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BENDIOCARBAMATE EFFECT ON THE CENTRAL NERVOUS SYSTEM IN THE CHICK EMBRYO

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ABSTRACT

The aim of the study was to investigate toxicity of bendiocarbamate (2,3-isopropylidene-dioxyphenyl methylcarbamate) to the central nervous system of the chick embryo. Bendiocarbamate was administered to chick embryos on day 3 (500 µg.egg⁻¹) and on day 10 (800 µg.egg⁻¹) of the incubation. The observations showed no microscopic changes in the central nervous system with the dose and day of incubation when bendiocarbamate was administered. The central nervous system was also investigated for caspase activity in relation to application of bendiocarbamate, and no differences in the number of cells with the caspase immunoreactivity were observed compared to the control.

Key words: bendiocarbamate; central nervous system; chick embryo

INTRODUCTION

The carbamate compounds are a class of cholinesterase-inhibiting pesticides (Farage-Elawar, 1990) and bendiocarb (2, 3-isopropylidene-dioxyphenyl methylcarbamate, BC) is the most widely used carbamate insecticide to control disease vectors (mosquitoes, flies, household and agricultural pests). Like other carbamate insecticides, BC is a reversible inhibitor of acetylcholinesterase (AChE). The blockage of AChE caused by BC persists for approximately 24 hours and, subsequently, the situation returns to normal because the insecticide does not accumulate in mammalian tissues (Siroťáková et al., 2005). However, concerning the exposure to BC it was observed that the substance can easily pass from mother to the developing embryo (Whyatt et al., 2003). Inhibition of AChE is linked with the pesticide mechanism of toxic action, irreversible or reversible bonding to the esteratic site of the enzyme and

potentiation of cholinergic action on the nervous system (Kristoff et al., 2006).

Acute intoxication with BC is manifested by tremor, tachycardia, mydriasis, hypersalivation, nausea etc. (Maraček and Antal, 2005).

The nervous system of the chick embryo is formed from the neural plate and the neural crest. Neuroblasts are visible from about the 2nd day of the incubation (2nd embryonic day, 2 ED) in the ventro-lateral part of the tube. Spinal nerves have developed by the 3 ED and regions of grey and white matter are recognizable by 3-4 ED. Dorsal and ventral horns can be seen in the grey matter from the 7 ED, and glial cells in the white matter. During following days the spinal cord becomes larger in transverse section and there is a change in a shape of the lumen from a longitudinal slit to an almost square or round shape (Bellairs and Osmond, 2005).

Currently the oral LD₅₀ of BC for hen is 137 mg/kg b.w. (WHO, 2007).

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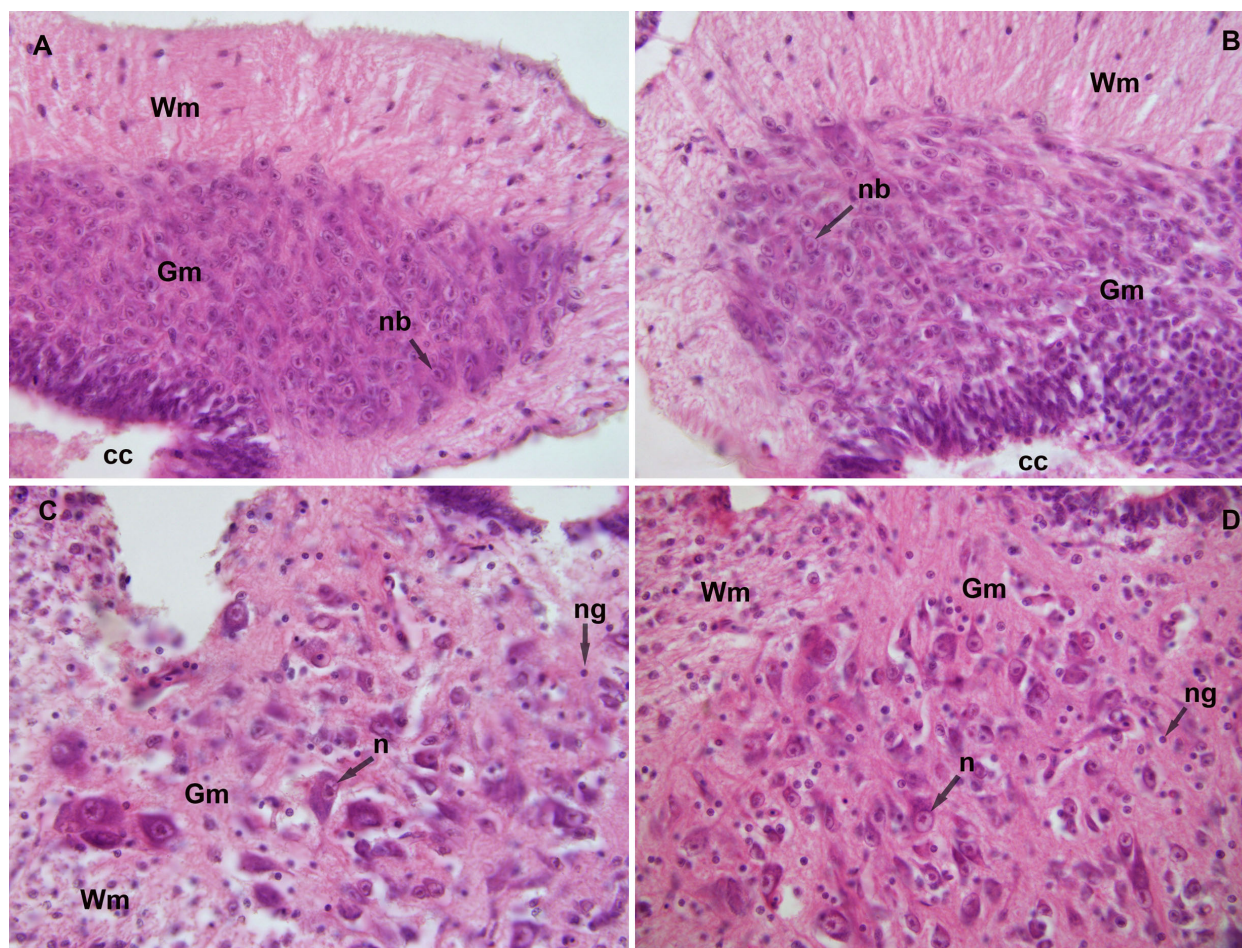
Chick embryos are more sensitive to toxic substances than mammalian embryos, because their action is not influenced by maternal metabolism. Therefore, the aim of the present study was to observe the effect of BC on chicken embryos.

MATERIAL AND METHODS

Fertile chicken eggs of Leghorn hybrid, spotted variant, were obtained from the animal farm Koleč, Institute of Molecular Genetics AS CR, Praha, Czech republic (80 eggs). They were incubated in a thermostat with forced circulation of air and the temperature maintained at 37.5 ± 0.5 °C and 60 % of relative humidity. Cytotoxicity to organs was determined by the application of BC on 3 ED and 10 ED. The eggs were opened by the modified „window technique“ (Jelínek, 1977). The application dose was 200 μ l per one egg, with acetone concentration equal to 10 μ l x 200 μ l⁻¹ of the application

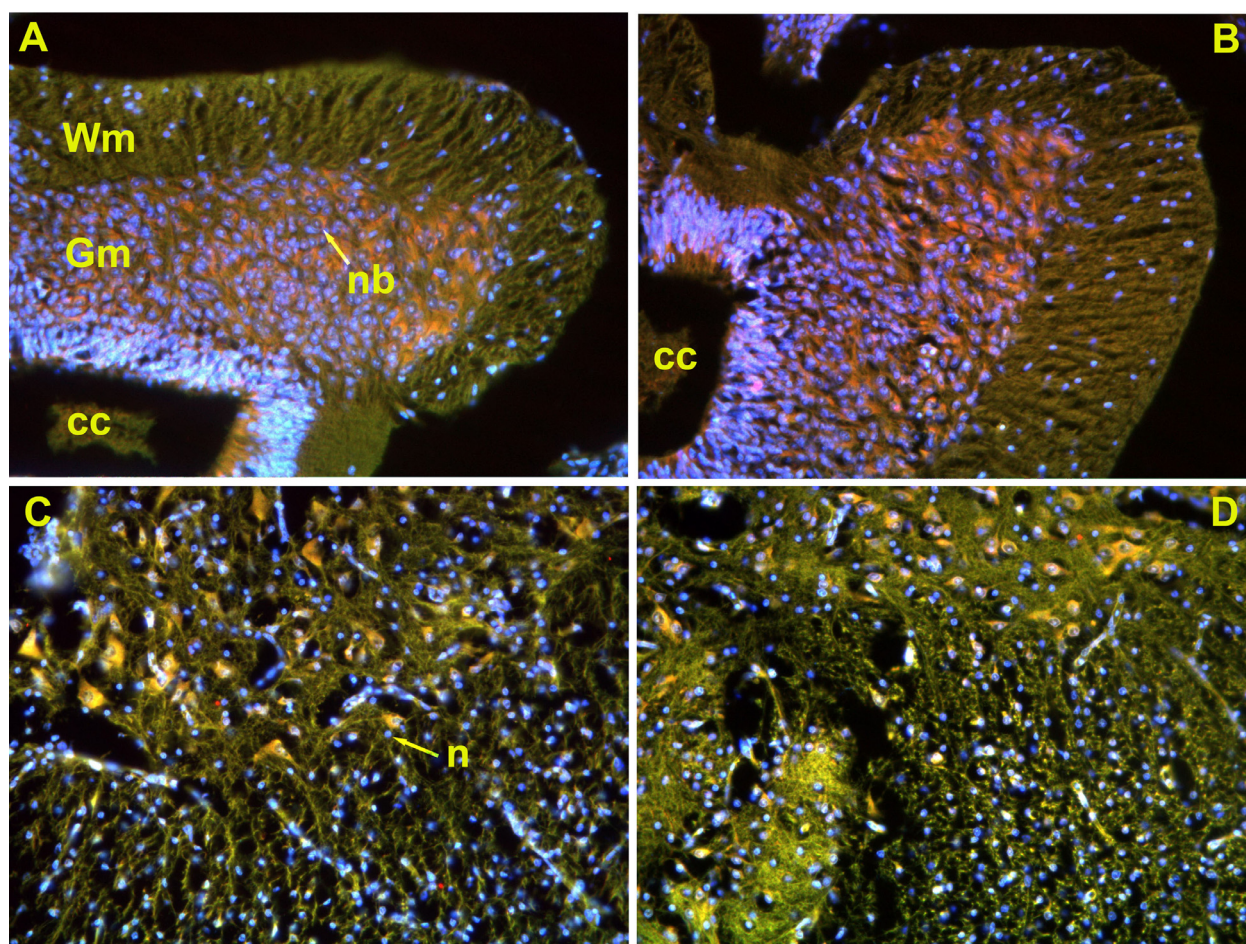
dose. The doses of BC applied to chicken embryos on 3 ED were 500 μ g/200 μ l/egg. The doses of BC applied to chick embryos on 10 ED were 800 μ g/200 μ l/egg. Identical volumes of the acetone solution were applied to control embryos: sterile water for tissue cultures (1:10), with acetone concentration 10 μ l x 200 μ l⁻¹ of the application dose. The embryos were inspected during the incubation and died embryos were eliminated from the experiment.

The chick embryos which were exposed to BC solution on 3 ED were dissected out of the membranes and weighted on 9 ED. Those, which were exposed to BC solution on the 10 ED were dissected out of the membranes and weighted on 17 ED. Subsequently, the embryos were fixed for 24 hours in Dents' solution (20 % dimethylsulfoxide and 80 % methanol) and routinely processed for histological examination. Neck was removed from fixed chick embryos. The respective parts of embryos were embedded in paraffin and after 24 hours



white matter (Wm); gray matter (Gm); central canal (cc); neuroblast (nb); neuron (n); neuroglia (ng) [H-E, 60x]

Fig. 1: Toxic action of BC on CNS of chicken embryos exposed on 3 ED (9 ED - A: control embryo, B: treated embryo; 500 μ g.egg⁻¹) and 10 ED (17 ED - C: control embryo, D: treated embryo; 800 μ g.egg⁻¹)



white matter (Wm); gray matter (Gm); central canal (cc); neuroblast (nb); nervous cell (n)
[stained immunohistochemically, 40x]

Fig. 2: Caspase activity of nervous cells (in white circle) after application of BC on 3 ED (9 ED - A: control, B: treated embryo; 500 µg.egg⁻¹) and 10 ED (17 ED - C: control, D: treated embryo; 800 µg.egg⁻¹)

were cut on a microtome (Leica RM 2265) to make 10 µm sections. To visualize microscopical changes in the central nervous system (CNS) the sections were stained with haematoxylin-eosin and the remaining sections were stained immunohistochemically for the caspase activity. The microscopic examination was carried out under Olympus BX 51 optical microscope using a dry objective with 60 x magnification. The caspase activity in the CNS was detected by the incubation in a primary mouse monoclonal antibody IgG 1 - Caspase-3/ CPP32 (BD Pharmingen) and a secondary antibody conjugated with Rhodamine Red dye (Jackson ImmunoResearch). The Rhodamine Red-conjugated antibody was red under a fluorescence microscope when using a suppression filter (465 nm) while the Hoechst 33258 stain was blue when using an excitation filter (420 nm). Autofluorescence in the fluorescein channel was used for tissue contrast. Microscopic examination was carried out using Leica

fluorescence microscope and a dry objective with 60 x magnification.

RESULTS

Organ toxicity

The microscopic findings in the CNS of chick embryos, exposed to BC on 3 ED and 10 ED, were negative when compared to the control. Part of the neck was sampled for this examination (including spinal cord cross section), and no histological changes were observed in the CNS as far as neurons and intercellular space were concerned (Fig. 1).

Caspase activity

We observed caspase activity in the viewing field of 887.5µm³ size with 450 nerve cells (one nerve cell/

2µm³). Chick embryos which were administered with BC on 3 ED at dose of 500 µg.egg⁻¹, showed no any cell with caspase activity in comparison with the control, where 1 cell (0,20 %) with the caspase activity was observed. In chick embryos which were administered with BC on the 10 ED at doses of 800 µg.egg⁻¹, one cell (0.20 %) with caspase activity was found in comparison to the control, which contained three (0,7 %) red stained nerve cells. In chick embryos that were exposed to BC on the 3 ED and 10 ED a lower caspase activity than in the control group was detected. Presence of apoptotic cells in the CNS after exposure to BC can be related to physiological elimination of excessive neurons at the generation of synapses (Fig. 2).

DISCUSSION

Organ toxicity

This study provides a first detailed analysis of bendiocarbamate toxicity in the chick embryo. For a while, no published data for any embryo species are available. Our experiment showed that the application of BC to chick embryos produced neither macroscopic nor microscopic changes in the CNS in comparison to the control after administration on 3 ED (500 µg.egg⁻¹), neither they occurred after application of BC (800 µg.egg⁻¹) on 10 ED.

A two-year study on dogs which received BC with food, revealed no changes in the weight of organs or any harmful effect of the pesticide on dog tissues. The daily dose used corresponded to 12.5 mg.kg⁻¹ body weight and the authors detected increased serum cholesterol and decreased bloodstream level of calcium (Baron, 1991).

Toxicity of bendiocarbamate to organs was investigated in adult rabbits which received BC *per os* at a dose of 5 mg.kg⁻¹.day⁻¹. In this study based on long-term (90 days) application of BC, the authors observed increased volume of cortex and decreased volume of thymus pulp. Moreover, morphometric analysis detected lower number of cells and also smaller diameter of cells in the thymus in comparison to the control (Flešárová et al., 2007).

In rat males, a significant increase in the incidence of nuclear cataract related to BC dose (20 and 200 mg.kg⁻¹; Environmental and Workplace Health, 2008) was observed.

Caspase activity

The experiment based on application of BC to chick embryos on 3 ED and 10 ED showed no increase in the number of cells with the caspase activity in comparison to the control.

Cell apoptosis occurs in chicken embryos for the

first time on 2 ED (somites and neural tube; Dawd and Hinchliffe, 1971). Cell apoptosis has an important role also in the nervous system. In the course of development of vertebrates the nerve cells are produced in excessive numbers and, therefore, cellular apoptosis involving 20-80 % of neurons is physiological. Foetal neurons thus compete for nerve growth factor (NGF) which ensures their survival and is produced not only by neurons but also by other cells. However, not all cells obtain the required quantity of NGF for their survival. Therefore, apoptosis adjusts the total number of produced neurons to such quantity, which is supported physiologically (Zakeri and Lockshin, 2002).

CONCLUSION

We conclude that bendiocarbamate does not possess a significant toxic potential, at least in the avian embryo. Nevertheless, large doses that would impair maternal metabolism could cause secondary problems to the developing embryo or fetus in mammals.

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