Original Scientific Paper

EFFECTS OF 3-NITROPROPIONIC ACID IN RATS: GENERAL TOXICITY AND FUNCTIONAL NEUROTOXICITY*

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3-Nitropropionic acid (3-NP) causes biochemical and morphological alterations in human and animal brain. Young adult male Wistar rats received 3-NP i. p. on five consecutive days and were investigated four weeks later (subacute treatment). Acute effects were investigated 24 h after one i. p. dose. Spontaneous or stimulus-evoked activity was recorded from cortical sensory foci, from subcortical nuclei and from the tail nerve, in urethane anesthesia. The subacutely treated rats were dissected and organ weights measured to study general toxic effects.

After subacute treatment, decrease was seen in the theta, and increase in the beta-2 and gamma, band of the spontaneous activity, dissimilarly in the cortical vs. subcortical sites. Latency of the sensory evoked potentials increased in all sensory foci after subacute treatment. Following acute treatment, amplitude of the somatosensory evoked potential decreased. The weight of the thymus decreased significantly in the treated rats.

Further studies could elucidate the link between biochemical effects of 3-NP and the observed functional neurotoxic changes.

KEY WORDS: *cortical evoked response, nerve action potential, organ weight, spontaneous cortical activity*

The toxin 3-nitropropionic acid (3-NP) is naturally present in leguminous plants used to feed animals and can poison grazing livestock (1). Human poisoning may come from consumption of foodstuffs (sugar cane, cereals, etc.) infested with moulds of the *Arthrinium* and *Aspergillus* genus producing 3-NP.

Acute encephalopathy followed by dystonia can develop in humans exposed to low doses of 3-NP (2). Toxin-treated experimental animals showed decreased motor performance (3) with degeneration found primarily in the striatum, but also in the hippocampus and thalamus (4). The damage caused by 3-NP is used as animal model of Huntington's disease (5, 6).

At the cellular level, 3-NP inhibits succinate dehydrogenase, a key enzyme of oxidative energy production (7), causing ATP levels in the brain to fall. This effect develops fast and is not limited to the sites of morphological damage (8). Since the function of the nervous system requires lots of energy, the mitochondrial damage is probably reflected in the electrical activity of the brain. Beyond that, 3-NP was found to act on N-methyl-D-aspartate (NMDA) receptors thereby inducing excitotoxicity (9) leading to further functional (and morphological) alterations. The measurement of these functional alterations could be convenient for following the development of the disease models (5,6), or for testing future drugs to be used in such models. The aim of this study was to test whether the applied neurophysiological methods were appropriate for the detection of functional neurotoxic changes caused by 3-NP in rats.

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METHODS

The effects of 3-NP were investigated in two different dosing regimens: subacute (the most frequently used scheme to model Huntington's disease) and acute (to represent immediate effects). The subacute experiment included ten-week-old male Wistar rats (ten in a group), receiving 3-NP i. p. in the doses of 10 mg kg^{-1} b.w. (low dose) or 15 mg kg^{-1} b.w. (high dose) for five consecutive days, and kept for four weeks before electrophysiological recording, similar to the experiment described by *Beal et al*. (10). The animals which received 3-NP i. p. as a single acute dose of 20 mg kg^{-1} were kept for 24 h before electrophysiological recording (4). Control animals were untreated.

For recording, the head of the animal - in urethane anaesthesia (1000 mg kg^{-1}; cf. 11) - was fixed in a stereotaxic instrument. The skull was opened to expose the left hemisphere; wounds were sprayed with 10 % lidocaine, and the exposed cortex was covered with warm paraffin oil. After recovery from the surgery (30 min) silver electrodes were placed on the dura over the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas (12). One steel needle electrode was inserted in the *caudato-putamen* (CAUD) and the *globus pallidus* (GPL; coordinates after Paxinos and Watson, 13). Spontaneous electrical activity (electrocorticogram, ECoG) was recorded from these sites simultaneously for six min, and the relative power spectrum of the frequency bands was determined by means of the Neurosys 1.11 software (Experimetria Ltd., UK).

Stimulus-evoked activity was then recorded via the same surface electrodes, and also from the tail nerve. Somatosensory stimulation was done using a pair of needles inserted into the whiskery part of the nasal skin, delivering rectangular electric stimuli (3 V to 4 V, 0.05 ms). Visual stimulation was performed by flashes (1 Hz, 60 lx) delivered by a flash generator via an optical fiber directly into the contralateral eye of the rat. For acoustic stimulation, sound clicks (1 Hz, 40 dB), were applied into the ear of the rat. Fifty stimuli of each modality per rat were applied and the evoked activity recorded. After averaging, latency and duration of the evoked responses was measured manually. Evoked activity of the tail nerve was taken by inserting a stimulating and a recording electrode pair, and applying double stimuli (3 V to 4 V, 0.02 ms, 1 ms to 10 ms inter-stimulus interval). From these data, conduction velocity (as described in reference 14, but performed at room temperature), and relative and absolute refractory period (15) were calculated.

After recording, the subacutely treated rats were killed with an overdose of urethane, and then dissected. Organ weights were measured, and relative organ weights were calculated on the basis of the brain weight, as an index of general toxic effect. The results were tested for significance using the two-sample *t*-test with $p<0.05$ or $p<0.01$ as a limit.

RESULTS

The most pronounced effect of subacute 3-NP treatment on the ECoG was the decrease in the theta activity seen in almost all cortical foci (Figure 2). This was less obvious in acute treatment (Figure

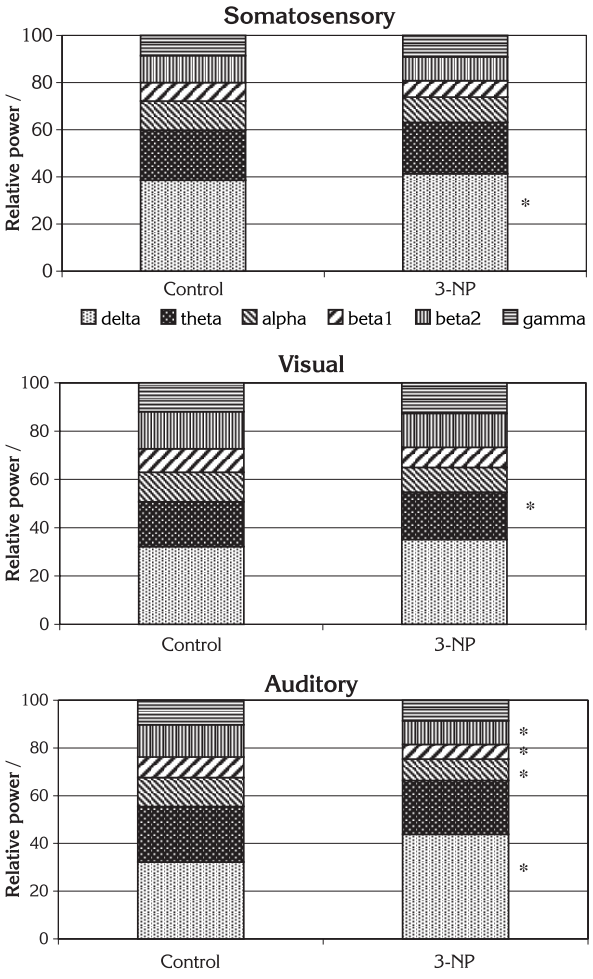

Figure 1 *Relative power spectrum of the spontaneous cortical activity (somatosensory, visual, and auditory focus) in rats acutely treated with 3-NP: 20 mg kg^{-1}. Ordinate: power spectrum of the standard ECoG bands (see insert in the uppermost panel for bar pattern). * $p<0.05$ vs. the same band in the control. (10 rats per group; 1 measurement per rat).*

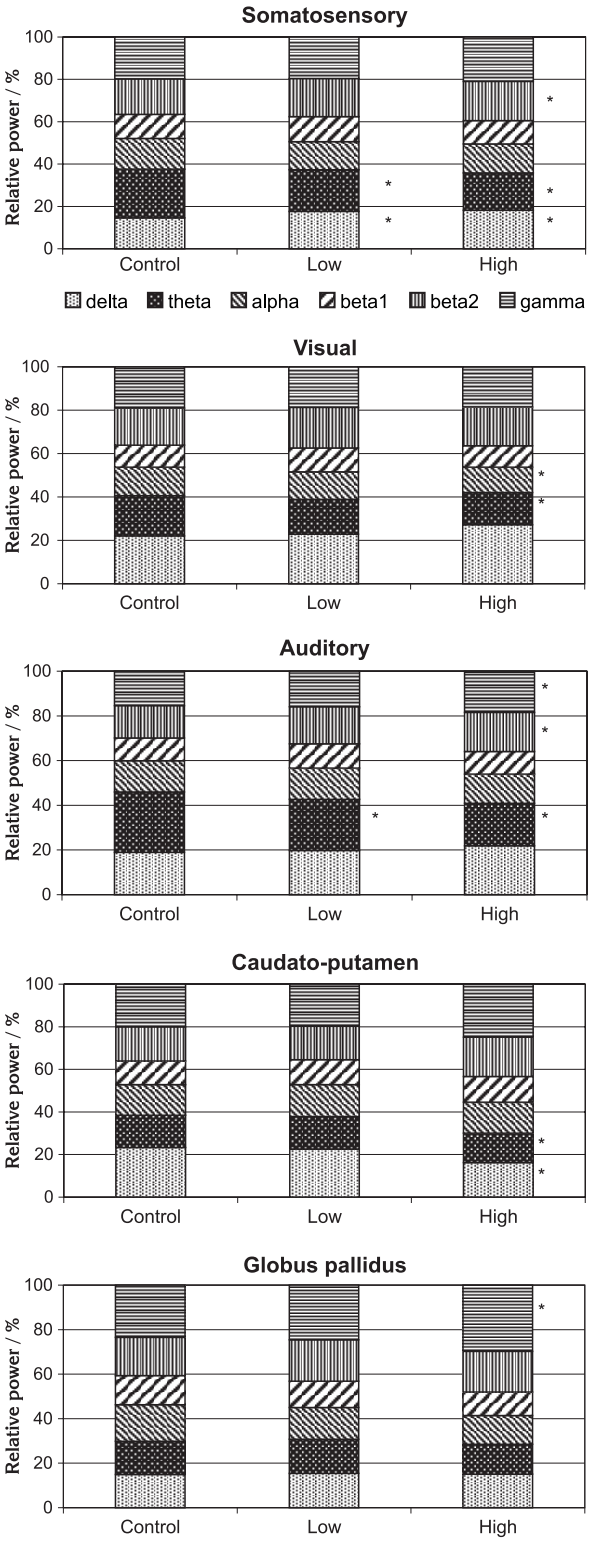

Figure 2 *Relative power spectrum of the spontaneous cortical activity (somatosensory, visual, and auditory focus) and subcortical activity (caudato-putamen and globus pallidus) in rats, subacutely treated with 3-NP: 10 mg kg^{-1} (low dose) and 15 mg kg^{-1} (high dose). Ordinate: power spectrum of the standard ECoG bands (see insert in the uppermost panel for bar pattern). * $p<0.05$ vs. the same band in the control. (10 rats per group; 1 measurement per rat).*

1). The increase in delta activity was significant only in the SS and CAUD area in subacute treatment, and in the SS and AUD area in acute treatment. In the alpha band, there was a massive decrease in the VIS area in both treated groups and, only in the acutely treated rats, also in the AUD area. In the fast bands (beta-1, beta-2, gamma) a significant increase was seen in the subacutely treated rats (SS, AUD, GPL) and a mild, but notable decrease in the acutely treated rats (AUD cortex).

The general slow-down of the cortical activity following subacute treatment with 3-NP was paralleled by the significant increase in the latency of sensory evoked potentials in all recorded cortical areas in both treated groups as opposed to control animals (Table 1). The change seemed to be dose-dependent, but high vs. low dose differences were not significant. The change in the duration of responses was slight. In acute 3-NP exposure, there was no consequent effect on the latency: a small decrease in the SS and AUD response, but significant increase in the VIS response. In contrast, the duration of potentials significantly increased in the SS and VIS, but not in the AUD area.

The parameters of the tail nerve action potential had no uniform trend. The relative refractory periods showed a mild, but not significant increase. The absolute refractory period slightly increased after subacute treatment, but decreased after acute treatment. A clear decrease was seen, however, in the conduction velocity, with evident dose dependence in the subacutely treated rats.

Data obtained from dissection following subacute treatment showed that 3-NP affected the main organs. The weights of organs decreased, except for the lung and the brain, but a significant decrease was only seen for the thymus (Table 2).

DISCUSSION

Rats treated with 3-NP showed a decrease in both spontaneous and stimulus-evoked cortical activity. By inhibiting mitochondrial oxidation, 3-NP depletes energy in the neurons, which results in a kind of tissue hypoxia. Similar effects were seen in volunteers exposed to low oxygen gas mixture, with the consequent shift of EEG toward lower frequencies (16). In humans with inherited or idiopathic mitochondrial dysfunction such as mitochondrial encephalomyopathy (ME), cortical

Table 1 *Latency and duration of the cortical evoked potentials (somatosensory, visual, and auditory focus) after subacute dosing with 3-NP (low dose = 10 mg kg^{-1}, high dose = 15 mg kg^{-1}) and acute dosing with 3-NP (20 mg kg^{-1}). *p<0.05 and **p<0.01 vs. control. (10 rats per group; 1 measurement per rat).*

GROUP	Somatosensory		Visual		Auditory focus	
	Latency/ms Mean±SD	Duration/ms Mean±SD	Latency/ms Mean±SD	Duration/ms Mean±SD	Latency/ms Mean±SD	Duration/ms Mean±SD
SUBACUTE						
Control	7,92±0,31	9,56±0,94	42,75±3,92	63,13±11,62	24,13±1,89	22,25±3,73
3-NP, low dose	8,59**±0,51	8,82±1,31	54,13**±2,75	55,00±11,17	27,25**±1,16	20,13±2,30
3-NP, high dose	8,83**±0,68	9,80±1,08	51,57**±5,41	56,43±11,50	27,88**±2,03	21,13±4,58
ACUTE						
Control	7,92±0,31	9,56±0,94	42,75±3,92	63,13±11,62	24,13±1,89	22,25±3,73
3-NP	7,22±0,46	12,05*±0,96	59,00*±5,41	99,67*±25,78	27,88±4,65	24,84±5,88

Table 2 *Relative organ weights (organ weight/brain weight) in controls and in the group receiving subacute treatment with 3-NP (15 mg kg^{-1}). *p<0.05 vs. control. (10 rats per group; 1 measurement per rat).*

GROUP	Liver Mean±SD	Lungs Mean±SD	Heart Mean±SD	Kidney Mean±SD	Spleen Mean±SD	Thymus Mean±SD	Adrenals Mean±SD
Control	6.81±1.12	0.91±0.10	0.59±0.06	1.36±0.02	0.31±0.02	0.28±0.03	0.036±0.006
3-NP	5.90±0.47	0.94±0.05	0.57±0.07	1.31±0.19	0.29±0.02	0.18*±0.02	0.035±0.003

functions are affected (17), which is manifest in slowed EEG activity (18) and in increased latency of certain visual evoked potential components (19, 20). In our study, subacute 3-NP administration, which impairs mitochondrial function, produced similar changes in the rats' cortical activity. Based on these, the aim of the study was achieved; the applied neurophysiological methods were found appropriate for the detection of functional neurotoxic changes caused by 3-NP mainly in subacutely treated rats.

Another effect, inhibition of astrocytic glutamate uptake of 3-NP (21) could potentially lead to increased cortical excitatory transmission and to an increase in cortical evoked potentials by causing imbalance between excitation and inhibition. This effect, however, was not observed in our experiments, probably because it was suppressed by the general effect of mitochondrial dysfunction caused by 3-NP.

The significant decrease in thymus weight suggests that 3-NP may affect the immune system.

The overall effect of 3-NP on the cortical activity is complex, involving elements of depression and excitation. Further studies should elucidate which of the known effects of 3-NP is specifically responsible for the observed effects, and how these are related to biochemical and morphological changes known to be caused by 3-NP.

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Sažetak

DJELOVANJE 3-NITROPROPIONSKE KISELINE NA ŠTAKORE: OPĆA TOKSIČNOST I FUNKCIJSKA NEUROTOKSIČNOST

Toksin 3-nitropropionska kiselina (3-NP) uzrokuje biokemijske i morfološke promjene u mozgu. U istraživanju su mladi muški Wistar štakori intraperitonealno primali 3-NP pet dana uzastopce. Djelovanje toksina promatrano je četiri tjedna kasnije (subakutna primjena). Akutni su učinci promatrani 24 sata nakon intraperitonealne primjene. Spontana i podražajno izazvana aktivnost mjerena je na osjetilnim žarištima korteksa, na supkortikalnim jezgrama te na repnome živcu, dok su životinje bile pod uretanskom anestezijom.

Nakon subakutnoga doziranja zamijećen je rast beta-2 i gama-valova kod spontane aktivnosti. Ova promjena nije bila istovjetna na kori i pod korom mozga. Stimulirana osjetilna aktivnost u ove je subakutne skupine pokazala povećanu latenciju u svim osjetilnim područjima.

U štakora koji su primili jednokratnu akutnu dozu amplituda evociranoga somatosenzornoga potencijala bila je manja. Masa prsne žlijezde bila je značajno niža u štakora koji su primili 3-NP. Potrebna su daljnja istraživanja koja bi razjasnila povezanost između biokemijskoga djelovanja 3-NP-a i zamijećenih neurotoksičnih funkcijskih promjena.

KLJUČNE RIJEČI: *spontana aktivnost korteksa, evocirani odgovor korteksa, potencijal živčanog djelovanja*

REQUESTS FOR REPRINTS:

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