CHANGES IN THE SPONTANEOUS AND EVOKED CORTICAL ACTIVITY OF RATS INDUCED BY TWO MITOCHONDRIAL TOXINS

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ABSTRACT: Mitochondrial toxins are an interesting group of neurotoxicants including certain heavy metals and a number of organic compounds. In case of manganese, brain is one of the target organs, resulting in compulsive and aberrant behaviour, emotional instability and hallucinations. Mn^{2+} ions are known to interfere with certain enzymes involved in the energy-producing mitochondrial oxidation. The organic toxin 3-nitropropionic acid (3-NP) is produced by various microfungi and is found in leguminous plants used as animal feed. 3-NP causes biochemical and morphological alterations in the brain. Humans exposed to 3-NP develop seizures and coma, followed by persisting abnormalities of motion. In this study we tried to elucidate to what extent neurophysiological measurements are suitable to detect functional changes caused by acute administration of these toxicants.

In anaesthetised male Wistar rats, a silver recording electrode was placed on the somatosensory projection area of the whiskers. The corresponding peripheral site was stimulated by weak electric pulses, and the cortical response was recorded. On the averaged records, amplitude and latency were measured. Spontaneous activity electrocorticogram (ECoG) was taken simultaneously, and the relative distribution of the activity over the bands (delta to gamma) was automatically generated.

On intraperitoned administration of Mn^{2+} (50 mg/kg) the amplitude of the evoked response showed first a decrease then an increase. In the spontaneous cortical activity, there was a parallel shift to lower frequencies but the correlation of the two changes was moderate. Acute administration of 3-NP (20 mg/kg, ip.) decreased the amplitude of the evoked response. Its effect on the spontaneous activity was below significance.

Due to the importance of these substances as environmental burden and as experimental tools, further investigation of their functional neurotoxic properties is justified.

KEY WORDS: Manganese, 3-nitropropionic acid, electrocorticogram, evoked potential, rat

INTRODUCTION

Manganese is a ubiquitous element, essential for living organisms in small amounts but toxic when overdosed (ATSDR, 2000). Exposure to abnormally high levels of

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manganese has traditionally been an occupational risk factor. Nowadays, Mn is present in the general environment, largely due to human activity. Methylcyclopentadienyl manganese tricarbonyl (MMT) is used as anti-knock petrol additive in certain countries (Lynam et al., 1999). Some Mn-compounds have widespread agricultural application as fungicides (Ferraz et al., 1988), and spent dry cells contribute to the Mn content of solid household waste.

In humans, the brain is among the organs most affected in chronic Mn exposure. Inorganic Mn can pass the blood-brain barrier (Ashner et al., 1999) and tends to deposit in the brain (Mena et al., 1967). The chronic disease resulting from long-term, high-dose occupational exposure to manganese resembles parkinsonism (Montastruc et al., 1994), with functional (Shinotoh et al., 1997) and structural (Yamada et al., 1986) damages to the dopaminergic systems.

In animals, manganese was found to interfere with CNS synaptic functions in several ways. 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, and the mitochondrial complex II (Malecki, 2001) and complex III (Zhang et al., 2003) were found to be inhibited by the presence of Mn. Mn^{2+} is known to block voltage-dependent Ca^{2+} channels of neurons and presynaptic endings (Nelson, 1986). Calcium currents of cortical neurons, induced by application of excitatory amino acids, were blocked by Mn^{2+} (Pumain et al., 1987). The release of the excitatory transmitters and of GABA, was reduced by moderate doses of Mn^{2+} (Takeda et al., 2003). Another effect of Mn^{2+}, inhibition of astrocytic glutamate uptake, can, on the contrary, enhance synaptic transmission in the cortex (Hazell and Norenberg, 1997).

The substance 3-nitropropionic acid (3-NP) is naturally found in *Astragalus* species *(Leguminosae)* thus intoxicating grazing animals (Johnson et al., 2000). Human intoxication may result from infestation of foodstuffs (sugar cane, cereals, etc.) with moulds of the *Anthirium* and *Aspergillus* genus producing 3-NP. Human exposure to 3-NP, even in low doses, causes acute encephalopathy followed by dystonia (Liu et al., 1992).

The morphological and functional effects of 3-NP intoxication have been replicated in animal experiments (Brouillet et al., 1999). Decrease of motor performance was seen (Teunissen et al., 2001) with degeneration of primarily the striatum but also the hippocampus and thalamus (McCracken et al., 2001). At the cellular level, 3-NP inhibits succinate dehydrogenase, a key enzyme of oxidative energy production (Coles et al, 1979) which effect develops fast and is not limited to the sites of morphological damage (Brouillet et al., 1998). Beyond that, 3-NP was found to act on NMDA receptors thereby inducing excitotoxicity (Pubill et al, 2001).

There are a few common points in the neurotoxicity of these two, chemically very different agents, primarily the effect on the mitochondrial energy production and on the dopaminergic structures. The aim of this work was to describe the effects of acute Mn^{2+} or 3-NP exposure on the spontaneous and stimulus-evoked cortical activity in rats and to find possible similarities in the effect of these two neurotoxicants.

METHODS

The experiments were done on adult male Wistar rats (ca. 350 g b.w.). In urethane (1000 mg/kg b.w.) anesthesia, the head of the rats was clamped in a stereotaxic frame and the left hemisphere was exposed. Silver recording electrodes were placed on the somatosensory projection area of the whiskers (barrel field). The corresponding peripheral site was stimulated by electric pulses (ca. 4 V, 0.05 ms, 1 Hz). The pattern of recording consisted of a five-minute epoch of electrocorticogram (ECoG) recording, followed by evoked potentials (EPs) recorded by applying one train of 20 stimuli to the whiskers. This pattern was repeated every 20 minutes. After 4-5 control records, 50 mg/kg Mn^{2+} ($MnCl_2$ dissolved in distilled water) or 20 mg/kg 3-NP (dissolved in distilled water and pH set to 6.0) was administered via a peritoneal cannula and the recording was continued for further ca. 2.5 hours. On the EPs, amplitude and peak latency was measured by hand after automated averaging. Form the ECoG records, band activity was automatically computed and the ECoG index (delta+theta/$beta_1$+$beta_2$) calculated.

RESULTS

Evoked potentials

The effect of Mn^{2+} was detectable mainly on the response amplitude. After injection of Mn^{2+}, an increase of the amplitude was seen which kept growing until the recording was finished and became significant (*Fig. 1A*). In parallel controls, not treated over the recording period, there was no trend of change. The latency of the first peak of the somatosensory EP was not influenced by the Mn treatment.

The effect of 3-NP was less manifest. With long delay after administration, the EP amplitude suddenly decreased (*Fig. 1B*). On the latency there was no noteworthy effect.

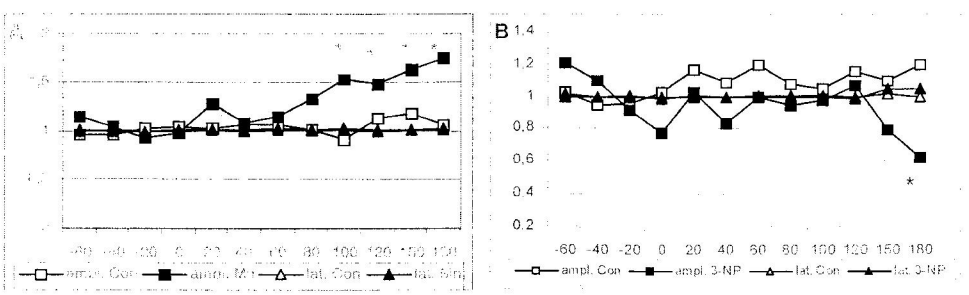

*Fig. 1. The effect of acute Mn^{2+} (50 mg/kg, ip, **A**) and 3-NP (20 mg/kg, ip, **B**) on the amplitude and latency of the somatosensory evoked potential. Abscissa: time, pretreatment control period indicated by negative values. Ordinate: amplitude and latency given in relative values (related to the mean value of the control period). *: $p<0.05$.*

Spontaneous activity

The effect of the two substances on spontaneous activity was of similar direction and magnitude (*Fig. 2, A* and *B*). The ECoG index increased in both cases, indicating the predominance of slow wave activity. With Mn^{2+}, the effect appeared within 40 minutes after administration while the action of 3-NP was seen only after ca. 1.5 hours.

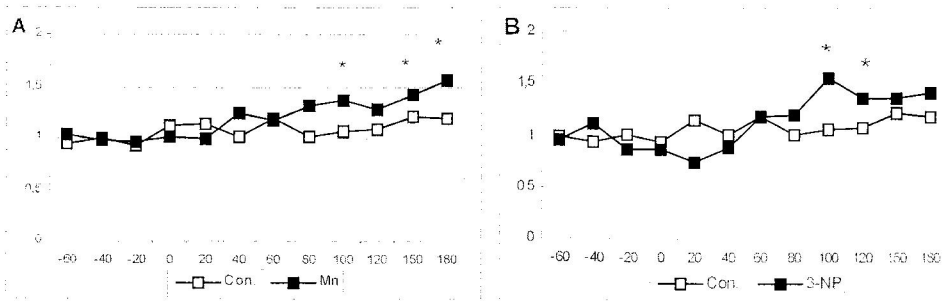

*Fig. 2. The effect of acute Mn^{2+} (**A**) and 3-NP (**B**) administration on the spontaneous cortical activity. Displayed as in Fig. 1, Ordinate: ECoG index value (see Methods). *: $p<0.05$.*

To see if there was any correlation between the effects of the agents on the spontaneous and evoked cortical activity, the numerical values of the two were plotted against each other. The correlation diagram obtained (*Fig. 3*) showed fair correlation in case of Mn^{2+} but practically no correlation in case of 3-NP.

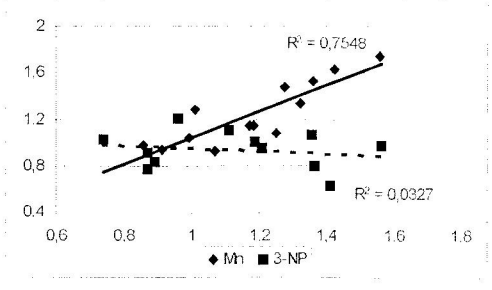

Fig. 3. Correlation diagram of the ECoG index (abscissa) and the evoked potential amplitude (ordinate) in case of Mn^{2+} and 3-NP treatment. Both axes are given in relative units. The correlation coefficients are inserted.

DISCUSSION

In the rats treated acutely with 3-NP, both spontaneous (preponderance of slow waves) and stimulus-evoked (decreased EP amplitude) cortical activities were de-

pressed. Although these two effects showed no correlation, they probably had a common cause, energy insufficiency due to impeded mitochondrial oxidation (Coles et al., 1979). In human volunteers exposed to hypoxia, EEG was shifted to lower frequencies (Van der Post et al., 2002). In cases of inherited or idiopathic mitchondrial dysfunction, cortical functions are likewise affected (Montirosso et al., 2002); in the EEG, the main abnormality was slowed activity (Sciacco et al., 2001). Alteration of certain visual EP components (Scaioli et al., 1998; Finsterer, 2001) was also seen in such patients.

Another effect, inhibition of astrocytic glutamate uptake by 3-NP (Tavares et al., 2001), 3-NP has in common with Mn^{2+} (Normandin and Hazell, 2002). In case of Mn, this leads to increased cortical excitatory transmission, probably explaining the increase of EP amplitude. In case of 3-NP, the effect is probably suppressed by the general effect of mitochondrial dysfunction. Mn, too, is a mitochondrial toxin (Malecki, 2001; Zhang et al., 2003), which effect was reflected in the slowed ECoG; in this aspect, the effect of the two substances was probably similar also in mechanism. On the contrary, the correlation of the alteration in the ECoG and EP parameters under Mn influence reflected no common mechanism but the accumulation of Mn in the brain.

Mn and 3-NP are both important as causes of environmental exposure and as tools in modelling the effect of the former, which justifies further studies of their neurotoxic effects.

REFERENCES

ASCHNER, M., VRANA, K. E., and ZHENG, W. (1999). "Manganese uptake and distribution in the central nervous system (CNS)." NeuroToxicol. 20:173–180.

ATSDR (2000). Toxicological Profile for Manganese. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, USA.

BROUILLET, E., CONDE, F., BEAL, M. F., and HANTRAYE, P. (1999). "Replicating Huntington's disease phenotype in experimental animals." Prog. Neurobiol. 59:427–468.

BROUILLET, E., GUYOT, M. C., MITOUX, V., ALTAIRAC, S., CONDE, F., PALFI, S., and HANTRAYE, P. (1998). "Partial inhibition of brain succinate dehydrogenase by 3-nitropropionic acid is sufficient to initiate striatal degeneration in rat." J. Neurochem. 70:794–805.

COLES, C. J., EDMONDSON, D. E., and SINGER, T. P. (1979). "Inactivation of succinate dehydrogenase by 3-nitropropionate." J. Biol. Chem. 254:5161–5167.

FERRAZ, H. B., BERTOLUCCI, P. H., PEREIRA, J. S., LIMA, J. G., and ANDRADE, L. A. (1988). "Chronic exposure to the fungicide maneb may produce symptomps and signs of CNS manganese intoxiation." Neurology 38:550–553.

FINSTERER, J. (2001). "Visually evoked potentials in respiratory chain disorders." Acta Neurol. Scand. 104:31–35.

HAZELL, A. S. and NORENBERG, M. D. (1997). "Manganese decreases glutamate uptake in cultured astrocytes." Neurochem. Res. 22:1443–1447.

HIRATA, Y., KIUCHI, K., and NAGATSU, T. (2001). "Manganese mimics the action of 1-methyl-phenylpyridinium ion, a dopaminergic neurotoxin, in rat striatal tissue slices." Neurosci. Lett. 311:53–56.

JOHNSON, J. R., ROBINSON, B. L., ALI, S. F., and BINIENDA, Z. (2000). "Dopamine toxicity following long term exposure to low doses of 3-nitropropionic acid (3-NPA) in rats." Toxicol. Lett. 116:113–118.

LIU, X., LUO, X., and HU, W., (1992). "Studies on the epidemiology and etiology of moldy sugarcane poisoning in China." Biomed. Environ. Sci. 5:161–177.

LYNAM, D. R., ROOS, J. W., PFEIFER, G. D., FORT, B. F., and PULLIN, T. G. (1999). "Environmental effects and exposures to manganese from use of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline." NeuroToxicol. 20:145–150.

MALECKI, E. A. (2001). "Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons." Brain Res. Bull. 55:225–228.

MCCRACKEN, E., DEWAR, D., and HUNTER, A.J. (2001). "White matter damage following systemic injection of the mitochondrial inhibitor 3-nitropropionic acid in rat." Brain Res. 892: 329–335.

MENA, I., MARIN, O., FUENZALIDA, S., and COTZIAS, G. C. (1967). "Chronic manganese poisoning: clinical picture and manganese turnover." Neurology 17:128–136.

MONTASTRUC, J. L., LLAU, M. E., RASCOL, O., and SENARD, J. M. (1994). "Drug-induced parkinsonism: a review." Fundam. Clin. Pharmacol. 8:293–306.

MONTIROSSO R., BRAMBILLA, D., FELISARI, G., SCLAUNICH, F., FILIPPONI, E., POZZOLI, U., and BRESOLIN, N. (2002). "Electrophysiological analysis of cognitive slowing in subjects with mitochondrial encephalomyopathy." J. Neurol. Sci. 15:3–9.

NELSON, M. T. (1986). "Interactions of divalent cations with single calcium channels from rat brain synaptosomes." J. Gen. Physiol. 87:201–222.

NORMANDIN, L. and HAZELL, A. S. (2002). "Manganese neurotoxicity: an update of pathophysiologic mechanisms." Metab. Brain Dis. 17:375–387.

PUBILL, D., VERDAGUER, E., CANUDAS, A. M., SUREDA, F. X., ESCUBEDO, E., CAMARASA, J., PALLAS, M., and CAMINS, A. (2001). "Orphenadrine prevents 3-nitropropionic acid-induced neurotoxicity in vitro and in vivo." Br. J. Pharmacol. 132:693–702.

PUMAIN, R., KURCEWICZ, I., and LOUVEL, J. (1987). "Ionic changes induced by excitatory amino acids in the rat cerebral cortex." Can. J. Physiol. Pharmacol. 65:1065–1077.

SCAIOLI, V., ANTOZZI, C., VILLANI, F., RIMLDI, M., ZEVIANI, M., PANZICA, F., and AVANZINI, G. (1998). "Utility of multimodal evoked potential study and electroencephalography in mitochondrial encephalomyopathy." Ital. J. Neurol. Sci. 19:291–300.

SCIACCO, M., PRELLE, A., COMI, G. P., NAPOLI, L., BATTISTEL, A., BRESOLIN, N., TANCREDI, L., LAMPERTI, C., BORDONI, A., FAGIOLARI, G., CISCATO, P., CHIVERI, L., PERINI, M. P., FORTUNATO, F., ADOBBATI, L., MESSINA, S., TOSCANO, A., NARTINELLI-BONESCHI, F., PAPADIMITRIOU, A., SCARLATO, G., and MOGGIO, M. (2001). "Retrospective study of a large population of patients affected with mitochondrial disorders: clinical, morphological and molecular genetic evaluation." J. Neurol. 248:778–788.

SHINOTOH, H., SNOW, B. J., CHU, N. S., HUANG, C. C., LU, C. S., LEE, C., TAKAHASHI, H., and CALNE, D. B. (1997). "Presynaptic and postsynaptic striatal dopaminergic function in patients with manganese intoxication: a positron emission tomography study." Neurology 48: 1053–1056.

TAKEDA, A., SOTOGAKU, N., and OKU, N. (2003). "Influence of manganese on the release of neurotransmitters in rat striatum." Brain Res. Rev. 965:279–282.

TAVARES, R. G., SANTOS, C. E., TASCA, C. I., WAJNER, M., SOUZA, D. O., and DUTRA-FILHO, C. S. (2001). "Inhibition of glutamate uptake into synaptic vesicles from rat brain by 3-nitropropionic acid in vitro." Exp. Neurol. 172:250–254.

TEUNISSEN, C. E., STEINBUSCH, H. W., ANGEVAREN, M., APPELS, M., DE BRUIJN, C., PRICKAERTS, J., and DE VENTE, J. (2001). "Behavioural correlates of striatal glial fibrillary acidic protein in the 3-nitropropionic acid rat model: disturbed walking pattern and spatial orientation." Neuroscience 105:153–167.

VAN DER POST, J., NOORDZIJ, L. A., DE KAM, M. L., BLAUW, G. J., COHEN, A. F., and VAN GERVEN, J. M. (2002). "Evaluation of tests of central nervous system performance after hypoxemia for a model for cognitive impairment." J. Psychopharmacol. 16:337–343.

YAMADA, M., OHNO, S., OKAYASU, I., OKEDA, R., HATAKEYAMA, S., WATANABE, H., USHIO, K., and TSUKAGOSHI, H. (1986). "Chronic manganese poisoning: a neuropathological study with determination of manganese distribution in the brain." Acta Neuropathol. (Berlin) 70: 273–278.

ZHANG, J., FITSANAKIS, V. A., GU, G., JING, D., AO, M., AMARNATH, V., and MONTINE, T. J. (2003). "Manganese ethylene-bis-dithiocarbamate and selective dopaminergic neurodegeneration in rat: a link through mitochondrial dysfunction." J. Neurochem. 84:336–46.