ELSEVIER

European Journal of Pharmacology 359 (1998) 55–58

ejp

Short communication

Mitochondrial toxin 3-nitropropionic acid evokes seizures in mice

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 7 August 1998; revised 28 August 1998; accepted 1 September 1998

Abstract

3-Nitropropionic acid, a potent inhibitor of succinate dehydrogenase which thus compromises cellular energy metabolism, evoked convulsions in mice in a dose-dependent manner. CD_{50} for clonic seizures was 158.5 (144.1–174.3) mg/kg. Tonic seizures were not observed. Broad-spectrum anticonvulsants, namely diazepam, phenobarbital and valproate, prevented the occurrence of 3-nitropropionic acid-induced seizures with ED_{50} of 4.9 (3.1–7.6), 33.1 (17.9–61.0) and 389.7 (351.2–432.3) mg/kg, respectively. Diphenylhydantoin-like drugs (diphenylhydantoin, and carbamazepine), anti-absence drugs (trimethadione and ethosuximide) and acetazolamide were ineffective. The characteristics of 3-nitropropionic acid-induced seizures resembled those of convulsions evoked by another mitochondrial toxin, aminooxyacetic acid.

Keywords: Seizure; Mitochondrial toxin; 3-Nitropropionic acid; Antiepileptic

1. Introduction

3-Nitropropionic acid is a widespread plant and fungal toxin that irreversibly inhibits succinate dehydrogenase, an enzyme which is present in both the Krebs cycle and the mitochondrial electron transport chain (Alston et al., 1977; Ludolph et al., 1991). The 3-nitropropionic acid-induced inhibition of the electron cascade in concert with the disruption of oxidative phosphorylation compromises cellular energy metabolism (Ludolph et al., 1991). When applied in rodents, 3-nitropropionic acid induces selective neuronal loss, predominantly in the basal ganglia, but rarely within the hippocampus or thalamus (Gould and Gustine, 1982; Ludolph et al., 1991; Beal et al., 1993; Bossi et al., 1993; Geddes et al., 1996; Miller and Zaborszky, 1997). The histopathological and biochemical features of 3-nitropropionic acid-evoked striatal neurodegeneration mimic those observed in the course of Huntington's disease (Beal et al., 1993), suggesting that defective energy metabolism might contribute to human neuropathology.

The effects of 3-nitropropionic acid and the biochemical profile of drug action are similar to those of another mitochondrial toxin—aminooxyacetic acid. Aminooxyacetic acid impairs cellular oxidation processes and diminishes ATP production via potent and irreversible inhibition of aspartate aminotransferase, an enzyme involved in the malate–aspartate shunt across mitochondrial membranes (Kauppinen et al., 1987). Aminooxyacetic acid was demonstrated to cause seizures in rodents and to evoke selective neuronal lesions resembling those of excitotoxic neurodegeneration (Turski et al., 1991; Urbanska et al., 1991, 1998).

In a view of our report on the convulsive properties of aminooxyacetic acid we hypothesized that 3-nitropropionic acid might also generate seizures. Preliminary data have confirmed this assumption (Blaszczak et al., 1996). Here we demonstrate the potent convulsant action of 3-nitropropionic acid in mice and describe the profile of protection afforded by common antiepileptics against 3-nitropropionic acid-induced seizures.

2. Materials and methods

2.1. Animals

The studies were carried out on male Albino–Swiss mice weighing 20–25 g. Animals were kept under standard

* Corresponding author. Department of Pharmacology and Toxicology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland. Tel./fax: +48-81-747-8924; E-mail: ewau@galen.imw.lublin.pl

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00648-7

laboratory conditions, with free access to food and water. The experiments were performed between 9.00 and 16.00 h.

2.2. Substances

The substances used in the study included: 3-nitropropionic acid, sodium valproate, carbamazepine and diphenylhydantoin (all Sigma), diazepam, phenobarbital, trimethadione, ethosuximide and acet-azolamide (all Polfa, Poland). Substances were dissolved in water or suspended in 1% Tween 80 (Sigma). 3-Nitropropionic acid was given intraperitoneally (i.p.). All anticonvulsants were administered i.p. at 10 min (sodium valproate), 20 min (ethosuximide), 30 min (diazepam, carbamazepine, trimethadione and acetazolamide) or 60 min (phenobarbital and diphenylhydantoin) prior to 3-nitropropionic acid, respectively, in a volume of 0.1 ml/10 g body weight.

2.3. Behavioral investigations

Each experimental group consisted of at least 8 animals. Behavioral observations were performed within 120 min following application of 3-nitropropionic acid. Mortality rate was evaluated at 120 min after 3-nitropropionic acid injection.

2.4. Statistics

The doses of a drug required to induce a seizure response in 50% and 97% of mice (CD_{50} and CD_{97}; convulsive dose) or mortality in 50% of mice (LD_{50}; lethal dose) were determined on the basis of data obtained from 3–4 experiments in which different doses of drug were studied. The dose of antiepileptic drug necessary to block convulsions in 50% of mice (ED_{50}; effective dose) was established in similar way. CD_{50}, CD_{97}, LD_{50} and ED_{50} values together with their confidence limits were estimated by computerized fitting of the data by linear regression analysis (Litchfield and Wilcoxon, 1949). Statistical comparisons of latency data were performed by means of one-way analysis of variance (ANOVA) followed by adjustment of P value by the Bonferroni method.

3. Results

3.1. Convulsions and mortality induced by 3-nitropropionic acid

Within 5–10 min of systemic application of 3-nitropropionic acid the animals became akinetic and displayed diminished responsiveness to external stimuli. When 3-nitropropionic acid was given at the doses of 100–120 mg/kg, occasional tremor or head twitches were observed. Convulsions occurred dose dependently after the administration of doses higher than 130 mg/kg of 3-nitropropionic acid (Table 1). The CD_{50} for clonus was 158.5 (144.1–174.3) mg/kg i.p. The mean latency to the onset of seizures correlated inversely with the dose of 3-nitropropionic acid (Table 1).

Table 1
Seizures and mortality evoked by 3-nitropropionic acid in mice

Dose (mg/kg)	Seizures		Mortality	
	n/N	Latency (min)	n/N	Latency (min)
100	0/8		0/8	
130	1/8	34.0	0/8	
150	3/8	28.7±5.5	0/8	
165	6/10	26.5±6.6	3/10	58.3±5.8
180	6/8	22.5±2.4	4/8	55.4±4.9
200	10/11	22.7±4.1	9/11	41.8±5.1
220	12/12	21.7±1.7	12/12	34.6±2.8

Data are expressed as means±S.D.; n/N = number of animals displaying seizures or dying (n) out of the total number of animals tested (N). Observation was carried out up to 120 min after injection of 3-nitropropionic acid.

Single seizure episodes started with clonic movements of the limbs, usually followed by the loss of body posture. In some cases body rotations along the longitudinal axis and paddling behavior appeared. Convulsions lasted no longer than 5–10 s each and could occur repeatedly, usually 3–5 times in an hour. Neither tonic seizures nor status epilepticus was noted. The mortality rate increased dose dependently (Table 1). The death of animals was not a direct result of convulsions. Rarely, following the application of the highest studied doses of 3-nitropropionic acid, some of the animals died without showing behavioral symptoms of seizures. The LD_{50} was 177.9 (166.2–190.4) mg/kg i.p. The mean latency to death after the injection of 3-nitropropionic acid decreased dose dependently (Table 1).

3.2. Effect of antiepileptic drugs on 3-nitropropionic acid-induced seizures and mortality

Diazepam (1.0–5.0 mg/kg) and valproate (250 mg/kg and more) increased the latency to the onset of 3-nitropropionic acid-induced seizures (Table 2). The prolongation of latency was dose dependent (Table 2).

Diazepam, phenobarbital and valproate afforded protection against seizures evoked by 3-nitropropionic acid (Table 2). Their respective ED_{50} values were 4.9 (3.1–7.6), 33.1 (17.9–61.0) and 389.7 (351.2–432.3) mg/kg i.p.

Diphenylhydantoin up to 50 mg/kg, carbamazepine up to 70 mg/kg i.p., trimethadione up to 400 mg/kg, ethosuximide up to 500 mg/kg and acetazolamide up to 1000 mg/kg did not alter the latency and occurrence of seizures induced by 3-nitropropionic acid (Table 2).

The latency to death following 3-nitropropionic acid injection was increased dose-dependently only by diazepam (1.0–5.0 mg/kg) and valproate (250 mg/kg and

Table 2
Effect of antiepileptic drugs on 3-nitropropionic acid-induced seizures and mortality in mice

Treatment (mg/kg)		Seizures		Mortality	
		n/N	Latency (min)	n/N	Latency (min)
Saline		8/8	19.0 ± 2.1	6/8	34.2 ± 2.2
Diazepam	1.0	8/8	24.6 ± 2.3[a]	8/8	46.6 ± 4.1[c]
	2.5	5/8	29.7 ± 3.0[c]	7/8	43.5 ± 2.5[b]
	5.0	4/8	30.0 ± 4.3[c]	8/8	47.0 ± 3.3[c]
	7.5	3/8	24.0 ± 2.5	6/8	40.3 ± 2.0
Saline		8/8	20.7 ± 2.7	8/8	35.0 ± 3.6
Phenobarbital	10.0	7/8	21.8 ± 3.9	7/8	32.7 ± 2.1
	30.0	4/8	20.0 ± 2.3	8/8	30.0 ± 5.0
	50.0	3/8	20.4 ± 4.1	6/8	32.0 ± 3.9
	70.0	2/8	19.6 ± 3.8	7/8	37.0 ± 3.4
Saline		8/8	18.7 ± 2.3	8/8	29.9 ± 2.5
Valproate	100.0	8/8	20.5 ± 3.4	8/8	29.3 ± 2.1
	250.0	6/8	26.2 ± 2.0[a]	8/8	39.5 ± 2.9[b]
	350.0	6/8	30.3 ± 3.6[c]	8/8	41.3 ± 4.2[c]
	400.0	3/8	31.6 ± 4.5[b]	7/8	45.0 ± 5.3[c]
	450.0	2/8	34.5 ± 2.9[b]	7/8	54.0 ± 4.1[c]
Saline		7/8	25.0 ± 3.2	8/8	31.9 ± 4.7
Carbamazepine	30.0	6/8	25.3 ± 4.1	8/9	33.0 ± 4.3
	50.0	5/8	29.2 ± 5.5	8/8	36.2 ± 5.7
	70.0	6/8	30.1 ± 5.2	7/8	38.0 ± 6.0
Saline		8/8	22.4 ± 2.1	7/8	33.2 ± 4.8
Diphenylhydantoin	10.0	7/7	21.1 ± 2.5	7/7	32.8 ± 3.2
	50.0	8/8	24.6 ± 3.7	8/8	35.0 ± 4.2
	100.0	7/8	27.5 ± 3.3	7/8	40.0 ± 5.1
Saline		10/10	24.2 ± 3.0	10/10	36.1 ± 2.9
Trimethadione	200.0	8/8	23.1 ± 2.8	8/8	37.3 ± 3.2
	400.0	7/8	24.5 ± 3.1	8/8	38.9 ± 4.0
Saline		9/9	23.9 ± 2.8	9/9	34.5 ± 3.7
Ethosuximide	200.0	7/8	23.5 ± 2.4	8/8	36.3 ± 2.5
	500.0	5/8	20.2 ± 3.1	7/8	35.1 ± 4.8
Saline		7/8	17.4 ± 2.9	7/8	40.0 ± 4.1
Acetazolamide	500.0	8/8	18.4 ± 4.0	7/8	36.3 ± 5.9
	1000.0	7/7	22.7 ± 4.4	7/7	35.7 ± 6.8

Animals were given 3-nitropropionic acid at the dose equal to CD_{97}, i.e., 220 mg/kg i.p. Anticonvulsants were injected i.p. 10 min (valproate), 20 min (ethosuximide), 30 min (diazepam, carbamazepine, trimethadione, acetazolamide) or 60 min (phenobarbital, diphenylhydantoin) before 3-nitropropionic acid. Data are expressed as means ± S.D. n/N = number of animals displaying seizure (n) out of the total number of animals tested (N). [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs. respective controls. Statistical comparisons of latencies were made by using a one-way analysis of variance (ANOVA), followed by the adjustment of P value by the method of Bonferroni.

more) (Table 2). None of the studied drugs diminished the occurrence of death after the application of 3-nitropropionic acid (Table 2).

4. Discussion

In the present report we describe the potent convulsive properties of 3-nitropropionic acid and characterize the anticonvulsive profile of antiepileptics effective in this novel model of seizures. The vast majority of previous studies have focused on 3-nitropropionic acid-related striatal degeneration and its behavioral consequences (Gould and Gustine, 1982; Ludolph et al., 1991; Beal et al., 1993; Brouillet et al., 1993; Miller and Zaborszky, 1997). Depressed motor activity, short episodes of hyperactivity and/or abnormal movements such as tremor, head bobbing, paddling and circling were noted as a result of single or daily repeated injection of 3-nitropropionic acid at the dose of 120 mg/kg (Gould and Gustine, 1982; Hamilton and Gould, 1987). Such behavior might precede the occurrence of experimental seizures or it can be related to the administration of convulsant in a subthreshold dose. We observed analogous behavioral changes in mice given 3-nitropropionic acid at the doses of 100–120 mg/kg.

One report mentioned the occurrence of convulsions following administration of 3-nitropropionic acid at 180 mg/kg in 3 out of 5 rats; however, this observation was not pursued further (Hassel and Sonnewald, 1995).

Here we demonstrate that 3-nitropropionic acid given systemically (130 mg/kg and more) triggered seizures in mice in a clear dose-dependent manner. In no case did the convulsions progress into tonic seizures, even after administration of high, lethal doses of 3-nitropropionic acid. The

lack of tonus following 3-nitropropionic acid application is different from the seizure profile characteristic for established convulsants. Pentylenetrazole and bicuculline, which impair γ-aminobutyric acid (GABA)-mediated inhibition, or excitatory amino acid receptor agonists *N*-methyl-D-aspartic acid (NMDA), kainate or α-amino-2,3-dihydro-5-methyl-3-oxo-isoxazole-propionate (AMPA) evoke tonic–clonic seizures, whereas the cholinomimetic pilocarpine induces limbic, and in higher doses, clonic–tonic convulsions (Meldrum, 1986; Turski et al., 1987, 1990; Mathis and Ungerer, 1992; Urbanska et al., 1998). The 3-nitropropionic acid-induced seizures resembled those caused by aminooxyacetic acid, which are also exclusively of the clonic type (Turski et al., 1991).

Among studied classical anticonvulsive drugs, diazepam, phenobarbital and valproate afforded protection against 3-nitropropionic acid-evoked seizures. The same anticonvulsants were also shown to potently block the occurrence of aminooxyacetic acid-induced convulsions (Turski et al., 1991). The doses of diazepam and valproate used to prevent seizures generated by 3-nitropropionic acid were almost two-fold higher than these needed to inhibit convulsions precipitated by aminooxyacetic acid, whereas phenobarbital was equally effective in both seizure models. Carbamazepine, diphenylhydantoin, ethosuximide, trimethadione and acetazolamide failed to suppress 3-nitropropionic acid-evoked convulsions. Likewise, seizures caused by aminooxyacetic acid were not susceptible to blockade by these antiepileptic drugs (Turski et al., 1991).

It is noticeable that none of the antiepileptics that were able to abolish 3-nitropropionic acid-induced seizures could prevent the mortality caused by this drug. However, diazepam and valproate, but not phenobarbital, elongated the latency to death after 3-nitropropionic acid administration. Possibly, the mechanisms governing the occurrence of seizures and mortality subsequent to the application of 3-nitropropionic acid are of a different nature.

In summary, we demonstrate that the mitochondrial toxin 3-nitropropionic acid is a powerful convulsant in mice. The seizures could be prevented by broad-spectrum anticonvulsants, whereas diphenylhydantoin-like and anti-absence antiepileptics were ineffective. The char-acteristics of 3-nitropropionic acid-induced seizures resemble those of convulsions evoked by another mitochondrial toxin, aminooxyacetic 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. Biochemistry 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, T., 1993. Neurochemical and histological characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J. Neurosci. 13, 4181–4192.

Blaszczak, P., Saran, T., Turski, W.A., Urbanska, E., 1996. AMPA/kainate receptors involvement in convulsant and proconvulsant properties of mitochondrial toxin 3-nitropropionic acid in mice. Soc. Neurosci. Abst. 22, 607.5.

Bossi, S.R., Simpson, J.R., Isacson, O., 1993. Age dependence of striatal neuronal death caused by mitochondrial dysfunction. NeuroReport 4, 73–76.

Brouillet, E., Jenkins, B.G., Hyman, B.T., Ferrante, R.J., Kowall, N.W., Srivastava, R., Roy, D.S., 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.

Geddes, J.W., Pang, Z., Wiley, D.H., 1996. Hippocampal damage and cytoskeletal disruption resulting from impaired energy metabolism. Implications for Alzheimers 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.

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.

Kauppinen, R.A., Sihra, T.S., Nicholls, D.G., 1987. Aminooxyacetic acid inhibits the malate–aspartate shuttle in isolated nerve terminals and prevents the mitochondria from utilizing glycolytic substrates. Biochem. Biophys. Acta 930, 173–178.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

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.

Mathis, C., Ungerer, A., 1992. Comparative analysis of seizures induced by intracerebroventricular administration of NMDA, kainate and quisqualate in mice. Exp. Brain Res. 88, 277–282.

Meldrum, B.S., 1986. Preclinical test systems for evaluation of novel compounds. In: Meldrum, B.S., Porter, R.J. (Eds.), Current problems in epilepsy. New anticonvulsant drugs. John Libbey, London, pp. 31–48.

Miller, P.J., Zaborszky, L., 1997. 3-Nitropropionic acid neurotoxicity: visualization by silver staining and implications for use as an animal model of Huntington's disease. Exp. Neurol. 146, 212–229.

Turski, L., Niemann, W., Stephens, D.N., 1990. Differential effects of antiepileptic drugs and beta-carbolines on seizures induced by excitatory amino acids. Neuroscience 39, 799–807.

Turski, W.A., Cavalheiro, E.A., Coimbra, C., Berzaghi, M.P., Ikonomidou-Turski, C., Turski, L., 1987. Only certain antiepileptic drugs prevent seizures induced by pilocarpine. Brain Res. Rev. 12, 281–305.

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., Ikonomidou, C., Sieklucka, M., Turski, W.A., 1991. Aminooxyacetic acid produces excitotoxic lesions in the rat striatum. Synapse 9, 129–135.

Urbanska, E.M., Dekundy, A., Kleinrok, Z., Turski, W.A., Czuczwar, S.J., 1998. Glutamatergic receptor agonists and brain pathology. In: Kostrzewa, R. (Ed.), Highly selective neurotoxins: basic and clinical applications. Humana Press, Totowa, NJ, pp. 329–354.