denote the start and finish of infusion with dizocilpine (150 nM). * $P < 0.05$ versus corresponding 150 min sample.

2.5. Assay of dopamine and metabolites

Dialysates were analysed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

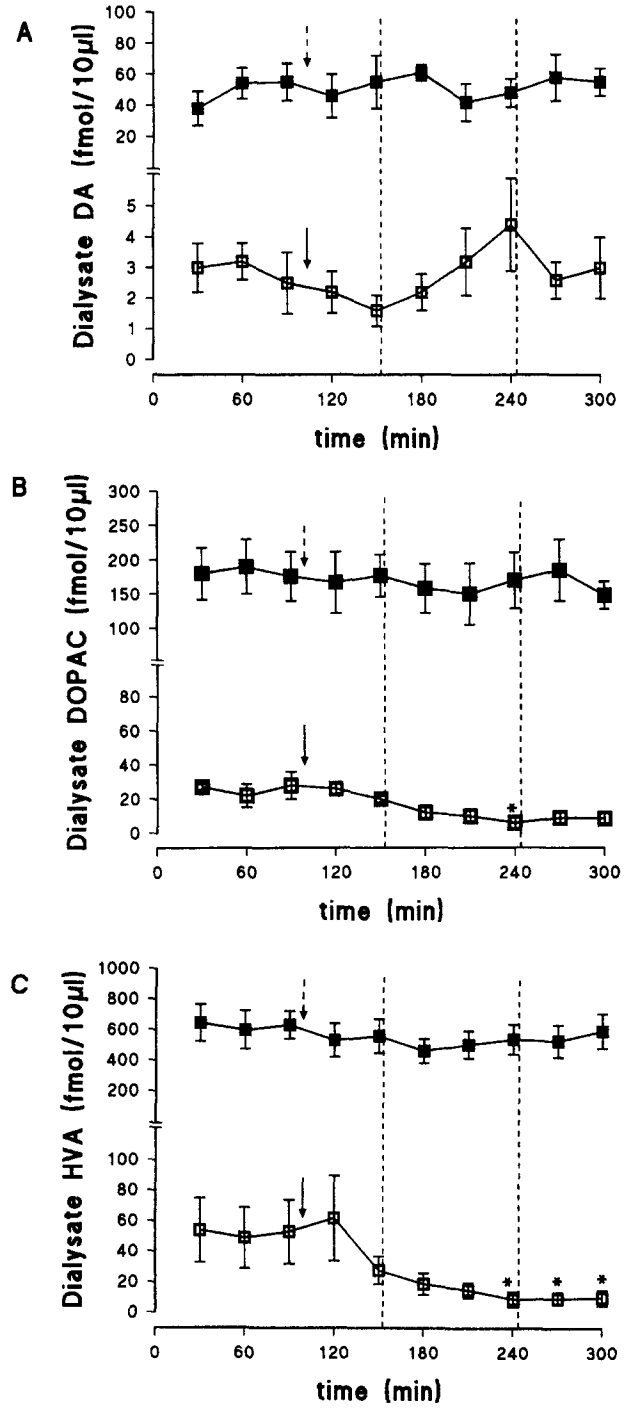

Fig. 2. Effect of CGP 40116 on basal nigral dialysate levels of dopamine and metabolites. Experimental details as for Fig. 1, except that vertical broken lines denote the start and finish of CGP 40116 (10 µM) infusion. * $P < 0.05$ versus corresponding 150 min sample.

using high-performance liquid chromatography with electrochemical detection (HPLC-ED). Measurements were generally carried out on the same day as collection, otherwise samples were stored at −80°C for a maximum of 2 days prior to analysis. The HPLC-ED system comprised a solvent delivery module (CMA model LC 250) and a C_{18}

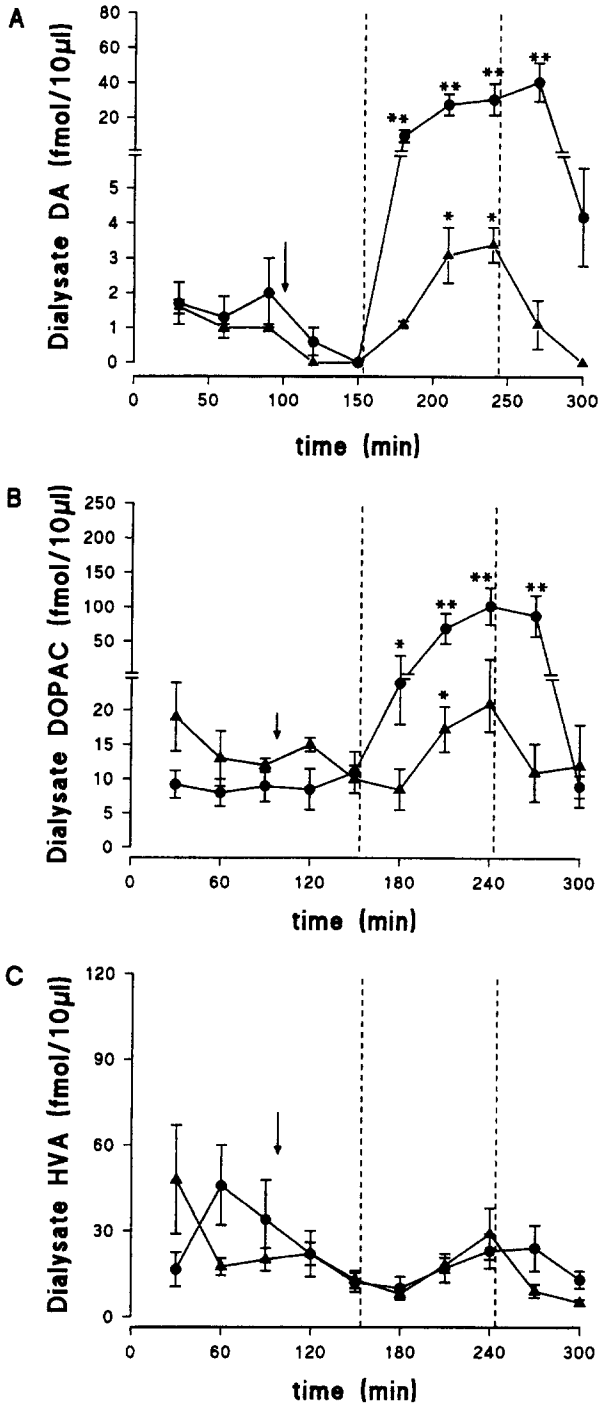

Fig. 3. Effect of dizocilpine on L-DOPA-induced increases in dopamine and metabolites in nigral dialysates. Animals were implanted with bilateral intranigral dialysis probes, injected with reserpine (4 mg/kg s.c.) and taken for dialysis 18 h later (0.5 µl/min). α-Methyl-*p*-tyrosine (200 mg/kg i.p.) was injected at the solid arrow. Between the vertical broken lines, rats were infused with L-DOPA (10 µM; solid triangles), or with L-DOPA (10 µM) plus dizocilpine (150 nM; solid circles). * $P < 0.05$, ** $P < 0.01$ versus corresponding 150 min sample (L-DOPA alone), or versus L-DOPA (L-DOPA plus dizocilpine). Each point is the mean ± S.E.M. of 8 determinations.

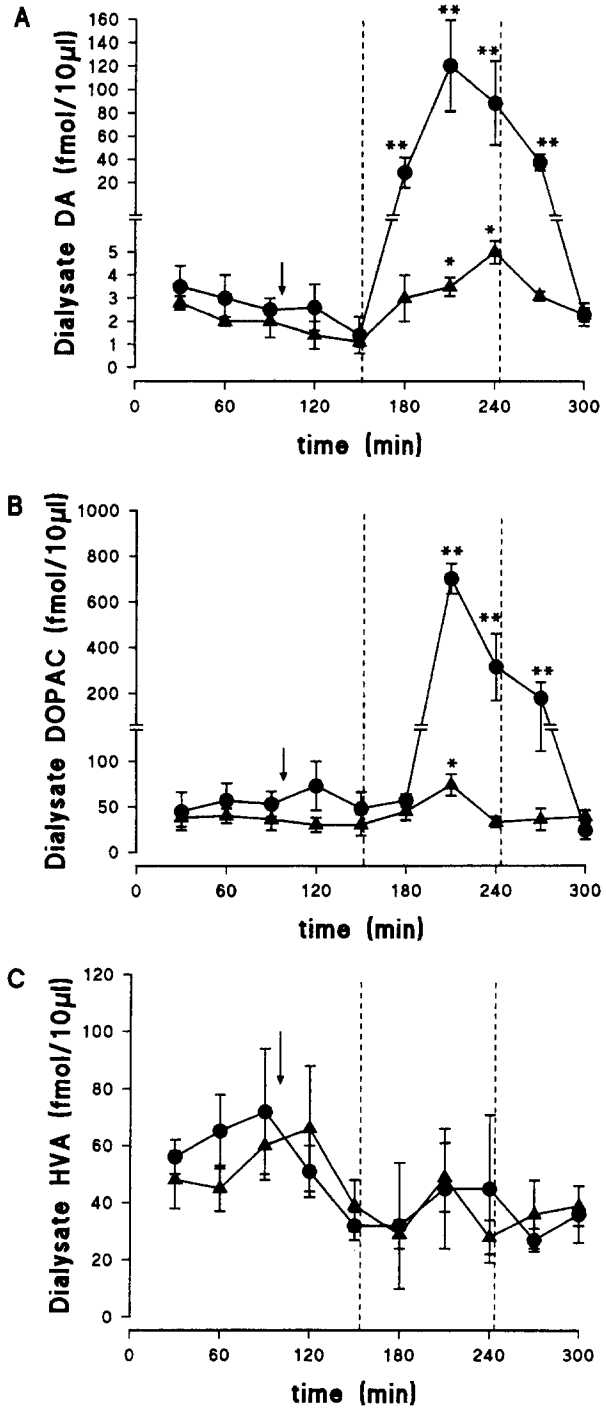

Fig. 4. Effect of CGP 40116 on L-DOPA-induced increases in dopamine and metabolites in nigral dialysates. Experimental details as for Fig. 3, except that vertical broken lines denote the start and finish of infusion with L-DOPA (10 µM; solid triangles) and L-DOPA (10 µM) plus CGP 40116 (10 µM; solid circles). * $P < 0.05$, ** $P < 0.01$ versus corresponding 150 min sample (L-DOPA alone), or versus L-DOPA (L-DOPA plus CGP 40116). Each point is the mean ± S.E.M. of 8 determinations.

reverse phase ODS column (3 μm particle size), coupled to a high-performance analytical cell (ESA, model 5014) with electrochemical detector (ECD, Coulochem 2). An isocratic separation was used as described previously (Biggs et al., 1992). Mobile phase (which was recirculated) consisted of (mM): sodium acetate 90, citric acid 35, EDTA 0.34 and sodium octylsulphonic acid 0.06 dissolved in 11% v/v methanol (pH 4.2). All mobile phase reagents were of analytical grade and were supplied by Fluka, Germany. Samples (10 μl) were processed automatically using a refrigerated autosampler (CMA model 200), coupled to the system injection port. ECD settings were as follows: electrode 1 set at −175 mV, electrode 2 set at +400 mV, channel 2 output set at 20 nA for a full-scale deflection. Data capture was achieved using a computer equipped with Drew Scientific chromatography software. The limit of detection for dopamine in substantia nigra pars reticulata dialysates was approximately 1 fmol/10 μl. Dialysates were not corrected for in vitro recovery.

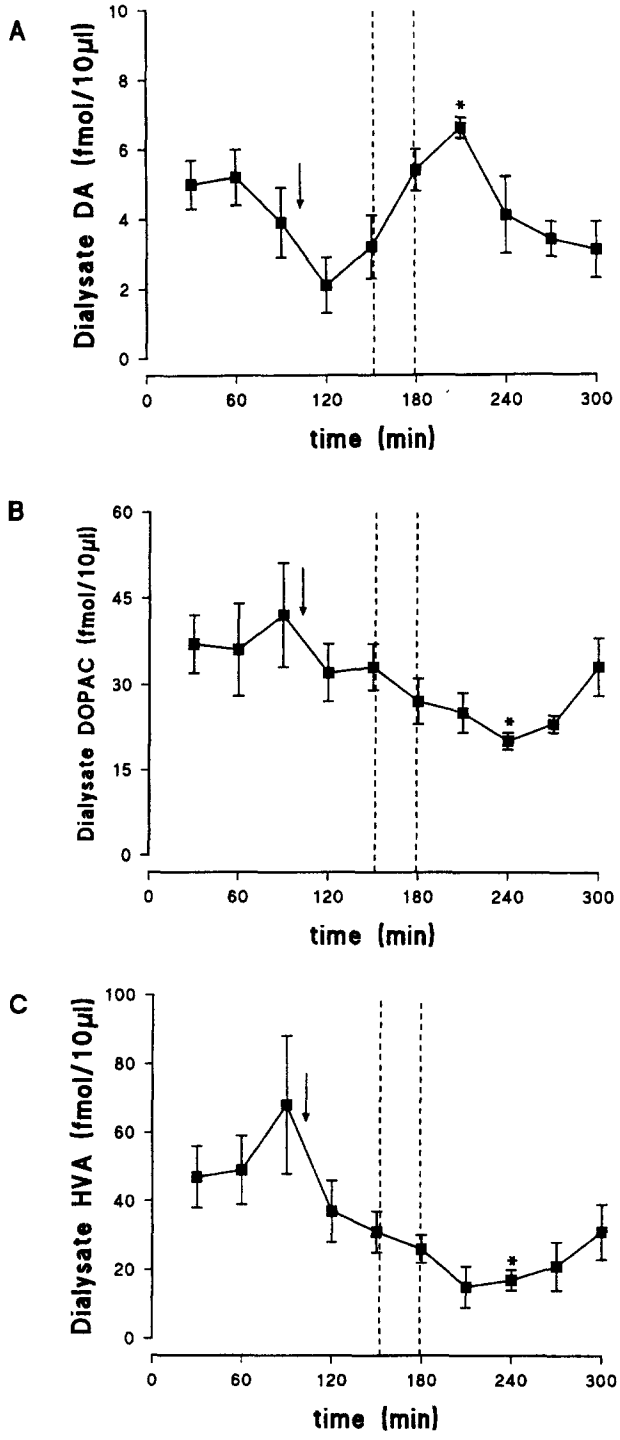

Fig. 5. Effect of high K^+ on nigral dialysate levels of dopamine and metabolites. Experimental details as for Fig. 3, except that ACSF containing high K^+ (100 mM) was applied between the vertical broken lines. * $P < 0.05$ versus 150 min sample.

2.6. Data analysis

All quantities of dopamine, DOPAC and HVA were calculated as fmol/10 μl dialysate. The Mann-Whitney *U*-test was used to compare analyte concentrations in dialysates prior to and after drug infusions, to determine statistical significance ($P < 0.05$).

3. Results

3.1. Effects of systemic reserpine and α-methyl-p-tyrosine

In monoamine-intact rats, baseline substantia nigra pars reticulata dialysate levels averaged 48 ± 10, 178 ± 39 and 650 ± 89 fmol/10 μl for dopamine, DOPAC and HVA respectively ($n = 8$). After 18 h pretreatment with reserpine (4 mg/kg s.c.), these levels were reduced markedly to 2.7 ± 0.8, 21 ± 5 and 34 ± 15 fmol/10 μl for dopamine, DOPAC and HVA respectively (Figs. 1 and 2). Acute administration of a single, high dose of α-methyl-*p*-tyrosine has previously been found to profoundly inhibit tyrosine hydroxylase and to lower dopamine content in the intact substantia nigra pars reticulata after only 45 min (Nissbrandt et al., 1989), but this treatment clearly had no further effect on dopamine outflow in the reserpine-treated substantia nigra pars reticulata during the first hour post-injection (Figs. 1 and 2).

3.2. Effects of intranigral infusion of dizocilpine or CGP 40116 in normal and reserpine plus α-methyl-p-tyrosine-treated rats

Infusion of dizocilpine (150 nM) or CGP 40116 (10 μM) for 90 min, in monoamine-intact rats, had no discernible effect on nigral extracellular concentrations of dopamine or its metabolites (Figs. 1 and 2). By contrast, dizocilpine reduced dopamine and metabolite levels to their limits of detection, in rats which had received both reserpine and α-methyl-*p*-tyrosine beforehand ($P < 0.05$; Fig. 1). In comparable experiments, intranigral infusion of

CGP 40116 similarly lowered DOPAC and HVA outputs ($P < 0.05$), but not that of dopamine (Fig. 2).

3.3. Effects of intranigral infusion of L-DOPA in reserpine plus α-methyl-p-tyrosine-treated rats

Infusion of the dopamine precursor L-DOPA (10 μM) resulted in a modest increase in the recoveries of dopamine and DOPAC ($P < 0.05$), but not HVA (Figs. 3 and 4). Peak levels of dopamine averaged 3.9 ± 0.3 fmol/10 μl dialysate. All of these biochemical parameters returned rapidly to baseline following cessation of L-DOPA application.

3.4. Effects of coinfusion of L-DOPA with dizocilpine or CGP 40116 in reserpine plus α-methyl-p-tyrosine-treated rats

When L-DOPA (10 μM) and dizocilpine (150 nM) were infused together in the substantia nigra pars reticulata of reserpine plus α-methyl-*p*-tyrosine-treated rats, dopamine recoveries rapidly rose from previously undetectable levels to a peak of 42 ± 9 fmol/10 μl, which was 12.1-fold higher than that reached with L-DOPA by itself ($P < 0.01$; Fig. 3A). We also observed a similarly robust increase in DOPAC concentration ($P < 0.01$; Fig. 3B), but no significant alteration in HVA output (Fig. 3C). These elevated releases of dopamine and DOPAC were more prolonged than those obtained with L-DOPA infusion alone.

When the experiments were repeated with CGP 40116 (10 μM) and L-DOPA (10 μM) coinfusion, a peak dopamine level of 121 ± 39 fmol/10 μl dialysate was reached, representing a 24.2-fold increase over the effect of L-DOPA alone ($P < 0.01$; Fig. 4A). There was a correspondingly marked elevation in extracellular DOPAC (Fig. 4B), but no change in HVA (Fig. 4C).

3.5. Effects of high K^+ infusion

Perfusion of nigras in reserpine plus α-methyl-*p*-tyrosine-treated rats, for 30 min with ACSF containing 100 mM K^+, increased dialysate levels of dopamine 3-fold (peak 6.9 ± 0.15 fmol/10 μl; $P < 0.05$; Fig. 5A). Small, concurrent decreases in DOPAC and HVA were also observed, consistent with a transient drop in dopamine catabolism (Fig. 5A and B).

3.6. Changes in motor behaviour

Reserpine-treated rats were characteristically quiescent and completely akinetic. Neither of the NMDA receptor antagonists, nor L-DOPA, affected the animals' behaviour when these compounds were administered singly to the substantia nigra pars reticulata. On the other hand, the prominent increases in interstitial dopamine elicited by NMDA receptor antagonist/L-DOPA coinfusions were accompanied by substantial behavioural arousal. The spatially restricted experimental cages used for the microdialysis did not permit forward locomotion, and so this behavioural stimulation was expressed as an increase in stereotyped activity, which included sniffing, vacuous chewing, forepaw treading and head weaving.

4. Discussion

The results of this study demonstrate that impairment of glutamate transmission at NMDA receptors in the dopamine-depleted substantia nigra pars reticulata greatly facilitates the bioavailability of dopamine newly synthesised from L-DOPA. We consider this finding to be of considerable functional significance, because recent evidence suggests the nigra may be of greater importance than the striatum as a site of L-DOPA decarboxylation, and hence dopamine-induced reversal of parkinsonian motor symptoms. For example, experiments in unilaterally 6-hydroxydopamine-lesioned rats have revealed that dopamine production from L-DOPA in the dopamine-depleted substantia nigra pars reticulata outweighs that in the corresponding striatum (Orosz and Bennett, 1992). Moreover, the time-course of the rotational behaviour evoked by systemic L-DOPA administration in this paradigm exactly matches the elevation in extracellular dopamine content in the substantia nigra pars reticulata, but not the shorter-lived increase in dopamine in the striatum (Robertson and Robertson, 1989). The latter authors confirmed that the rise in substantia nigra pars reticulata dopamine levels was functionally important, by demonstrating that intranigral injection of the dopamine D_1 receptor antagonist, SCH 23390, completely blocked L-DOPA-induced circling. The present study endorses this view, since the massive increase in dopamine output we observed in the reserpine-treated substantia nigra pars reticulata, when either dizocilpine or CGP 40116 were co-infused with L-DOPA, was invariably accompanied by pronounced motor activation. These data therefore provide the first biochemical explanation for the behavioural observation that NMDA receptor antagonists synergistically improve the antiakinetic action of L-DOPA in monoamine-depleted rats (reviewed by Starr, 1995).

How dizocilpine and CGP 40116 effect such a massive increase in extracellular dopamine concentration is not yet clear, but a number of possibilities present themselves. Firstly, they could directly stimulate dopamine release from the vesicles or cisterns of the dopamine neuronal dendrites, which arborize through the substantia nigra pars reticulata and represent the source of the dopamine recovered by dialysis in the present experiments (Groves and Linder, 1983; Wassef et al., 1981). These nigral storage pools of dopamine are broadly susceptible to the same external influences as their axon terminal counterparts in

the striatum, although there are some important differences (Kalivas and Duffy, 1991; Santiago and Westerink, 1991). For instance, dendritic dopamine is more resistant to depletion by reserpine and 6-hydroxydopamine than is terminal dopamine, as near-total (98%) reductions of dopamine in the striatum are accompanied by approximately 80% reductions in dopamine content in the nigra (Elverfors and Nissbrandt, 1991; Robertson and Robertson, 1989). Although tissue contents of dopamine were not determined in the present study, a substantial diminution in nigral dialysate levels of dopamine to about 6% of normal is taken to indicate that combined treatment with reserpine and α-methyl-*p*-tyrosine severely compromised dopamine synthesis and storage in the substantia nigra pars reticulata (Figs. 3 and 4), in keeping with earlier studies (Elverfors and Nissbrandt, 1991; Dluzen and Liu, 1994).

Whilst the application of high K^+ via the probe showed it was still possible to evoke a depolarization-induced release of dopamine, albeit from a severely depleted pool (Fig. 5A; Fairbrother et al., 1990), we saw no evidence of increased dopamine release with either dizocilpine (150 nM) or CGP 40116 (10 μM) infused alone. If anything, these treatments caused a further fall in the release of dopamine and/or its metabolites in reserpine-treated rats, although they had no effect whatsoever on basal releases in dopamine-intact rats. Given that the recovery rates from our probes are in the order of 10%, we estimate the interstitial concentrations reached by dizocilpine and CGP 40116 to be no greater than 15 nM and 1 μM respectively, which we calculate are roughly equivalent to those reached by systemic doses of the compounds potentiating L-DOPA in behavioural experiments (Kaur et al., 1994; Maj et al., 1993). Thus, in spite of numerous reports of high doses of NMDA receptor antagonists liberating dopamine in dialysis experiments in other parts of the brain (e.g. Whitton et al., 1992b), there is no evidence that dizocilpine or CGP 40116 is able to do this in the substantia nigra pars reticulata at the exceedingly small concentrations employed here.

According to Zhang et al. (1992, 1993), dizocilpine is capable of increasing the firing rates of nigrostriatal dopamine cells by inhibiting local γ-aminobutyric acid (GABA) circuits in the substantia nigra pars reticulata. However, we consider it unlikely that this mechanism contributes to the enormous increase in dopamine recovered with L-DOPA/NMDA receptor antagonist mixtures in the present study, since somatodendritic dopamine output is reported to be relatively insensitive to action potential frequency (Kalivas and Duffy, 1991; Nissbrandt et al., 1985), and also because dopamine formation from exogenous L-DOPA is allegedly independent of dopaminergic neuronal activity (Mizoguchi et al., 1993).

Another way of stimulating dopamine release in the substantia nigra pars reticulata is to inhibit dopamine reuptake (Robertson et al., 1991). The behavioural profile of dizocilpine closely resembles that of the psychostimulant phencyclidine (PCP), and both compounds bind with high affinity to the so-called PCP-1 site within the open cation channel linked to the NMDA receptor (Young and Fagg, 1990). PCP also potently inhibits dopamine uptake via the so-called PCP-2 site (Rothman, 1994), for which dizocilpine has a very low affinity ($IC_{50} > 40$ mg/kg; Maurice et al., 1991). Consequently we do not believe that dizocilpine (or CGP 40116), at the very low concentrations used here, had any effect on the dopamine transporter in the substantia nigra pars reticulata. This suspicion is reinforced by our finding that neither of the NMDA receptor antagonists increased dopamine recovery in the substantia nigra pars reticulata of dopamine-intact rats (Fig. 1A and 2A). Furthermore, when both NMDA receptor antagonists were infused in conjunction with L-DOPA, they caused a simultaneous surge in the outflow of DOPAC (Fig. 3B and 4B), whereas nomifensine did not do this (Robertson et al., 1991). We attribute this difference to the fact that DOPAC is formed by the enzymic oxidative deamination of dopamine intraneuronally, so that an increase in DOPAC output is more in keeping with an elevated synthesis and metabolism of dopamine within the dendrites, and not with dopamine reuptake inhibition, which would prevent dopamine gaining access to intraneuronal catabolic sites.

Lastly, we have to consider that NMDA receptor blockade might have increased the somatodendritic uptake of L-DOPA and/or its rate of bioconversion to dopamine by the enzyme aromatic L-amino acid decarboxylase (EC 4.1.1.28, AAAD). Neurochemical evidence suggests that AAAD activity is undiminished by combined treatment with reserpine and α-MPT (Dluzen and Liu, 1994), but that AAAD is under inhibitory control from dopamine autoreceptors (Hadjiconstantinou et al., 1993; Zhu et al., 1992). Furthermore, it is known that glutamate suppresses dopamine synthesis in striatal synaptosomes (Chowdhury and Fillenz, 1991) and that NMDA receptor antagonists increase it (Scatton et al., 1970; Svensson et al., 1991; Van Zwieten-Boot and Noach, 1975), although the mechanism is believed to involve tyrosine hydroxylase rather than AAAD. Nevertheless, given that glutamatergic tone is raised in the substantia nigra pars reticulata of dopamine-depleted animals (Albin et al., 1989), we hypothesise that dopamine synthesis is held in a suppressed state by pathologically overactive glutamatergic inputs to the substantia nigra pars reticulata, acting via NMDA receptors linked to AAAD, and that removing this restraint with NMDA receptor antagonists discloses the true dopamine synthetic potential of the nigrostriatal dopamine neurones. It will be necessary to extract and measure AAAD to provide direct support for this proposal, but in the meantime the fact that NMDA antagonism increased nigral dialysate levels of DOPAC in parallel with dopamine, but not those of HVA, is strongly suggestive of a presynaptic site of action of dizocilpine and CGP 40116, involving an increase in the rates of synthesis and breakdown of dopamine.

Future work will determine the applicability of these

observations to other classes of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) receptor antagonists, and in other models of parkinsonism, and hopefully provide us with a firm biochemical basis with which to recommend the use of these compounds as adjuncts to L-DOPA in the management of Parkinson's disease in man.

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