REVIEW ARTICLE

# 3-NITROPROPIONIC ACID AND SIMILAR NITROTOXINS

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*Summary:* 3-Nitropropionic acid as well as 3-nitro-1-propanol and its β-D-glucopyranoside (miserotoxin) are the plant and fungal toxins reported to interrupt mitochondrial electron transport resulting in cellular energy deficit. These nitrotoxins induce neurological degeneration in ruminants and humans. 3-Nitropropionic acid-intoxicated rats serve as the animal model for Huntington's disease.

***Key words:*** *Plant toxin; Fungal toxin; Nitrotoxin; 3-nitropropionic acid; Miserotoxin; Milkvetch*

## Introduction

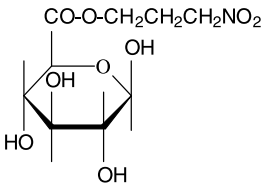
The nitrotoxins, 3-nitropropionic acid, 3-nitro-1-propanol and 3-nitro-1-propyl-β-D-glucopyranoside called miserotoxin, were found in many leguminous plants. These compounds are toxic principle of various milk vetches (*Astragalus* spp.) that are distributed worldwide. These poisonous plants are often a reason of intoxication of cattle, sheep, and horses in the United States and Canada (33,49,50). 3-Nitropropionic acid is sometimes produced on moldy crops as for example sugarcane or peanuts in amounts sufficient to cause severe neurological disorders when consumed by humans (45,39). 3-Nitropropionic acid is produced by the fungus *Arthrinium* spp. (6). Many synthetically prepared nitro-compounds have also considerable toxicity to both invertebratea and vertebratea, but because these compounds are not natural origin, there are not the aim of this article.

## Chemistry

3-Nitropropionic acid, is white solid, m.p. 65-68 °C, soluble in water (0.1 g/ml $H_2O$), ethanol, ether and chloroform, insoluble in petroleum. Dissociation exponent (pK) = 3.79 at 25 °C. 3-Nitro-1-propanol, liquid of density 1.21 g/cm$^3$ is an aglycone of miserotoxin. Miserotoxin, 3-nitro-1-propyl-β-D-glucopyranoside (I) is native compound in many plants of spp. *Astragalus*.

3-Nitropropionic acid was prepared for the first time by Lewkovitsch (J.Prakt. Chem.20, 169, 1879) by reaction of sodium salt of 3-iodopropionic acid with sodium nitrite. Free form of 3-nitropropionic acid is easy available by reaction of 3-iodopropionic acid with $AgNO_2$ and is very stabile (63).

Oehrlein et al. (43) prepared 3-nitro-1-propanal by 1,4-nitrite addition to acrolein and described several acetals of 3-nitro-1-propanal, as well as 3-nitro-1-propanol and some of its ethers. 3-Nitro-1-propanol has been obtained by borane-dimethylsulfide reduction of both 3-nitro-1-propanal and 3-nitropropionic acid.

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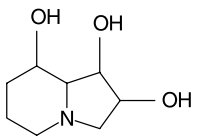



II

## Biological effects

### *Mitochondrial toxin and inhibitor of succinate dehydrogenase*

Mitochondria produce most of energy in animal cells by a process called oxidative phosphorylation. At this procedure electrons are passed along a series of respiratory enzyme complexes located in the inner mitochondrial membrane, and the energy released by this electron transfer is used to pump protons across the membrane. The resultant electrochemical gradient enables another complex, ATP-synthase, to synthesize the energy carrier ATP (55). 3-Nitropropionic acid irreversible inactivates succinate dehydrogenase (SDH) in mitochondria and caused a defect in cellular energy metabolism and is called as mitochondrial toxin (2,20). SDH is inactivated in all cells, but very important is mainly inhibition of SDH in neurons, because it is connected with some mitochondrial diseases and with mitochondrial encephalomyopathies (22,51). After 3-nitropropionic acid administration to animals the SDH activity

was reduced according to a similar time-course, most prominently in the cerebellum and least sharply in the thalamus. Activity of SDH remained significantly reduced in most areas of brain, except thalamus, for up to five days after dosing (47). Serial 1H-NMR spectroscopy study in primates shows that 3-nitropropionic acid-induced SDH inhibition following systematic injection similarly affects all brain regions (21).

### *Hypothermia*

The administration of 3-nitropropionic acid to animals leads to hypothermia. A single dose of 3-nitropropionic acid in dose 30 mg/kg s.c. to adult male Sprague-Dawley rats experienced a progressive hypothermia, which reached a loss of 3 °C or more in core body temperature by 3 hours after dosing (21). Hypothermic effect is connected with mitochondrial blockade of succinate dehydrogenase and reduction of cellular energy (54).

### *Animal model of Huntington's disease*

Huntington's disease (HD) is a neurodegenerative disorder characterized by selective loss of neurons in the basal ganglia. Although the gene defect connected with mitochondrial dysfunction has recently been identified (28, 56), the mechanism by which it leads to neuronal degeneration remains unknown. It was hypothesized that a defect in oxidative phosphoprylation may lead to slow, excitotoxic neuronal degeneration in this illness. It is well known that the neuronal function and survival depend on a continuous supply of glucose and oxygen, used to generate ATP through glycolysis and mitochondrial respiration, as well as that a perturbation in energy metabolism during conditions such as ischemia and brain trauma may cause irreversible neuronal injury (65). An age-related decline in energy metabolism also may contribute to neuronal loss during normal aging, as well as in neurodegenerative diseases (8). Recent studies with 3-nitropropionic acid have shown that it can produce striking similarities to the neuropathologic and neurochemical features of HD in both rodents and primates (30). If such a mechanism is indeed relevant to the pathogenesis of HD, the agents that can improve oxidative phosphorylation might prove to be efficacious. It was found that both coenzyme $Q_{10}$ and nicotinamide can ameliorate striatal lesions produced by mitochondrial toxins in vivo (7).

Previous studies in primates have shown that chronic systemic administration of the 3-nitropropionic acid replicates most of the motor, cognitive, and histopathological features of one neurodegenerative disease, HD (19). In HD, uncontrollable involuntary movements, psychiatric abnormalities and a loss of intellectual functions (dementia) are the three major manifestations. Involuntary movements, such as chorea, result from abnormalities in basal ganglia which are located deep in the brain and regulate motor movements. One of these structures called striatum shows a decreased volume in HD. The atrophy is due to degeneration of a particular subpopulation of the neurons called medium-size spiny neurons located within the striatum (17). Dementia and psychiatric abnormalities are due to degeneration of neurons outside the basal ganglia. A loss of neurons in the cerebral cortex is particularly prominent in Alzheimer's disease. The mechanism of the degeneration is not fully understood, but there is not doubt that 3-nitropropionic acid is an indirect excitotoxin (67). It has been also demonstrated that it exacerbates N-methyl-D-aspartate toxicity and this synergism may be relevant to the neuronal death observed in neurodegenerative dosorders (29). Fukuda et al. (26) demonstrated that astrocytes are more vulnerable than neurons to 3-nitropropionic acid-induced cellular $Ca^{2+}$ overload and toxicity.

The morphological and histochemical effects of 3-nitropropionic acid were examined in cultured murine embryonal carcinoma cells. This compound caused a dose-dependent inhibition of cell proliferation at concentrations above 1.05 mM and was lethal at 4.2 mM. Morphological changes included gross swelling of the cells, swelling of mitochondria and accumulation of organellar debris within the cytoplasm. 3-Nitropropionic acid inhibited succinate dehydrogenase but not of malate, isocitrate or glucose-6-phosphate dehydrogenases, resulting in a decrease in intracellular ATP (45).

### *Neurotoxicity*

3-Nitropropionic acid-intoxicated rats frequently serve as the animal model for HD (53,57). This acid can be characterized as excitotoxic compound and impaired mitochondrial energy component which produced lesions in the striatum, hippocampus, and corpus callosum but not in the cortex (7,9,19). Excitotoxic effect of 3-nitropropionic acid is different from excitotoxic effect of quinolinic acid (52). Frim et al. (24) have found that nerve growth factor, delivered biologically by the implantation of a genetically altered fibroblast cell-line, can protect locally against striatal degeneration induced by infusions of high doses of glutamate receptor agonists and reduced the size of adjacent striatal 3-nitropropionic acid lesions. The role of the glutamatergic system in the convulsant action of 3-nitropropionic acid was studied in mice by Urbanska et al. (62). The occurence 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-5H-2,3-benzodiazepine (GYKI 52466). Motor deficits and striatal neuron degeneration produced by systemic 3-nitropropionic acid administration may be protected by 2-amino-6-trifluoromethoxy-benzothiolate (Riluzole) (31). Riluzole is a glutamate release inhibitor (35,36) which is used clinically in patients with amyotrophic lateral sclerosis (64).

Systemic administration of 3-nitropropionic acid produced lateral striatal, hippocampal CA1 and CA3 lesions in the rat brain (60). Behrens et al. (11) found that 3-nitropropionic acid produces gradual neuronal degeneration characterized by cell body shrinkage and DNA fragmentation. Addition of glutamate antagonists during 3-nitropropionic acid exposure did not reduce neuronal death. However, addition of the macromolecular synthesis inhibitors cycloheximide, emetine or actinomycin D markedly reduced neuronal death. Their results do not exclude that 3-nitropropionic acid can induce excitotoxicity in more intact systems, but raise the additional possibility that this nitrotoxin may also act to induce neuronal apoptosis. Pang and Geddes (44) found that in cultured rat hippocampal neurons, 3-nitropropionic acid-induced cell death occurs through two distinct pathways. One involves activation of the NMDA receptor, which leads to a rapid necrotic death. The other is a delayed, apoptotic death, which is independent on N-methyl-D-aspartate (NMDA) receptor activation (23,59,66). Bonfocco et al. (16) before now demonstrated that glutamate-induced cell death could be either apoptotic or necrotic depending on the intensity of stimuli or the ability of neurons to recover mitochondrial membrane potential (5) and Boireau et al. (15) demonstrated that 3-nitropropionic acid exacerbates GABA release evoked by glucose deprivation in rat brain slices. It is uncertain whether the presumed apoptosis did indeed result from glutamate receptor activation or merely reflected background apoptosis (1,61). Moderate levels of extracellular glutamate shift the cell death pathway from apoptosis to necrosis. It seems to be excitotoxicity that may display both apoptic and necrotic features (48).

### *Animal toxicity*

Many milk vetches are toxic for animals and cattle. Horses and sheep have also been poisoned. Poisoning results in pulmonary emphysema and spinal cord demyelinization, commonly occurs in cattle. Milk vetch toxins act quickly with most animals that ingest lethal doses. They die in 4 to 24 hours, but more often, animals die within 3 or 4 hours after eating the plant. One kg of green milk vetch may be a lethal dose for a 500-kg cow. These acutely poisoned animals generally show severe respiratory distress and muscular weakness progressing to paralysis such that affected animals fall after the slightest excitement. The heart rate is often extremely high. Although animals appear bright, they quickly die. The exact cause of death in this case has not been determined, but it is thought to be caused by fatal cardiovascular effects of nitrotoxins. It is true, that in some species of *Astragalus*, for example *Astragalus bisulcatus* or *A. lentiginosus*, whose growth in Canada, also other very toxic chemical, indolizidine alkaloid swainsonine (II) was observed, which cause locoism in cattle, horse, and sheep (40) and it is not challenged that biological effects of nitrotoxins and swainsonine may be cumulated. Swainsonine also causes teratogenic deformities in lambs, calves, and goals (18).

Chronic intoxication most often occur in cattle and sheep from grazing any of the toxic varieties of nitrotoxin-containing *Astragalus* species slowly over a period of several days or weeks. Poisoned animals have respiratory problems, rapid respiration and as intoxication progresses, respiration develops a wheezing or roaring sound. These animals develop extensive pulmonary emphysema and demyelinization of the posterior spinal cord. When forced to move rapidly, animal may collapse and die.

The toxicity of miserotoxin in rats after peroral administration is low and $LD_{50}$ is more than 2.5 g/kg but 3-nitro-1-propanol is at least 30 times more toxic ($LD_{50}$ = 77 mg/kg) (38). Extensive toxicity of misorotoxin for cattle is caused by the fact, that miserotoxin is quickly hydrolyzed to 3-nitro-1-propanol in the rumen of cattle (38).The nitrocompounds 3-nitro-1-propanol and 3-nitropropionic acid were shown to be equally toxic when injected intraperitoneally into male Wistar rats. The $LD_{50}$ for 3-nitro-1-propanol was 0.58 mmol/kg (60 mg/kg) and for 3-nitropropionic acid it was 0.56 mmol/kg (67 mg/kg) (48).

### *Behavioral toxicity*

The behavioral effects of a single subcutaneous injection of both 3-nitro-1-propanol and 3-nitropropionic acid dissolved in saline solution were studied in mice and rats, respectively. Clinical signs included depression, abnormal motor activity, and recumbency (27). No differences between 3-nitro-1-propanol and 3-nitroproiponic acid were observed.

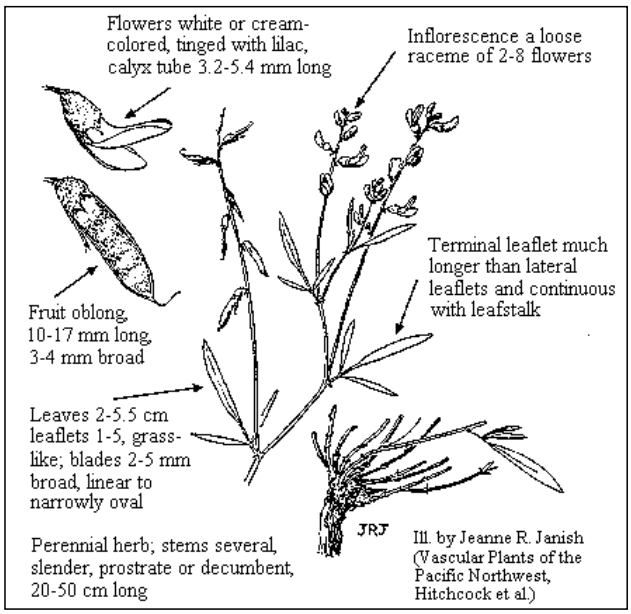
The SDH activity, demonstrated histochemically in frozen brain section, was markedly reduced in intoxicated mice and rats but the changes in activity of another mitochondrial flavoprotein enzyme, α-glycerophosphate dehydrogenase, was not observed.

### *Natural sources*

Nitrotoxins were found in many species and varieties of *Astragalus* plants. The most important poisonous plants are different species of milk vetches, whose grow throughout much of North America. Commonly widespread strain, the meadow milk vetch (*Astragalus diversifolius*) is given on Fig. 1. Most of the poisonous species grow on the meadows, deserts, and forests in the Rocky Mountain States. Milk vetches emerge from late April to June, depending on elevation and snowmelt. As plants mature and dry, they decrease in toxicity until they are nontoxic when they are dry. The loss of toxicity may be due to newly observed specific oxidase that catalyses the oxygen-dependent oxidation of 3-nitropropionic acid to malonate, semialdehyde, nitrate and nitrite (32). Recently nitrotoxins were found also in plants of spp. *Astragalus* which growth in Europe (59). The poisonous substance in milk vetches is the β-D-glucoside of 3-nitro-1-propanol, called miserotoxin (I). This compound is

hydrolysed to its aglycon, more toxic 3-nitro-1-propanol, in a stomach of ruminants (69), and this compound is further metabolized to 3-nitropropionic acid (41). A bacterium capable of metabolizing 3-nitro-1-propanol and 3-nitropropionic acid has been isolated from a mixed ruminal population (3). This ruminal bacterium was characterized as nonmotile gram-positive bacterium which not produce spores (4). The biotransformation of 3-nitro-1-propanol to 3-nitropropionic acid via 3-nitropropanal was also implemented *in vitro* by horse liver alcohol dehydrogenase (12).

**Fig. 1:** Meadow milkvetch *(Astragalus diversifolius var. diversifolius).*




## Hazard for humans

We found also some information about human toxicity of reviewed nitrotoxins. Moldy sugarcane poisoning, an acute fatal food poisoning of unknown etiology, has occurred in 13 provinces in China. The epidemiological characteristics and clinical features were described. Evidence from laboratory studies indicates that 3-nitropropionic acid produced by the fungus *Arthrinium* spp. is the etiological factor of this food poisoning (36). The biosynthesis of 3-nitropropionic acid is probably realized by enzymic decarboxylation of L-nitrosuccinate (6). Ingestion of 3-nitropropionic acid in moldy sugar cane causes brain damage in children. As has been account previously, the mechanism of 3-NPA toxicity is thought to be inhibition of energy production, leading to ATP depletion and excitotoxicity (12). Beal et al. (10) reported that the marker of hydroxyl radical production was attenuated in CuZn-superoxide dismutase transgenic mice. Additionaly, recent studies have suggested involvement of reactive oxygen species and oxidative stress in the 3-nitropropionic acid induced neurotoxicity (13,14, 25,68).

From this information and from the accidental animal intoxications as well as from the mechanism of their toxic action we can concluded that both 3-nitro-1-propanol and 3-nitropropionic acid represent very dangerous compounds with high degree of hazard for human being (47). Since the preparation of these compounds in laboratory is not overwhelming problem (43), we must consider 3-nitro-1-propanol as well as 3-nitropropionic acid to be potentially misuse toxic compounds with deadly effect.

## Conclusions

Both 3-nitropropionic acid and 3-nitro-1-propanol are the most important representatives of nitrotoxins, which are toxic principles of many leguminous plants. Namely plants of some *Astragalus* spp. contain considerable amount of these toxins and there are often a reason of intoxication of cattle, sheep, and horses. Nitrotoxins have deadly effect and may be very dangerous for human being, too.

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