OPINION STATEMENT

by Prof. Elena Borisova Dzhambazova, Ph.D.
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on a dissertation presented to a scientific jury appointed by order No. RD-38-632/02.12.2022 of the Rector of Sofia University "St. Kliment Ohridski" for acquiring the educational and scientific degree "Doctor" in the professional field: 4.3. Biological Sciences (Animal and Human Physiology)
Doctoral candidate: **Bilyana Mariyanova Ilieva**
Dissertation topic: "**Obestatin inotropic effect on frog heart (*Pelophylax ridibundus*). Role of autonomic innervation**"
Scientific supervisor: **Prof. Hristo Gagov, PhD**

The doctoral student has submitted all the required documents according to the Law for the Development of Academic Staff in the Republic of Bulgaria, and the Regulations for the terms and conditions for acquiring the educational and scientific degree "Doctor" at Sofia University "St. Kliment Ohridski". The originality of the presented dissertation work has been established according to the legal order for the absence of plagiarism.

Actuality and significance of the developed topic

The topic of the dissertation is related to establishing the participation of autonomic nerve endings in the effect of the peptide obestatin, discovered in 2005, on the contractile activity of a frog's heart. The significance of the development is determined by the fact that this relatively recently identified mediator is still the subject of intensive research, and its influence on various organs and functions is the subject of discussion due to the fact that its receptor is still unknown. Numerous literature data have been collected demonstrating the structure, biological activity, and changing levels of obestatin in response to obesity, diabetes, and cardiovascular disease. There is evidence that the peptide is involved in the modulation of intestinal motility, protection against gastrointestinal diseases, an anti-inflammatory effect on the vascular endothelium, and cardio- and neuroprotective effects. However, the therapeutic potential and mechanism of action of obestatin remain unclear, and there is no standardized method for measuring its levels. The studies conducted on the involvement of autonomic nerve endings in the effect of obestatin were based on an experimental model "isolated heart", allowing the application of pharmacological substances directly to the organ. After creating the experimental model, showing the doctoral student's dexterity and precision in terms of methodology, methodically planned activities were successively performed, showing her in-depth knowledge of cardiac regulation. A pharmacological approach was used by successively specific inhibition of catecholamine synthesis, blocking of vesicular monoamine transport in nerve endings, blocking of adrenaline reuptake, and elimination of autonomic

neurons from 6-hydroxydopamine. As a result of labor-intensive experiments, the mechanism of the positive inotropic effect of obestatin was established, by activating the secretion of adrenaline from sympathetic nerve fibers.

Characteristics of the dissertation and the abstract

The dissertation contains 123 pages. It is written in a very good scientific style and solid, grammatically correct Bulgarian language. It is constructed according to the accepted standard scheme for this category of scientific works, containing all the necessary sections: Introduction - 1 page, Literature review - 47 pages; Purpose and tasks - 1 page; Materials and methods - 5 pages, Results - 17 pages, Discussion - 17 pages, Conclusions - 1 page, Scientific contributions - 1 page, Literature - 17 pages. There is a logical connection between the sections. A list of abbreviations used has also been prepared, which makes reading easier. The dissertation is illustrated with 25 figures and 3 tables. In the end, lists of three publications and 5 participations in scientific forums are presented.
The abstract, in Bulgarian (36 pages) and English (32 pages) version, reflects the main highlights and results of the doctoral work, illustrated with figures, followed by conclusions and contributions.

Degree of knowledge of the state of the problem by the doctoral student

The literature review has a volume of 47 pages, it is written concisely, but sufficiently detailed and thorough. It includes data on the anatomy and physiology of the frog heart, the formation and structure of obestatin, and the physiological effects of the peptide on various organs and systems. For the preparation of the literary review, doctoral student Bilyana Marianova Ilieva used many key publications (284 in the entire work, of which only 2 are in Bulgarian), which emphasizes her good knowledge of the topic of the dissertation. The literature review is illustrated with 9 figures and 2 tables. 2 more figures are included in the Materials and Methods section, where the chemicals used, the object of study, the experimental set-up, and the treatment scheme are described.
Purpose and tasks. The purpose of the dissertation work is clearly defined, and 5 formulated tasks follow from it. They focused on monitoring the effect of obestatin on the contractile activity of the isolated frog heart after a specific pharmacological challenge.
The results and the discussion occupy a total of 34 pages. The results are summarized and presented in 14 figures and 1 table. The discussion of the obtained data is in a separate section. The skillful interpretation and commenting of the results, and the assumptions about the potential mechanisms of action with references to literature sources make a very good impression, which shows the deep knowledge of the problem and the high quality of the discussion.
The conclusions drawn at the end summarize the obtained data in detail. The Ph.D. student formulates a contribution to the development of a pharmacological approach to identify the target tissue of physiological regulation by a catecholamine-dependent mechanism.

Conclusion

The dissertation presents original data on the effect of obestatin on the contractile activity of an isolated frog heart after a specific pharmacological effect. 3-iodo-tyrosine was shown to reduce the positive inotropic effect of obestatin, and treatment with reserpine and desipramine completely abolished it. The positive inotropic effect of obestatin on the contractile activity of an isolated frog heart occurs mainly by activating the secretion of adrenaline from the sympathetic autonomic nerve endings in the heart muscle wall. Vesicular transport and reuptake are essential for adrenergic signaling in the presence of the investigated peptide. Chemical sympathectomy with 6-OHDA eliminated the effect of obestatin, but also exerted a nonspecific positive inotropic effect most likely due to the generation of reactive oxygen radicals in cardiomyocytes. The data from the present research will have mainly theoretical significance and can potentially be the basis for future research in this direction. The Ph.D. student, Assistant Professor Bilyana Ilieva, has implemented a large experimental program by applying a labor-intensive methodology. She has a very good theoretical background and knows the literature in her field. In connection with the dissertation, assistant professor Ilieva has 3 published scientific papers in journals with IF - two original articles in Current Topics in Pharmacology (IF = 0.261 for 2020, SJR=0.135, Q4) and one review in Acta Zoologica Bulgarica (IF = 0.448 for 2020, SJR=0.237, Q4). She is the lead co-author of all publications. I want to point out that Bilyana Ilieva is the co-author of 5 more articles outside of the dissertation work in journals with an impact factor and has a total of 15 citations. She has grown to the level of an accomplished young scientist and can plan and conduct research and analyze the results obtained.

In conclusion, I consider that Bilyana Ilieva's dissertation work, abstract and scientific publications meet the minimum scientific requirements of the
Law for the Development of Academic Staff in the Republic of Bulgaria, and the Regulations for the terms and conditions for acquiring the educational and scientific degree "Doctor" at Sofia University "St. Kliment Ohridski". Based on the above, I give my positive assessment of the dissertation work, and I propose to the respected scientific jury to award Bilyana Marianova Ilieva the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences, scientific specialty "Animal and Human Physiology".

23 January 2023
Sofia

Signed:
(Prof. Elena Dzhambazova, Ph.D.)