

REVIEW

by Professor Reni Emil Kalfin, PhD, Head of the Scientific Department

"Biological Effects of Natural and Synthetic Substances"

at the Institute of Neurobiology - BAS

on a thesis for awarding the educational and scientific degree "Doctor"

Professional direction 4.3. "Biological Sciences"

PhD Program "Animal and Human Physiology"

Author: Assistant Bilyana Mariyanova Ilieva

Form of doctoral studies: Independent PhD program

Department: „ANIMAL AND HUMAN PHYSIOLOGY“ at the Faculty of Biology of Sofia University "St. Kliment Ohridski"

Thesis title: „*Obestatin inotropic effect on frog heart (Pelophylax ridibundus). Role of autonomic innervation*“

Supervisor:

Professor Hristo Stefanov Gagov, PhD

Faculty of Biology at Sofia University "St. Kliment Ohridski"

1. Brief biographical data and description of scientific interests

Bilyana Ilieva was born in the city of Sofia on February 16th, 1982. In 2007, she graduated with full honors in the specialty "Molecular Biology" with a professional qualification in "Animal and Human Physiology" at the Faculty of Biology of Sofia University "St. Kliment Ohridski". The PhD student has acquired a post-graduate qualification "Teaching Methodology in Biology". After completing her higher education, she worked for one year as a specialist-biologist at the Institute of Neurobiology, Bulgarian Academy of Sciences. Since February 2008 and at present time, assistant Bilyana Ilieva conducts laboratory exercises for bachelor and master students at the Faculty of Biology, Sofia University. In February 2019, Bilyana was enrolled in an independent PhD program in the specialty "Physiology of Animals and Humans", and three years later she was dismissed with the right of defense.

According to the attached documents, assistant Bilyana Ilieva speaks English at a good level. She participated in a total of 7 funded research projects, where she studied the mechanisms and action of the hormone obestatin in the heart of a frog; the

regulation of hepatic and renal diamine oxidase by nitric oxide, aminoguanidine and testosterone in the rat; the role of algotoxins as a risk factor for human health.

2. Actuality of the PhD thesis subject

Despite the great progress in the treatment and diagnosis of cardiovascular diseases, they continue to be one of the main problems not only for the medical and scientific communities, but also for the whole society. Sufficiently is to mention their ever-increasing frequency worldwide as a result of urbanization with its accompanying lifestyle stresses, immobility, changes in nutrition with an increase in the proportion of foods with a high glycemic index, high content of lipids and fatty acids, alcohol abuse and smoking habits. In statistics published last year, it was established that Bulgaria is at the first place in the European Union in terms of premature death from cardiovascular diseases, which indicates the need for timely diagnostics, the search for new effective means of natural origin with good biological tolerance for the treatment of cardiovascular diseases.

In this regard, PhD thesis is devoted to an important problem: it studies the influence of the strength and speed of heart contractions by the hormone obestatin and the mechanisms that determine its positive inotropic effect. All this makes the topic of the dissertation contemporary in a scientific and scientific-applied sense and justifies the need for the conducted research.

3. Knowledge on the subject

The question about the knowledge on the subject by assistant Bilyana Ilieva finds its answer in the literature review presented by her. At the beginning of the literature review, she traces the physiology and anatomy of the frog heart and the regulation of cardiac activity in vertebrates. Bilyana Ilieva focused her attention on the peptide obestatin, discovered in 2005, which reduces food intake and reduces body weight. The doctoral student thoroughly presents the formation and structure of obestatin, its effects and physiological functions. Bilyana concludes that despite more than 15 years of research, the influence of obestatin on various organs and its mechanism of action cause many discussions, which is due to both its still unknown receptor and the possible degradation of its molecule to smaller fragments that are supposed to have effects of their own. The literature review of the thesis gives me a reason to positively evaluate the creative and analytical approach of the PhD student Bilyana Ilieva and her knowledge on the thesis subject.

4. Research methodology

Bilyana used an experimental frog "isolated heart" model for the in vitro studies with obestatin, which model provides an opportunity to study the effects of pharmacological substances, hormones and other mediators directly on the isolated organ. For the conduction of these studies, the Bulgarian company Stokes has developed a specialized software program TENZU, which allows the recording of heart activity and the storage of the obtained data in electronic form. An auxiliary program TENZOGRAPH has been developed for additional visualization of the obtained data. Since the recording system amplifies the received signal three times, the actual force of heart contractions in the thesis is expressed in millinewtons after reducing the recorded amplified amplitude three times.

5. Characterization of the PhD thesis and assessment of the contributions

The PhD thesis reviewed by me is written on 115 non-standard pages with a classic structure as follows: introduction (1 page), table of contents (2 pages), abbreviations (1 page), literature review (47 pages), purpose and tasks (1 page), materials and methods (5 pages), results (17 pages), discussion (17 pages), conclusions and contributions (2 pages). The PhD thesis contains a total of 24 figures and 3 tables. The list of cited literature contains 284 sources, of which 2 are in Cyrillic and 282 in Latin language. The structure of the individual parts is appropriate and the subheadings correspond to the logic of the work and the commented results. The PhD thesis is written legibly, in very good scientific language.

The aim of the dissertation is to provide an answer to the question of how obestatin realizes its positive inotropic effect in the heart of a frog - directly on cardiomyocytes or indirectly through the autonomic nervous system, as well as to establish the mechanism underlying this effect.

The set tasks are 5 in number and correspond to the goal formulated above.

The "Results" and "Discussion" chapters are the most important part of the PhD student's own research.

Bilyana found that in the presence of obestatin, vesicular transport and reuptake were essential for adrenergic signaling, and chemical elimination of sympathetic innervation abolished the positive inotropic effect of obestatin in frog heart preparations.

The PhD student managed to answer the main question posed for consideration in the dissertation, namely that the positive inotropic effect of obestatin on preparations of

isolated frog heart is carried out by activating the secretion of adrenaline from the axons of sympathetic autonomic neurons located in the heart muscle wall.

The discussion of the experimental results shows the PhD student's thorough and up-to-date knowledge on cellular biochemistry and experimental physiology. The literature cited is sufficient and modern, it shows the in-depth knowledge on the thesis subject.

Everything said above is proof of the sufficient amount of material processed and described in detail by Bilyana, which gives me reason highly to assess the author's skills under the guidance of Prof. Hristo Gagov to examine the obtained results in the context of the known facts from the literature, to discover the cause-effect relationships between the studied parameters and accurately to formulate the conclusions.

The contribution of the dissertation noted by the PhD student is her own work, obtained with the support and assistance of her supervisor. Upon completion reading the PhD thesis, the reader is left with the impression that this work is not finished. This is because the work provides perspective for future research in several directions. This is the place to congratulate the supervisor of the PhD student, who in addition to being a well-known scientist, has many years of experience in supervising PhD students.

6. Scientometric data of the PhD student

According to the attached certificate from the Department "PhD Students" at the Faculty of Biology - Sofia University, Bilyana Ilieva has successfully passed all exams from her individual PhD training.

She has published three articles related to the PhD thesis, all with quartile Q4, from which Bilyana receives a total of 36 points and exceeds the minimum required 30 points in Professional direction 4.3. Biological Sciences. It is worth to note that in all articles related to the dissertation, Bilyana Ilieva is the first author, which shows her personal participation in the PhD thesis research. She has 5 participations in scientific meetings on the PhD thesis subject, where she is also the first author.

7. PhD thesis abstract

The abstract of the PhD thesis is written on 36 non-standard pages and meets the requirements. In a summarized form, it reflects the goal and tasks of the thesis, the methods used, the obtained own results analytically described and interpreted, the author's conclusions and contributions made.

8. Recommendations and questions

My recommendation is that the introduction should be placed after the content in the structure of the dissertation, and marked with its page number.

I have a few questions for the PhD student: 1). Do the obtained results have practical significance? 2). What are the directions for a future research? 3). Apart from the assumption of reactive oxygen forms generation, what according to the PhD student accounts for the non-specific positive inotropic effect of the neurotoxin 6-hydroxydopamine on isolated frog heart preparations?

CONCLUSION

The PhD thesis submitted for defense on a topic: „*Obestatin inotropic effect on frog heart (Pelophylax ridibundus). Role of autonomic innervation*“ contains original scientific results that have been promoted through five communications in scientific forums and published in three well-written scientific papers.

The dissertation shows that the PhD student Bilyana Ilieva possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Physiology of Animals and Humans", demonstrating qualities and skills for independent scientific research. The results of the dissertation make a valuable original scientific contribution to elucidate the mechanism of hormone obestatin action in the heart and identify the target tissue for physiological regulation by a catecholamine-dependent mechanism. All this gives me the conviction that today our scientific jury shall elect a new doctor - a continuation of the long-lasting tradition in the Bulgarian physiology.

Given the above, I have a completely positive assessment of the conducted scientific research, presented by the above-reviewed PhD thesis, its abstract, achieved results and contributions, and I will vote positively as a member of the Scientific Jury for the awarding of the educational and scientific degree "Doctor" of Bilyana Marianova Ilieva in the scientific specialty "Physiology of Animals and Man" in professional direction 4.3. "Biological Sciences".

March 5th, 2023

Reviewer:

Professor Reni Kalfin, PhD