PEER REVIEW

From Prof. Jana Tchekalarova
Head of the Department “Behavioral Neurobiology”
Institute of Neurobiology, Bulgarian Academy of Sciences

Member of the Scientific Jury, selected by Rector's Order No. RD-38-632 / 02.12.2022.

According to the procedure for the defense of the thesis on the topic: "Inotropic effect of obestatin on the heart of a frog (Pelophylax ridibundus). Role of vegetative innervation" for the acquisition of an educational and scientific degree "DOCTOR" in a professional direction 4.3. "Biological Sciences".

Author of the dissertation: Bilyana Marianova Ilieva, doctoral student, doctoral program "Animal and Human Physiology" at the Department of Animal and Human Physiology, Faculty of Biology /BF/, SU "Kliment Ohridski" and with supervisor: prof. Dr. Hristo Gagov.

Brief biographical data. Bilyana Marianova Ilieva holds a master's degree from the Faculty of Arts of the University of St. Kliment Ohridski" , speciality in "Animal and Human Physiology". She has one year of work experience as a biologist at the Institute of Neurobiology, Bulgarian Academy of Sciences and 16 years as an assistant in the BF of SU "St. Kliment Ohridski".

The structure of the dissertation. The dissertation contains 115 pages and the standard structure for this type of scientific work: Introduction /1 page/ Literature review /48 pages/, Goals and tasks /1 page/, Materials and methods /5 pages/ , Results /17 p./, Discussion /17 p./, Conclusions /1 p./, Contributions /1 p./, Scientific publications and participation in simposiums /1 p./, Bibliography /18 p./. The dissertation work is illustrated with 25 figures and 3 tables.

Literature review. The completed literature review is comprehensive and shows that the doctoral student possesses skills for summarizing and analyzing literary data with an idea of

posing problematic questions that require clarificatio. A detailed review of the anatomy and physiology of the frog heart, cardiac regulation in vertebrates, and the structure, distribution, and physiological effects of obestatin in the gastrointestinal tract, cardiovascular system, and nervous system is presented. The PhD student reviewed in details the role of this endogenous peptide in certain diseases such as obesity and diabetes. At the end of the review, the involvement of obestatin in the pathophysiology of cancer is discussed. The PhD student concludes that the literature data regarding the subject of the dissertation, obestatin, are not yet fully understood, including its receptors, stability, and certain physiological effects. On the other hand, the analysis shows that, although there is still no clarity regarding the physiological stimuli for secretion of the peptide, its plasma levels rise under pathological conditions. In this way, through the literature review, the doctoral student logically formulates a main goal of the study, to be solved by implementing experimental tasks.

Purpose and tasks. The PhD student formulated a goal related to investigating the role of autonomic nervous regulation in the effects of obestatin on the contractile activity of the frog heart and the mechanisms mediating it. In order to realize the goal, a total of five tasks have been set, which are clearly and adequately formulated.

Materials and methods. Methodical approaches have been chosen that are appropriate and consistent with the goal and set tasks, which are precisely described, i.e. experimental model "isolated heart" to study in vitro the effects of obestatin, applying a pharmacological approach with a range of different antagonists to establish the mechanism of action of peptide and registration of cardiac activity through a specialized software program. The applied approach to the implementation of the assigned tasks makes a good impression. As a recommendation, although this information is available in the results, instead of presenting historical data on the development of the "isolated heart" method, a brief description of each inhibitor's mechanism of action and how the respective doses used were selected in the experiment is recommended.

The results follow the course of the set goal and tasks. They are well presented and illustrated with 1 table and 11 figures. The consistent presentation of the results for each experiment with figures comparing the effects of the blocker and obestatin combination versus the blocker's own effect and figures comparing the effects of the control, obestatin and the blocker and obestatin combination is impressive. I would recommend, in the presence of statistical reliability, that the values of p = be presented not only in the text below the figures, but also in the text of the results.

The discussion of the results is done according to their presentation in the separate sub-chapters of the Results. An excellent impression is made by the interpretation of the own data according to the results available in the scientific literature.

Conclusions and Contributions. A total of 5 conclusions and one contribution related to the described results are systematized. I agree with the conclusions drawn and one contribution, but it is advisable to point out its confirmatory or original character.

The cited literature covers a total of 284 sources. With regard to citations, it is appropriate that they be agreed in the text and finally in the literature reference. In this case, in the dissertation, since the citations are given in the text not by numbers, but by the surname of the first author and year, the numbers should not be placed in the order of their presentation in alphabetical order in Cited Literature.

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Publications on the subject of the dissertation and personal contribution of the doctoral student.

Three articles have been published on the subject of the dissertation - two published in an international journal with an impact factor and one article in a Bulgarian journal, in which part of the results of the dissertation are reported. In all three articles, Bilyana Ilieva is the first author, which confirms her personal contribution to the development of the dissertation work. No citation data is presented for these articles, which is understandable given that they were published in the last two years. Results of the dissertation work have been presented at two international and three national scientific forums, which are on the topic of the dissertation work and at which the doctoral student is also the first author.

Critical notes and recommendations. Since the dissertation is mainly focused on investigating the role of the vegetative system in the effects of obestatin on the contractile function of the frog heart, I believe that the paragraph of the Literature Review, which reviews the available data on the effects of the peptide on cardiovascular activity, should is placed at the end of the review with an emphasis on the unknown and still unclear actions of the peptide in this system, which is a prerequisite for formulating the purpose of the research in the Dissertation work.

I would make an encouraging recommendation to the PhD student to continue and expand the issue, including research on the role of this biologically active peptide in the CNS in experimental rodent models. Also, it is advisable to emphasize in the dissertation the translational significance of the established mechanisms by which obestatin realizes its inotropic effects on a frog heart preparation.

Conclusion. The dissertation work presented to me for review is dedicated to a current topic, and the goals and tasks set in it were realized through extensive experimental work by means of adequate methodical approaches. Establishing the mechanism of inotropic action of the biologically active peptide obestatin in the heart of a frog can be the basis for further studies on this peptide in other organs and systems in experimental models of rodents. The fact that levels

of the peptide are increased under pathological conditions suggesting its relevance in such conditions and opportunities for future developments with translational relevance using knowledge of its mechanism of action in frog cardiac muscle. The efforts made by the doctoral student to develop the topic deserve high praise. Therefore, I consider that the dissertation work of Bilyana Ilieva is original and fully complies with the Law on the Development of the Academic Staff in the Republic of Bulgaria, the regulations for its implementation and the regulations of SU "St. Kliment Ohridski" for awarding the educational and scientific degree "Doctor".

Due to the above, I give my positive assessment and recommend the members of the Honorable Scientific Jury to award Bilyana Marianova Ilieva the educational and scientific degree "Doctor" in professional direction 4.3. Biological Sciences, Science Major: Physiology.

27. 02. 2023 г. Signature.....

(Prof. Dr. Jana Tchekalarova)