# ACUTE EFFECTS OF TWO MITOCHONDRIAL TOXINS, 3-NITROPROPOINIC ACID AND MALONIC ACID, ON THE SPONTANEOUS AND EVOKED CORTICAL ACTIVITY IN RATS

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ABSTRACT: Mitochondrial toxins are becoming important tools in modelling human neurological diseases. 3-nitropropionic acid (3-NP) is a natural substance found in some weeds, and in foodstuffs infested by certain moulds causing occasional human intoxication with nervous system manifestations. Malonic acid (MA) is also found both in plants and animals. 3-NP causes an irreversible block of mitochondrial succinate dehydrogenase while the effect of MA is reversible. In our study, both toxicants were given acutely to young adult male Wistar rats and certain functional changes were observed. In the somatosensory cortical evoked potentials, 3-NP reduced the amplitude in the first but increased in the second evoked response obtained by double-impulse stimulation. MA caused no reduction of amplitude and its effect was more rapid. The spontaneous activity was slowed down in 3-NP- but not in MA-treated rats. The discrepancies indicate that some effects result possibly not from the mitochondrial action of the substances, which may be relevant for human disease models.

KEY WORDS: 3-nitropropionic acid, malonic acid, corticogram, evoked potentials, rat

## INTRODUCTION

The neurotoxic substance 3-nitropropionic acid (3-NP) is naturally found in certain leguminous plants, intoxicating grazing animals (Johnson et al., 2000). Human intoxication may result from infestation of foodstuffs (sugar cane, cereals, etc.) with moulds producing 3-NP. Exposure to 3-NP, even in low doses, causes acute encephalopathy followed by dystonia (Liu et al., 1992). The damages seen in systemically or locally 3-NP-treated experimental animals – both in systemic or intracerebral application – are decreased motor performance (Teunissen et al., 2001) and degeneration in the striatum, hippocampus, and thalamus (McCracken et al., 2001), provide the base of modelling Huntington's disease in animals by 3-NP administra-

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tion. At cellular level, 3-NP inhibits succinate dehydrogenase, a key enzyme of oxidative energy production (Coles et al., 1979).

Malonic acid (MA) also naturally occurs in some plant and animal tissues. MA is a reversible inhibitor of mitochondrial succinate dehydrogenase (Kim, 2002) and is applied in modelling disease similarly to 3-NP (Maragos et al., 2002). In contrast to 3-NP, however, MA has always been applied in form of local microinjections.

It is likely that the energetic insufficiency induced by either of these substances in the brain has a general effect, also reflected in the spontaneous and/or stimulus-evoked activity of the central and peripheral nervous system. The aim of the present study was to find evidence of this effect by means of the complex neuro-functional investigation methodology available in our department (Dési and Nagymajtényi, 1999).

## METHODS

The experiments were performed in young adult male Wistar rats (ca. 350 g b.m.) in the GLP certified neurotoxicological laboratory (certification No. 3011/48/2003) of the Department. In urethane (1000 mg/kg ip.) anesthesia, the rats were placed in a stereotaxic instrument, the skull was opened over the left hemisphere and silver electrodes were placed on the primary somatosensory (SS), visual (VIS), and auditory (AUD) areas. First, electrocorticogram (ECoG) was recorded for 5 minutes. In this epoch, the power spectrum was obtained and the so-called ECoG index, the ratio of the slow and fast bands [delta+theta/beta$_1$+beta$_2$] calculated. Next, sensory evoked potentials (EPs) were recorded in the SS and VIS sites. Fifty evoked potentials of each modality were recorded, at a stimulation frequency of 1 Hz (using double pulses of various inter-stimulus intervals for SS stimulation). After averaging, the amplitude and the onset latency was measured. As for other details of the stimulation and recording procedure, see Dési and Nagymajtényi (1999). Three complete sets of control records were taken in 20 min intervals. Then, 3-NP (20 mg/kg b.m.) or MA (600 mg/kg b.m.) was intraperitoneally injected and further records were taken for 1.5–2 hours. The given doses were considered equitoxic, based on the published $LD_{50}$ data (Pass et al., 1985; Sigma-Aldrich, 2003). From the measured data of the individual animals, group mean (n=10) was calculated. The alterations induced by the substances in the measured neurophysiological parameters were expressed as relative changes, taking the average of the pre-administration period (first 3 measurements) as reference. After finishing the above investigations, the rats were sacrificed by an overdose of urethane.

In the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

## RESULTS

In the 3-NP-treated rats, a slowly developing increase of the ECoG index signalized a growing preponderance of slow waves (*Fig. 1A*). In the MA-treated animals, no similar effect was seen (*Fig. 1B*).

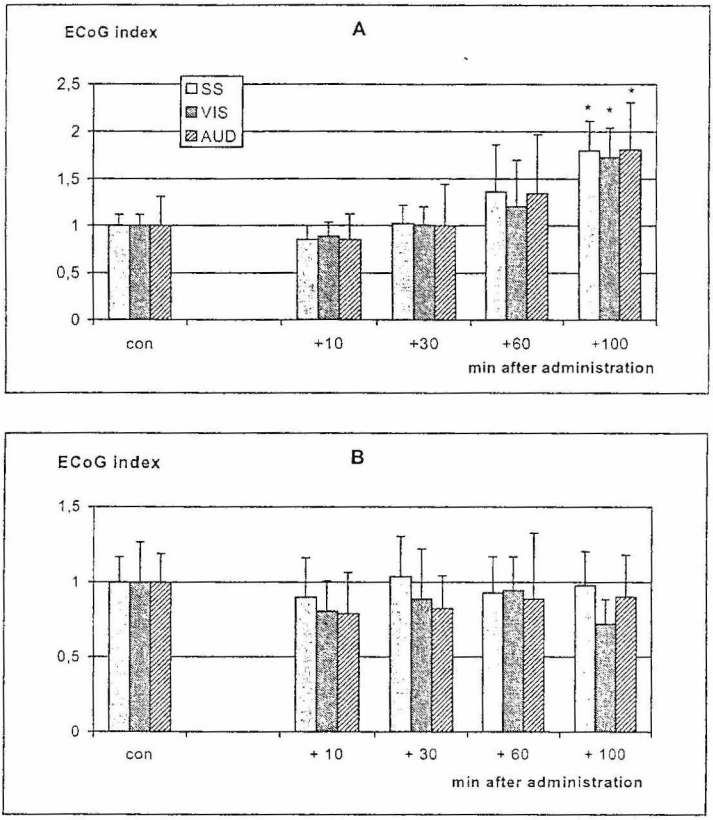


*Fig. 1. Effect of acutely administered 3-NP (20 mg/kg b.m., **A**) and MA (600 mg/kg b.m., **B**) on the spontaneous cortical activity. Ordinate: ECoG index in relative units, normalized to the control (mean+SD, n=10). SS, VIS, and AUD: somatosensory, visual, and auditory, respectively; con = mean values of the pre-administration records. *p<0.05 vs. control.*

The effects of the two agents on the SS evoked potentials elicited by double-pulse stimulation were partly similar. In case of 3-NP (*Fig. 2A*), the amplitude of the first EP decreased slightly after administration. The amplitude of the second EP, with 300 and 240 ms inter-stimulus interval, started to increase after 3-NP and this

trend became significant to the end of recording. In case of MA (*Fig. 2B*), the amplitude of the first EP showed a moderate non-significant increase. The increase of the second EP amplitude was strongest with the 240-ms inter-stimulus interval. The effect of MA on the EP amplitude seemed to develope and disappear faster than in case of 3-NP. Neither of the two substances affected significantly the latency.

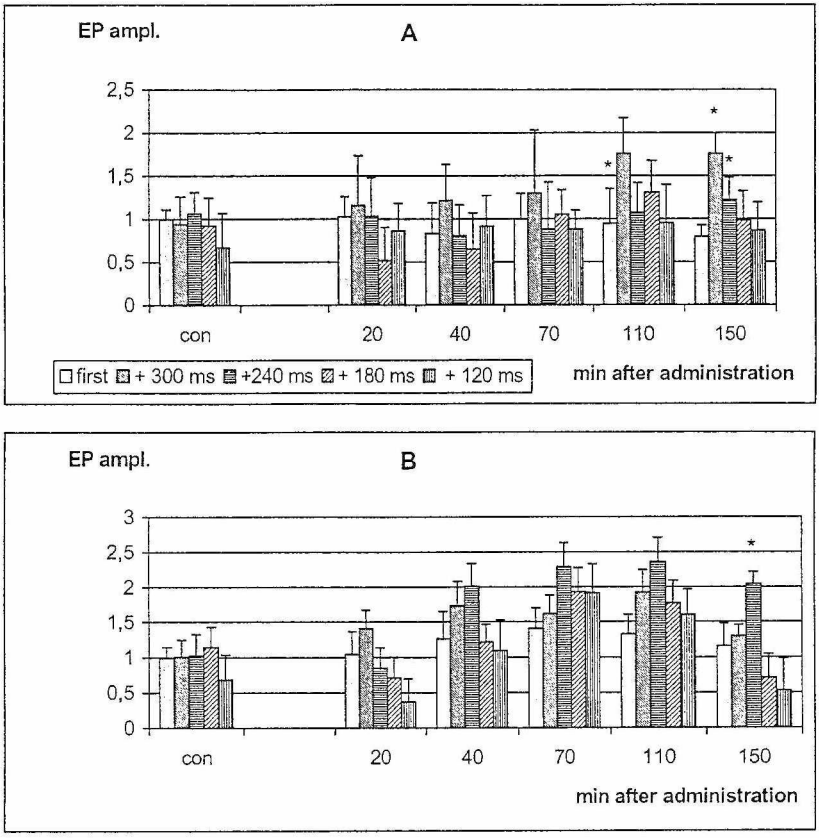


*Fig. 2. Effect of acutely administered 3-NP (A) and MA (B) on the amplitude of the somatosensory evoked potentials. Ordinate: amplitude in relative units normalized to the pre-administration "control first". Insert: first potentials and second potentials with the inter-stimulus intervals given. *p<0.05 vs. the first evoked potential.*

In the 3-NP-treated rats, the amplitude of the VIS EP showed a significant decrease followed by an increasing trend. At the same time, its latency was hardly altered (*Fig. 3A*). The effect of MA on the latency (*Fig. 3B*) was also insignificant. The decrease of the amplitude appeared later than with 3-NP.

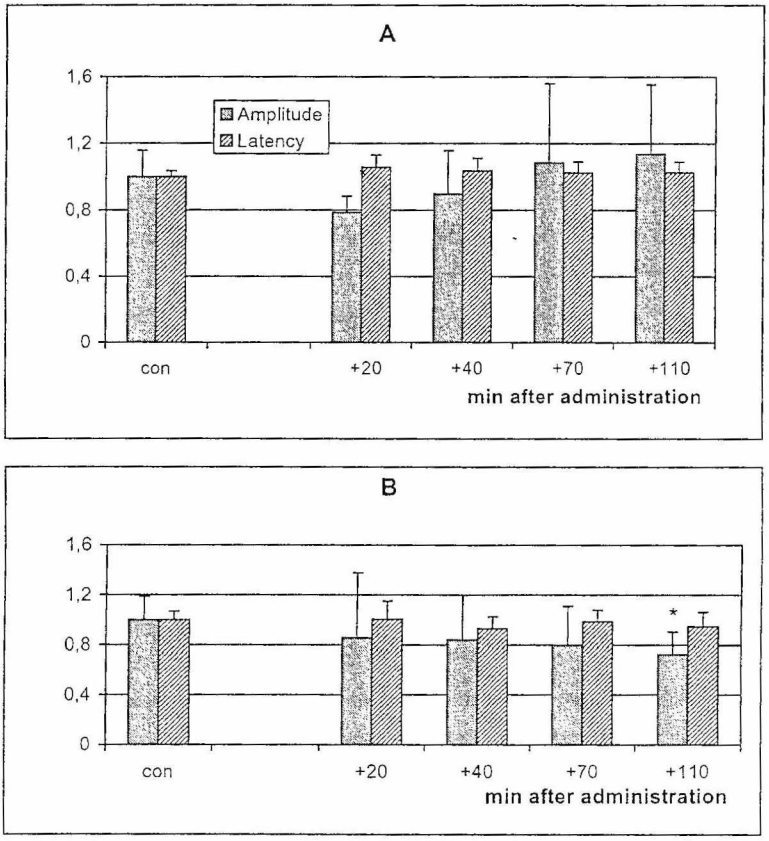


*Fig. 3. Effect of acutely administered 3-NP (A) and MA (B) on the amplitude and latency of the visual evoked potential (relative units normalised to the control). Other details as in* Fig. 1.

## DISCUSSION

The alterations of the SS EP to i.p. injection of 3-NP and MA were alike, which indicated that MA, applied only locally up to now (Greene et al., 1993), was also effective when given systemically. The amplitude increase of the second evoked potential in double-pulse stimulation indicated some kind of disinhibition. For 3-NP, such an effect was previously described (Szabó et al., 2004). The effect of MA was, at comparable doses, qualitatively similar, indicating the possibility of a common mechanism, more exactly a disinhibition resulting from the mitochondrial damage caused by both substances. In human patients with mitochondrial encephalomy-

opathy, paired-pulse somatosensory stimulation delivered to the median nerve revealed strongly reduced intracortical inhibition (Liepert et al., 2001). As the connection of mitochondrial disorders and increased cortical excitability has also been described (Rosing et al., 1985), it can be supposed that double-pulse stimulation is suitable to reveal and/or to follow up the action of mitochondrial toxins in the brain in a sensitive and specific way, which could contribute to improve disease models based on these toxins.

In case of the VIS EP obtained with single-pulse stimulation, only the amplitude showed a significant change. The decrease caused by 3-NP was fast and transient, with a subsequent slight insignificant increase. The effect of MA developed more slowly. The decrease was probably a result of the direct mitochondrial effect of both agents, and the slight increase seen with 3-NP was probably due to a secondary glutamatergic hyperactivity (Tavares et al., 2001).

The effect on the spontaneous cortical activity, shown here by means of ECoG index, was dissimilar with 3-NP and MA. The cholinergic neurons in the basal forebrain are major sources of ACh regulating the level of cortical activity (Fournier et al., 2004). The block of glutamate uptake by 3-NP might led to gradual desensitization of the receptors on these neurons, and so, to reduced ACh release. Why the same was not observed in MA-treated rats, remained an open question requiring further studies. They should reveal the details of the mechanism behind the phenomena described here and their relevance in the disease model.

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# FUNCTIONAL NEUROTOXIC EFFECTS TO ACUTE APPLICATION OF MALONIC ACID IN RATS

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ABSTRACT: Malonic acid (MA) is a natural substance with mitochondrial toxic activity which makes it important in modelling chronic human neurological diseases like Huntington's disease. The aim of this study was to investigate the effects of acutely administered MA on the spontaneous activity and evoked potential in the cortex, and action potentials in a peripheral nerve. Young adult male Wistar rats were anesthetized with urethane and received 600 mg/kg b.m. MA ip. Spontaneous activity and evoked potentials in the somatosensory, visual and auditory cortical areas as well as the compound action potentials of the tail nerve, elicited by electrical stimulation, were recorded before and after MA administration. In the spontaneous activity, a transient decrease was seen in the delta, and an increase in the beta band. In the cortical evoked potentials, both amplitude and duration were affected. There was no considerable effect on nerve conduction velocity. Experiments of this kind may contribute to the background of modelling certain human neurological diseases.

KEY WORDS: Malonic acid, electrocorticogram, evoked potentials, peripheral nerve, rat

## INTRODUCTION

Malonic (MA) acid is a dicarboxylic acid naturally occurring in leguminous plants and also detected in animal tissues (Kim, 2002). MA is a reversible inhibitor of mitochondrial succinate dehydrogenase (Kim, 2002), and is used, beside other mitochondrial toxins, in animal models of chronic human neurological diseases, first of all Huntington's disease (Borlongan et al., 1997; Greene et al., 1993). In the experiments published up to now, MA was applied by intracerebral microinjection and its effects were studied in the context of the morphological and functional damages relevant to animal models of the above mentioned diseases (Greene and Greenamyre, 1995). It is, however, of interest what functional alterations in the cortical activity develop under the influence of MA and how these can be utilized in studies based on the disease models. In the present study, MA was given to rats by acute intraperitoneal injection and alterations in the spontaneous and evoked cortical

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