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Łukasz M. Kołodziejczyk*, Magdalena Puzik, Agnieszka Greń,
Marta Batoryna, Grzegorz Formicki, Edyta Kapusta

Department of Animal Physiology and Toxicology, Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Kraków, Poland; *lukasz.kolodziejczyk@up.krakow.pl

Does benzo[*a*]pyrene affect the embryonic development of the heart?

Introduction

The chicken embryo and the avian *in ovo* model are one of the prominent experimental procedures in several tests for toxins and the preclinical testing of drugs. It is also the oldest known embryological protocol of basic developmental research (Davey, Tickle, 2007). It is well documented that many milestone discoveries on the embryology of vertebrates and on the organogenesis of brains or hearts were made using this model organism (Le Douarin, 1998; Pardanaud et al., 2001).

Benzo[*a*]pyrene is a polycyclic aromatic hydrocarbon that is a well-known carcinogen, teratogen, and neurotoxin widely present in urban air pollution, cigarette smoke, and certain kinds of foods, i.e. smoked fish, smoked meat, etc. The problem of intoxication with this substance is actually one of the most urgent in big developing cities, such as Cracow (*European Environment Agency*…, 2016). There are many reports confirming its toxicity for mice and other mammals; however, knowledge on its impact on birds is still limited. Whereas, birds are an important element of the typical urbicenosis, and basic knowledge on the biology of this group of vertebrates suggests that they may be a useful biotest for this stress factor (Lee, Shim, 2007). The effect of benzopyrenes on the developing heart of vertebrates is unknown and requires effective update. The embryos of birds exhibit several opportunities to perform physiological experiments on the heart (Tazawa et al., 1994).

The aim of presented paper is to determine the main action of benzo[*a*]pyrene on selected parameters of the heart muscle of chicken embryos in the *in ovo* developmental model, with special attention to the antioxidative defence mechanisms and the bioelectric properties of heart rhythm.

Material and methods

We used chicken embryos of the race 'Ross 308' to verify the influence of benzo[*a*] pyrene on physiological and biochemical parameters of the heart muscle. Fertilised chicken eggs were obtained from a certified farm (Łężkowice, Poland) and incubated in an automated incubator (HEKA, Germany) at 37.5°C. The benzo[*a*]pyrene in an organic oil solution (Sigma Aldrich, USA) was injected *in ovo* on the 6th day of the incubation into the yolk at the following doses of 1 mg/kg weight of eggs; 0.5 mg/kg w. e. and 0.1 mg/kg w. e. The intact eggs and eggs injected with the organic oil were used as control groups. On the 14th day of the incubation, eggs were opened in order to examine embryos and achieve tissues for further analyses.

We performed the electrocardiography of embryos using AsCARD AMBER equipment (Aspel, Poland) and 4 extremital copper electrodes. We also estimated the weight of hearts *post mortem* using a laboratory balance (Rad Wag, Poland). Finally, we determined the concentration of reduced glutathione (GSH) according to Ellman's method and malonyldialdehyde (MDA) using TBARS MDA Assay in the heart tissue. To perform all spectrophotometric measurements, we used a Sunrise Absorbance Reader (Tecan, Austria).

The quantitative data was analysed statistically using Shapiro-Wilk tests and Student tests with the significance level at $p < 0.05$.

Results and discussion

The electrocardiography performed on the 14th day of incubation does not show any important changes in the heart rate and rhythm in relation to controls (Fig. 1). In the electrocardiogram basal sinus rhythm without evident QRS intervals was visualised, which is typical for immature bird hearts (Yoshiyama, Kanke, 2005).

The increased weight of the heart muscle was observed in the embryos treated with a dose of 1 mg/kg w. e. In this group, the average heart weight accounted 211 mg. In contrast, the average heart weight in control groups was approximately equal to 120 mg. (Fig. 2A). This result may suggest that the higher doses of benzo[*a*]pyrene increase blood retention in the systemic circulation, which results in the heart hypertrophy. A similar effect was described in several pathologies connected with increased vascular resistance (Pardanaud et al., 2001). In groups contaminated with lower doses of benzo[*a*]pyrene, heart weight did not differ significantly from the control and intact eggs.

We determined a statistically significant increase of the GSH concentration in the heart tissue from the group injected with 1 mg/kg w. e. In the case of the lower benzo[*a*]pyrene doses, the level of GSH was similar to the controls (Fig. 2B).

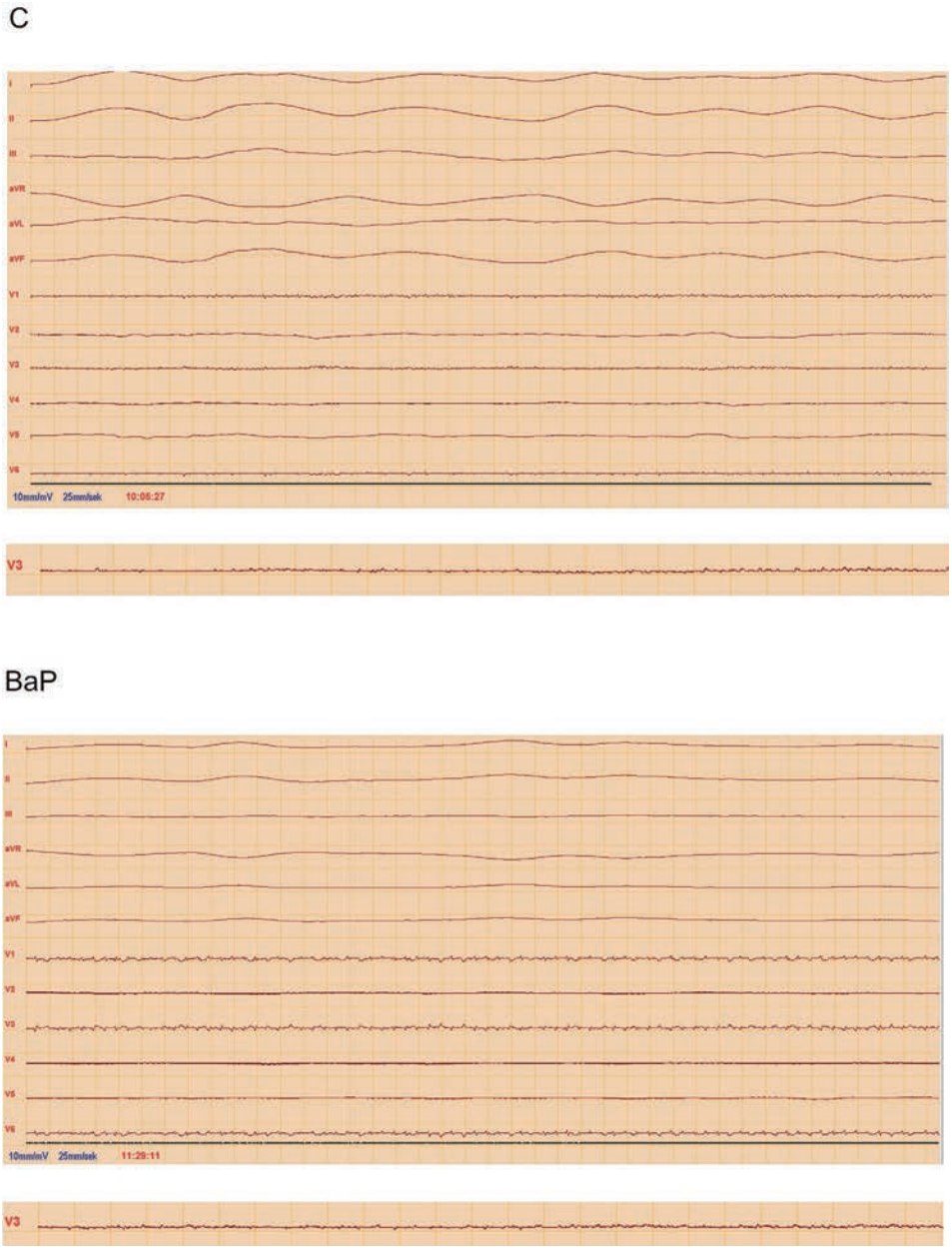

Fig. 1. Examples of electrocardiograms of chicken embryos on the 14th day of incubation. C – control; BaP – an individual contaminated with benzo[*a*]pyrene

Differences in the MDA concentrations in all experimental groups were not statistically significant in relation to the controls (Fig. 2C).

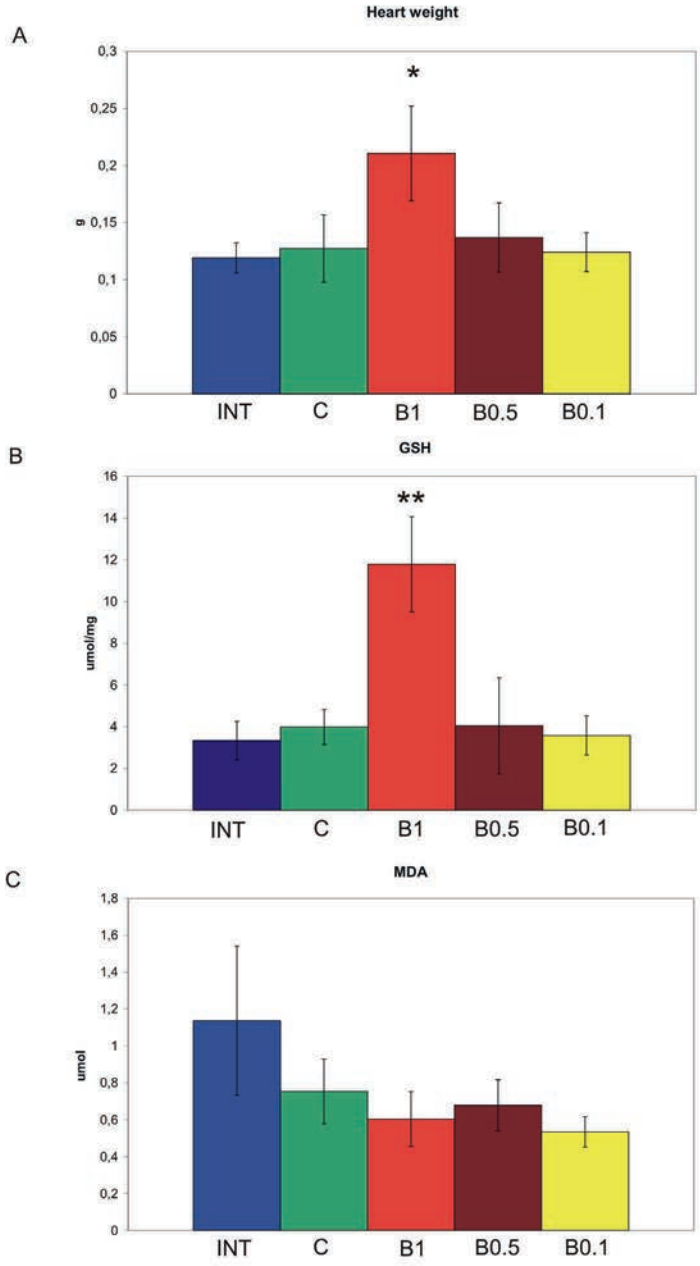

Fig. 2. A – the effects of benzo[*a*]pyrene on the heart weight *post mortem* of chicken embryos; B – the effects of benzo[*a*]pyrene on the concentration of reduced glutathione in the heart tissue; C – the effects of benzo[*a*]pyrene on the concentration of malonyldialdehyde in the heart tissue; INT – intacts; C – control; B1 – 1 mg/kg w. e. of benzo[*a*]pyrene; B0.5 – 0.5 mg/kg w. e. of benzo[*a*]pyrene; B0.1 – 0.1 mg/kg w. e. of benzo[*a*]pyrene; significant differences between the experimental and control groups are indicated with asterisks (*$p < 0.05$, **$p < 0.01$); the error bars denote the standard deviation of the mean value; n = 6

These results may suggest that benzo[*a*]pyrene is a toxicological stress factor, which activates the glutathione synthesis in the organism in response to the acute poisoning and may activate other mechanisms of the glutathione-dependent antioxidative defence. It has been proven that some neurotoxins (i.e. acrylamide) involve varied disturbances and defence responses in the antioxidative system of the chicken embryo's brain (Batoryna et al., 2017; 2018).

Conclusion

We conclude that the subacute dose of benzo[*a*]pyrene is a stress factor, which strongly activates the glutathione-dependent antioxidative defence and probably do not affect the heart conducting system of the chicken embryo; however, the influence of this substance on the morphology and biochemistry of the developing heart requires further examination.

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Abstract

Benzo[*a*]pyrene is a polycyclic aromatic hydrocarbon with a well-proven toxic effect on animal cells and tissues. We used a chicken *in ovo* developmental model to verify its influence on selected parameters of the heart rhythm and on the antioxidative defence in the heart tissue. We determined that the dose of 1 mg/kg weight of eggs of benzo[*a*]pyrene strongly activates the glutathione-dependent antioxidative system, but it did not significantly affect the heart conducting system of the chicken embryo. We postulate that further

study on the benzo[*a*]pyrene action during embryonic development of birds is recommended.

Key words: benzo[*a*]pyrene, ecg, embryo, GSH, heart, MDA

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Czy benzo[*a*]piren wpływa na rozwój zarodkowy serca?

Streszczenie

Benzo[*a*]piren jest wielopierścieniowym węglowodorem aromatycznym o dobrze znanym toksycznym działaniu na komórki i tkanki zwierząt. Wykorzystaliśmy model rozwoju zarodków kury in ovo do weryfikacji wpływu tej substancji na rytm serca oraz wybrane parametry układu antyoksydacyjnego w mięśniu sercowym. Wykazaliśmy, że dawka 1 mg/kg masy jaj silnie aktywuje zależny od glutationu mechanizm antyoksydacyjny, ale jak się wydaje nie wpływa znacząco na układ przewodzący serca zarodków kury. Konieczne są dalsze badania wpływu benzo[*a*]pirenu na rozwój zarodkowy ptaków.

Słowa kluczowe: benzo[*a*]piren, EKG, zarodek, GSH, serce, MDA

Information on the authors

Łukasz M. Kołodziejczyk
He is a PhD student at the Department of Animal Physiology and Toxicology in the Institute of Biology, Pedagogical University of Cracow. His scientific interests are developmental biology and comparative anatomy.

Magdalena Puzik
She is a graduate of bioinformatics in the Institute of Biology, Pedagogical University of Cracow.

Agnieszka Greń https://orcid.org/0000-0003-2383-1096
She is a professor at the Department of Animal Physiology and Toxicology in the Institute of Biology, Pedagogical University of Cracow.

Marta Batoryna
She is a PhD student at the Department of Animal Physiology and Toxicology in the Institute of Biology, Pedagogical University of Cracow.

Grzegorz Formicki https://orcid.org/0000-0001-9964-6132
He is a professor at the Department of Animal Physiology and Toxicology in the Institute of Biology, Pedagogical University of Cracow.

Edyta Kapusta https://orcid.org/0000-0002-4350-5514
She is a laboratory specialist at the Department of Animal Physiology and Toxicology in the Institute of Biology, Pedagogical University of Cracow.