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ARTICLE

Altered stimulus frequency and intensity dependence of the somatosensory evoked potential in rats after acute application of two mitochondrial toxins

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ABSTRACT Mitochondrial toxins are a special group of toxicants with nervous system effects. The resulting nervous system damage could be detected and followed-up by means of functional biomarkers but these still have to be worked out. In this work, adult male Wistar rats were anesthetized with urethane, the left hemisphere was exposed, and a silver recording electrode was placed on the projection area of the whiskers. The whisker pad was stimulated with electric square pulses and the cortical response was recorded. The intensity of the stimulus was varied between 25% and 100% (just supramaximal), and its frequency, between 1 and 10 Hz. Control records were taken, then one of the agents (3-nitrporopionic acid, a mitochondrial toxin of microfungal origin: 20 mg/kg b.w.; or manganese, a heavy metal: 50 mg/kg b.w. in chloride form) was injected ip. and further records were taken. Both agents had an effect on the latency, but on the amplitude, only Mn. Of the relationships between stimulation settings and evoked potential parameters, frequency dependence of latency had the clearest alteration on application of Mn or 3-NP. Such effects may have the potency to be developed to functional biomarkers, applicable in practical toxicology or in animal research.
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KEY WORDS

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rat
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A number of agents - environmental xenobiotics, drugs etc. - act on the human and animal nervous system. Even if the effect is seldom strong enough to cause overt symptoms, long-term exposure leads to subclinical alterations. A modern way to detect such alterations is the use of biomarkers, that is, measurements that indicate exposure to a chemical, the effect of such exposure, or susceptibility to the (usually toxic) effect of such an exposure (Hayes 2001). For neuro-functional alterations, chemical biomarkers are not ideal (Manzo et al. 1996), so the development of markers based on electrophysiological recording may be a promising field of investigation. In this study, two different neurotoxicants, both belonging to the mitochondrial toxins, were used.

Manganese is essential for living organisms in small amounts but toxic when overdosed. Exposure by excess amounts of Mn is typically occupational (metal industries, dry cell manufacturing, use of organo-Mn fungicides) but population-level exposure can also be observed, via drinking water or due to the petrol additive MMT (ATSDR 2000). Long-term exposure causes manganism (an occupational disease resembling Parkinson's disease, characterized by dopaminergic abnormalities) seen mostly in workers exposed to airborne Mn (Bowler et al. 2006), and modelled in animals (Yu et al. 2003). Tyrosine hydroxylation, a crucial step of dopamine synthesis, was blocked by Mn in vitro (Hirata et al. 2001) possibly by a mechanism depending on inhibition of mitochondrial function (Zhang et al. 2003). There are, however, not much reports on Mn effects on spontaneous or evoked cortical activity. Only a few authors found neurological (EEG and/or evoked potential) disturbances following occupational (Sinczuk-Walczak et al. 2001; Sjögren et al. 1996) or accidental (Hernandez et al. 2003) Mn exposure. In our earlier studies, behavioural and electrophysiological changes were found in rats after several weeks oral Mn exposure (Vezér et al. 2005).

The toxin 3-nitropropionic acid (3-NP) is naturally present in leguminous plants, occasionally poisoning grazing livestock (James et al. 1980). Human poisoning may come from consumption of foodstuffs infested with certain moulds (*Arthrinium , Aspergillus, Penicillium* spp.) producing 3-NP (Liu et al. 1989; Peraica and Domijan 2001). Most human intoxications were described in China after consumption of mould-infested sugar cane stalks (by children as a delicacy: Liu et al. 1992). The brain damage observed in the victims initiated the application of 3-NP in animal modelling of Huntington's disease (Alexi et al. 2000). Functional deficits described from experimental animals treated with 3-NP involve motility changes (Koutouzis et al. 1995) and low memory performance (Teunissen et al. 2001). Previous works from our

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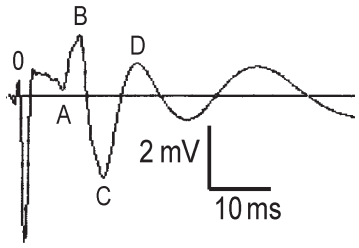

Figure 1. Measurements on the averaged somatosensory evoked potential. Onset latency was determined between 0 and A (stimulus artefact and the start of the main wave); and peak-to-peak amplitude, between B and C.

lab (Szabó et al. 2005) proved that the nervous system effects of 3-NP can be detected by electrophysiological methods.

The common point in the action of the two agents, most probably contributing to their neurotoxicity, is mitochondrial inhibition. 3-NP acts on succinate dehydrogenase (Coles et al. 1979) which is part of mitochondrial complex II. Mn inhibits both complex II (Malecki, 2001) and III (Zhang et al. 2003). The resulting energetic insufficiency may well be reflected in the electrical activity of the nervous system, similarly to what was described in mitochondrial encephalopathy (Scaioli et al. 1998) or experimental hypoxia (Van der Post et al. 2002). Based on this, and on previous experience (Szabó et al. 2003), the aim of the present work was to investigate whether the relationship of the parameters of somatosensory stimulation (intensity and frequency) and somatosensory cortical evoked potential (latency and amplitude) is altered by acute application of 3-NP or Mn.

Materials and Methods

The two toxicants were acutely given to adult (ca. 300 g) male Wistar rats ip. The rats were first anesthetized by ip. injection of urethane (1000 mg/kg b.w.), and the left hemisphere was exposed. Following recovery, a recording electrode was placed on the projection area of the whiskers in the primary somatosensory cortex. A pair of electrodes was inserted in the contralatertal whisker pad, to deliver 0.05 ms wide square electric pulses as stimuli, and cortical evoked potentials (EP) were recorded. The just-supramaximal stimulus strength (around 3 – 5 V) was determined first and taken as 100%. Then, series of 50 stimuli each were given with 25, 50, 75 and 100% strength at 1 Hz stimulation frequency, and 100% stimuli at 2, 5 and 10 Hz. This sequence was repeated 3 times with 30 min interval for control (lines a–c in Figs. 2, 3 and 4). Then, one of the test substances was injected ip. and further 4 records were taken (lines d–f in the figures). Mn was given in form of $MnCl_2$ dissolved in distilled water so that the dose for the pure metal was 50 mg/kg b.w, and the injection volume, 1 ml/kg. 3-NP was given in 20 /kg b.w. dose, also dissolved in distilled water (doses found to have acute effect:

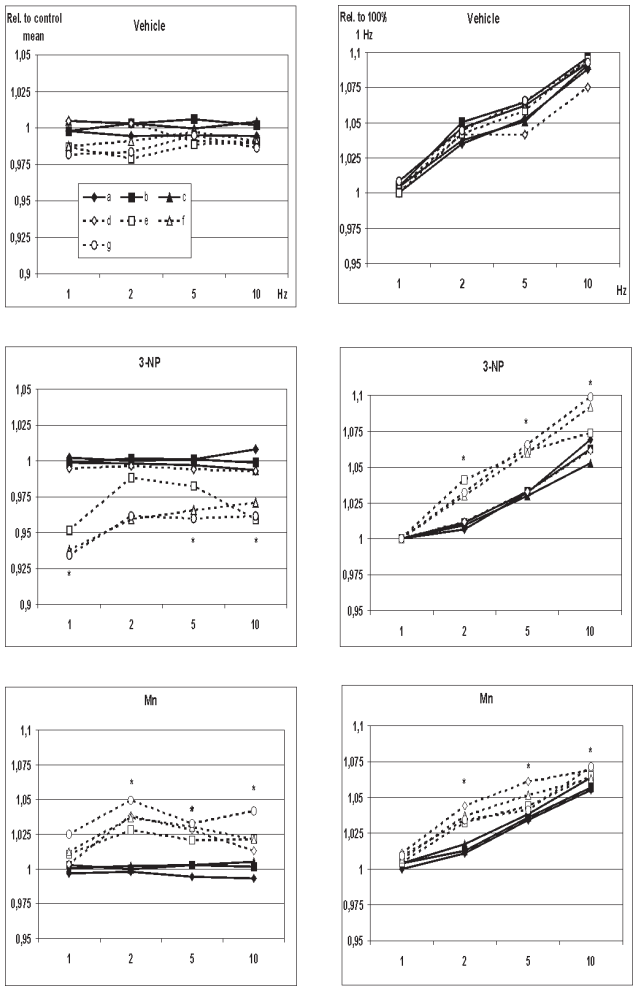

Figure 2. Dependence of the latency of the somatosensory evoked potential on the frequency of stimulation. Abscissa, frequency; ordinate, normalized latency values. Left column: normalized to the mean of the control period (first 3 series), right column: normalized to the value obtained by 1 Hz stimulation (100% intensity). Insert: line symbols for the records: a, 0 min; b, 30 min; c, 60 min (control period); d, 90 min; e, 120 min; 150 min; f, 180 min (administration period). *: p<0.05 after vs. before administering the agent.

Pecze et al. 2004; Szabó et al. 2005). Both substances were tested in 8 rats, and another 8 were used as vehicle-treated parallel controls. 3-NP was obtained from Sigma-Aldrich, and $MnCl_2$, from Reanal.

After the last recording, the rats were sacrificed by an overdose of urethane. The principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed throughout.

The recorded EPs were averaged, and their onset latency and peak-to-peak amplitude was measured (Fig. 1). Recording, storage and analysis of the EPs was done by the Neurosys 1.11 software (Experimetria, Budapest). To eliminate individual variation, first of all in the EP amplitudes, the

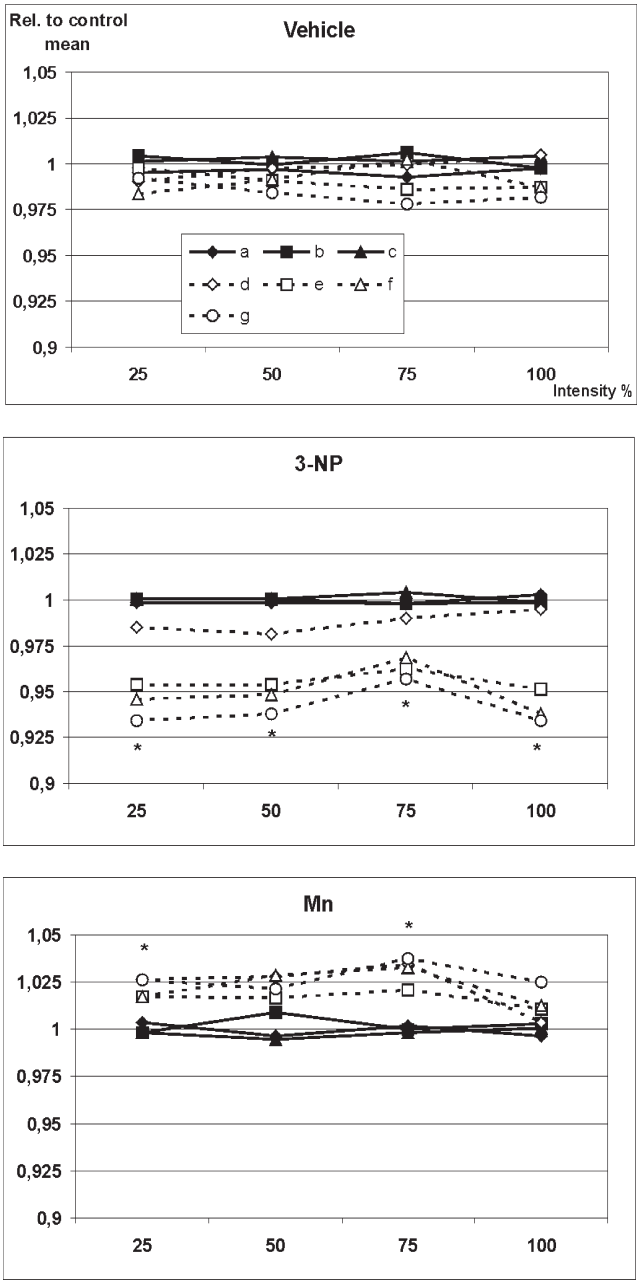

Figure 3. Dependence of the latency of the somatosensory evoked potential on the intensity of stimulation. Abscissa, intensity; ordinate, latency values normalized to the mean of the control period (first 3 series). *: p<0.05 after vs. before administering the agent.

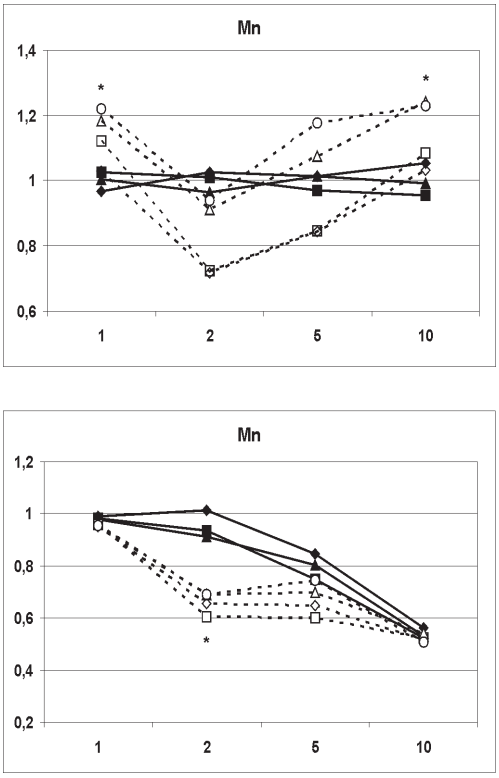

Figure 4. Dependence of the amplitude of the of the somatosensory evoked potential on the frequency of stimulation. Abscissa, frequency; ordinate, amplitude values normalized to the mean of the control period (top), and to the value obtained by 1 Hz stimulation (bottom). *: p<0.05 after vs. before administering the agent.

measured values were normalized. The reference base was either the mean of the control period (first 3 series), separately for each stimulus setting. The other way was to normalize to the value obtained by 1 Hz stimulation (100% intensity) in the same series. These normalized data were plotted against the intensity (25 to 100%) or the frequency (1 to 10 Hz) of stimulation and any difference between the resulting plots before vs. after administration of the toxicant was sought for. The significance of before-after differences was tested by two-sample t-test.

Results

In the parallel control (vehicle treated) animals, and in the treated animals in the control period, the latency and amplitude data normalized to control mean and plotted against frequency or intensity of stimulation gave a more or less horizontal line around one (Fig. 2 top left); while after normalizing to 1 Hz stimulation, an oblique relationship was seen (Fig. 2 top right).

EP latency was altered by both agents tested. Compared to control mean, 3-NP caused a shortening of latency in the treated animals (Fig. 2 mid left), which developed with some delay (line d not different from lines a–c) and reached full size at 10 Hz earlier (line e) than at lower stimulus rates. When normalized to 1 Hz stimulation, a steeper frequency-dependent increase of the latency was seen (Fig. 1 mid right). The effect of Mn was lengthening which appeared faster than the effect of 3-NP (Fig. 2 bottom left). The frequency dependence became somewhat steeper also here (Fig. 2 bottom right).

Shortening of EP latency on 3-NP treatment, and lengthening on Mn, was also seen when values recorded at different stimulus strengths were plotted (Fig. 3). On the basis of control records, the changes were similar to those seen as a function of frequency. The intensity dependence itself was, however, not significantly altered (not shown).

On the EP amplitude, only Mn had significant effect (that of 3-NP was observable but less characteristic). Related to control mean, the amplitude gradually increased at 1 and 10 Hz, at 2 Hz and partly 5 Hz, first a clear decrease was seen which changed to an increase later (Fig. 4 top). When normalized to 1 Hz stimulation, the decrease of amplitude after injection of Mn was steeper between 1 and 2 Hz but not at higher frequencies.

Discussion

The changes in the latency and amplitude of the EPs obtained by electrical stimulation of the whisker pad were similar to those observed in earlier works of the Department (Pecze et al. 2004; Szabó et al. 2005) which confirmed also that such acute experiments with short time span are suitable for examining neurotoxic effects. Of especial importance is in this context the stability of the measured and calculated parameters in the parallel control animals and during the control periods, indicating that urethane anaesthesia itself was not responsible for the observed effects.

First of all the shortened EP latency in the 3-NP treated animals suggested that the mechanism responsible is specific, beyond the energetic crisis caused by mitochondrial inhibition. Cortical EPs are due to specific afferent pathways working with glutamatergic excitation. 3-NP is known to inhibit the glial uptake of glutamate (Tavares et al. 2001) resulting in more intense cortical response. 3-NP also affects GABAergic transmission (Erecinska and Nelson 1994) which can contribute to the mentioned effect.

Why the effect of Mn, another mitochondrial toxin, on the latency was dissimilar, was probably due to its other effects. On the one hand, Mn inhibits both the glial uptake (Hazell and Norenberg 1997) and the breakdown of glutamate (Normandin and Hazell 2002); on the other hand, Mn^{2+} ions are inorganic Ca-channel blockers (Büsselberg, 1995), also in cortical neurons (Pumain et al. 1987) – the final effect of which can be slower but higher EPs.

Of all relationships tested, the frequency dependence of the EP latency seemed to be the best choice in terms of a possible biomarker. All the more so, because it is known that natural stimulation of the whiskers at 1 or 10 Hz frequency induces qualitatively different cortical activation (Moore 2004). Practical application in health protection is more likely in case of Mn, where the development of neurological consequences of occupational Mn exposure can possibly be followed-up by non-invasive functional tests. For other metals, the sensitivity of such tests has been already published (lead – Bleecker et al. 2003; mercury – Chang et al. 1995; Lamm and Pratt 1985).

In case of 3-NP, application in the animal model of Huntington's disease is more likely, to check the development of the damage serving as model.

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