Biomed. Papers 148(2), 209–211 (2004)

COMPARISON OF CHANGES IN ACHE ACTIVITY IN THE BRAIN OF THE LABORATORY RAT AFTER SOMAN AND TABUN INTOXICATION

Petr Hájek[a], Dáša Slížová[a], Otakar Krs[a], Jiří Bajgar[b]

[a] *Department of Anatomy, Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic*
[b] *Department of Toxicology, Purkyne Military Medical Academy, Hradec Králové, Czech Republic*
e-mail: hajekp@lfhk.cuni.cz

Received: September 20, 2004

Keywords: Cholinesterase inhibitors/Soman/Tabun/Rat/Histochemistry/AChE

The aim of this study was to compare changes in activity of acetylcholinesterase (AChE) in the brain and motor endplates of rat after administration of soman and tabun. We took brain and diaphragm from laboratory rats administered a median lethal dose (LD_{50}) of soman or tabun. Enzyme activity of AChE was studied in selected structures of brain and in motor endplates in the diaphragm. Histochemical detection of AChE by Karnovski and Roots with simultaneous histochemical detection of alkaline phosphatase in case of brain sections was used. The highest activity of AChE in the control group was found in the striatum, amygdaloid nuclei, substantia nigra, superior colliculi, and motor nuclei of cranial nerves V, X a XII. LD_{50} of both nerve agents dramatically decreased the activity of AChE in the structures studied – both brain and diaphragm. After intoxication by either agent, activity in above mentioned nuclei was characterized as low or focally moderate. Very low activity was seen in some structures (CA3 field of hippocampus, some nuclei of the tegmentum and cerebellar cortex). We found minimal differences in the histochemical picture of soman or tabun intoxication, apart from the striatum and the superior colliculi which showed stronger inhibition by tabun.

INTRODUCTION

Organophosphates (OP) are derivatives of phosphoric acid, which specifically attack the active surface of cholinesterase in addition to effects induced by other toxic agents. They block the function of cholinesterases even in very low concentrations[1, 2]. In spite of their high toxicity, a plethora of these substances is used in industry, e.g. as pesticides and insecticides. Those characterized by median lethal dose (LD_{50}) at the level of milligrams/kg are used as nerve agents in chemical warfare[3].

Sarin, soman and tabun pass easily through the blood–brain barrier. This is why their most serious effect is central respiratory insufficiency caused by inhibition of acetylcholinesterase (AChE) in the reticular formation of the pons and the medulla oblongata. Another OP, e.g. VX may differ in stronger effect on the motor endplates of the diaphragm and heart[4].

Target enzymes of OP are cholinesterases. Acetylcholinesterase is localized in erythrocytes, muscle cells and in nerve cells. Inhibition of AChE leads to accumulation of acetylcholine and the inhibited enzyme undergoes only slow spontaneous reactivation[1, 4–6].

A key feature of the histochemical picture of OP intoxication is strong decrease in cholinesterase activity almost throughout the brain and other organs. In addition, the effect of OP includes histopatological lesions, which are not specific to these agents and can be found after intoxication by other toxicants: hemorrhages, perivascular and pericellular oedema in brain, heart, liver and lungs[7].

MATERIALS AND METHODS

The cholinesterase inhibition in the brain and in the motor endplates of the diaphragm was studied histochemically on the rat model. We compared a control group of rats intoxicated by soman or tabun.

The animals were administered D_{50} of soman or tabun and killed after 30 min. The brains were removed, quickly frozen and cut on a cryostat to 20 µ slices. The simultaneous histochemical detection of AChE by Karnovsky and Roots and AP (alkaline phosphatase) was used. Concurrently we made a set of sections stained by hematoxylin – eosin for the assesment of histopathological changes. For identification of brain structures we used histochemical atlases of the rat brain[8].

The diaphragm was taken and studied for AChE activity in the motor endplates.

Light microscope Olympus BH2 and digital camera Olympus Camedia C-3030 were used for microphotographic documentation.

Fig. 1. Section through striatum at the level of *1.6* mm rostrally to bregma. Control, incubated for AChE.

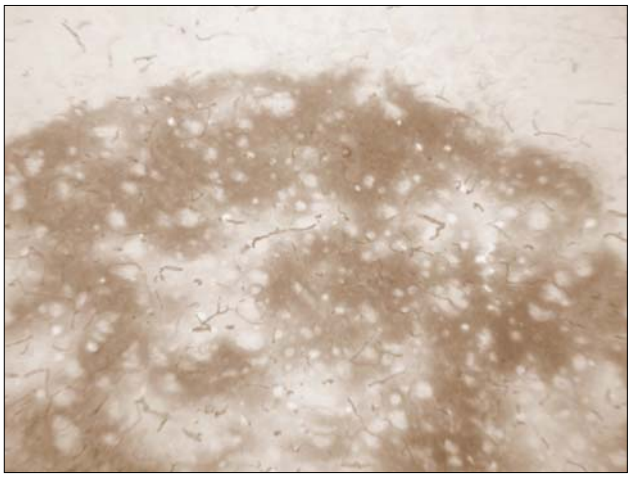

Fig. 2. Section through striatum at the level of *1.6* mm rostrally to bregma. Soman intoxication, AChE + AP.

Fig. 3. Section through striatum at the level of *1.6* mm rostrally to bregma. Tabun intoxication, AChE + AP.

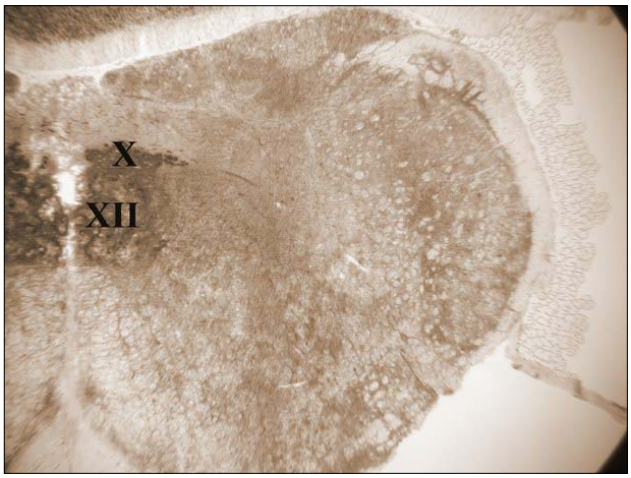

Fig. 4. Control section through oblongata with high activity of motor nuclei X and XII, AChE.

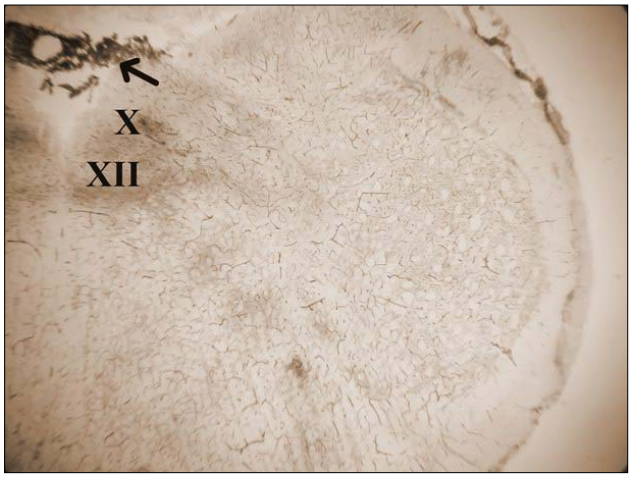

Fig. 5. Corresponding section after tabun intoxication. Nuclei X and XII show moderate activity. Choroid plexus of the 4th ventricle expresses high AP activity (arrow). AChE + AP.

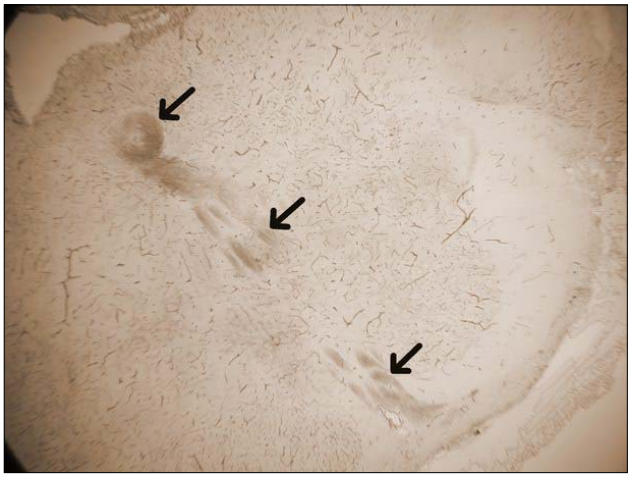

Fig. 6. Section through brainstem at the level of intraparenchymal course of cranial nerve VII (arrows), after tabun intoxication. AChE + AP.

RESULTS

LD_{50} of both nerve agents dramatically decreases the activity of AChE in the structures studied. Differences were observed in particular in the nuclei of the brain.

In the control group the highest activity was found in the striatum, basolateral amygdaloid nuclei, substantia nigra – compact part, superior colliculi – superficial gray and motor nuclei of cranial nerves V, X a XII. High activity was expressed in the globus pallidus, thalamus (laterodorsal nuclei), zona incerta, hippocampus and dentate gyrus, reticular formation, pontine nuclei, cerebellar cortex – granular layer, septal nuclei, gracile and cuneate nuclei. Moderate activity was found in the periaqueductal gray, red nucleus, hypothalamus, while low activity was found in the cerebral cortex.

No more than moderate activity in the striatum and dorsal nucleus of vagus nerve was detected after soman intoxication. Low activity was shown in amygdala, substantia nigra, superior colliculi, motor nuclei V and XII. Spots of low or zero activity were found in other structures of the gray matter (CA3 field of hippocampus, tegmentum and reticular formation, pontine nuclei, cerebellar cortex, laterodorsal nuclei of thalamus, zona incerta, periaqueductal gray, septal nuclei).

Tabun intoxication led to similar morphological findings as intoxication with soman. Stronger inhibition by tabun was found in striatum and superior colliculi.

In the diaphragm we found a dramatic drop in AChE activity after both soman and tabun.

DISCUSSION

Our preliminary results document a similar histochemical picture after both soman and tabun intoxication. There are only slight differences in location and intensity of histochemical product. However, the course of intoxication is more serious in the case of soman intoxication due to its fast dealkylation of enzyme-inhibitor complex. For this reason soman remains the focus of interest of toxicologists[6]. In our next experiments we want to study the situation after using antidotes and to quantify AChE activity[9, 10].

REFERENCES

1. Bajgar J. Intoxikace organofosforovými inhibitory cholinesteráz: účinek, diagnóza a terapie. In: Novinky v medicíně 34. Praha: Avicenum, 1985. p. 7–40.
2. Matoušek J. Vysoce toxické organické sloučeniny fosforu. In: Extrémně toxické nízkomolekulární syntetické jedy – sborník ze sympozia toxikologické chemie Československé společnosti chemické ČSAV. Hradec Králové: VLVDÚ 1979. p. 7–39.
3. Prymula R. Biologický a chemický terorismus – informace pro každého. Praha: Grada – Avicenum, 2002
4. Bajgar J. The influence of inhibitors nad other factors on cholinesterases. Sborník vědeckých prací LFUK-HK 34. Praha: SPN, 1991.
5. Patočka J, Fusek J, Bajgar J. Interakce organofosfátů a karbamátů s cholinesterázami in vitro. In: Extrémně toxické nízkomolekulární syntetické jedy – sborník ze sympozia toxikologické chemie Československé společnosti chemické ČSAV. Hradec Králové: VLVDÚ 1979. p. 41–59.
6. Kassa J, Fusek J. (2002) The influence of oxime selection on the efficiacy of antidotal treatment of soman-poisoned rats. Acta Med 45, 19–27.
7. Tryphonas L, Clement JG. (1995) Histomorphogenesis of soman-induced encephalocardiomyography in Sprague-Dawley rats. Toxicol Pathol *23*, 393–409.
8. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press, 1987.
9. Hammond PI, Jelacic T, Padilla S, Brimijoin S. (1996) Quantitative, video-based histochemistry to measure regional effects of anticholinesterase pesticides in rat brain. Anal Biochem *241*, 82–92.
10. Ma T, Cai Z, Wellman SE, Ho IK. (2001) A quantitative histochemistry technique for measuring regional distribution of acetylcholinesterase in the brain using digital scanning densitometry. Anal Biochem *296*, 18–28.