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An attempt to assess functionally minimal acetylcholinesterase activity necessary for survival of rats intoxicated with nerve agents

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ABSTRACT

Acetylcholinesterase (AChE, EC 3.1.1.7) is an important enzyme for cholinergic nerve transmission. The action of toxic organophosphates such as nerve agents is based on AChE inhibition. The death following acute nerve agent poisoning is due to central or peripheral respiratory/cardiac failure.

Therefore, the changes in AChE activity following nerve agents acting predominantly on the central (sarin, soman) or peripheral (VX) level were studied. It is known that AChE activity in different structures exists in relative excess. Female Wistar rats intoxicated with sarin, soman, and VX in different doses ($0.5–2.0 \times LD_{50}$) were divided into groups of survived and died animals. AChE activities in diaphragm, brain parts (pontomedullar area, frontal cortex, basal ganglia, in some cases other parts of the brain) were determined and the rest of activity (in %) was correlated with survival/death of animals. More precise elucidation of action of nerve agents and the assessment of minimal AChE activity in different organs compatible with the survival of organism poisoned with nerve agents were the aims of this study.

1. Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) is an important enzyme for cholinergic nerve transmission. It splits neuromediator acetylcholine released at the cholinergic synapses, either peripheral or central. It is distributed in different organs of the body of mammals but it was also found in non-mammals, bacterias, some plants, etc., where its function has not been fully elucidated yet. Inhibition of AChE is a basic mechanism of action of highly toxic organophosphates such as nerve agents [1–3]. However, there is different action of these compounds: some of them are acting more peripherally, and action of others is more characterized by their central effect; these two main target sites can be combined, however, selective central effect at different brain structures was demonstrated for both groups [4–8]. From different brain parts studied, pontomedullar area is an interesting structure and it was suggested that AChE activity in this structure could be important for survival of animals intoxicated with nerve agents [4,9,10]. The death following acute nerve agent poisoning is due to the central or peripheral respiratory/cardiac failure [11,12].

AChE activity/inhibition is different in various tissues. It is most expressed for cholinesterases in the transport system (blood) according to principle “first come, first served” [14]. The AChE inhibition in other target tissues (peripheral and central nervous system) is very dependent on the type of the agent [2]. Simultaneously, it is known that AChE activity differs for various organs. It is known that AChE exists in excess in different structures [2,5] including brain. The brain is very heterogeneous but well and complex

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organized structure of the body [13]. Generally, the brain AChE activity is many times higher than it is necessary for its physiological function.

We tried to determine the minimal AChE activity compatible with the survival of organism poisoned with nerve agents. Nerve agents were used as a model compounds because their basic mechanism of action is AChE inhibition. There are two aims of this study:

- to elucidate more precisely mechanism of action of nerve agents and
- to assess minimal AChE activity in different parts of the brain of survived and died animals intoxicated with nerve agents.

2. Material and methods

2.1. Chemicals

Nerve agents (sarin, soman, VX) of minimally 98% purity were obtained from Military Technical Institute of Protection (Brno, Czech Republic). All other chemicals were products of Sigma–Aldrich (Czech Republic).

2.2. Animals

Female Wistar rats, weighing from 200 to 220 g, were purchased from BioTest (Konarovice, Czech Republic). The animals were maintained in an air-conditioned room ($22 \pm 2\,^{\circ}C$ and $50 \pm 10\%$ r.h., with light from 7 a.m. to 7 p.m.), and were allowed free access to standard chow and tap water. Housing of animals was realized in the Central Vivarium of the Faculty of Military Health Sciences under veterinary control. All the experiments were performed under permission and supervision of the Ethic Committee of the Medical Faculty of Charles University, and Faculty of Military Health Sciences, Hradec Kralove (Permission No. 153/02) according to Section 17 of the Czech Law No. 207/2004, permission of responsible person 0001/94–M 699.

2.3. Intoxication

PRESENT experiment: A single dose of $1.0 \times LD_{50}$ of sarin, soman or VX was injected (saline solutions) i.m. Control rats were treated with saline. All rats were killed by decapitation 30 min after nerve agent intoxication. For biochemical experiments, 6 animals per group, and for histochemical examination, 4 animals per group were used. A typical course of poisoning – development of salivation, disturbed ventilation and convulsions – was observed in intoxicated animals. Moreover, the results published previously were used for further analysis [4,5,9,10,15]. The numbers of animals and doses of nerve agents including literature sources are shown in Table 1.

2.4. Biochemical examinations

After decapitation, the brain parts (frontal cortex/FC/, dorsal septum/S/, hypothalamus/HTh/, thalamus/Th/, pontomedullar area containing mostly nuclei gigantocellulares/PM/and basal ganglia/BG/) were prepared and frozen. After thawing, tissue was homogenized (1:10, distilled water, Ultra-Turrax homogenizer) and homogenates were used for enzymatic analysis. The diaphragm was homogenized (1:10, distilled water) and the homogenate was used for biochemical determination of AChE activity.

AChE activity was determined using the method of Ellman et al. [16], as described elsewhere [4]. Acetylthiocholine was used as substrate (Tris–HCl buffer pH 7.6) The results were expressed as μcat/g wet weight tissue [Unit/L (kg) = $16.67 \times$ cat/L (kg)] or as % of control values. UVIKON 752 spectrophotometer was used for determination of absorbancy at 412 nm.

2.5. Histochemical examination

Four animals in each group were used for histochemical examinations. After decapitation, the brain was removed, rapidly frozen and cut into series of 20 μm sections in a cryostat. For neuroanatomical mapping according to the rat brain atlas [17], AChE detection [18] was used. The method is based on hydrolysis of artificial substrate acetylthiocholine (the same as in biochemical examination) and detection of the released reaction product (thiocholine). For digital microphotography, Olympus BH2 light microscope and digital camera Olympus Camedia C-3030 Zoom were used. Quantitative evaluation was made using software 3-D Doctor [19,20]. The pictures were transposed to grayscales with density distribution (expressed in pixels) ranging from 1 to 256. Lower pixel number indicates high activity, higher number shows inhibition. Because the density is of linear scale, the difference (256 – determined density) gives us information on the AChE activity. This pixel density was compared in control and intoxicated animals for each structure examined in absolute and relative (%) values.

2.6. Statistical evaluation

Biochemical results were evaluated using Student's *t*-test and differences were considered significant when $P < 0.05$. The mean values (AChE activity) were compared with quantitative results of histochemical evaluation using regression analysis.

3. Results

In the dose range of $0.5–2 \times LD_{50}$, from total number (120) of experimental animals 61 rats died and 59 survived (Table 1). In soman-poisoned animals, the activity of AChE in the pontomedullar area determined using biochemical method was about 10% for died animals and 16% for survived animals, respectively. Similar results for AChE activity in this area determined by quantitative histochemical method were 10% and 17–32% of control values. For sarin-poisoned animals, AChE activity in the same area of died rats was about 7–10% while in survived animals the activity was higher (18–25% of control values). AChE activity in the pontomedullar area in VX-poisoned animals was slightly different for survived animals (about 20%) or died animals (about 10–20%). However, following VX intoxication, marked differences in AChE activity in