ELSEVIER

European Journal of Pharmacology 386 (1999) 217–226

ejp

www.elsevier.nl/locate/ejphar

Striatal dopamine depletion and behavioural sensitization induced by methamphetamine and 3-nitropropionic acid

Oweikumo L. Eradiri, Michael S. Starr *

Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK

Received 3 June 1999; received in revised form 18 October 1999; accepted 29 October 1999

Abstract

The neurotoxic effects of methamphetamine (4 × 5 mg/kg i.p. at 2-h intervals) and 3-nitropropionic acid (20 mg/kg i.p. on days 1–4 and 6–9, saline on day 5), administered alone or in combination (3-nitropropionic acid as above and methamphetamine on day 5), were investigated in rats 1 week after the last injection. Neither methamphetamine nor 3-nitropropionic acid on their own altered brain dopamine levels, but in combination, they selectively lowered dopamine in the terminal regions of the corpus striatum and nucleus accumbens. Methamphetamine depleted 5-hydroxytryptamine (5-HT) in the striatum, while 3-nitropropionic acid depleted 5-HT in the accumbens and substantia nigra, but a combination of the two toxins failed to lower 5-HT in any of these brain regions. Measurements of aromatic L-amino acid decarboxylase activity disclosed no change in the capacity to decarboxylate L-3,4-dihydroxyphenylalanine in any region with any of the treatments, but a lowered capacity to decarboxylate 5-hydroxytryptophan in the nigra after all three treatments. Methamphetamine evoked characteristic hyperactivity and stereotypy in the animals, whereas 3-nitropropionic gave rise to early hypermotility followed by hypoactivity. At 1 week after treatment with 3-nitropropionic/methamphetamine, rats exhibited normal spontaneous motor behaviour, a poor response to dopamine D_1 receptor stimulation and an exaggerated response to dopamine D_2 receptor agonists. These results show that combined systemic treatment with methamphetamine and 3-nitropropionic acid partially depletes dopamine in the basal ganglia, rendering the animals supersensitive to dopamine D_2 receptor activation without altering their spontaneous locomotion.

Keywords: Methamphetamine; 3-Nitropropionic acid; Neurotoxicity; Dopamine; 5-HT, 5-Hydroxytryptamine; Aromatic L-amino acid decarboxylase; Motor behaviour

1. Introduction

3-Nitropropionic acid is an irreversible inhibitor of succinic dehydrogenase (mitochondrial complex I; Gould and Justine, 1982). When administered either systemically or focally to experimental animals, 3-nitropropionic acid compromises mitochondrial function, thereby causing impairment of energy metabolism and a selective degeneration of neurones in the basal ganglia (Ludolph et al., 1991; Beal et al., 1993; Brouillet et al., 1993; Wüllner et al., 1994). The caudate–putamen is particularly vulnerable to mitochondrial poisons like 3-nitropropionic acid, especially the γ-aminobutyric acidergic (GABAergic) output neurones (Hassel and Sonnewald, 1995; Reynolds et al., 1997; Tsai et al., 1997; Araujo and Hilt, 1998). The resultant loss of striatal cells following 3-nitropropionic acid intoxication leads to pronounced decrements in the animal's pattern of motor behaviour, giving rise to the proposition that toxins like 3-nitropropionic acid may be used to mimic certain human hypokinetic disorders (Koutouzis et al., 1994a,b; Borlongan et al., 1995a,b; Nakao and Brundin, 1997; Araujo and Hilt, 1998).

3-Nitropropionic acid is not only injurious towards cell bodies in the striatum, but also damages fibres of passage and axon terminals (Nakao and Brundin, 1997). Sonsalla et al. (1997) demonstrated that intrastriatal infusion of a similar, reversible mitochondrial toxin, malonate, targetted the terminals of nigrostriatal dopamine fibres and caused a retrograde destruction of dopamine cell bodies in the corresponding substantia nigra. Indeed, it was found that dopamine terminals in the striatum of adult rats were more susceptible to energy impairment than were the resident GABA-containing neurones (Zeevalk et al., 1997). The

* Corresponding author. Tel.: +44-171-753-5901; fax: +44-171-753-5902.

E-mail address: mstarr@cua.ulsop.ac.uk (M.S. Starr)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00776-1

high sensitivity of dopamine neurones to modest energy failure has also been clearly demonstrated in mesencephalic cell cultures (Marey-Semper et al., 1995; Zeevalk et al., 1995), and it is this feature of dopamine cells that could underlie their propensity for degeneration in idiopathic Parkinson's disease.

The mechanism of 3-nitropropionic acid-induced neurotoxicity has been proposed to involve the release of glutamate (Erecinska and Nelson, 1994; Tsai et al., 1997) and the activation of NMDA-type glutamate receptors (Wüllner et al., 1994), since it can be allayed by NMDA receptor antagonists (Riepe et al., 1992). Indeed, it has been shown using primary cultures of striatal neurones that 3-nitropropionic exacerbates NMDA-induced neurotoxicity (Greene et al., 1998). Similarly, with cultures of mesencephalic dopamine neurones, which were found to be damaged in a synergistic fashion by a combination of glutamate and rotenone, an alternative inhibitor of mitochondrial complex I (Marey-Semper et al., 1995). It would not be surprising, therefore, to find that agents which promote the release of excitatory amino acids, for example the amphetamines, are also capable of interacting with inhibitors of mitochondrial energy metabolism to cause exaggerated cell death (Albers et al., 1996; Bowyer et al., 1996).

The damage inflicted on dopamine neurones by amphetamines is, in fact, well-documented (Seiden and Ricuarte, 1987) and the possibility of using amphetamine- or methamphetamine-induced toxicity as a model for Parkinson's disease has not passed unnoticed (for review see Ossowska, 1994). At toxic dose levels, methamphetamine elicits the overflow of large quantities of dopamine and glutamate, which correlate directly with the extent of the accompanying neuropathology (O'Dell et al., 1993; Stephans and Yamamoto, 1996). The release of these neurotransmitters is undoubtedly associated with the perturbation of energy supplies caused by methamphetamine (Chan et al., 1994), as the toxicity it causes can be attenuated by pretreatment with either dopamine (Buening and Gibb, 1974; Hotchkiss and Gibb, 1980; Sonsalla et al., 1986a,b; O'Dell et al., 1993) or NMDA receptor antagonists (Sonsalla et al., 1989). Conversely, if energy metabolism is additionally compromised by the intrastriatal (Albers et al., 1996) or systemic administration (Bowyer et al., 1996) of a succinic dehydrogenase inhibitor, then the neurodegenerative effects of systemic methamphetamine or amphetamine on the nigrostriatal dopamine system are seen to be markedly increased.

The purpose of this study was to determine whether systemic treatment with a combination of 3-nitropropionic acid and methamphetamine caused deficits in brain amines consonant with changes in the animal's motor behaviour. We have therefore measured tissue concentrations of dopamine and 5-hydroxytryptamine (5-HT) and their synthesizing enzyme aromatic L-amino acid decarboxylase in the basal ganglia. In addition, we have monitored changes in the spontaneous motor activity of 3-nitropropionic acid and/or methamphetamine-treated animals, as well as their responsiveness to directly and indirectly acting D_1 and D_2 dopamine receptor agonists.

2. Materials and methods

2.1. Animals and neurotoxin treatment

Male Wistar albino rats (A.R. Tuck) weighing 180–240 g at the outset of the experiment were used. The animals were housed in groups of six at 22 ± 1°C and allowed free access to rat chow and water. Experiments were conducted between 0800 and 1800 h and each animal was used once only. All experiments were conducted in accordance with the Animals (Scientific Procedures) 1986 Act UK and with institutional guidelines. Rats received methamphetamine, 3-nitropropionic acid or a combination of the two agents according to the schedules listed in Table 1, while control rats received saline.

2.2. Behavioural assessments

Rats were inspected visually for overt changes in their patterns of spontaneous motor behaviour after receiving the toxin treatments described above. At 5 days after

Table 1
Neurotoxin injection schedule

Experiment				
Time	Methamphetamine alone	3-Nitropropionic acid alone	3-Nitropropionic acid plus methamphetamine[a]	Control
Day 1	saline	20 mg/kg	20 mg/kg	saline
Day 2	saline	20 mg/kg	20 mg/kg	saline
Day 3	saline	20 mg/kg	20 mg/kg	saline
Day 4	saline	20 mg/kg	20 mg/kg	saline
Day 5	4×5 mg/kg	saline 4×1 ml/kg	Methamphetamine 4×5 mg/kg	saline 4×1 ml/kg
Day 6	saline	20 mg/kg	20 mg/kg	saline
Day 7	saline	20 mg/kg	20 mg/kg	saline
Day 8	saline	20 mg/kg	20 mg/kg	saline
Day 9	saline	20 mg/kg	20 mg/kg	saline

[a]All doses refer to 3-nitropropionic acid unless otherwise indicated.

receiving the last toxin injection, rats were placed singly onto the floor of a Perspex observation box ($610 \times 610 \times 305$-cm^3 high), without prior acclimatization, and their gross motor activity recorded every 10 min for 2 h by an overhead Microwave Doppler Module (model 8960, R.S. Components, London, UK). The sensitivity of the Doppler sensor was adjusted so as to detect horizontal locomotion and rearing, but not stereotypy. Each unit was connected to an amplifier and a timer, with motor counts being displayed on an integrated liquid crystal display (the whole assembly was fabricated in this laboratory). In some experiments, the time (min) spent grooming was determined by a trained observer using a stopwatch. The presence or absence of other behavioural elements was also noted, but was not quantified.

To determine the responsiveness of toxin-treated rats to dopaminergic agents, rats were first habituated to the test apparatus for 2 h, then injected with a dopamine agonist and observed for a further 2 h. Drugs investigated included the selective D_1 dopamine receptor agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine hydrochloride (SKF 38393, 30 mg/kg i.p.), the selective D_2 dopamine receptor agonist *N*-*n*-propyl-*N*-phenylethyl-*p*(3-hydroxyphenyl)ethylamine hydrochloride (RU 24213, 5 mg/kg s.c.), the mixed D_1/D_2 dopamine receptor agonist apomorphine (0.5 mg/kg s.c.) and the dopamine-releasing agent (+)-5-methyl-10,11-dihydro-5*H*-dibenzo-(*a*,*d*)-cyclohepten-5,10-imine (MK 801, 0.1–0.5 mg/kg i.p.). All drug doses were selected on the basis of published literature reports. Results were expressed as motor counts per 10 min and min grooming in 2 h.

2.3. Preparation of tissue samples

Seven days after receiving the last toxin injection, rats were sacrificed by decapitation with a guillotine and their brains removed into ice-cold saline. The corpus striatum, substantia nigra and nucleus accumbens were dissected out and homogenised in 1 ml (nigra and accumbens) or 3 ml (striatum) ice-cold 0.25 M sucrose. One hundred microliters of each homogenate were stored frozen in mini-Eppendorf tubes at −80°C for subsequent protein assay (Bradford, 1976), while the remainder was centrifuged for 10 min at $3000 \times g$. Twenty microliters of supernatant were taken for the assay of aromatic L-amino acid decarboxylase, while blanks for the enzyme assay were used to determine tissue levels of dopamine and 5-HT.

2.4. Assay of aromatic L-amino acid decarboxylase activity and tissue levels of dopamine and 5-HT

The enzyme assay was based on the procedure described by Nagatsu et al. (1979). Twenty microliters of tissue supernatant were added to 380 μl of the following incubation mixture (mM): ascorbic acid, 0.18; EDTA, 0.11; HEPES hemisodium salt buffer (pH 7.2), 50; 2-mercaptoethanol, 1; pargyline, 0.11; pyridoxal-5′-phosphate, 0.01; and either L-3,4-dihydroxyphenylalanine (L-DOPA; 0.53 mM; to determine DOPA decarboxylase), 5-hydroxytryptophan (5-HTP; 0.042 mM; to determine 5-HTP decarboxylase), or double-deionised water (blanks). The incubation was carried out for 10 min at 37°C and the reaction stopped by the addition of 80 μl ice-cold 0.5 M perchloric acid, containing 0.5 nM isoprenaline as internal standard. The mixture was then centrifuged for 10 min at $3000 \times g$ and 1 μl aliquots of the supernatant assayed by high performance liquid chromatography with electrochemical detection. DOPA decarboxylase activity was expressed as nmol dopamine/mg protein/10 min and 5-HTP decarboxylase activity as nmol 5-HT/mg protein/10 min. Tissue levels of dopamine and 5-HT were obtained by assaying 1 μl of each supernatant blank.

2.5. High performance liquid chromatography

One microliter of each aromatic L-amino acid decarboxylase supernatant and blank (to give basal tissue levels) was diluted to 100 μl with water and 20 μl assayed for dopamine and 5-HT by standard high performance liquid chromatography with electrochemical detection. Samples were injected via a rheodyne (model 7125, Cotati, CA, USA) connected via a guard cell (set at +0.5 V; model 5020, ESA, USA) to a C_{18} reverse-phase column (particle size 3 μm; Rainin, Woburn, USA), and then to an analytical cell (set at +0.4 V; model 5100A, ESA, USA) and electrochemical detector (set at −0.17 V; Coulochem model 5100A, ESA, USA). The mobile phase was pumped at 1 ml/min and consisted of (mM): anhydrous sodium acetate, 90; octanesulphonic acid, 0.06; EDTA, 0.34; dissolved in 5.5% v/v methanol. Results were analyzed by a desk-top computer equipped with ChromPerfect software and expressed as pmol/mg protein.

2.6. Statistical analysis

All results were expressed as means ± S.E.M. for at least $n = 6$ determinations. Tissue levels of amines were compared with controls by analysis of variance (ANOVA) and post-hoc by Dunnett's *t*-test, while behavioural data were analysed by one- or two-way ANOVA, with repeated measures where appropriate. Significance was set at $P < 0.05$.

2.7. Drugs and materials

Methanol (Rathburn, Scotland) and other chromatography chemicals (Fluka, Gillingham, UK) were of the highest grade. General chemicals used for the enzyme incubation mix were obtained from Sigma (Poole, UK). Pargyline hydrochloride, isoprenaline hydrochloride, L-DOPA, 5-HTP, apomorphine hydrochloride (Sigma, Poole, UK), *N*-*n*-propyl-*N*-phenylethyl-*p*(3-hydroxyphenyl)ethylamine

hydrochloride (RU 24213; Roussel, Romainville, France), 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine hydrochloride (SKF 38393; Research Biochemicals, Natick, USA) and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo-(*a*,*d*)-cyclohepten-5,10-imine (MK 801; Research Biochemicals, Natick, USA) were supplied as shown. Drugs were administered in double-deionized water in a dose volume of 1 ml/kg.

3. Results

3.1. Biochemical determinations

One week after completing a series of injections of methamphetamine or 3-nitropropionic acid alone (see Table 1), tissue levels of dopamine were found not to be significantly altered in the nucleus accumbens, substantia nigra or corpus striatum (Fig. 1). Combined treatment with methamphetamine and 3-nitropropionic (Table 1), however, selectively lowered the dopamine contents of nucleus accumbens (47.2%, $P < 0.05$) and corpus striatum (69.0%, $P < 0.05$), while sparing the substantia nigra.

The tissue concentrations of 5-HT appeared to be more susceptible to depletion by these neurotoxins (Fig. 2). Methamphetamine alone decreased 5-HT levels in the corpus striatum (66.8%, $P < 0.05$), while 3-nitropropionic acid alone was similarly effective in reducing the amounts of 5-HT in the nucleus accumbens (58.5%, $P < 0.05$) and substantia nigra (85.2%, $P < 0.01$). 3-nitropropionic/methamphetamine combination, on the other hand, failed to diminish 5-HT in the substantia nigra or corpus striatum, and the downwards trend we detected in the nucleus accumbens was not statistically significant ($P > 0.05$; Fig. 2).

Fig. 3 shows the activity of DOPA decarboxylase remained unchanged as the result of neurotoxin treatment. By contrast, the activity of 5-HTP decarboxylase in the nucleus accumbens, but not in the other structures, was significantly decreased by roughly similar amounts following methamphetamine (48.0%, $P < 0.01$), 3-nitropropionic acid (37.3%, $P < 0.01$) and the mixture of the two toxins (40.5%, $P < 0.01$; Fig. 4).

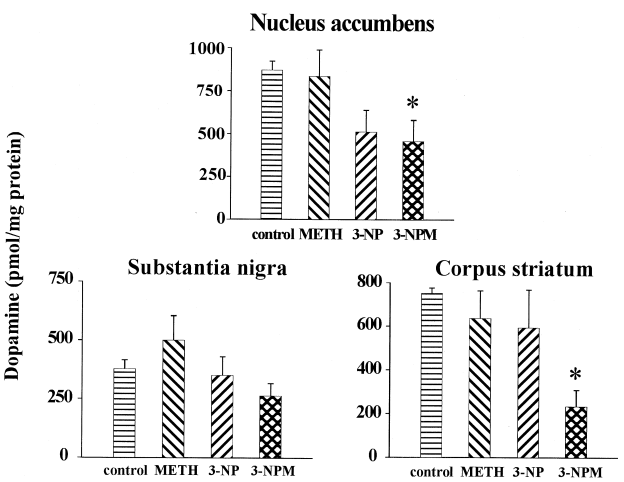

Fig. 1. Effects of pretreatment with methamphetamine, 3-nitropropionic acid or a combination of the two neurotoxins on brain levels of dopamine. Treatments were as follows: methamphetamine, 4×5 mg/kg i.p. at 2-h intervals; 3-nitropropionic acid, 20 mg/kg i.p. on days 1–4 and 6–9, with 1 ml/kg i.p. saline on day 5; combined treatment as for 3-nitropropionic acid but methamphetamine (4×5 mg/kg i.p. at 2-h intervals) given on day 5; control, as for dual toxin treatment but saline (1 ml/kg i.p.) substituted for the toxin injections. Neurochemical measurements were made 7 days after the last injection. $^{*}P < 0.05$ vs. corresponding control by Dunnett's *t*-test; METH, methamphetamine; 3-NP, 3-nitropropionic acid; 3-NPM, 3-nitropropionic acid plus amphetamine.

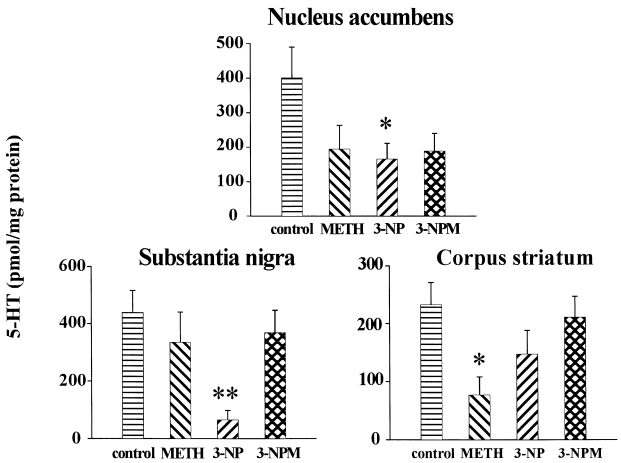

Fig. 2. Effects of pretreatment with methamphetamine, 3-nitropropionic acid or a combination of the two toxins on brain levels of 5-HT. Experimental details as for Fig. 1. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. corresponding control by Dunnett's *t*-test.

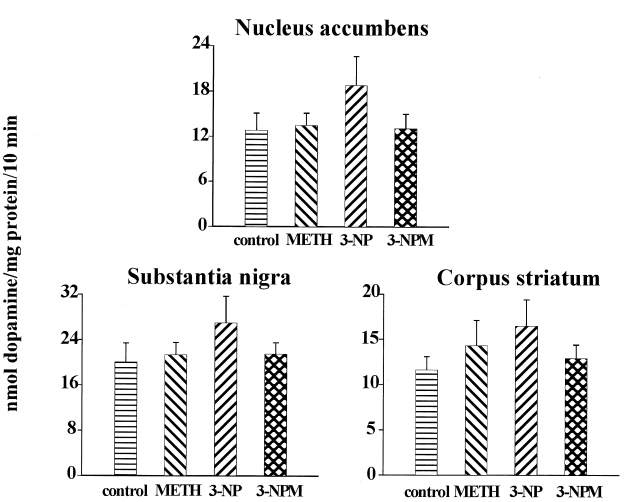

Fig. 3. Effects of pretreatment with methamphetamine, 3-nitropropionic acid or a combination of the two toxins on brain levels of aromatic L-amino acid decarboxylase, using L-DOPA as substrate. Other experimental details as for Fig. 1.

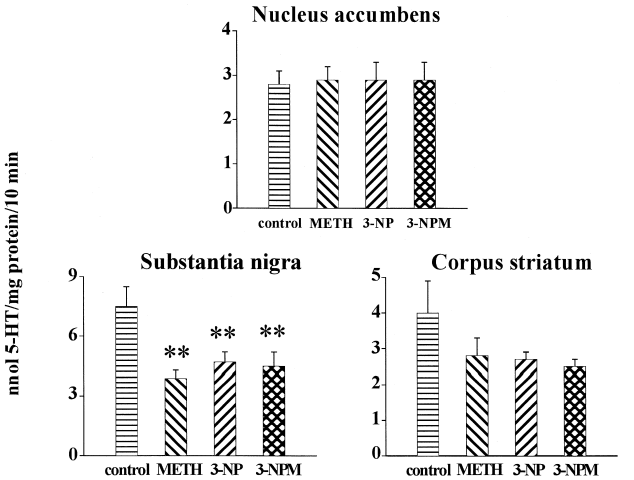

Fig. 4. Effects of pretreatment with methamphetamine, 3-nitropropionic acid or a combination of the two toxins on brain levels of aromatic L-amino acid decarboxylase, using 5-HTP as substrate. Other experimental details as for Fig. 1. $^{**}P < 0.001$ vs. corresponding control by Dunnett's *t*-test.

3.2. Acute behavioural changes

Within minutes of receiving methamphetamine, 5 mg/kg i.p., rats exhibited characteristic locomotor hyperactivity together with pronounced stereotyped movements, which included grooming, sniffing the floor, head weaving, back pedalling and biting. This behavioural syndrome lasted about 6 h after the last methamphetamine dose. The dose regimen of 4×5 mg/kg methamphetamine (2 h apart) resulted in 8.3% fatalities within 24 h, after which the surviving rats appeared normal.

Rats injected with 3-nitropropionic acid, 20 mg/kg i.p., became hyperactive after about 20 min and were observed to sniff and rear more. This behaviour subsided after 2–3 h, but recurred with every subsequent daily dose of 3-nitropropionic acid. No fatalities occurred with eight repeated injections of 3-nitropropionic acid.

The acute behavioural effects of daily dosing with 20 mg/kg 3-nitropropionic acid for 8 days, interspersed with the administration of 4×5 mg/kg (2 h apart) methamphetamine on day 5, were a synthesis of the individual treatments described above. Initially, fatalities amounted to 17.7%, although we later found that if the rats were held in a cool room (about 14°C) after methamphetamine injection, to protect them against the harmful effects of methamphetamine-induced hyperthermia, then no deaths occurred (see Bowyer et al., 1992). Of the surviving 79 rats, six exhibited hindlimb dysfunction and were not used further. The remaining 73 animals were all hypoactive on the day after the last injection of 3-nitropropionic acid, preferring to sit still when placed into a new environment rather than explore. However, this hypoactivity wore off over the next few days and by the time the first quantitative measure of spontaneous activity was made, 1 week after completion of treatment, the rats looked no different

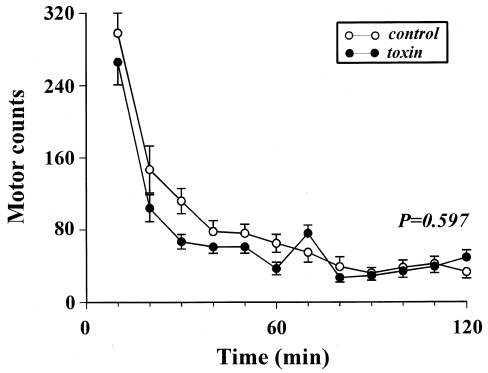

Fig. 5. Spontaneous motor activity of 3-nitropropionic/methamphetamine-treated and saline control rats, 1 week after the last injection (see Table 1 for injection schedule). Rats were placed singly onto the floor of a Perspex test box, without prior acclimatization, and their gross motor activity measured every 10 min for 2 h by overhead Doppler sensors (see Section 2). Statistical significance shown for comparison of two time–response plots by two-way ANOVA with repeated measures.

from controls. This is illustrated in Fig. 5, which shows that 3-nitropropionic acid/methamphetamine-treated rats ($n = 34$) were behaviourally indistinguishable from controls ($n = 33$; two-way interaction $F(11,799) = 0.84$, $P = 0.597$ by repeated measures ANOVA).

3.3. Alterations in the response of 3-nitropropionic acid / methamphetamine-treated rats to dopaminergic drugs

The D_1 dopamine receptor agonist SKF 38393, 30 mg/kg i.p., initiated a significant, though short-lived hyperlocomotion in 3-nitropropionic acid/methamphetamine-treated rats ($F(17,125) = 3.808$, $P < 0.001$ by one-way ANOVA), but not in control rats ($F(17,125) = 1.71$, $P = 0.055$; Fig. 6). However, two-way ANOVA revealed no significant difference in the time–response curves for the two groups (two-way interaction $F(23,311) = 1.175$,

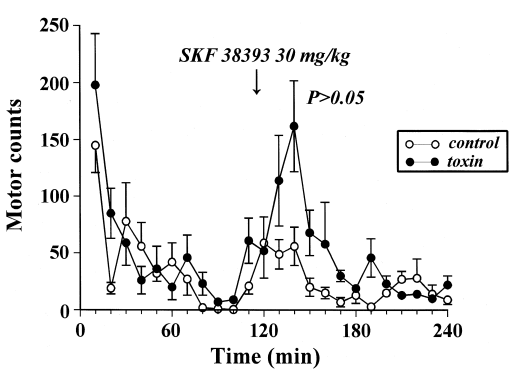

Fig. 6. Effects of the D_1 dopamine receptor agonist SKF 38393 on motor activity of 3-nitropropionic acid/methamphetamine-treated and saline control rats, tested 1 week after the last injection (see Table 1 for injection schedule). Experimental details as for Fig. 5, except that SKF 38393 (30 mg/kg i.p. at the arrow) was injected after 2 h and motor activity recorded for a further 2 h. Statistical analysis by two-way ANOVA with repeated measures shows responses of toxin-treated and control rats were not significantly different.

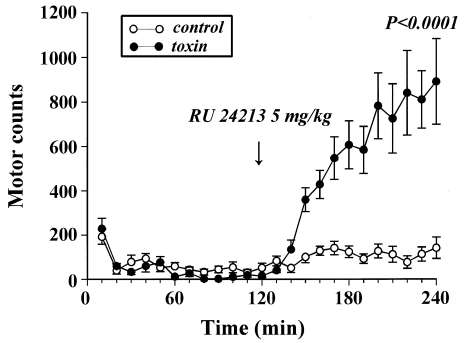

Fig. 7. Effects of the D_2 dopamine receptor agonist RU 24213 on motor activity of 3-nitropropionic acid/methamphetamine-treated and saline control rats, tested 1 week after the last injection (see Table 1 for injection schedule). Details as for Fig. 6, except that RU 24213 (5 mg/kg s.c.) was injected at the arrow. Statistical analysis by two-way ANOVA with repeated measures shows responses of toxin-treated and control rats were significantly different.

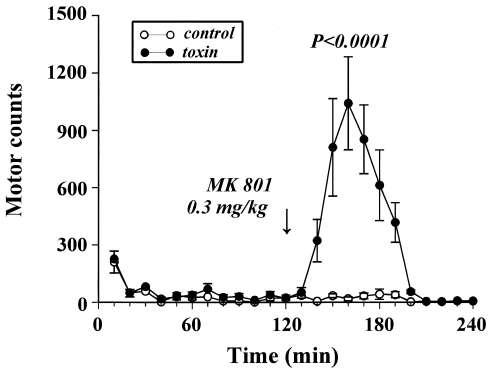

Fig. 9. Effects of the dopamine-releaser MK 801 on motor activity of 3-nitropropionic acid/methamphetamine-treated and saline control rats, tested 1 week after the last injection (see Table 1 for injection schedule). Details as for Fig. 6 except that MK 801 (0.3 mg/kg i.p.) was injected at the arrow. Statistical analysis by two-way ANOVA with repeated measures shows responses of toxin-treated and control rats were significantly different.

$P = 0.267$). After saline injection, control rats groomed spontaneously for 3.3 ± 0.9 min over a 2-h period; this behaviour was significantly increased by SKF 38393 to 17.5 ± 3.2 min (ANOVA $F(1,11) = 18.65$, $P < 0.001$). Comparable values for 3-nitropropionic acid/methamphetamine-treated rats amounted to 3.8 ± 1.0 min after saline and 15.3 ± 2.0 min after receiving the D_1 dopamine receptor agonist (ANOVA $F(1,11) = 22.28$, $P < 0.001$). Thus, while SKF 38393 increased grooming in both sets of animals, there was no indication that this behaviour was facilitated after combined neurotoxin treatment (ANOVA $F(1,11) = 0.38$, $P = 0.89$).

Fig. 7 shows that the D_2 dopamine receptor agonist RU 24213, 5 mg/kg s.c., evoked a particularly weak increase in motor activity in habituated control rats ($F(17,142) = 3.28$, $P = 0.007$), which was enormously potentiated by 3-nitropropionic acid/methamphetamine treatment (two-way interaction $F(18,189) = 4.22$, $P < 0.001$).

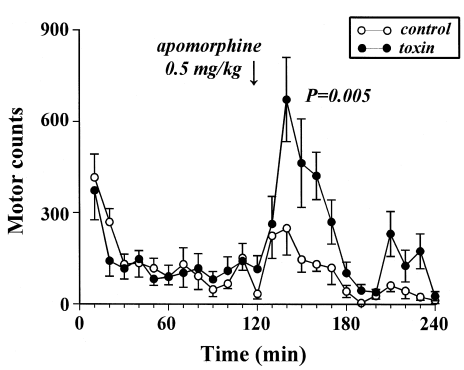

Fig. 8. Effects of the D_1 /D_2 dopamine receptor agonist apomorphine on motor activity of 3-nitropropionic acid/methamphetamine-treated and saline control rats, tested 1 week after the last injection (see Table 1 for injection schedule). Details as for Fig. 6 except that apomorphine (0.5 mg/kg s.c.) was injected at the arrow. Statistical analysis by two-way ANOVA with repeated measures shows responses of toxin-treated and control rats were significantly different.

The direct D_1/D_2 dopamine receptor agonist apomorphine, 0.5 mg/kg s.c., elicited a small and transient hypermotility in control rats ($F(17,143) = 2.99$, $P = 0.01$; Fig. 8). Here, too, the effect of 3-nitropropionic acid/methamphetamine was to greatly amplify the magnitude, but not the duration of the dopaminergic response (two-way interaction $F(23,383) = 1.99$, $P = 0.005$), and the biphasic nature of the apomorphine response can now be clearly seen (Fig. 8).

Surprisingly, the dopamine-releasing agent MK 801, 0.3 mg/kg i.p., was unable to evoke an increase in motor behaviour in habituated control rats ($F(17,89) = 1.03$, $P = 0.445$; Fig. 9). A lower dose of MK 801 (0.1 mg/kg) was also inactive, while a higher dose (0.5 mg/kg) caused hindlimb abduction and postural abnormalities. In 3-nitropropionic acid/methamphetamine-treated rats, however, 0.3 mg/kg MK 801 gave rise to pronounced hypermotility (two-way interaction $F(18,189) = 4.22$, $P < 0.001$).

4. Discussion

The present study shows that treating rats systemically with a combination of 3-nitropropionic acid and methamphetamine, according to the protocol outlined in Table 1, gave rise to a pattern of dopamine depletion in the corpus striatum (69.0%) and nucleus accumbens (47.3%) that was not evident with the component treatments alone, while sparing dopamine cell bodies in the substantia nigra. It would appear that the Wistar strain of rats we employed in these experiments may be unusually resistant to the dopamine-depleting effects of multiple injections of methamphetamine, since our dose regimen of 4×5 mg/kg at 2-h intervals was without effect on brain dopamine 1 week later, whereas other investigators have previously

noted marked depletions (54–78%) in striatal dopamine 3–7 days after administering four injections of 2.5–7.5 mg/kg methamphetamine, 2 h apart, to rats (O'Dell et al., 1993; Ohmori et al., 1996; Abekawa et al., 1997) and mice (Sonsalla et al., 1989). This strain and/or species variation in the sensitivity to methamphetamine-induced neurotoxicity is considered to be less important than the fact that methamphetamine interacted with normally ineffective injections of 3-nitropropionic acid (20 mg/kg for 8 days) to give a selective depletion of terminal dopamine in the basal ganglia, more especially in the striatum than in the accumbens.

3-Nitropropionic acid is also toxic towards dopamine neurones, but the potency of this effect is highly dependent upon its manner of presentation. Thus, intrastriatal infusion of micromolar amounts of 3-nitropropionic acid has been found to cause a loss of tyrosine hydroxylase and a near total depletion of tissue dopamine (Beal et al., 1993; Brouillet et al., 1993). Similar results, plus retrograde dopamine cell degeneration in the substantia nigra, have also been described following intrastriatal deposition of the mitochondrial toxin malonate (Albers et al., 1996; Zeevalk et al., 1997). These findings contrast sharply with the absence of any reduction in dopaminergic parameters (i.e., dopamine levels, synthetic enzymes and dopamine cell numbers) noted with parenteral 3-nitropropionic acid (Beal et al., 1993; Brouillet et al., 1993; Bowyer et al., 1996; present study). These differences in the neurotoxicity of 3-nitropropionic acid according to its route of delivery are further emphasised in the presence of amphetamine. Studies with systemically administered 3-nitropropionic acid provide clear evidence of a synergistic interaction between the dopamine neurotoxicities of 3-nitropropionic acid and methamphetamine (present study) or amphetamine (Bowyer et al., 1996), whereas the combined neurotoxic effects of local inhibition of striatal energy metabolism and systemic methamphetamine appear to be additive rather than cooperative (Albers et al., 1996).

Methamphetamine also has a well-known propensity for depleting 5-HT (Ricuarte et al., 1980) and the 5-HT reductions we observed in the corpus striatum of rats, one week after multiple methamphetamine treatment (4×5 mg/kg), are broadly in line with those recently described by Abekawa et al. (1997). The 5-HT-depleting effects of repeated daily injections of 3-nitropropionic acid exhibited a different profile to methamphetamine, with significant reductions occurring in the substantia nigra and nucleus accumbens. As noted by Brouillet et al. (1993), however, 5-HT in the corpus striatum was resistant to depletion by 3-nitropropionic acid. Surprisingly, no changes in 5-HT concentrations were detected in the substantia nigra or corpus striatum of rats receiving both toxins, suggesting the two toxic agents afforded a mutual *protection* against each other's 5-HT-depleting qualities in these structures. The results for 5-HT in the nucleus accumbens were more equivocal, for although dual treatment with the toxins appeared to lower the 5-HT content of this brain region, this effect was not statistically significant. With the 3-nitropropionic acid/methamphetamine model, therefore, the combined action of the two neurotoxins is to aggravate the depreciation of dopamine and to ameliorate the diminution of 5-HT in the basal ganglia.

Histological verification of cellular damage was not made in the present study, although decrements in the activity of the enzyme aromatic L-amino acid decarboxylase would be expected to reveal gross destruction of dopaminergic or 5-HTergic elements (Lloyd and Hornykiewicz, 1970; Gjedde et al., 1993). In fact, decarboxylase activity remained remarkably steady with most of the neurotoxin protocols employed here, suggesting that any loss of monoamines was due to interference with their storage rather than to the elimination of the neurones themselves. The notable exception was the substantia nigra, which suffered a loss of 5-HTP decarboxylase after treatment with methamphetamine and/or 3-nitropropionic acid, which may signify that 5-HT afferents from the raphé are particularly vulnerable to these neurotoxins (Ricuarte et al., 1980). Even if this were the case, the stability of nigral 5-HT content following dual toxin administration (Fig. 2), leads us to believe that 5-HT-mediated synaptic transmission in the substantia nigra has not been compromised. It will be a simple matter to test this claim by measuring extracellular 5-HT levels in toxin-treated rats, by means of nigral microdialysis.

The differential modification of DOPA and 5-HTP decarboxylases by the neurotoxins employed here, supports the contention that aromatic L-amino acid decarboxylase exists in different isoforms with different kinetic characteristics and distribution in the brain (Sims et al., 1973; Berry et al., 1987), in spite of molecular biological evidence to the contrary (Albert et al., 1987). This issue has generated much controversy over the years, with the weight of evidence beginning to favour the notion that L-DOPA and 5-HTP are normally decarboxylated independently of each other. Improving our understanding of the way in which L-DOPA is converted to dopamine in the brain is of particular relevance to Parkinson's disease, where the decarboxylation step may become rate-limiting and therefore determine the bioavailability of dopamine formed from exogenous L-DOPA, and consequently, the success or otherwise of dopamine-replacement therapy.

The dopamine-selective toxicities of methamphetamine and 3-nitropropionic acid described above are believed to be mediated by the release of excitatory amino acids. Stephans and Yamamoto (1996) recently showed that methamphetamine stimulates the overflow of glutamate in microdialysis experiments, supporting the contention by Sonsalla et al. (1989) that methamphetamine-induced neurotoxicity can be ameliorated by the non-competitive NMDA receptor antagonist, MK 801. The same is probably true for 3-nitropropionic acid, whose toxicity is likewise proposed to involve the release of glutamate

(Erecinska and Nelson, 1994) and the activation of NMDA receptors (Riepe et al., 1992; Wüllner et al., 1994). Tsai et al. (1997) reported that seven daily injections of 3-nitropropionic acid (10 mg/kg i.p.) massively lowered the striatal concentration of glutamate on day 8, consistent with the development of neuronal and/or glial dysfunction. We have not been able to detect any such change in glutamate content in the basal ganglia, 1 week after the cessation of single or combined neurotoxin treatment (Eradiri and Starr, unpublished data), which suggests to us that the early decrements in glutamate noted by Tsai et al. (1997) were short-lived and had completely recovered by this time. At any rate, the significance of alterations in the tissue content of glutamate is dubious at best, given the ubiquity of the amino acid and its widespread distribution across both neurotransmitter and metabolic pools.

The characteristic behavioural syndrome we observed with methamphetamine, which was dominated by locomotor hyperactivity and stereotypy, has been extensively investigated and is widely attributed to the massive release of monoamines in the brain (Robinson and Becker, 1986). Behavioural investigations with 3-nitropropionic acid, on the other hand, have revealed a biphasic response pattern, consisting of an acute hyperactivity shortly after 3-nitropropionic acid administration, followed days later by hypomotility (Hamilton and Gould, 1987; Koutouzis et al., 1994a,b; Borlongan et al., 1995a,b; Guyot et al., 1997; Reynolds et al., 1997). Severe intoxication with 3-nitropropionic acid leads to the animals becoming recumbent and having difficulty moving their hindlimbs, effectively dragging themselves about with the aid of their forelimbs. Such a model can have little value for the purposes of behavioural investigation. Borlongan et al. (1995a) found that the onset of the hypoactive stage corresponded to the appearance of visible lesions in the striatum, indicating the motor deficiencies were related to neuronal degeneration in the basal ganglia. In the present work, rats which had been injected with 3-nitropropionic acid exhibited the early hyperlocomotion and subsequent bradykinesia seen in previous studies. However, the animals went on to make a full recovery, with the exception of a few rats (6/79) additionally treated with methamphetamine. The spontaneous activity and outward appearance of the majority (73/79) of 3-nitropropionic acid/methamphetamine-treated rats were consequently indistinguishable from saline-treated control animals, in spite of reduced dopamine levels in the striatum, suggesting this treatment provides a safe and effective way of producing an animal with a partial nigrostriatal dopamine lesion.

Although the reduction in striatal dopamine in 3-nitropropionic acid/methamphetamine-treated rats was insufficient to impair spontaneous movements, it nevertheless resulted in enhanced motor responses of these animals to selected dopamine receptor agonists, suggestive of the development of postsynaptic dopamine receptor supersensitivity. Contrary to earlier reports in naive animals, the selective D_2 dopamine receptor agonist RU 24213 (Euvrard et al., 1980), the mixed D_1/D_2 dopamine receptor agonist apomorphine and the dopamine releaser MK 801 (Whitton, 1997), at doses that would normally be regarded as behaviourally effective, did not stimulate motility in our saline-treated control rats (Starr and Starr, 1994). This apparent behavioural anomaly, like the resistance to toxin treatment mentioned above, could be a further manifestation of dopaminergic insufficiency in these particular animals. This could be related to the multiple injection treatment regime, which may have caused a repeated stress-related release of dopamine (Keefe et al., 1993) and hence, a desensitization and/or down-regulation of postsynaptic dopamine receptors. Whatever the reason, all of the agonists elicited robust motor responses following combined 3-nitropropionic acid and methamphetamine treatment, indicating a degree of sensitization had taken place. The fact that this heightened responsiveness did not extend to the locomotion or grooming evoked by the selective D_1 dopamine receptor agonist SKF 38393 (Setler et al., 1978), suggests that only the D_2 and not the D_1 dopamine receptors in the brain had become supersensitive. One explanation is that only the postsynaptic D_2 receptor population in the striatum was upregulated to compensate for the partial damage that 3-nitropropionic acid had caused to the nigrostriatal dopamine neurones. This proposal is suppported by the recent finding that another mitochondrial toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), differentially increased D_2 and not D_1 dopamine receptor binding in mouse striatum (Tanji et al., 1999).

Another possibility is that systemic 3-nitropropionic acid selectively damaged the executive pathways of the striatum which express the D_1 dopamine receptor. A study by Koutouzis et al. (1994b) lends weight to this argument. These authors found that 3-nitropropionic acid caused a modest down-regulation of striatal D_1 dopamine receptors, which they attributed to a deleterious effect of the toxin on striatonigral GABA output neurones, since these are preferentially endowed with D_1 dopamine receptors (Gerfen et al., 1990). Thus, by impairing dopamine release and sparing the D_2 receptor-bearing striatopallidal output pathway, 3-nitropropionic acid treatment results in an animal with a preferentially enhanced sensitivity to D_2 dopamine receptor stimuli.

The biphasic motor response of dual 3-nitropropionic acid and methamphetamine-treated rats to apomorphine is worth noting, as it resembles that elicited by apomorphine in unilaterally 6-hydroxydopamine-lesioned rats. In the latter model, apomorphine-induced contraversive circling is normally monophasic, but becomes biphasic with repeated apomorphine challenge, with peaks occurring at about 10 and 40 min (Oberlander et al., 1980), corresponding to the differential activation of dopamine D_1 and D_2 dopamine receptors (Coward, 1983). The biphasic response to apomorphine in our 3-nitropropionic acid/methamphetamine model differs in several important re-

spects: (a) no pharmacological kindling was required; (b) the peaks were temporally displaced (20 and 90 min); and (c) no involvement of dopamine D_1 receptors appears likely (although this requires confirmation).

In summary, treating rats systemically with a combination of the neurotoxins 3-nitropropionic acid and methamphetamine preferentially lowers dopamine levels in the dopamine terminal regions of the basal ganglia (striatum > accumbens), rendering them more sensitive to D_2 dopamine receptor stimulation, without causing long-term deficits in their spontaneous behaviour. Animals thus treated could provide a useful model for investigating the effects of partial degeneration of the nigrostriatal dopamine neurones, and in turn, improve our understanding of neurological disorders such as Parkinson's disease.

Acknowledgements

This work was supported by grants from the Medical Research Council and The Sir Jules Thorn Charitable Trust, and by an Overseas Research Scholarship to O.L. Eradiri.

References

Abekawa, T., Ohmori, T., Koyama, T., 1997. Tolerance to the neurotoxic effect of methamphetamine in rats behaviorally sensitized to methamphetamine or amphetamine. Brain Res. 767, 34–44.

Albers, D.S., Zeevalk, G.D., Sonsalla, P.K., 1996. Damage to dopaminergic nerve terminals in mice by combined treatment of intrastriatal malonate with systemic methamphetamine or MPTP. Brain Res. 718, 217–220.

Araujo, D.M., Hilt, D.C., 1998. Glial cell line-derived neurotrophic factor attenuates the locomotor hypofunction and striatonigral neurochemical deficits induced by chronic systemic administration of the mitochondrial toxin 3-nitropropionic acid. Neuroscience 82, 117–127.

Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R., Hyman, B.T., 1993. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J. Neurosci. 13, 4181–4192.

Borlongan, C.V., Koutouzis, T.K., Freeman, T.B., Cahill, D.W., Sanberg, P.R., 1995a. Behavioral pathology induced by repeated systemic injections of 3-nitropropionic acid mimics the motoric symptoms of Huntington's disease. Brain Res. 697, 254–257.

Borlongan, C.V., Koutouzis, T.K., Randall, T.S., Freeman, T.B., Cahill, D.W., Sanberg, P.R., 1995b. Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. Brain Res. Bull. 36, 549–556.

Bowyer, J.F., Tank, A.W., Newport, G.D., Slikker, W., Ali, S.F., Holson, R.R., 1992. The influence of environmental temperature on the transient effects of methamphetamine on dopamine levels and dopamine release in rat striatum. J. Pharmacol. Exp. Ther. 260, 817–824.

Bowyer, J.F., Clausing, P., Schmued, L., Davies, D.L., Binienda, Z., Newport, G.D., Scallet, A.C., Slikker, W., 1996. Parenterally administered 3-nitropropionic acid and amphetamine can combine to produce damage to terminals and cell bodies in the striatum. Brain Res. 712, 221–229.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brouillet, E., Jenkins, B.G., Hyman, B.T., Ferrante, R.J., Kowall, N.W., Srivastava, R., Samanta, R.D., Rosen, B.R., Beal, M.F., 1993. Age-dependent vulnerability of the striatum to the mitochondrial toxin 3-nitropropionic acid. J. Neurochem. 60, 356–359.

Buening, M.K., Gibb, J.W., 1974. Influence of methamphetamine and neuroleptic drugs on tyrosine hydroxylase activity. Eur. J. Pharmacol. 26, 30–34.

Chan, P., Di Monte, D.A., Luo, J.-J., DeLanney, L.E., Irwin, I., Langston, J.W., 1994. Rapid ATP loss caused by methamphetamine in mouse striatum: relationship between energy impairment and dopaminergic neurotoxicity. J. Neurochem. 62, 2484–2487.

Coward, D.M., 1983. Apomorphine-induced biphasic circling behaviour in 6-hydroxydopamine-lesioned rats: a pharmacological kindling phenomenon. Naunyn-Schmideberg's Arch. Pharmacol. 323, 49–53.

Erecinska, M., Nelson, D., 1994. Effects of 3-nitropropionic acid on synaptosomal energy and transmitter metabolism: relevance to neurodegenerative brain diseases. J. Neurochem. 63, 1033–1041.

Euvrard, C., Ferland, L., Di Paulo, T., Beaulieu, M., Labrie, F., Oberlander, C., Raynaud, J., Boissier, J.R., 1980. Activity of two new potent dopaminergic agonists at the striatal and pituitary levels. Neuropharmacology 19, 379–386.

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, J., Sibley, D.R., 1990. D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429–1432.

Gjedde, A., Léger, G.C., Cumming, P., Yasuhara, Y., Evans, A.C., Guttman, M., Kuwabara, H., 1993. Striatal L-DOPA decarboxylase activity in Parkinson's disease in vivo: implications for the regulation of dopamine synthesis. J. Neurochem. 61, 1538–1541.

Gould, D.H., Justine, D.L., 1982. Basal ganglia degeneration, myelin alterations, and enzyme inhibition induced in mice by the plant toxin 3-nitropropionic acid. Neuropathol. Appl. Neurobiol. 8, 377–393.

Greene, J.G., Sheu, S.-S., Gross, R.A., Greenamyre, J.T., 1998. 3-Nitropropionic acid exacerbates *N*-methyl-D-aspartate toxicity in striatal culture by multiple mechanisms. Neuroscience 84, 503–510.

Guyot, M.-C., Palfi, S., Stutzmann, J.M., Mazière, M., Hantraye, P., Brouillet, E., 1997. Riluzole protects from motor deficits and striatal degeneration produced by systemic 3-nitropropionic acid intoxication in rats. Neuroscience 81, 141–149.

Hamilton, B.F., Gould, D.H., 1987. Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. Acta Neuropathol. 72, 286–297.

Hassel, B., Sonnewald, U., 1995. Selective inhibition of the tricarboxylic acid cycle of GABAergic neurons with 3-nitropropionic acid in vivo. J. Neurochem. 65, 1184–1191.

Hotchkiss, A.J., Gibb, J.W., 1980. Long-term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in rat brain. J. Pharmacol. Exp. Ther. 214, 257–262.

Keefe, K.A., Sved, A.F., Zigmond, M.J., Abercrombie, E.D., 1993. Stress-induced dopamine release in the neostriatum: evaluation of the role of action potentials in nigrostriatal dopamine neurons or local initiation by endogenous excitatory amino acids. J. Neurochem. 61, 1943–1952.

Koutouzis, T.K., Borlongan, C.V., Freeman, T.B., Cahill, D.W., Sanberg, P.R., 1994a. Intrastriatal 3-nitropropionic acid: a behavioural assessment. NeuroReport 5, 2241–2245.

Koutouzis, T.K., Borlongan, C.V., Scorcia, T., Creese, I., Cahill, D.W., Freeman, T.B., Sanberg, P.R., 1994b. Systemic 3-nitropropionic acid: long-term effects on locomotor behaviour. Brain Res. 646, 242–246.

Lloyd, G., Hornykiewicz, O., 1970. Parkinson's disease: activity of L-dopa decarboxylase in discrete brain regions. Science 170, 1212–1213.

Ludolph, A.C., He, F., Spencer, P.S., Hammerstad, J., Sabri, M., 1991. 3-Nitropropionic acid — erogenous animal neurotoxin and possible human striatal toxin. Can. J. Neurol. Sci. 18, 492–498.

Marey-Semper, I., Gelman, M., Lévi-Strauss, M., 1995. A selective

toxicity toward cultured mesencephalic dopaminergic neurons is induced by the synergistic effects of energetic metabolism impairment and NMDA receptor activation. J. Neurosci. 15, 5912–5918.

Nakao, N., Brundin, P., 1997. Effects of α-phenyl-*tert*-butyl nitrone on neuronal survival and motor function following intrastriatal injections of quinolinate or 3-nitropropionic acid. Neuroscience 76, 749–761.

Nagatsu, T., Yamamoto, T., Kato, T., 1979. A new and highly sensitive voltammetric assay for AADC activity by high performance liquid chromatography. Anal. Biochem. 199, 160–165.

Oberlander, C., Dumont, C., Boissier, J.R., 1980. Time course of apomorphine-induced circling behaviour after striatal dopamine receptor denervation. Eur. J. Pharmacol. 62, 107–110.

O'Dell, S.J., Weihmuller, F.B., Marshall, J.F., 1993. Methamphetamine-induced dopamine overflow and injury to striatal dopamine terminals: attenuation by dopamine D_1 or D_2 antagonists. J. Neurochem. 60, 1792–1799.

Ohmori, T., Abekawa, T., Koyama, T., 1996. The role of glutamate in behavioral and neurotoxic effects of methamphetamine. Neurochem. Int. 29, 301–307.

Ossowska, K., 1994. The role of excitatory amino acids in experimental models of Parkinson's disease. J. Neural Transm. [P.D. Sect] 8, 39–71.

Reynolds, N.C., Lin, W., Cameron, C.M., Roerig, D.L., 1997. Differential responses of extracellular GABA to intrastriatal perfusions of 3-nitropropionic acid and quinolinic acid in the rat. Brain Res. 778, 140–149.

Ricuarte, G.A., Schuster, C.R., Seiden, L.S., 1980. Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res. 193, 153–163.

Riepe, M., Hori, N., Ludolph, A.C., Carpenter, D.O., Spencer, P.S., Allen, C.N., 1992. Inhibition of energy metabolism by 3-nitropropionic acid activates ATP-sensitive potassium channels. Brain Res. 586, 61–66.

Robinson, T.E., Becker, J.B., 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11, 157–198.

Seiden, L.S., Ricuarte, G.A., 1987. Neurotoxicity of methamphetamine and related drugs. In: Meltzer, H.Y. (Ed.), Psychopharmacology: The Third Generation of Progress. Raven Press, New York, pp. 359–368.

Setler, P.E., Sarau, H.M., Zirkle, C.L., Saunders, H.L., 1978. The central effects of a novel dopamine agonist. Eur. J. Pharmacol. 50, 419–430.

Sims, K.L., Davis, G.A., Bloom, F.E., 1973. Activities of 3,4-dihydroxy-L-phenylalanine and 5-hydroxy-L-tryptophan decarboxylases in rat brain: assay characteristics and distribution. J. Neurochem. 20, 449–464.

Sonsalla, P.K., Gibb, J.W., Hanson, G.R., 1986a. Nigrostriatal dopamine actions on the D2 receptors mediate methamphetamine effects on the striatonigral substance P system. Neuropharmacology 25, 1221–1230.

Sonsalla, P.K., Gibb, J.W., Hanson, G.R., 1986b. Roles of D_1 and D_2 dopamine receptor subtypes in mediating the methamphetamine-induced changes in monoamine systems. J. Pharmacol. Exp. Ther. 238, 932–937.

Sonsalla, P.K., Nicklas, W.J., Heikkila, R.E., 1989. Role for excitatory amino acids in methamphetamine-induced nigrostriatal dopaminergic toxicity. Science 242, 398–400.

Sonsalla, P.K., Manzino, L., Sinton, C.M., Liang, C.-L., German, D.C., Zeevalk, G.D., 1997. Inhibition of striatal energy metabolism produces cell loss in the ipsilateral substantia nigra. Brain Res. 773, 223–226.

Starr, M.S., Starr, B.S., 1994. Comparison of the effects of NMDA and AMPA antagonists on the locomotor activity induced by selective D_1 and D_2 dopamine agonists in reserpine-treated mice. Psychopharmacology 114, 469–476.

Stephans, S., Yamamoto, B., 1996. Methamphetamine pretreatment and the vulnerability of the striatum to methamphetamine neurotoxicity. Neuroscience 72, 593–600.

Tanji, H., Araki, T., Nagasawa, H., Itoyama, Y., 1999. Differential vulnerability of dopamine receptors in the mouse brain treated with MPTP. Brain Res. 824, 224–231.

Tsai, M.J., Goh, C.C., Wan, Y.L., Chang, C., 1997. Metabolic alterations produced by 3-nitropropionic acid in rat striata and cultured astrocytes: quantitative in vitro ^{1}H nuclear magnetic resonance spectroscopy and biochemical characterization. Neuroscience 79, 819–826.

Whitton, P.S., 1997. Glutamatergic control over brain dopamine release in vivo and in vitro. Neurosci. Biobehav. Rev. 21, 481–488.

Wüllner, U., Young, A.B., Penney, J.B., Beal, M.F., 1994. 3-Nitropropionic acid toxicity in the striatum. J. Neurochem. 63, 1772–1781.

Zeevalk, G.D., Derr-Yellin, E., Nicklas, W.J., 1995. NMDA receptor involvement in toxicity to dopamine neurons in vitro caused by the succinate dehydrogenase inhibitor 3-nitropropionic acid. J. Neurochem. 64, 455–458.

Zeevalk, G.D., Manzino, L., Hoppe, J., Sonsalla, P., 1997. In vivo vulnerability of dopamine neurons to inhibition of energy metabolism. Eur. J. Pharmacol. 320, 111–119.