

ORIGINAL ARTICLE

Influence of different ways of euthanasia on the activity of cholinesterases in the rat

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Summary

We studied the influence of four methods of euthanasia (decapitation, exsanguination via cardiocentesis following ether anaesthesia, Nembutal anaesthesia, or immersion in a CO₂ atmosphere) on the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the brain and in the blood of mature female rats. A significant decrease was observed in the activity of AChE in the whole blood in the group treated with Nembutal. There were no significant changes in the activity of BChE with any method of euthanasia. Euthanasia in a CO₂ atmosphere was the best technique with respect to the results indicating lack of affect on the activity of AChE and BChE in the periphery and the brain, as well as from the point of view of the welfare of the animals.

Key words: animal welfare; carbon dioxide; Ellman's method; ether; Nembutal; pentobarbital; anesthesia

INTRODUCTION

Euthanasia is commonly performed in laboratory animal experiments. The method of euthanasia may affect the results of such studies and therefore it must be compatible with the research objectives including

subsequent tissue analyses. Our present study was performed to evaluate the effects of four methods of euthanasia (decapitation alone, exsanguination via cardiocentesis following ether anaesthesia, Nembutal anaesthesia, or immersion in a CO₂ atmosphere) on the activity of acetylcholinesterase (AChE, 3.1.1.7) and butyrylcholinesterase (BChE, 3.1.1.8) in the brain and in the blood of mature rats.

Determination of AChE activity is the key tool for diagnosis of intoxication with AChE inhibitors (organophosphorous and carbamate insecticides, nerve agent weapons), as well as diseases of the nervous system (Alzheimer's disease, myasthenia gravis). Moreover, determination of AChE activity is used for the evaluation of activity of novel AChE reactivators and candidates for therapy of Alzheimer's disease (Bajgar et al. 2007, Kuča et al. 2007, Musílek et al. 2007).

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MATERIAL AND METHODS

Animals

Adult females of albino Wistar rats weighing 180 to 210 g were used throughout this study. They were housed six in a cage, in a temperature controlled (20 °C to 24 °C) environment with 12 h light/dark cycles (lights on from 06:00 to 18:00 h) and had free access to food and water. The animals were allotted to four groups (six animals in each group) for selected methods of euthanasia. The animals were handled under the supervision of the Ethics Committee of the Faculty of Military Health Sciences in Hradec Králové, Czech Republic.

Chemicals

Ether (Sigma Aldrich, Germany),
Nembutal (pentobarbital) (Abbott Laboratories, North Chicago, USA),
CO₂ (Technoplyn Linde, Czech Republic).

Experimental setup

Group number 1 was euthanized via decapitation and blood was collected from the cervical vessels. Groups 2–4 were euthanized under total anaesthesia (ether, Nembutal, CO₂) and blood was collected by cardiocentesis. A portion of blood was centrifuged and the serum was frozen at –80 °C. The brain was collected from all the animals and frozen at –80 °C.

Biochemical examinations

The activity of AChE was measured in homogenized brain and fresh whole blood. The activity of BChE was measured also in the homogenized brain and plasma.

The fresh whole blood was haemolysed (1:20, 0.02M Tris-HCl buffer, pH 7.6; 5 min) and the activity of AChE was determined immediately.

The brain was removed from the cranial cavity, and frozen. After thawing, tissue was homogenized (1:10, 0.02M Tris-HCl buffer, pH 7.6; DI 25 basic homogeniser, IKA WERKE, Germany) and the homogenates were used for enzymatic analysis.

Cholinesterases (ChE) activity was determined in the whole blood, plasma and brain using the method of Ellman et al. (1961) as described elsewhere. Acetylthiocholine was used as substrate together with 0.1M Tris-HCl buffer, pH 7.6. The results were expressed as $\mu\text{cat/g}$ wet weight tissue. A Helios Alpha spectrophotometer (Thermo Electron Corporation, USA) was used for determination of absorbancy at 436 nm. The activity was expressed as μmol of substrate hydrolyzed (60 min/kg) wet weight tissue.

Statistical evaluation

Enzyme activities in tissue homogenates were expressed as the mean \pm standard deviation and statistical difference were tested by the t-test.

RESULTS

Results are summarized in the following figures (1–4). There was only one group with a significant difference of activity of AChE in the whole blood. We observed decreased activity after Nembutal (pentobarbital) administration.

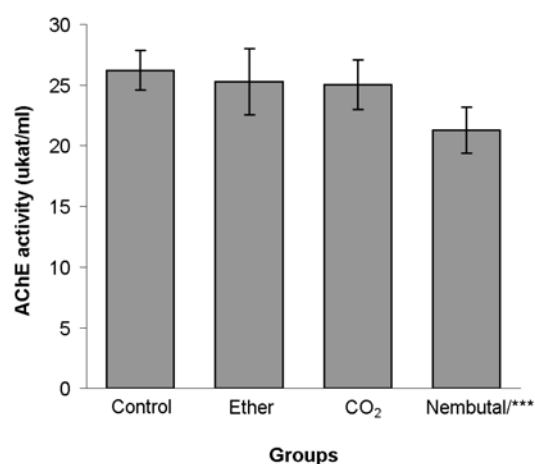


Fig. 1. **Activity of AChE in the whole blood.** There is one significant difference between the control group and the group where we used Nembutal anaesthesia. Also the t-test noted a significant difference (value of t-test 0,000693; ***) in the Nembutal group.

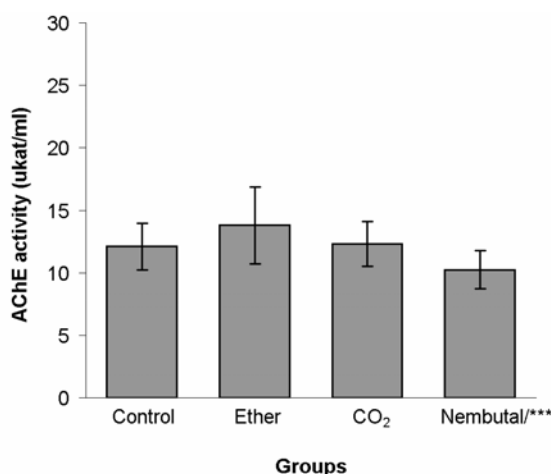


Fig. 2. **Activity of AChE in the plasma.** There is no significant difference between the control group and the particular methods of euthanasia. Also the t-test did not note any significant difference.

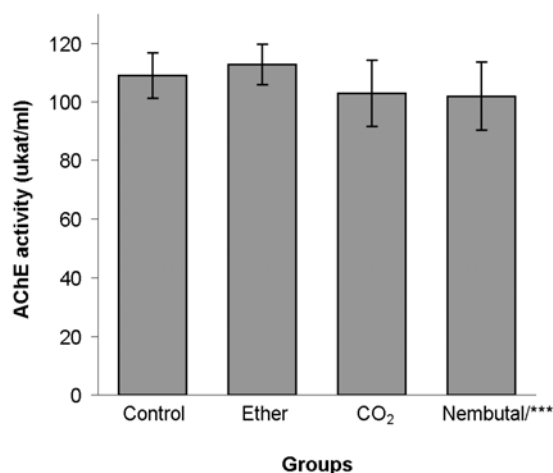


Fig. 3. **Activity of AChE in the brain.** There is no significant difference between the control group and the particular methods of euthanasia. Also the t-test did not note any significant difference.

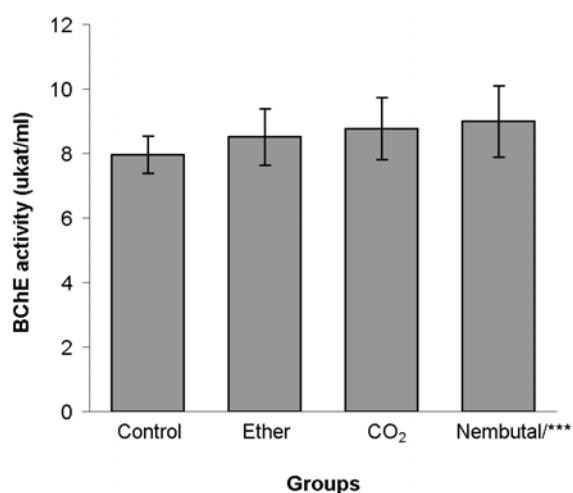


Fig. 4. **Activity of BChE in the whole blood.** There is no significant difference between the control group and the particular methods of euthanasia. Also the t-test did not note any significant difference.

DISCUSSION

This results of this study demonstrated that the methods of euthanasia chosen have no significant influence on AChE activity. We noted a significant decrease of AChE activity in the whole blood in the group anesthetised with Nembutal. There was no decreased activity of AChE in the brain in the same group of animals. We expected direct inhibition of AChE activity after exposure to Nembutal. However, probably the activity in the brain remains unchanged

during the short exposure to Nembutal. The ability of Nembutal to influence ChE activity in the tissues should be tested in also *in vitro* experiments.

According to the “Recommendations for euthanasia of experimental animals” (Close et al. 1996) not all methods used in our experiment are acceptable for euthanasia. Ether is an irritant to the mucous membranes, and may be stressful to the animals as it elevates catecholamines (Breazile and Kitchell 1969, Green et al. 1987, Blackshaw et al. 1988). Exsanguination should only be carried out after the animal has been rendered insensible by another method because of the stress associated with extreme hypovolaemia and the pain of incising the deeper blood vessels (Close et al. 1996).

Carbon dioxide (CO₂) is the most commonly used and acceptable agent for euthanasia of laboratory rodents (Close et al. 1996, Hackbarth et al. 2000, Conlee et al. 2005).

On the other hand, there are reports describing distress during euthanasia induced by CO₂ inhalation (Thurauf et al. 1991, Barbaccia et al. 1996).

The Australian and New Zealand Council for the Care of Animals in Research and Teaching (ANZCCART) suggest the administration of CO₂ is best preceded by an inhalation anaesthetic, such as isoflurane (Fenwick in Reilly 2001).

In conclusion, we suggest euthanasia in a CO₂ atmosphere was the best among the tested techniques both from the point of view of animal welfare and the absence of effects on activity of AChE and BChE in the periphery and brain.

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