REVIEW

Of the member of the dissertation council for the dissertation of *Oleg V Vetrovoy* on the topic: "*Long-term effects of prenatal hypoxia-induced impairment of rat brain development*", submitted for the degree of *doctor of biological sciences in the scientific speciality 1.5.4. Biochemistry*

The thesis presented by Oleg V Vetrovoy deals with a fundamental developmental problem afflicting many individuals – the long-term consequences of hypoxia and ischaemia, both pre- and post-natal, which can extend not only through childhood with developmental disorders but into later life, and ultimately can even be a contributor to occurrence of neurodegenerative disease. This is a growing research field that has been much underexplored, and it is therefore critical to understand the molecular mechanisms that can underlie these changes both pre- and early post-natal, as well as in later life. In a significant series of studies published in quality scientific journals Oleg V Vetrovoy has explored some of these mechanisms at a fundamental level, in particular addressing effects at the level of the genome. The work is novel and extends to exploring potential therapeutic regimes such as hypoxic postconditioning. There is an excellent balance of the work ranging from animal and cell models to underlying causative mechanisms at the molecular level. The thesis is clearly written and illustrated in good scientific style. Statistical analysis was appropriately performed and justify the data obtained. Although the command of English in the translated version of the thesis is not perfect in some places, it does not affect the readability and clarity of the scientific message.

The Introduction to the thesis covers the importance of the underlying research topic, the consequences, both short- and long-term, of hypoxia, which can extend into later life. Also, the role of underlying stress factors affecting various systems of the organism, particularly glucocorticoids, have been highlighted. The key regions of the brain, e.g. the hippocampus, that are involved in the changes are critically examined. The particular novel contribution of the work in the thesis is the analysis of epigenetic changes to brain chromatin and of particular transcription factors. In this respect the thesis represents a comprehensive overview of the current state of the field from the molecular level to the whole organism. The large diversity of the physiological and pathological consequences of prenatal hypoxia, particularly during early prenatal development, are thoroughly reviewed and discussed.

The main purpose and tasks of the dissertation are clearly specified. A particular novel focus of the present scientific work is in studying the effects of prenatal hypoxia on epigenetic (DNA and histone) modifications including the role of the hypoxia-inducible transcription factor HIF-1α. The work is broad-ranging and comprehensive, extending from study of effects of the

maternal stress response to effects of prenatal hypoxia on specific neuronal systems, spatial memory and neuronal loss. Modern analytical methods are employed in all aspects of the work.

Chapter 1 focuses at the molecular level on the changes induced in rat brain by prenatal hypoxia and identifies the methylation changes on histone H3 in specific brain regions. Of particular novelty are the age-related changes that are observed which are clearly illustrated in the accompanying figure and these changes are particularly relevant to understanding the mechanisms underlying the long-term implications of early hypoxia and maternal stress. A significant group of publications by the candidate in international peer-reviewed journals support the conclusions of this chapter.

Chapter 2 explores the role of hypoxia-inducible factor 1α (HIF-1α) in the effects of hypoxia on cell functions and particularly on cell energy metabolism via the pentose phosphate pathway (PPP). The author has also shown that an increase in HIF-1α levels induces a decrease of the main enzyme of the PPP- glucose-6-phospate dehydrogenase (G6PD). Application of the drug topotecan, which regulates gene expression and precludes formation of HIF-1α, was shown to have an opposite effect. Moreover, postconditioning with mild hypobaric hypoxia also had a protective effect both at the levels of HIF-1α accumulation and G6PD expression. The latter approach can be a valuable therapeutic strategy to protect cells and organisms against harmful effects of severe hypoxia both in the prenatal and postnatal periods. These conclusions have been well documented in the published papers in high-impact scientific journals.

Chapter 3 presents a detailed analysis of the effect of prenatal hypoxia on the hormonal levels (corticosterone) in maternal blood and on the expression of glucocorticoid receptors (GRs) in the hippocampus and peripheral tissues of the offspring. The data solidly demonstrate that maternal stress caused by hypoxia and the increase in maternal corticosterone levels lead to long-lasting changes in expression of GRs and affects a variety of parameters in the developing organism. These could be reversed by administration to pregnant dams of an inhibitor of corticosterone synthesis, metyrapone.

Chapter 4 is dedicated to an extensive analysis of the changes in the glutamate metabolism system in the hippocampus caused by prenatal hypoxia. As clearly shown by the results obtained in this study prenatal hypoxia impairs not only the system of synthesis and catabolism of this mediator but also expression of its receptor which inevitably leads to disruption of neuronal connections leading to memory deficits observed in animals during postnatal life. A very important observation relates to the fact that, despite reduced sensitivity in the hippocampus towards acute hypoxic episodes in postnatal life manifested as reduced formation of lipid peroxidation products, in general this defence system did not have sufficient capacity to protect hippocampal neurons against premature death which weakened the neuronal

network and led to early cognitive decline. These findings open therapeutic opportunities towards development of compensatory treatments of the consequences of prenatal hypoxia by balancing glutamate metabolism either pharmacologically or via physiological means such as hypoxic postconditioning.

The Summary chapter presents a comprehensive and critical analysis of the results obtained in this work and their place in global knowledge on this subject. The conclusions are substantiated by the data and provide a significant advance in our knowledge on how prenatal hypoxia affects brain development and functions at the biochemical and behavioural levels. The thesis is thoroughly and well cited and provides a wide background for further reading on the topic.

There are some questions to raise after reading the thesis:

1. It is known that topotecan induces apoptosis of cancer cells but in the dissertation work it was shown to reduce apoptosis in the hippocampus of rats that survived prenatal hypoxia. How can this specific response be explained?

2. How can it be explained that there was a decrease of HIF-1α in the hippocampus of two-week old rat pups that survived prenatal hypoxia on embryonic days 14-16 but further there was an increase observed at the age of 3 months? Which cellular events could lead to such changes taking into account that HIF-1α was shown in the previously published papers to be increased also in the embryonic brain?

3. How can the epigenetic changes in chromatin structures found in the hippocampus after prenatal hypoxia be related to the increase in the expression levels of glutamate receptors in later life?

Other comments on the thesis which do not diminish the significance of this work are:

1. There is a lack of graphical presentation of original results in Chapter 1 showing changes in the DNA methylation and histone methylation or acetylation during rat normal lifespan or after severe prenatal hypoxia. From Figure 1 summarizing such results it is difficult to see the exact levels of the changes in each particular case, which is discussed in this chapter, without referring to the published papers.

2. A very interesting aspect of the work dedicated to a protective effect of mild hypoxic postconditioning is only briefly described in Chapter 2 and not mentioned in the Methods sections.

3. The Methods section also only names the techniques used in the study not providing underlying biochemical background and reasons for selecting them.

4. There are no error bars and significance indications in Fig 4, A.

5. Discussing the effect of hypoxia in women and newborn children, it is not correct to refer to corticosterone but to cortisol (pages 173, 174).

In summary, the thesis and the work presented therein represent a substantial and novel contribution to a fundamental medical problem – early-life hypoxia or ischemia, their potential lifelong consequences and underlying molecular mechanisms as well as the potential methods for treatment. The work is presented clearly and concisely. This scientific work and the thesis itself, accompanied by internationally recognized scientific publications, are of excellent quality and represent a very significant contribution to this growing and important scientific field. The results of this work have been extensively presented in prestigious national and international scientific conferences. I therefore unreservedly declare that:

The dissertation of **Oleg V Vetrovoy** on the topic: "**Long-term effects of prenatal hypoxia-induced impairment of rat brain development**" meets the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". **The applicant Oleg V Vetrovoy deserves to be awarded the academic degree of doctor of biological sciences in the scientific speciality 1.5.4. Biochemistry.** No violations of paragraphs 9 and 11 of the specified Order have been detected.

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