ELSEVIER

European Journal of Pharmacology 370 (1999) 251–256

ejp

AMPA/kainate-related mechanisms contribute to convulsant and proconvulsant effects of 3-nitropropionic acid

Ewa M. Urbanska [a,b,*], Piotr Blaszczak [a], Tomasz Saran [a], Zdzislaw Kleinrok [a], Waldemar A. Turski [a,b]

[a] *Department of Pharmacology and Toxicology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland*
[b] *Department of Clinical Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland*

Received 19 October 1998; received in revised form 23 February 1999; accepted 26 February 1999

Abstract

The role of the glutamatergic system in the convulsant and proconvulsant action of a mitochondrial toxin, 3-nitropropionic acid, was studied in mice. The occurrence of 3-nitropropionic acid-induced seizures was inhibited by the α-amino-2,3-dihydro-5-methyl-3-oxo-isoxazole-propionate (AMPA)/kainate receptor antagonists, 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione disodium (NBQX) and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine HCl (GYKI 52466), with ED_{50} of 14.1 (7.9–25.2) and 7.2 (5.3–9.6) mg/kg, respectively. The *N*-methyl-D-aspartate (NMDA) receptor antagonists, dizocilpine (MK-801) and 3-(2-carboxypiperazine-4-yl)propenyl-1-phosphonic acid (CPPene), were ineffective. Moreover, 3-nitropropionic acid given in a subthreshold dose potently enhanced seizures generated by intracerebroventricular administration of AMPA and kainate, lowering their CD_{50} from 0.98 (0.83–1.17) and 0.73 (0.64–0.83) to 0.55 (0.45–0.66) ($P < 0.001$) and 0.58 (0.51–0.65) ($P < 0.05$) nmol, respectively. In contrast, NMDA action was not changed by 3-nitropropionic acid application. We conclude that AMPA/kainate-mediated events are involved in proconvulsive and convulsive effects of 3-nitropropionic acid.

Keywords: 3-Nitropropionic acid; Mitochondrial toxin; Seizure; Excitatory amino acid receptor agonist; Excitatory amino acid receptor antagonist; NMDA receptor; Non-NMDA receptor

1. Introduction

3-Nitropropionic acid is a naturally occurring toxin demonstrated to impair energy metabolism via irreversible inhibition of a mitochondrial complex II component, succinate dehydrogenase (Alston et al., 1977; Ludolph et al., 1991). Accidental ingestion of 3-nitropropionic acid has been associated with neurological disease in humans and animals (Ludolph et al., 1991). Experimental application of 3-nitropropionic acid evoked a selective neuronal loss closely resembling lesions observed in the course of Huntington's disease (Gould and Gustine, 1982; Ludolph et al., 1991; Beal et al., 1993; Geddes et al., 1996). It has been proposed that a deranged energy supply leads to increased vulnerability of cells to excitatory amino acid receptor agonists (Henneberry et al., 1989).

The search for an involvement of the glutamatergic system in 3-nitropropionic acid toxicity has yielded contradictory results. *N*-methyl-D-aspartate (NMDA) receptor antagonists either did not abolish the occurrence of 3-nitropropionic acid-induced lesions in vivo (Beal et al., 1993) or diminished them on chronic administration (Wenk et al., 1996). In vitro, NMDA receptor antagonists marginally reduced 3-nitropropionic acid-evoked changes of membrane potential, and, alone or in combination with an α-amino-2,3-dihydro-5-methyl-3-oxo-isoxazole-propionate (AMPA)/kainate antagonist, mitigated morphological lesions caused by 3-nitropropionic acid (Riepe et al., 1992; Riepe et al., 1994). An NMDA receptor antagonist diminished 3-nitropropionic acid toxicity for cultured cerebellar neurons and mesencephalic dopaminergic neurons (Weller and Paul, 1993; Zeevalk et al., 1995). Others, however, have demonstrated that NMDA receptor antagonists did not attenuate 3-nitropropionic acid toxicity in striatal cultures (Fink et al., 1996).

* Corresponding author. Fax: +48-81-747-8924; E-mail: ewau@galen.imw.lublin.pl

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00147-8

Glutamatergic receptor activation might contribute to the pathogenesis of epilepsy (see for review Meldrum, 1994; Urbanska et al., 1998b). Experimental application of NMDA and AMPA/kainate agonists precipitates seizures, whereas glutamatergic antagonists act as powerful anticonvulsants against a variety of experimental seizures induced chemically and electrically (Meldrum, 1994; Urbanska et al., 1998b). We have recently demonstrated the potent convulsive properties exerted by 3-nitropropionic acid in mice (Urbanska et al., 1998a). Therefore, the aim of this study was to investigate the role of glutamatergic system in the convulsive and proconvulsive action of 3-nitropropionic acid.

2. Materials and methods

2.1. Animals

The studies were carried out on male Albino–Swiss mice weighing 20–25 g. The animals were kept under standard laboratory conditions, with free access to chow pellets and water. The experiments were performed between 9:00 and 16:00 h. Each experimental group consisted of at least 8 animals. Behavioral observations were recorded (a) within 2 h following peripheral application of a convulsive dose of 3-nitropropionic acid, (b) within 1 h after intracerebroventricular (i.c.v.) administration of convulsants. Mortality rate was evaluated at 2 h after systemic 3-nitropropionic acid injection. All animal experiments were performed according to the Lublin Medical University School ethical guidelines.

2.2. Drugs

3-Nitropropionic acid (Sigma) was dissolved in water, the pH being adjusted to 7.2, and was injected intraperitoneally (i.p.) at the dose of 100 or 220 mg/kg. Dizocilpine maleate (MK-801; RBI) was dissolved in water and given i.p. in doses of 0.2–0.5 mg/kg. 3-(2-Carboxypiperazine-4-yl)propenyl-1-phosphonic acid (CPPene; RBI) was dissolved in water and administered i.p. at doses of 10.0 and 20.0 mg/kg. 6-Nitro-7-sulphamoyl-benzo [*f*]quinoxaline-2,3-dione disodium (NBQX) (Tocris), and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine HCl (GYKI 52466) (a generous gift from Dr. I. Tarnawa; Institute for Drug Research, Budapest, Hungary) were dissolved in a minimum quantity of 1N NaOH, the pH was adjusted to 8.0 and 7.4, the drugs being administered i.p. in doses of 5.0–40.0 and 5.0–20.0 mg/kg, respectively. NBQX was administered simultaneously with 3-nitropropionic acid (given in the dose of 220 mg/kg, i.e., equal to CD_{97}, as established in the preliminary studies), MK-801 at 10 min, and CPPene and GYKI 52466 at 30 min before the convulsant. The injection volume was 0.1 ml/10 g body weight.

NMDA, kainic acid and α-amino-2,3-dihydro-5-methyl-3-oxo-isoxazole-propionate (AMPA) (all 3 substances from RBI) dissolved in a minimum quantity of 1 N NaOH, with the pH of their solutions adjusted to 7.2, were injected i.c.v. in doses of 0.6–1.2, 0.4–1.0 and 0.4–1.2 nmol, respectively.

2.3. Intracerebroventricular administration of glutamatergic agonists

Intracerebroventricular injections of NMDA, kainate and AMPA were performed according to the method previously described (Turski et al., 1991). Briefly, drug solutions were injected free-hand with the use of Hamilton syringe into the left cerebral ventricle of unanesthetized mice, in a volume of 5 μl. The subthreshold dose of 3-nitropropionic acid, i.e., 100 mg/kg (equal to 75% of its CD_{16}) was given i.p., 30 min prior to the i.c.v. injection of the excitatory amino acid receptor agonist.

Table 1
Effect of glutamatergic ionotropic receptor antagonists on the latency to seizures and death following the application of 3-nitropropionic acid in mice

Treatment (mg/kg)		Latency (min)	
		Seizures	Death
3-Nitropropionic acid		21.2 ± 3.0	34.6 ± 3.9
3-Nitropropionic acid + MK-801	0.2	34.1 ± 2.9[c]	46.1 ± 5.8[a]
	0.4	35.8 ± 3.4[c]	52.3 ± 4.0[b]
	0.5	40.3 ± 4.2[c]	53.5 ± 5.4[c]
3-Nitropropionic acid		22.6 ± 3.4	43.5 ± 2.5
3-Nitropropionic acid + CPP-ene	10.0	23.8 ± 2.9	45.1 ± 3.5
	20.0	26.3 ± 3.9	48.8 ± 4.7
3-Nitropropionic acid		24.1 ± 2.3	38.7 ± 4.2
3-Nitropropionic acid + NBQX	5.0	25.8 ± 3.8	54.2 ± 7.4[b]
	15.0	29.8 ± 2.8[a]	61.3 ± 4.9[c]
	25.0	32.5 ± 3.1[b]	58.4 ± 6.1[b]
	40.0	34.0	73.2 ± 9.5[c]
3-Nitropropionic acid		22.7 ± 4.4	41.7 ± 5.1
3-Nitropropionic acid + GYKI 52466	5.0	22.4 ± 3.9	42.0 ± 6.5
	7.5	41.5 ± 4.6[c]	60.6 ± 9.1[b]
	10.0	49.0	64.5 ± 5.2[c]
	20.0	43.0	72.0 ± 12.8[c]

All animals were given 3-nitropropionic acid at the dose equal to its CD_{97}, i.e., 220 mg/kg i.p. NBQX was administered simultaneously with 3-nitropropionic acid, MK-801 at 10 min, GYKI 52466 and CPPene at 30 min before the convulsant.

Latency data are expressed as means ± S.D., wherever the number of animals displaying positive effect, i.e., seizures or death was > 1.

Statistical comparisons of latencies were performed with the one-way analysis of variance (ANOVA), followed by adjustment of the *P* value by the method of Bonferroni.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

2.4. Statistics

The doses of excitatory amino acid receptor agonist inducing a seizure response in 16, 50 or 97% of the mice (CD_{16}, CD_{50} and CD_{97}; convulsive dose) were determined based on the data obtained from 3-4 experiments performed with different doses of drug. The dose of the excitatory amino acid receptor antagonist necessary to block 3-nitropropionic acid convulsions in 50% of mice (ED_{50}; effective dose) was established in a similar way. CD_{16}, CD_{50}, CD_{97}, and ED_{50} values, together with their confidence limits, were estimated by computerized fitting of the data using linear regression analysis (Litchfield and Wilcoxon, 1949). Statistical comparisons of latencies were performed based on a one-way analysis of variance (ANOVA) followed by adjustment of the P value by the method of Bonferroni.

3. Results

3.1. Behavioral effects of 3-nitropropionic acid

Peripheral application of 3-nitropropionic acid in the dose of 220 mg/kg i.p., equal to its CD_{97}, evoked clonic seizures with a latency of 21–24 min (Table 1). Typically, a seizure episode lasting 5–10 s, included clonic limb movements, paddling behaviour and loss of body posture.

Table 2
Effect of glutamatergic ionotropic receptor antagonists on the occurrence of 3-nitropropionic acid-induced seizures and mortality in mice

Treatment (mg/kg)		Seizures (n/N)	Mortality (n/N)
3-Nitropropionic acid		9/9	7/9
3-Nitropropionic acid + MK-801	0.2	9/9	8/9
	0.4	6/8	7/8
	0.5	6/8	8/8
3-Nitropropionic acid		8/8	8/8
3-Nitropropionic acid + CPP-ene	10.0	7/8	8/8
	20.0	6/8	8/8
3-Nitropropionic acid		9/9	8/9
3-Nitropropionic acid + NBQX	5.0	6/8	7/8
	15.0	5/8	7/8
	25.0	3/8	6/8
	40.0	1/8	5/8
3-Nitropropionic acid		9/9	9/9
3-Nitropropionic acid + GYKI 52466	5.0	7/8	8/8
	7.5	4/8	8/8
	10.0	1/8	8/8
	20.0	1/8	6/8

All animals were given 3-nitropropionic acid at the dose equal to its CD_{97}, i.e., 220 mg/kg i.p. NBQX was administered simultaneously with 3-nitropropionic acid, MK-801 at 10 min, GYKI 52466 and CPPene at 30 min before the convulsant.
n/N: number of animals displaying seizures or death (n) out of the total number of subjects tested (N).

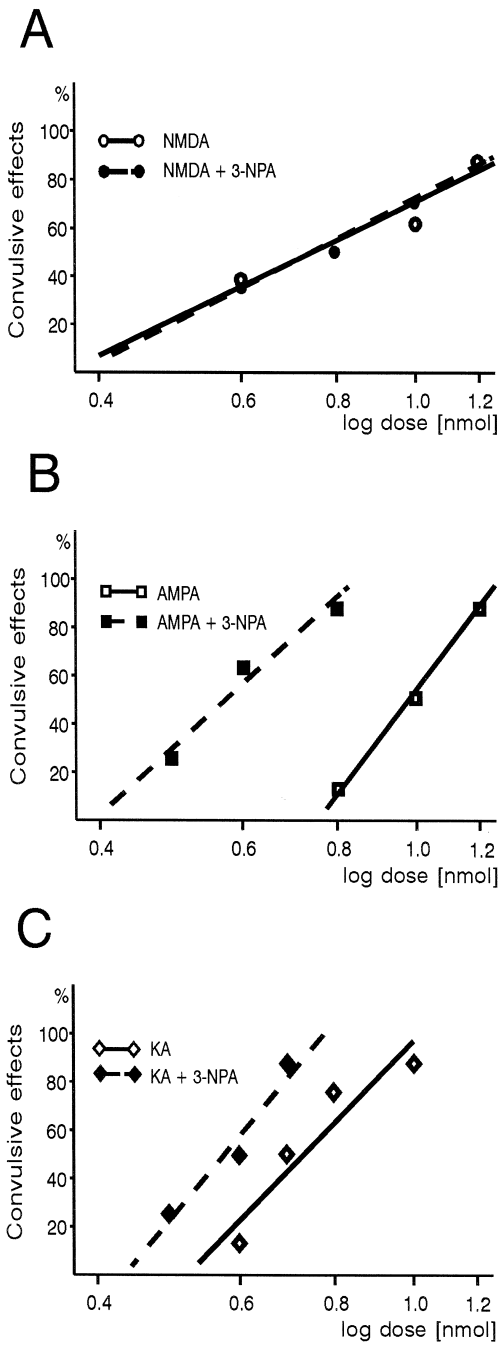

Fig. 1. Effect of 3-nitropropionic acid on clonic seizures evoked by NMDA (A), AMPA (B) and kainate (C). 3-Nitropropionic acid was administered in the dose of 100 mg/kg, i.e., equal to 75% of its CD_{16}, at 30 min before i.c.v. injection of glutamate agonist. Data are presented as percentages of animals displaying seizures. Probit–log dose regression curves were calculated using GraphPAD software. (A): NMDA: $y = 155.4x - 396.4$, $r = 0.97$; NMDA + 3-nitropropionic acid: $y = 168.3x - 432.7$, $r = 0.97$. (B): AMPA: $y = 414.9x - 1192.0$, $r = 0.99$; AMPA + 3-nitropropionic acid: $y = 292.9x - 758.7$, $r = 0.98$. (C): KA: $y = 521.0x - 1433.8$, $r = 0.99$; KA + 3-nitropropionic acid: $y = 405.8x - 1069.9$, $r = 0.97$.

This was followed by a period of hypoactivity. Seizure attacks usually occurred repetitively, 3–5 times/h. Tonic seizures were not observed and death of animals was not a

direct result of convulsions. The mean latency to death was 35–44 min (Table 1).

3.2. Effects of glutamatergic antagonists upon 3-nitropropionic acid-induced seizures

The latency to the onset of 3-nitropropionic acid-induced seizures was increased by MK-801 (0.2 mg/kg i.p. and more), NBQX (15.0 and 25.0 mg/kg i.p.) and GYKI 52466 (7.5 mg/kg i.p.) but not by CPP-ene (Table 1). The latency to death following application of 3-nitropropionic acid was increased by MK-801 (> 0.2 mg/kg and more), NBQX (5.0 mg/kg and more) and GYKI 52466 (7.5 mg/kg and more) but not CPP-ene. (Table 1). Neither severity nor duration of a single seizure episode was changed by the application of excitatory amino acid receptor antagonists.

MK-801, given up to 0.5 mg/kg i.p., and CPPene, used up to 20 mg/kg i.p., did not affect the occurrence 3-nitropropionic acid-induced seizures (Table 2). In contrast, NBQX and GYKI 52466 displayed potent anticonvulsant activity with ED_{50} of 14.1 (7.9–25.2) and 7.2 (5.3–9.6) mg/kg i.p., respectively (Table 2).

3.3. Effects of peripheral 3-nitropropionic acid application on convulsions evoked by i.c.v. injection of glutamatergic agonists

3-Nitropropionic acid injected at the dose of 100 mg/kg i.p. 30 min before the test lowered the threshold for clonic seizures evoked by i.c.v. administration of AMPA and kainate but not NMDA (Fig. 1). The CD_{50} for clonic seizures induced by AMPA and kainate was diminished from 0.98 (0.83–1.17) and 0.73 (0.64–0.83) to 0.55 (0.45–0.66) ($P < 0.001$) and 0.58 (0.51–0.65) ($P < 0.05$) nmol, respectively (Table 3).

Table 3
Effect of 3-nitropropionic acid on action of glutamatergic ionotropic receptor agonists administered i.c.v. in mice

Treatment		CD_{50} (nmol)
NMDA		0.73 (0.55–0.97)
NMDA + 3-nitropropionic acid	100	0.73 (0.55–0.99)
AMPA		0.98 (0.83–1.17)
AMPA + 3-nitropropionic acid	100	0.55 (0.45–0.66)[b]
Kainate		0.73 (0.64–0.83)
Kainate + 3-nitropropionic acid	100	0.58 (0.51–0.65)[a]

Animals were given 3-nitropropionic acid at the dose of 100 mg/kg, i.e., equal to 75% of its CD_{16}, at 30 min before the i.c.v. injection of glutamatergic agonist.
The doses of excitatory amino acid receptor agonists inducing a seizure response in 50% of the mice (CD_{50}; convulsive dose) presented here together with their confidence limits were estimated by computerized fitting of the data by linear regression analysis (Litchfield and Wilcoxon, 1949).
[a] $P < 0.05$.
[b] $P < 0.001$.

4. Discussion

This study extends our recent report on the convulsive activity displayed by the mitochondrial toxin 3-nitropropionic acid (Urbanska et al., 1998a), and indicates that the excitatory amino acids system is involved in the convulsant and proconvulsant action of 3-nitropropionic acid. A competitive AMPA/kainate antagonist, NBQX, and a non-competitive one, GYKI 52466, inhibited seizures evoked by systemic application of 3-nitropropionic acid and prolonged the latency to their onset. In contrast, CPP-ene, a competitive NMDA antagonist, and MK-801, a non-competitive one, did not affect the occurrence of 3-nitropropionic acid convulsions. MK-801, however, increased seizure latency. Moreover, peripherally given 3-nitropropionic acid potently reduced the threshold for seizures precipitated by i.c.v. administration of AMPA and kainate, but not by NMDA. These results implicate a prevailing involvement of AMPA/kainate-, but not of NMDA-mediated, events in the convulsive and proconvulsive properties of 3-nitropropionic acid.

Recent data indicate a role of the glutamatergic system in the neurotoxic action of 3-nitropropionic acid in vitro and in vivo, but the precise role of NMDA vs. AMPA/kainate receptor stimulation in 3-nitropropionic acid neurotoxicity is not clearly defined. 3-Nitropropionic acid inhibits succinate dehydrogenase, thus diminishing mitochondrial synthesis of ATP (Alston et al., 1977). Energy depletion was shown to cause partial neuronal depolarization and subsequent relief of the voltage-dependent block of NMDA receptor ion channels (Henneberry et al., 1989) which could suggest that mitochondrial impairment after 3-nitropropionic acid application may lead to over-activation of the excitatory amino acid system.

MK-801 was demonstrated to attenuate 3-nitropropionic acid related neuronal damage, e.g., in cortical explant culture (Ludolph et al., 1992; Riepe et al., 1994), striatal cultures (Fink et al., 1996), cultured cerebellar granule neurons (Weller and Paul, 1993), or cultured hippocampal neurons (Pang and Geddes, 1997). Among other NMDA antagonists, D-2-amino-phosphonopentanoic acid (APV) was shown to delay but not prevent 3-nitropropionic acid neurotoxicity in cultured cerebellar granule neurons (Weller and Paul, 1993). APV, 3-(±)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and 7-Cl-kynurenic acid only partially reversed the late depolarization evoked by 3-nitropropionic acid in pyramidal neurons of hippocampal slices (Riepe et al., 1992; Riepe et al., 1995). Others have observed that neither kynurenic acid, nor DL-2-amino-7-phosphonoheptanoic acid (APH), used alone or concomitantly, was able to reduce the neuronal loss caused by 3-nitropropionic acid in striatal cultures (Fink et al., 1996).

Thus, the vast majority of reports indicating effectiveness of the NMDA antagonists against 3-nitropropionic acid-evoked neurotoxicity in vitro are based on results obtained with MK-801 whereas other NMDA antagonists seem to be much less effective.

The AMPA/kainate antagonist, 6,7-dichloroquinoxaline-2,3-dione (CNQX), was shown to either partially antagonise the action of 3-nitropropionic acid in hippocampal slices (Riepe et al., 1992) or to have no effect in cortical explants (Riepe et al., 1994). However, there are a number of reports that CNQX potentiates the protective action of MK-801 against 3-nitropropionic acid in various preparations in vitro (Ludolph et al., 1992; Riepe et al., 1994; Zeevalk et al., 1995), indicating the involvement of the AMPA/kainate system in 3-nitropropionic acid toxicity.

There are only sparse data concerning the neurotoxic effects of 3-nitropropionic acid in vivo in the context of glutamatergic system activity. A single dose of MK-801 did not affect the neurodegeneration evoked by intrastriatal injection of 3-nitropropionic acid in rats (Beal et al., 1993). We found that a single administration of an NMDA antagonist, MK-801 or CPPene, did not affect the occurrence of seizures induced by i.c.v. application in mice. Only the latency to seizures and to death due to 3-nitropropionic acid was prolonged by MK-801. Nevertheless, MK-801 even at higher doses, corresponding to these preventing electroconvulsions, reflex seizures or seizures induced by 4-aminopyridine (Urbanska et al., 1991; Fragoso-Veloz and Tapia, 1992; Meldrum, 1994) did not influence the occurrence of 3-nitropropionic acid-evoked seizures. Moreover, the selective NMDA antagonist, CPPene, did not change the time to the onset of convulsions or to death. It cannot be excluded that the partial activity of MK-801 against 3-nitropropionic acid seizures is not related to its antagonism at NMDA receptors. In fact, MK-801 action appears also to involve dopaminergic, serotonergic and noradrenergic systems (Clineschmidt et al., 1982; Löscher and Honack, 1992).

In contrast to NMDA antagonists, the AMPA/kainate ones, NBQX and GYKI 52466, displayed effective and potent protection against 3-nitropropionic acid-induced seizures. 3-Nitropropionic acid consistently enhanced seizures generated by i.c.v. administration of AMPA and kainate, but not of NMDA. Others have shown that peripheral application of 3-nitropropionic acid 12 h before the test enhanced the NMDA-induced neuronal loss in rat striatum (Simpson and Isacson, 1993). In our study, the convulsive action of i.c.v. NMDA was not potentiated by 3-nitropropionic acid administered i.p. 0.5 h earlier. However, NMDA applied intrastriatally in contrast to i.c.v. injection did not evoke seizures.

3-Nitropropionic acid did not affect seizures induced by NMDA and NMDA antagonists were ineffective against 3-nitropropionic acid-evoked seizures what implies that the convulsive and proconvulsive activity of 3-nitropropionic acid as opposed to the neurotoxic action of 3-nitropropionic acid, does not involve activation of the NMDA system. The present data suggest a prominent contribution of AMPA/kainate- but not of NMDA-related mechanisms to the convulsant and proconvulsant effects of 3-nitropropionic acid.

3-Nitropropionic acid, being an irreversible inhibitor of succinate dehydrogenase and mitochondrial complex II, potently depletes neuronal sources of ATP and causes very profound metabolic derangement. According to the hypothesis proposed by Zeevalk and Nicklas (1991), severe metabolic stress might activate not only NMDA receptors, but also a major proportion of AMPA/kainate ones, rendering NMDA antagonists unable to offer sufficient protection.

In summary, our findings suggest the involvement of AMPA/kainate glutamatergic mechanisms in seizures triggered by 3-nitropropionic acid administration, as well as in the proconvulsive action of this mitochondrial toxin. In contrast, NMDA-mediated events do not appear to play an important role in the convulsive and proconvulsive activity of 3-nitropropionic acid.

References

Alston, T.A., Mela, L., Bright, H.J., 1977. 3-Nitropropionate, the toxic substance of *Indigofera*, is a suicide inactivator of succinate dehydrogenase. Proc. Natl. Acad. Sci. U.S.A. 74, 3767–3771.

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.

Clineschmidt, B.V., Martin, G.E., Bunting, P.R., Papp, M.L., 1982. Central sympathomimetic activity of (±)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine (MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug Dev. Res. 2, 135–146.

Fink, S.L., Ho, D.Y., Sapolsky, R.M., 1996. Energy and glutamate dependency of 3-nitropropionic acid neurotoxicity in culture. Exp. Neurol. 138, 298–304.

Fragoso-Veloz, J., Tapia, R., 1992. NMDA receptor antagonists protect against seizures and wet-dog shakes induced by 4-aminopyridine. Eur. J. Pharmacol. 221, 275–280.

Geddes, J.W., Pang, Z., Wiley, D.H., 1996. Hippocampal damage and cytoskeletal disruption resulting from impaired energy metabolism. Implications for Alzheimer disease. Mol. Chem. Neuropathol. 28, 65–74.

Gould, D.H., Gustine, 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.

Henneberry, R.C., Novelli, A., Cox, J.A., Lysko, P.G., 1989. Neurotoxicity at the *N*-methyl-D-aspartate receptor in energy-compromised neurons: An hypothesis for cell death in aging and disease. Ann. NY Acad. Sci. 568, 225–233.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–111.

Löscher, W., Honack, D., 1992. The behavioural effects of MK-801 in rats: involvement of dopaminergic, serotonergic and noradrenergic systems. Eur. J. Pharmacol. 215, 199–208.

Ludolph, A.C., He, F., Spencer, P.S., Hammerstad, J., Sabri, M., 1991.

3-Nitropropionic acid—exogenous animal neurotoxin and possible human striatal toxin. Can. J. Neurol. Sci. 18, 492–498.

Ludolph, A.C., Seelig, M., Ludolph, A., Novitt, P., Allen, C.N., Spencer, P.S., Sabri, M.I., 1992. 3-Nitropropionic acid decreases cellular energy levels and causes neuronal degeneration in cortical explants. Neurodegeneration 1, 155–161.

Meldrum, B.S., 1994. The role of glutamate in epilepsy and other CNS disorders. Neurology 44 (suppl. 8), S14–S23.

Pang, Z., Geddes, J.W., 1997. Mechanisms of cell death induced by mitochondrial toxin 3-nitropropionic acid: acute excitotoxic necrosis and delayed apoptosis. J. Neurosci. 17, 3064–3073.

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.

Riepe, M., Ludolph, A., Seelig, M., Spencer, P.S., Ludolph, A.C., 1994. Increase of ATP levels by glutamate antagonists is unrelated to neuroprotection. Neuroreport 5, 2130–2132.

Riepe, M.W., Hori, N., Ludolph, A.C., Carpenter, D.O., 1995. Failure of neuronal ion exchange, not potentiated excitation, causes excitotoxicity after inhibition of oxidative phosphorylation. Neuroscience 64, 91–97.

Simpson, J.R., Isacson, O., 1993. Mitochondrial impairment reduces the threshold for in vivo NMDA-mediated neuronal death in the striatum. Exp. Neurol. 121, 57–64.

Turski, W.A., Dziki, M., Urbanska, E., Calderazzo-Filho, L.S., Cavalheiro, E.A., 1991. Seizures induced by aminooxyacetic acid in mice: pharmacological characteristics. Synapse 7, 173–180.

Urbanska, E., Dziki, M., Kleinrok, Z., Czuczwar, S.J., Turski, W.A., 1991. Influence of MK-801 on the anticonvulsant activity of antiepileptics. Eur. J. Pharmacol. 200, 277–282.

Urbanska, E., Blaszczak, P., Saran, T., Kleinrok, Z., Turski, W.A., 1998a. Mitochondrial toxin 3-nitropropionic acid evokes seizures in mice. Eur. J. Pharmacol. 359, 55–58.

Urbanska, E.M., Czuczwar, S.J., Kleinrok, Z., Turski, W.A., 1998b. Excitatory amino acids in epilepsy. Rest. Neurol. Neurosci. 13, 25–39.

Weller, M., Paul, S.M., 1993. 3-Nitropropionic acid is an indirect excitotoxin to cultured cerebellar granule neurons. Eur. J. Pharmacol. 248, 223–228.

Wenk, G.L., Danysz, W., Roice, D.D., 1996. The effect of mitochondrial failure upon cholinergic toxicity in the nucleus basalis. Neuroreport 7, 1453–1456.

Zeevalk, G.D., Nicklas, W.J., 1991. Mechanisms underlying initiation of excitotoxicity associated with metabolic inhibition. J. Pharmacol. Exp. Ther. 257, 870–878.

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.