

O P I N I O N

from

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Regarding dissertation work on the topic: "Inotropic effect of obestatin on frog (*Pelophilax ridibundus*) heart." Role of autonomic innervation".

Author of the dissertation: assistant professor Bilyana Marianova Ilieva - doctoral student of independent preparation, for awarding the educational and scientific degree "Doctor" in the scientific field 4.3. Biological Sciences, the scientific specialty "Animal and Human Physiology".

1. Brief introduction for the candidate: Asst. Bilyana Marianova Ilieva completed her higher education at Sofia University «St. Kliment Ohridski», Faculty of Biology in 2004 and received an educational and qualification degree "Bachelor" majoring in Biology. In the period 2007-2008, she worked at the Bulgarian Academy of Sciences, Institute of Neurobiology, and her main scientific activity as a biologist was in the field of mediator mechanisms of drug effects. In February 2008, after successfully passing a competition, she was appointed as an assistant in the Department of "Animal and Human Physiology" at the Faculty of Biology, Sofia University "St. Kliment Ohridski», Sofia.

During her teaching experience in the department, Assistant Professor Bilyana Ilieva performed her official duties in the following areas - educational and scientific. She participates in the organization and conduct of practical classes with 3rd and 4th year students, majoring in "Molecular Biology", "Biology and Chemistry", "Ecology and Environmental Protection", "Biotechnologies", "Biology and Geography ", with Master's students in "Animal Physiology" and "Pharmacy". The inhibition of their synthesis, their secretion and their reuptake, as well as the importance of adrenergic innervation on the effects of these mediators, have been investigated sequentially. The scientific activity of Assistant Professor Bilyana Ilieva is realized with publications in our and foreign scientific publications. Some of them are indexed and referenced in world famous databases with scientific information - Web of Science and Scopus. She presented evidence of active participation in national and international conferences, where she presented her scientific achievements. She also participated in scientific projects, some of which were on the subject of her dissertation.

Simultaneously with her routine teaching activity, Bilyana Ilieva works hard to increase her professional qualification and personal competence. She uses written and spoken English B1 level. Has a good command of Microsoft Office products, document and image processing, graphic techniques, working with design software and graphic design, creating and maintaining

real-time audio and conferencing network training. Possesses excellent communication skills - skills for team work and independent work, ability to listen during contact with students during laboratory classes with them.

2. Relevance and significance of the problem of the dissertation work.

The dissertation work of Assistant Professor Bilyana Ilieva is dedicated to a current scientific problem, closely related to one of the important physiological functions (effects) of the recently identified peptide hormone obestatin related to the expression of the ghrelin gene, on the cardiovascular system, the importance of adrenergic innervation on the contractile activity of the heart myocardium, the cardioprotective effects of this mediator, as well as its potential therapeutic application in medicine. An important aspect in this direction is the development of a pharmacological approach to identify the target tissue through which physiological regulations are carried out by a catecholamine-dependent mechanism by studying the sequential inhibition of their synthesis, secretion and reuptake, as well as the importance of adrenergic innervation on the effects of this hormone. Data to date suggest that the role of obestatin in cardiovascular control is still unclear and controversial, sparking much discussion. The actuality of the problem and the practical benefit of the obtained results are the basis of the development of the present dissertation work.

3. Structure of the dissertation work. The structure of the dissertation on "Inotropic effect of obestatin on the heart of the frog *Pelophylax ribibundus*). Role of autonomic innervation", meets the accepted requirements for this category of scientific works and is presented in a mature professional and literary language and style. The dissertation is written on 115 pages, contains all mandatory sections, as well as publications related to the dissertation and is illustrated with the necessary number of figures and tables. The scientific work is focused on studying the participation of autonomic nerve endings in the effect of obestatin on the contractile activity of the heart of a frog (*Pelophylax ridibundus*) and investigates the mechanism by which this is realized. The bibliographic reference covers 284 sources, of which 2 are in Cyrillic and 282 in Latin. Of the total number of cited literary sources, 77.5% are from the last 10-12 years. Based on the conducted research, 5 scientifically based conclusions and 1 original contribution with potential therapeutic application in medical practice were made. The scientific output of the conducted research is realized in 3 scientific publications included in the dissertation work and 5 participations in scientific conferences and forums on the subject of the dissertation work.

4. Literary awareness. The literature review is presented on 65 standard pages. It is structured properly and is thorough, targeted and rich in information reflecting data on the structure and metabolic actions of obestatin on various organs, the established physiological effects and its mechanism of action. The morphofunctional features of the frog heart, as well as the mechanisms regulating cardiac activity, are also described in detail. The motivated overview reflects the broad awareness of Assistant Professor Bilyana Ilieva in the various aspects of the problem and the creative interpretation of literary data. This shows a critical approach to existing information and gives an analytical character to literary awareness.

5. Purpose and tasks of the research. The purpose of the scientific research is formulated clearly and comprehensibly and is in accordance with the title of the dissertation work and the literature review - to establish the participation of vegetative nerve endings in the physiological effect of obestatin on the contractile activity of a frog heart and the mechanism by which this is realized. The five tasks set to realize the purpose of the research are specific and correctly formulated. Their implementation guarantees the intended purpose.

6. Material and methods. The studies were conducted with an experimental model of an "isolated perfused heart" of a frog, and the preparation was modified and adapted to the specific needs of the study. The dissertation student points out that this preparation is extremely useful for studying the effects of pharmacological substances, hormones and other mediators directly on the organ, which is directly related to the research tasks. The experiments carried out, the chemicals used and the statistical processing of the data are subsequently described. A certain number of indicators have been selected that are directly related to the tasks set.

7. Results and Discussion. The results of the conducted research are presented on 16 pages and are very well illustrated with original figures and tables. The section contains a lot of original data obtained on the basis of the used set of modern methods and techniques. Concrete results have been obtained, which guarantee the achievement of the set goal and tasks. They have been processed statistically, clearly documenting the information from the conducted research and giving adequate answers with theoretical and scientific-applied significance. Based on the obtained results of the conducted experiments, certain regularities of important scientific and applied importance were established - presence or absence of an inotropic effect of obestatin on cardiac activity - in particular the amplitude (strength of heart contractions), in the presence and absence of certain pharmacological and biological active substances and inhibitors. The presence of a catecholamine-dependent mechanism, through which the physiological effect of obestatin on cardiac activity is realized, has been established. The analyzes used are a logical prerequisite for the credibility of discussion and formation of scientifically sound conclusions.

The discussion was conducted consistently, thoroughly and correctly, with the own results being interpreted and explained skillfully and at the same time compared with the data from other authors' research on the problem under consideration. In this regard, the results that aim to bring more clarity to the well-expressed positive inotropic effect of obestatin deserve attention. This effect of obestatin occurs through the activation of adrenergic receptors, mainly ventricular β -adrenoceptors. This occurs through the release of adrenaline from the varicose veins of the sympathetic axons, which leads to the observed positive inotropic effect of obestatin.

Based on the obtained results, 5 well-grounded and highly informative conclusions were made, which fully correspond to the experimental results. 1 original contribution is presented to the dissertation work, as well as a developed pharmacological method for identifying the target tissue, by means of which physiological regulations are carried out according to a catecholamine-dependent mechanism by studying the sequential inhibition of their synthesis, secretion and reuptake.

In connection with the topic of the dissertation work, 3 scientific articles have been published, printed in prestigious scientific journals. The presented contribution of an original nature has both

fundamental and scientific-applied significance for the scientific specialty "Animal and Human Physiology"

8. Conclusion.

In conclusion, I will note that the set goal and tasks of the scientific research have been successfully fulfilled. The dissertation submitted for review on the topic: "Inotropic effect of obestatin on frog heart." (Pelophilax ridibundus). Role of the vegetative innervation", developed by Assistant Professor Bilyana Marianova Ilieva for awarding the educational and scientific degree "Doctor" in the scientific direction 4.3. Biological sciences, the scientific specialty "Physiology of animals and man" is an independent, up-to-date and purposeful scientific development, which in terms of structure and content fully meets the criteria of the National Academy of Medical Sciences and the regulations for its application for obtaining the scientific degree "Doctor". That is why I give a highly positive assessment of the conducted scientific research and the obtained results, and **I strongly recommend to the members of the scientific jury to award the scientific degree "Doctor" in the scientific specialty "Physiology of animals and man" to Assistant Professor Bilyana Marianova Ilieva** in the scientific direction 4.3. Biological Sciences, the scientific specialty "Animal and Human Physiology", Department "Animal and Human Physiology" at the Faculty of Biology, Sofia University « St. Kliment Ohridski», Sofia.

08.02.2023

Sofia

Signature:.....

(Prof. Dr. Ya. Iliev, PhD)