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Effect of 2-Nitropropane on Chemical Neurotransmission, Spontaneous and Evoked, in the Sartorius Muscle of the Frog

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Abstract

In this project we studied the effect of 2-nitropropane in spontaneous and evoked chemical neurotransmission, using as a model the frog neuromuscular junction, in vitro. The experiments were carried out in the sciatic nerve-sartorius muscle preparation. Our results show that neurotransmission was inhibited, manifesting as a decrease in the amplitude and instantaneous frequency of miniature end-plate potentials and the reduction of end-plate potentials amplitude of the neuromuscular junction, evoked by nerve stimulation. Because 2-nitropropane showed inhibitory effects at peripheral level, we recommend that in work environments where there is exposure to organic solvents, neurological clinical examinations must be performed to detect abnormalities or neurological damage the earliest.

Keywords: 2-Nitropropane; Solvents; Synaptic transmission; Neuromuscular junction; Frog; Organic solvents; Sartorius muscle

Introduction

Organic solvents are chemically different compounds with one common feature: They dissolve fats, oils, resins, cellulose, acetate, and cellulose nitrate, which makes them widely used in industry. Most often organic solvents are used in paint and lacquer industries, in production of pesticides, explosives, plastic, rubber, cellulose, air conditioners, in pharmaceutical industry, and in leather industry [1]. Millions of people around the world are exposed to industrial organic solvents in the manufacturing sectors. Some individuals drink solvents, for example alcohol dependence and alcohol abuse or harmful use cause substantial morbidity and mortality [2]. Solvents are neurotoxic substances that are detrimental to the functioning of the nervous system [3-5]. High-level occupational exposure to volatile organic solvents may elicit various neurotoxic effects [6]. It has previously been shown that some solvents modulate inhibitory transmission and affect synaptic transmission [7,8].

Ultrastructural examination of a peripheral nerve biopsy, showed axonal degeneration and unusual lesions of the myelin, when a 35-years-old man had prolonged occupational exposure to solvents such as carboxylate, triethylbenzene, xylene, and dichloromethane [9]. The 2-nitropropane (2-NP) is a nitroalkane containing a NO2 at the 2 position. It is used to produce oils, dyes and paints, mainly. Under conditions of 2-NP intoxication, symptoms manifest as neurological disorders of the central and peripheral nervous systems, characterized by encephalopathy, neuropathy, muscle weakness, personality changes, etc. [10-14]. In this respect, the frog neuromuscular synapse (sciatic nerve-sartoriusmuscle preparation) is a widely used model to study the functional aspects of chemical neurotransmission and the effects of a wide variety of xenobiotics on this form of neurotransmission, and to establish their mechanisms of neurotoxicity. Also with this model have been carried out other scientific studies [15-17].

The purpose of this study was to investigate the effect of 2-NP on spontaneous and evoked neurotransmission, in vitro, having found an inhibitory neurotoxic effect, which manifested as decreased amplitude (mV) and instantaneous frequency of the miniature end-plate potentials (MEPPs), and the amplitude of end-plate potentials (EPPs). The effects of 2-NP on synaptic transmission Have Not Been Previously Studied at the neuromuscular junction.

Materials and Methods

Chemicals

The following substances were purchased from Sigma Chemical Co. (Dahlia Tubers, St Louis, Missouri, USA): Sodium chloride, potassium chloride, calcium chloride, magnesium chloride, tris-HCl, tris-base, neostigmine methyl sulfate and D-tubocurarine. The 2-NP was purchased from Sigma-Aldrich Chemical S. A. C. V. (Toluca, Estado de Mexico, Mexico). Remaining chemicals were of analytical reagent grade.

Getting preparation

The frog is sacrificed by decapitation for the dissection of the neuromuscular preparation (sciatic nerve-sartorius muscle), and spinal cord must be destroyed to avoid reflex muscle contractions, thus facilitating dissection. The skin of the legs is removed and proceeds to expose the spine referred to in the two last nerve roots, such as the sciatic nerve originates in the frog, and includes the innervation of the sartorius. The muscle is dissected without damaging the edges, because in these we can see a layer of cells that are easily found in synaptic contact sites, between the nerve terminal and muscle fiber. The dissection is carried out in the stereomicroscope, with the preparation immersed in normal Ringer solution (Table 1). Once obtained, the preparation was placed in a lucite chamber with the muscle immersed in Ringer's solution, fixed to the bottom of it (on a layer of Silgard). The muscle is adjusted to the length that presents in situ. The sciatic nerve was placed on a pair of electrodes, in a separate compartment of the

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chamber, which is covered with a transparent glass in order to keep wet the preparation during the experiment.

Electrophysiological techniques

The resting membrane potential (RMP) of the postsynaptic membrane was recorded intracellularly using a micropipette filled with 3 M KCl, with 10-20 megahoms resistance. Recordings were obtained in control conditions (normal Ringer) and during exposure to 2-NP (20 mg/mL) in order to evaluate the effect of solvent on the RMP, and to investigate if there is any effect on the ionic conductances which determine the value of this potential. Registration of the MEPPs was performed intracellularly in the area corresponding to the neuromuscular junction, using micropipettes with the same characteristics as described above. The preparation should remain submerged in Ringer's solution containing 50µg/mL of neostigmine methylsulfate (an anticholinesterase that blocks acetylcholinesterase, favoring the amplification of the MEPPs). This is done for methodological purposes regarding the resolution of the MEPPs. In addition, records were obtained in control conditions (absence of 2-NP) and with the preparation exposed to 4, 8, 12, 16 and 20 mg/mL of 2-NP.

Collection and recording of the EEPs

The EEPs were generated by stimulation of the sciatic nerve with the preparation immersed in Ringer's solution containing low concentration of calcium and magnesium (Table 1). In this condition, the release of neurotransmitter is reduced, allowing that the amplitude of the EEPs fluctuate in the sub-threshold range, to avoid generating action potentials in the muscle cell, which would avoid the intracellular recording. The frequency of stimulation was 1 Hz, and the EEPs were recorded in control conditions and during the exposure of the preparation to 2-NP (same pattern of concentrations for the MEPPs).

Analysis

In all cases, the records obtained were digitized and stored for later analysis. We used an analog-digital converter (TL-125 Labmaster, Axon Instruments Inc., USA).

Statistical analysis

Data were analyzed by one-way analysis of variance, and are expressed as the mean ± standard error of the mean. A p-value less than 0.05 was considered statistically significant (*).

Results

Effect of 2-NP on the RMP: A first aspect to consider was to investigate whether 2-NP is able to alter the RMP of muscle cells, because variations in this parameter, per se, can affect the amplitude of post-synaptic potentials. Thus, we found that exposure of muscle cells to 2-NP did not alter the RMP of these (Table 2), which fluctuated around - 90 mV, which coincides with the normal values for this type of cells.

Effect of 2-NP on the MEPPs: Our neuromuscular preparation, in vitro, was viable and functional for several hours, so that the register values of the MEPPs (frequency and amplitude) remained statistically unchanged (Figure 1). Table 3 shows the average results of 8 experiments in the absence of 2-NP (control), during times similar to those established for observing the effect of this. As can be seen, for at least 18 minutes the MEPPs retain their amplitude and frequency.

SUBSTANCE	NORMAL RINGER (mM)	RINGER [↓ Calcium - ↑ Magnesium (mM)]
NaCl	112	109
CaCl	1.8	0.8
KCl	2	2.1
MgCl	0	6
Tris-HCl	2.16	2.16
Tris-base	0.83	0.83

Table 1: Ringer's solution used in the experimental protocol to record MEPPs and EEPs, first and second column respectively.

EXPERIMENT	CONTROL [mean ± standard error (SE)]	2-NP [mean ± standard error (SE)]
1	- 90 ± 2.56	- 89.4 ± 2.34
2	- 89.4 ± 3.15	- 89.4 ± 2.36
3	- 87.5 ± 3.22	- 90 ± 1.52

Table 2: Values of RMP obtained in muscle cells in control conditions and exposed to 20 mg/mL of 2-NP. There is no statistically significant difference between both conditions. The values are given as the mean ± standard error (SE) (n = 10 for each experiment).

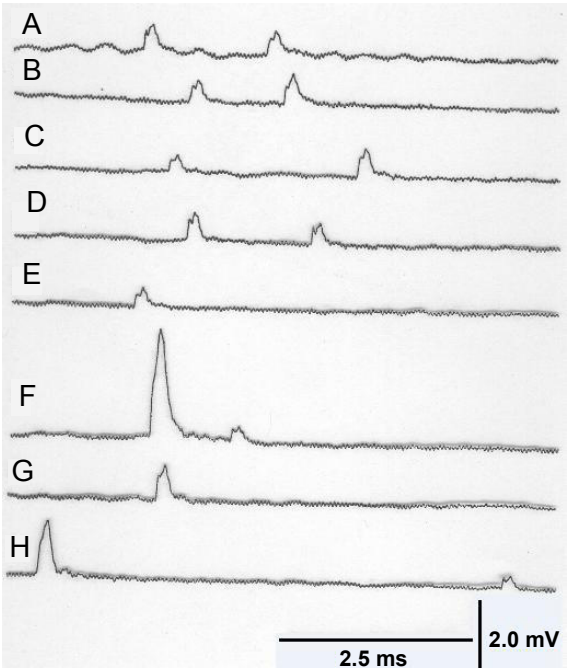

Figure 1: The registered MEPPs are observed for a period longer than 18 minutes, in untreated cells. Each stroke (A-H) shows the representative MEPPs occurred at intervals of 3 minutes each.

However, when the preparation was exposed to 2-NP decreased both the amplitude and instantaneous frequency (Table 4, Figure 2). In all experiments in which the preparation was exposed to 2-NP the MEPPs were not observed with concentrations above 16 mg/ml.

Effect of 2-NP on the EEPs: In the series of experimentsstablished to study this effect, we found that the solvent in a concentration equal to 4 mg/ml, inhibited evoked synaptic transmission, which manifested as reduced amplitude of the EEPs, and a blockade of nerve impulse, since failures in the corresponding record were observed (Figure 3). Complete blockage occurred during the first two minutes of exposure to 2-NP, occurring earlier in comparison to what happened to the MEPPs.

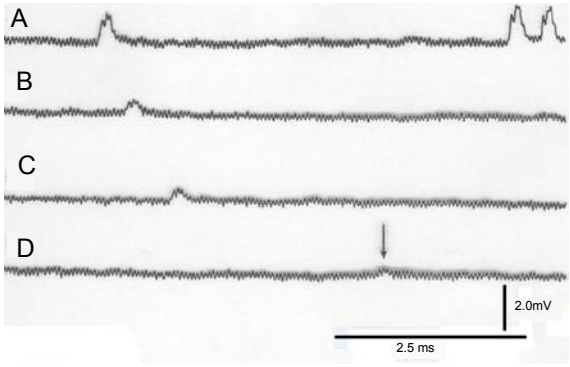

Figure 2: Effect of 2-NP on the amplitude of the MEPPs. A: Potentials recorded in control conditions. B-D: Scan that shows potentials recorded after exposing the preparation to different concentrations of 2-NP (4, 8, and 12 mg/mL, with an exposure time equal to 3 minutes for each concentration of solvent). The arrow in D indicates a potential barely perceptible. MEPPs no longer appeared after 12 minutes (16 mg/mL of 2-NP), record not shown.

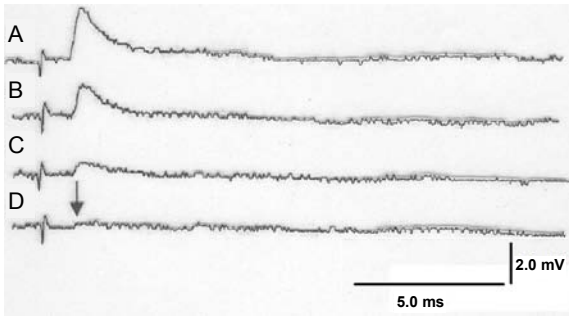

Figure 3: Effect of2-NP on the EEPs generated by nerve stimulation. A: Evoked potential recorded in control conditions, B-D: Records obtained after 1, 2 and 3 minutes after exposing the preparation to 2-NP (4 mg/mL), respectively. The arrow indicates the absence of evoked potential, as it should have been generated by the stimulus, showing only the artifact itself.

TIME (minutes)	MEPPs AMPLITUDES (mV) [mean ± standard error (SE)]	FREQUENCY (N, mean)
3	1.535 ± 0.427	52
6	1.534 ± 0.420	58
9	1.539 ± 0.555	49
12	1.538 ± 0.482	50
15	1.538 ± 0.511	53
18	1.537 ± 0.519	56

Table 3: MEPPs amplitudes [mean ± standard error (SE)] recorded during a period equal to 18 minutes in control conditions (absence of 2-NP), measured at intervals of 3 minutes. There were no significant differences between each period (n = 8 for each time interval).

CONCENTRATION OF 2-NP (mg/mL)	MEPPs AMPLITUDES (mV) [mean ± standard error (SE)]	INSTANTANEOUS FREQUENCIES (N)
4	0.931 ± 0.264 *	546
8	0.729 ± 0.267 *	503
12	0.502 ± 0.149 *	404
16	0.233 ± 0.121 *	101

Table 4: Average amplitudes of the MEPPs [mean ± standard error (SE)] recorded in the presence of 2-NP (4, 8, 12 and 16 mg/mL), measured at intervals of 3 minutes for each concentration. After the final solvent concentration (16 mg/mL), the MEPPs did not occur after 12 minutes elapsed. Also, the respective amplitudes decreased significantly in each time interval, compared with the average amplitudes of control group (see Table 3) (n = 8 for each time interval).

Discussion

The purpose of this research was to study the possible effects of the solvent 2-NP on chemical neurotransmission at the neuromuscular junction of the frog, in vitro. Organic solvents are compounds that dissolve fats and oils [1]. Millions of people are exposed to industrial organic solvents. These are neurotoxic substances that are detrimental to the functioning of the nervous system [3]. In assessing different effects of 2-NP at the neuromuscular junction of the frog, we note that the RMP of muscle cells was not altered, suggesting that the ionic conductances that determine their value (in the skeletal muscle is mainly the leakage of K+) are not affected at least in the protocol established in this investigation. This is important because it is then possible to distinguish effects at synaptic transmission level. However, under conditions of exposure to 2-NP decreased the amplitude and the instantaneous frequency of the MEPPs. In most experiments, when the preparation was exposed to the solvent, the MEPPs were no longer observable with concentrations greater than 16 mg/mL of 2-NP. This indicates the occurrence of a depressing effect exerted on synaptic spontaneous neurotransmission, event consistent with previous studiesin which indicates that some solvents decrease neuron excitability [18]. Similarly, the same effect was seen on the EEPs.

The difference in the time course of effects on MEPPs and EPPs suggests some effects of 2-NP at presynaptic membrane on events related to exocytosis, because the fundamental difference between transmission spontaneous and evoked is the source of calcium required for the phenomenon of exocytosis. The interaction of the neurotransmitter with the receptor and post-synaptic events are similar in both types of transmission. Additionally, decreases of MEPPs amplitudes can a priori result from either pre- or post-synaptic mechanisms. Pre-synaptic mechanisms that can lead to a decrease in the amplitudes of MEPPs are those exerted by hemicholinium and vesamicol, the former decreasing the uptake of choline by the motor nerve terminal and the latter blocking the storage of synthesized acetylcholine by the synaptic vesicles. However, these blockers need, characteristically, a long time (sometimes more than one hour) in order to affect the amplitude of MEPPs. However, the effects of 2-NP on MEEPs were observed in the time scale of minutes. This indicates, therefore, that the effects of 2-NP on MEEPs was probably of a post-synaptic nature, and still probably, of a non-competitive nature.

Our suggestion takes into account that it is known that hydrophobic substances, as it is the case of 2-NP, can block the muscle nicotinic receptor function in a non-competitive manner, interacting with hydrophobic pockets known to exist in the mentioned receptor. In another sense, it is important to mention that there was no effect of 2-NP by exposing the preparation to concentrations below 4 mg/mL. Then, in the present study we disclosed concentrations of solvent (2-NP) with which adverse effects are observed in chemical neurotransmission, and concentrations at which these effects are no longer observed, at least in our protocol and experimental model (*in vitro*).This finding will save time and effort to those who seek to inquire further into this line of research, since we need knowledge about the molecular mechanisms that led to the results reported in this project, which remain still unknown.

Our results should call the attention of public health professionals, they should be alert to potential health damaging effects among workers exposed to 2-NP, and effective methods to control exposure to this solvent should be implemented at worksite. Being concise, our findings are relevant because there are sufficient similarities between the functioning of the frog neuromuscular junction and that of the

human being, by which the obtained results can be extrapolated [19-21]. Therefore, the 2-NP could cause serious disturbances in the peripheral nervous system in humans, among others.

Conclusion

The 2-NP is an organic solvent capable of inhibiting synaptic neurotransmission at both pre- and post-synaptic sites, as is showed in the results obtained in this study, *in vitro*, evidenced by the decrease in amplitude and instantaneous frequency of MEPPs and decreased amplitude of EEPs evoked by nerve stimulation. However, the 2-NP did not affect the RMP.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Jovanović J, Jovanović M (2004) Neurotoxic effects of organic solvents among workers in paint and lacquer manufacturing industry. Med Pregl 57: 22-25.
2. Schuckit MA (2009) Alcohol-use disorders. Lancet 374: 492-501.
3. Gopal KV (2008) Audiological findings in individuals exposed to organic solvents: case studies. Noise Health 10: 74-82.
4. Jovanović JM, Jovanović MM, Spasić MJ, Lukić SR (2004) Peripheral nerve conduction study in workers exposed to a mixture of organic solvents in paint and lacquer industry. Croat med J 45: 769-774.
5. Wilson MP, Hammond SK, Nicas M, Hubbard AE (2007) Workers exposure to volatile organic compounds in the vehicle repair industry. J Occup Environ Hyg 4: 301-310.
6. Herpin G, Gargouri I, Gauchard GC, Nisse C, Khadhraoui M, et al. (2009) Effect of chronic and subchronic organic solvents exposure on balance control of workers in plant manufacturing adhesive materials. Neurotox Res 15: 179-186.
7. Belmeguenai A, Botta P, Weber JT, Carta M, De Ruiter M, et al. (2008) Alcohol impairs long-term depression at the cerebellar parallel fiber-Purkinje cell synapse. J Neurophysiol 100: 3167-3174.
8. Basavarajappa BS, Ninan I, Arancio O (2008) Acute etanol suppresses glutamatergic neurotransmission through endocannabinoids in hippocampal neurons. J Neurochem 107: 1001-1013.
9. Vital A, Vital C, Arnaud A, Moesch C, Lagueny A (2006) Axonal degeneration with unusual lesions of the myelin in an occupational neuropathy. Ultrastruc Pathol 30: 261-266.
10. Linz DH, de Garmo PL, Morton WE, Wiens AN, Coull BM, et al. (1986) Organic solvent-induced encephalopathy in industrial painters. J Occup Med 28: 119-125.
11. van Vliet C, Swaen GM, Volovics A, Tweehuysen M, Meijers JM, et al. (1990) Neuropsychiatric disorders among solvent-exposed workers. First results from a dutch case-control study. Int Arch Occup Environ Health 62: 127-132.
12. Morrow LA, Ryan CM, Golstein G, Hodgson MJ (1989) A distinct pattern of personality disturbance following exposure to mixtures of organic solvents. J Occup Med 31: 743-746.
13. Szyrocka-Szwed K (1989) Evaluation of the nervous system with reference to the bioelectric activity of the brain and neural conduction in workers exposed to organic solvents. Med Pr 40: 342-349.
14. Polakowska B (1989) Currents views on the pathological changes in the nervous system of personas chronically exposed to organic solvents. Wiad Lek 42: 970-974.
15. Thams S, Brodin P, Plantman S, Saxelin R, Kärre K, et al. (2009) Classical major histocompatibility complex class I molecules in motoneurons: new actors at the neuromuscular junction. J Neurosci 29: 13503-13515.
16. de Bivort BL, Guo HF, Zhong Y (2009) Notch signaling is required for activity-dependent synaptic plasticity at the Drosophila neuromuscular junction. J Neurogenet 23: 395-404.
17. Korkut C, Ataman B, Ramachandran P, Ashley J, Barria R, et al. (2009) Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. Cell 139: 393-404.
18. Mameli M, Botta P, Zamudio PA, Zucca S, Valenzuela CF (2008) Ethanol decreases Purkinje neuron excitability by increasin GABA release in rat cerebellar slices. J Pharmacol Exp Ther 327: 910-917.
19. Leite LF, Gomez RS, Fonseca Mde C, Gomez MV, Guatimosim C (2011) Effect of intravenous anesthetic propofol on synaptic vesicle exocytosis at the frog neuro muscular junction. Acta Pharmacol Sin 32: 31-37.
20. Lou F, Dittrich M, Stiles JR, Meriney SD (2011) Single-pixel optical fluctuation analysis of calcium channel function in active zones of motor nerve terminals. J Neurosci 31: 11268-11281.
21. Nurullin LF, Mukhitov AR, Tsentsevytsky AN, Petrova NV, Samigullin DV, Malomouzh AI, et al. (2011) Voltage-dependent P/Q-type calcium channels at the frog neuromuscular junction. Physiol Res 60: 815-823.