ELSEVIER

European Journal of Pharmacology 403 (2000) 229–233

ejp

www.elsevier.nl/locate/ejphar

Short communication

Proconvulsive effects of the mitochondrial respiratory chain inhibitor — 3-nitropropionic acid

Grzegorz Haberek [a], Tomasz Tomczyk [a], Beata Zuchora [a], Marian Wielosz [a], Waldemar A. Turski [a,b], Ewa M. Urbanska [a,b,*]

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

Received 20 April 2000; received in revised form 12 July 2000; accepted 17 July 2000

Abstract

The role of impaired mitochondrial function in processes leading to the generation of seizures was studied in mice. An inhibitor of mitochondrial complex III, 3-nitropropionic acid, which is known to evoke convulsions per se, and was used here in subthreshold dose, enhanced seizures generated by electric current and application of 4-aminopyridine. In contrast, 3-nitropropionic acid did not affect convulsions induced by γ-aminobutyric acid (GABA) receptor antagonists — bicuculline, pentylenetetrazol and picrotoxin, glycine antagonist — strychnine, cholinomimetic drug–pilocarpine, and kynurenine aminotransferase inhibitor — aminooxyacetic acid. It is hypothesised that deranged mitochondrial metabolism renders the central nervous system more susceptible to factors inducing seizures via direct depolarization.

Keywords: 3-Nitropropionic acid; Mitochondrial toxin; Seizure; GABA (γ-aminobutyric acid); 4-Aminopyridine; Electroconvulsion

1. Introduction

Compromised brain oxidative phosphorylation seems to occur during ischemia or hypoglycemia, as well as during chronic neuropathologies such as Huntington's disease or in the course of convulsive disorders (Lees, 1993; Wallace et al., 1994). Possibly, the energy deficit reflects the diminished availability of substrates for the synthesis of ATP and may result from aging, genetic predisposition or accumulation of environmental poisons (Lees, 1993; Greene and Greenamyre, 1996).

The 3-nitropropionic acid, a natural mitochondrial toxin, impairs energy metabolism via irreversible inhibition of succinate dehydrogenase (Alston et al., 1977; Ludolph et al., 1991). This interference with mitochondrial complex III leads to a reduced formation of cellular ATP (Alston et al., 1977; Ludolph et al., 1992). Experimental evidence indicates that application of 3-nitropropionic acid may have a dual effect. Chronic administration of relatively low doses of 3-nitropropionic acid causes a specific chemical, behavioural and neuropathological outcome reminiscent of Huntington's disease (Beal et al., 1993; Borlongan et al., 1997). Recently, we have shown that acute application of higher doses of 3-nitropropionic acid in mice induces clonic convulsions relatively resistant to the action of anticonvulsants (Urbanska et al., 1998, 1999). Only broad-spectrum antiepileptics, but not anti-absence or diphenylhydantoin-like drugs, are able to effectively prevent the occurrence of seizures (Urbanska et al., 1998). Alteration in excitatory amino acid transmission seems to be involved in the convulsive activity of 3-nitropropionic acid. Seizures generated by 3-nitropropionic acid can be inhibited by glutamate receptor antagonists of the non-*N*-methyl-D-aspartate (non-NMDA) type, but not by NMDA ones (Urbanska et al., 1999). Moreover, 3-nitropropionic acid lowers the threshold for convulsions evoked by intracerebral injection of excitatory amino acid receptor agonists, α-amino-3-hydroxy-5-methyloisoxazolo-4-propionate (AMPA) and kainate, but not by NMDA itself (Urbanska et al., 1999).

The aim of the present study was to evaluate the effect of 3-nitropropionic acid administration on the susceptibil-

* Corresponding author. Department of Pharmacology and Toxicology, Medical University School, Jaczewskiego 8, 20-090 Lublin, Poland. Tel.: +48-81-747-8924; fax: +48-81-747-8646.
E-mail address: ewau@galen.imw.lublin.pl (E.M. Urbanska).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00510-0

ity to convulsions evoked by electrical stimulation, interference with cellular K^+ currents, γ-aminobutyric acid (GABA)-, glycine- and acetylcholine-mediated neurotransmission, and alterations in kynurenic acid synthesis.

2. Materials and methods

2.1. Animals

The experimental subjects were male Albino–Swiss mice weighing 20–25 g. Animals were kept under standard laboratory conditions, with food and water ad libitum. Convulsive tests were performed between 9:00 and 16:00 h. Each experimental group included at least eight mice. The Ethical Committee of the Medical University in Lublin approved all animal studies presented here.

2.2. Drugs

The 3-nitropropionic acid (Sigma) was dissolved in water, the pH being adjusted to 7.2 and injected intraperitoneally (i.p.) at the dose of 100 mg/kg, i.e. 75% of its CD_{16} dose (Urbanska et al., 1999). The 4-aminopyridine (RBI), picrotoxin, pentylenetetrazol and strychnine (all Sigma) were dissolved in water. Aminooxyacetic acid (Sigma) was dissolved in water, the pH being adjusted to 7.2 with 1N NaOH. Bicuculline (Fluka) was dissolved in a minimum quantity of glacial acetic acid, and the final volume was made up with water, the pH being adjusted to approximately 5.5. Pilocarpine (Sigma) was dissolved in water and given to animals pretreated with *N*-methylscopolamine (Sigma) in a dose of 1 mg/kg s.c., 30 min earlier. Bicuculline, pentylenetetrazol and aminooxyacetic acid were administered subcutaneously (s.c.), whereas all other convulsants were injected i.p. The injection volume was 0.05 or 0.1 ml/10 g body weight for drugs administered s.c. or i.p., respectively.

2.3. Chemically induced seizures

The 3-nitropropionic acid, in a dose of 100 mg/kg i.p., was injected 20 min prior to 4-aminopyridine, bicuculline, pentylenetetrazol, picrotoxin and strychnine, 10 min before aminooxyacetic acid and concomitantly with pilocarpine. The timing of 3-nitropropionic acid administration was based on the average latency to the occurrence of seizures following injection of the respective convulsant, and was chosen to ensure the maximum effect of 3-nitropropionic acid, corresponding to the onset of seizures. Observation of convulsive behaviour was performed for 60 min following administration of 4-aminopyridine, bicuculline, pentylenetetrazol, picrotoxin and strychnine, for 2 h after application of aminooxyacetic acid, and for 3 h after injection of pilocarpine. The episodes of clonic, tonic or limbic seizures were recorded. Mortality rate was evaluated 4 h after the administration of convulsant.

2.4. Electroconvulsions

The 3-nitropropionic acid was given i.p., in a dose of 100 mg/kg, 30 min before electroconvulsive shock. Electroconvulsions were induced with a Hugo Sachs generator (Germany), delivering an alternating current (50 Hz; 0.2 s stimulus) via ear-clip electrodes. Tonic extension of the hind limbs was considered an end-point.

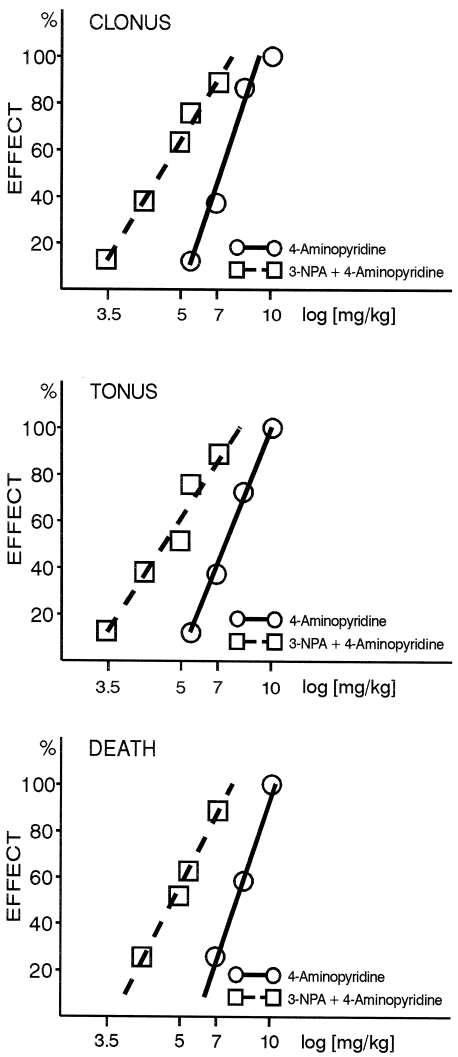

Fig. 1. Effect of 3-nitropropionic acid administration on convulsions and mortality evoked by 4-aminopyridine in mice. The 3-nitropropionic acid was administered i.p., in a dose of 100 mg/kg, 20 min before 4-aminopyridine. CD_{50}, LD_{50} and CS_{50} values, together with their confidence limits, and statistical comparisons were estimated by computerized fitting of the data using linear regression analysis of quantal log dose–probit functions (Litchfield and Wilcoxon, 1949). Probit–log dosage regression curves were plotted with the use of GraphPAD software. (Upper panel) Clonic convulsions — 4-aminopyrydine: $y = 488.5 \times - 370.3$, $r = 0.99$; 4-aminopyridine + 3-nitropropionic acid (3-NPA): $y = 257.2 \times - 128.1$, $r = 0.99$. (Middle panel) Tonic convulsions — 4-aminopyridine: $y = 390.8 \times - 292.0$, $r = 0.99$; 4-aminopyridine + 3-NPA: $y = 250.7 \times - 126.0$, $r = 0.98$. (Lower panel) Mortality — 4-aminopyridine: $y = 480.8 \times - 383.9$, $r = 0.99$; 4-aminopyridine + 3-NPA: $y = 323.9 \times - 188.0$, $r = 0.99$.

Table 1
The effect of 3-nitropropionic acid on chemically induced seizures

Treatment	Seizures		Mortality
	Clonic	Tonic	
Bicuculline	2.3 (2.0–2.6)	2.4 (2.1–2.7)	2.5 (2.2–2.8)
Bicuculline + 3-nitropropionic acid	2.2 (1.9–2.5)	2.3 (2.0–2.7)	2.4 (2.1–2.7)
Pentylenetetrazol	56.1 (47.6–66.1)	69.7 (59.7–81.5)	69.7 (59.7–81.5)
Pentylenetetrazol + 3-nitropropionic acid	51.8 (45.1–59.5)	62.9 (54.3–72.8)	64.6 (55.8–74.8)
Picrotoxin	2.5 (2.1–3.1)	6.9 (5.2–9.2)	5.3 (3.2–8.8)
Picrotoxin + 3-nitropropionic acid	2.7 (1.8–3.9)	5.9 (4.0–8.6)	4.4 (3.2–6.2)
Strychnine	n.o.	0.65 (0.53–0.79)	0.68 (0.56–0.82)
Strychnine + 3-nitropropionic acid	n.o.	0.62 (0.55–0.69)	0.66 (0.55–0.80)
Aminooxyacetic acid	78.6 (62.5–98.9)	n.o.	> 115.0
Aminooxyacetic acid + 3-nitropropionic acid	84.3 (65.5–107.9)	n.o.	> 115.0
	Limbic–clonic		
Pilocarpine	306.5 (279.4–336.2)		346.0 (313.3–382.0)
Pilocarpine + 3-nitropropionic acid	286.0 (260.9–314.9)		322.8 (293.0–355.5)

Data are presented as CD_{50} and LD_{50} values, with confidence limits in parentheses, i.e. the doses of convulsants (in mg/kg) evoking convulsions or mortality in 50% of studied animals, respectively. The 3-nitropropionic acid (100 mg/kg i.p.) was injected 20 min prior to 4-aminopyridine, bicuculline, pentylenetetrazol, picrotoxin and strychnine, 10 min before aminooxyacetic acid and concomitantly with pilocarpine. n.o. — not occurring.

2.5. Statistics

The dose of convulsant evoking seizure response or mortality in 50% of mice (CD_{50} and LD_{50}; convulsive dose and lethal dose) and the current inducing tonic–clonic seizures in 50% of animals (CS_{50}; electroconvulsive threshold) were determined based on the data obtained from three to four experiments performed with different doses of drug or different currents. CD_{50}, LD_{50} and CS_{50} values, together with their confidence limits, and statistical comparisons were estimated by computerized fitting of the data using linear regression analysis of quantal log dose–probit functions (Litchfield and Wilcoxon, 1949). Probit–log dosage regression curves were plotted with the use of GraphPAD software.

3. Results

The 3-nitropropionic acid, administered in a dose of 100 mg/kg, lowered the CS_{50} value from 4.8 (4.3–5.2) to 3.8 (3.5–4.1) mA ($P < 0.001$). Administration of 3-nitropropionic acid enhanced the convulsive activity of 4-aminopyridine, as its CD_{50} for clonic seizures was lowered from 7.2 (6.2–8.3) to 4.9 (4.2–5.8) mg/kg ($P < 0.001$) and for tonic seizures from 7.3 (6.6–8.2) to 5.0 (4.2–6.0) mg/kg ($P < 0.001$). The LD_{50} of 4-aminopyridine was diminished, reaching 5.4 (4.7–6.2) vs. 7.8 (7.2–8.5) mg/kg in the control group ($P < 0.001$) (Fig. 1).

In contrast, 3-nitropropionic acid did not affect the occurrence of clonic seizures, tonic seizures and mortality caused by bicuculline, pentylenetetrazol, picrotoxin and strychnine, tonic seizures and mortality produced by strychnine, limbic–clonic seizures and mortality induced by pilocarpine, and clonic seizures evoked by aminooxyacetic acid. Respective CD_{50} and LD_{50} values are shown in Table 1.

4. Discussion

The mitochondrial toxin, 3-nitropropionic acid, administered peripherally in a subthreshold dose, is able to facilitate the occurrence of seizures in some experimental animal models. The 3-nitropropionic acid lowers the threshold for convulsions generated by electric current and application of 4-aminopyridine. In contrast, 3-nitropropionic acid does not affect seizures evoked by the kynurenine aminotransferase inhibitor — aminooxyacetic acid, the GABA receptor antagonists — bicuculline, pentylenetetrazol, and picrotoxin, the glycine antagonist — strychnine and the cholinomimetic agent — pilocarpine.

We have previously shown that 3-nitropropionic acid, an irreversible inhibitor of mitochondrial complex III, is a strong convulsant itself (Urbanska et al., 1998). Convulsions evoked by 3-nitropropionic acid are exclusively clonic, appear not earlier than 20–25 min after peripheral administration and are susceptible to blockade by some antiepileptic drugs (Urbanska et al., 1998). The occurrence of seizures may be also prevented by non-NMDA receptor antagonists such as 6-nitro-7-sulphamoyl-benzo(*f*)quinoxaline-2,3-dion disodium salt (NBQX) and 1-(4-aminophenyl)-4-methyl-7,8-methylenodioxy-5*H*-2,3 benzodiazepine HCl (GYKI 52466). Moreover, 3-nitropropionic acid was found to diminish the threshold for convulsions induced by intracerebrally given AMPA and kainate, but not NMDA (Urbanska et al., 1999). It was hypothesised that 3-nitropropionic acid application, via a deranged mitochon-

drial energy status, initiates a cascade of events including enhanced excitatory transmission (mediated mainly via non-NMDA receptors) and culminates in the generation of seizures (Urbanska et al., 1999). Data shown here indicate that 3-nitropropionic acid may also amplify other epileptogenic mechanisms.

The 3-nitropropionic acid was found to lower the threshold for electroconvulsions. Generalized tonic–clonic seizures induced by maximal electroshock serve as a screening model for the evaluation of potential anticonvulsant drugs (Löscher and Schmidt, 1988). In this model, the current intensity is a multiple of the convulsive threshold. The electroconvulsive threshold is assumed to disclose more information concerning the initiation of seizure phenomena, as opposed to maximal electroshock, which also models the spread of seizure within the brain (Löscher and Schmidt, 1988). Since the influx of Na^+ ions and neuronal depolarization are probable triggers of electroconvulsions (Löscher and Schmidt, 1988), it might be suggested that 3-nitropropionic acid renders the cells more susceptible to excessive depolarization, leading to seizures.

The 4-aminopyridine is a convulsant that blocks fast K^+ channels, thus preventing membrane repolarization and increasing the neuronal depolarization time (Yamaguchi and Rogawski, 1992). Generalized tonic–clonic convulsions induced by 4-aminopyridine are similar in their appearance to electroconvulsions. The augmented release of excitatory neurotransmitters into the synaptic cleft due to presynaptic depolarization is implicated as a causative factor in 4-aminopyridine-evoked seizures (Buckle and Haas, 1982). The 3-nitropropionic acid potently amplified the action of 4-aminopyridine and lowered the threshold for tonic–clonic seizures and mortality evoked by this drug. The observed enhancement of 4-aminopyridine action by 3-nitropropionic acid further supports the concept that 3-nitropropionic acid may increase susceptibility to excitatory neurotransmission and potentiate depolarization-related events.

Interestingly, 3-nitropropionic acid did not affect other mechanisms of seizure generation investigated here. The 3-nitropropionic acid did not alter clonic–tonic convulsions generated by pentylenetetrazol, bicuculline and picrotoxin. These three convulsants bind to different domains of the $GABA_a$ receptor complex and reduce inhibitory transmission (De Deyn et al., 1992). Strychnine, an antagonist of the strychnine-sensitive glycine receptor, evokes brief, intense tonic–clonic seizures similar to maximal electroshock-induced convulsions, which, in contrast to them, are highly resistant to anticonvulsant drugs (De Deyn et al., 1992). The threshold for seizures generated by strychnine was not changed by 3-nitropropionic acid. Pilocarpine is a cholinergic agonist causing limbic convulsions in rodents (Turski et al., 1983), and its convulsive activity was also not affected by 3-nitropropionic acid administration. Similarly, clonic seizures induced by aminooxyacetic acid, a potent inhibitor of kynurenic acid synthesis (Turski et al., 1991), were not altered by application of 3-nitropropionic acid. Thus, administration of a subthreshold dose of the mitochondrial inhibitor, 3-nitropropionic acid, does not affect seizure activity related to a diminished GABA-ergic influence, increased strychnine-sensitive glycinergic transmission, cholinergic system activity or impaired synthesis of the endogenous excitatory amino acid receptor antagonist, kynurenic acid.

High energy demands together with a low ability to accumulate energy-rich substrates make neurones extremely sensitive to energy deficits (Lees, 1993; Greene and Greenamyre, 1996). A decline in the ATP production following 3-nitropropionic acid application may enhance neuronal sensitivity to directly depolarizing proconvulsive events. It might be hypothesised that deranged mitochondrial metabolism, either genetically determined or evoked by various endogenous or exogenous factors, renders the central nervous system more susceptible to convulsions triggered by enhanced excitatory neurotransmission and depolarization-related events.

Acknowledgements

This study was supported by grants from Medical School in Lublin and INCO-Copernicus PL-963338.

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.

Borlongan, C.V., Nishino, H., Sanberg, P.R., 1997. Systemic, but not intraparenchymal, administration of 3-nitropropionic acid mimics the neuropathology of Huntington's disease: a speculative explanation. Neurosci. Res. 28, 185–189.

Buckle, P.J., Haas, H.L., 1982. Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. J. Physiol. (London) 326, 109–122.

De Deyn, P.P., D'Hooge, R., Marescau, B., Pei, Y.Q., 1992. Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants. Epilepsy Res. 12, 87–110.

Greene, J.G., Greenamyre, J.T., 1996. Bioenergetics and glutamate excitotoxicity. Prog. Neurobiol. 48, 613–634.

Lees, G.J., 1993. Contributory mechanisms in the causation of neurodegenerative disorders. Neuroscience 54, 287–322.

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

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.

Löscher, W., Schmidt, D., 1988. Which animal model should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 2, 145–181.

Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.J., Kleinrok, Z., Turski, L., 1983. Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological study. Behav. Brain Res. 9, 315–335.

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

Urbanska, E.M., Blaszczak, P., Saran, T., Kleinrok, Z., Turski, W.A., 1999. AMPA/kainate-related mechanisms contribute to convulsant and proconvulsant effects of 3-nitropropionic acid in mice. Eur. J. Pharmacol. 370, 251–256.

Wallace, D.C., Lott, M.T., Shoffner, J.M., Ballinger, S., 1994. Mitochondrial DNA mutations in epilepsy and neurological disease. Epilepsia 35, S43–S50.

Yamaguchi, S., Rogawski, M.A., 1992. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. Epilepsy Res. 11, 9–16.