ADHD – What Is the Meaning of Sex-dependent Incidence Differences?

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Abstract: There is a clear experience in clinical practice: boys with a diagnosis of ADHD are clearly in greater numbers than girls. It is noteworthy that even in the "older" review articles, the cause of sex-dependent incidence is not mentioned. If we accept the neurodevelopmental hypothesis of such disorder, then the possible genetic predisposition breaks down into two separate groups. On the genome of an individual with ADHD and on the genome of the parents. However, it cannot be overlooked that the incidence of ADHD (3–7%) corresponds to the incidence and sex differences of the number of newborns born at a certain risk (premature birth, immaturity, hypotrophy, hypoxic-ischemic syndrome, low birth weight, etc.). This association of possible genetic predisposition with "external" risks in the genesis of ADHD raises the question of whether a higher incidence of ADHD, as well as higher morbidity and mortality in males, are a) the norm and the female is privileged, or b) the female is the norm and the male is handicapped. The picture of ADHD includes various cognitive dysfunctions with one possible cause in norepinephrine and dopamine insufficiency. Experimental work shows that in response to stress females release more catecholamines in the CNS than males. Since catecholamines stimulate membrane Na^+ K^+ ATPase activity, this means both the value of the membrane potential and the threshold for activation is increased. Females are more successful in responding to and adapting to a stressful situation due to their higher production of noradrenaline in the CNS.

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Introduction

Despite great research efforts in ADHD treatment options, there are still many issues that have not been fully clarified yet. Here we try to extend our previous discussions on this topics (Mourek and Pokorný, 2021). If we look only at research conclusions that are not too old, a clear clinical experience prevails: boys with a diagnosis of ADHD are evidently more common in outpatient clinics than girls. At the same time, the authors do not hesitate to state the mutual incidence ratio of ADHD, which at that time was 10:1 (Bouček and Pidrman, 2005). With the onset of work that began to emphasize the genetic link (cause) of ADHD, the index (ratio) of sex-dependent ADHD began to change gradually, either in the sense that differences were not mentioned (e.g. Du Rietz et al., 2018), or only in the notes without specifying (Rubia, 2018). It is noteworthy that even in the "older" comprehensive studies on ADHD, the possible mechanism behind the differences in sex-dependent incidence of this disease is not mentioned at all (Tarver et al., 2014), or only touched (Konrad and Eickhoff, 2010). If genetic analysis was done, the sex difference was omitted and only male patients were studied (Swanson et al., 2000), though the work concerned newborns (Lou et al., 2004) or the young generation (Paclt et al., 2010). Contemporary papers admit the sex ratio ranging from 2:1 to 10:1 (Ramtekkar et al., 2010; Willcutt, 2012; Mowlem et al., 2019). Gender differences were not found in impulsivity, academic performance, social functioning, fine motor skills, parental education, or parental depression. However, compared with ADHD girls, ADHD boys displayed lower intellectual impairment, higher levels of hyperactivity, and higher rates of other externalizing behaviors (Gaub and Carlson, 1997).

Genetic predisposition

With the advancing therapeutic experience, the authors became interested in the real causes of the difference in the incidence of ADHD in males and females. The genetic predisposition breaks down into two separate groups. One is the genome of an individual with ADHD and the other is the genome of the parents. Studies on the genetic factors in the genesis of ADHD brought about several fundamental facts.
A) ADHD occurs in 3–7% (elsewhere 3–5%) children (according to anamnesis).
B) At the same time, there is a long-standing and "unshakable" fact that the same – or similar percentage – corresponds to the high-risk newborns (premature birth, immaturity, hypotrophy, hypoxic-ischemic syndrome, low birth weight, etc.). This connection of both "external" risks on the genesis of ADHD syndrome with a possible genetic predisposition is currently respected (Momany et al., 2017; Saez et al., 2018; Shaw et al., 2020).

At the same time, there is a long-established fact that the male gender has – compared to the female – a lifelong handicap: males have a higher mortality rate practically throughout their life, i.e. from the neonatal period to death. Since birth, there is also a higher morbidity, a higher incidence of prematurity and respiratory

distress syndrome (Steen et al., 2014; Pongou, 2015). Also more males are born with a birth defect (Zdravotnická ročenka České republiky, 2015).

We can therefore ask whether these facts constitute a "physiological" norm, i.e. the female gender is the norm or it is privileged by nature. This is not a pun, but a serious question of deeper biological significance, including the developmental aspects of the facts described above. The idea of the role of X and Y chromosomes in the sex-dependent differences in morbidity and mortality of males and females (i.e. not only in newborns) is related to the number of genes (Y chromosome contains only 50, while X approximately 3,000!). However, other pressing issues arise.

To date, the idea of ADHD as a neurodevelopmental disease is generally accepted, namely with a reflection on noradrenaline and dopaminergic signalling. This hypothesis reflects results of the therapy that has been using drugs blocking their reuptake. However, it is still difficult to link this fact to possible recessive elements on the Y chromosome. In this regard, a targeted genetic search (Šerý et al., 2015) is in place. Parallel findings of a lower volume of some areas of the CNS (central nervous system) (basal ganglia, corpus callosum, etc.) (Rubia, 2018; Tang et al., 2019) raise more and more questions. The smaller volume can be explained by lower number of neurons, their smaller volume, less numerous glial elements, reduced dendrification and reduced interneuronal connections or a smaller intercellular spaces. These structural changes may be related to the individual handicap from the beginning of life (low birth weight, immaturity, etc.) and can bring various defects in cognitive functions. Manifestation of ADHD symptoms can therefore be either a specific or completely general reaction.

Metabolic disorders

It is necessary to mention also the research findings that links and interprets ADHD to metabolic disorders. Changes in functional relationship between the glial and neuronal compartments has been mentioned (Russell et al., 2006) as well as the imbalance (mostly in terms of increase) of oxygen radical production (Kul et al., 2015; Sezen et al., 2016). The defective activity of mitochondria (without gender discrimination) and the resulting possible insufficiency in ATP production may be highly significant (Verma et al., 2016), suggesting an impairment of all mitochondrial functions, including their scavenger capacity.

Catecholamines

The catecholamine network in the CNS is very extensive, surprisingly, despite of the relatively small number of catecholaminergic neurons (locus coeruleus, substantia nigra and VTA – area tegmentalis ventralis) (Smeets and Gonzales, 2000; Mravec and Kiss, 2004). Axons of these neurons can form up to several thousand (!) synapses. Very specific property is also their ability (limited) of self-regeneration – probably with justified significance (Bjorklund and Stenevi, 1979). Catecholamines are – from a developmental point of view – a very strong conservative element.

The acknowledged fact about the combined effects of genetic predisposition + multifactorial epigenetic effects (during pregnancy, childbirth and early postnatal period) on the ADHD manifestation was finalized in the therapeutic area with treating ADHD as a noradrenaline and dopamine insufficiency.

The major problem of experimental analysis of the ADHD syndrome is the absence of a relevant animal model. Our research was therefore aimed at the already suggested hypothesis of the role of catecholamines in ADHD. The study is entirely based on laboratory rats (Wistar type). Animal studies are always argued whether the results apply to humans. However, we know – and this is a general experience – that animal experiments are a good steppingstone for further research.

Dopamine beta-hydroxylase (DBH) (E.C. 1.14.17.1) is an enzyme that converts dopamine to norepinephrine. This enzyme has been identified in synaptic vesicles of sympathetic nerve terminals, blood plasma, adrenal medulla, etc. Although it can hydrolyse various substrates, its activity in noradrenaline synthesis can be considered by far the most common and biologically most important. The fact that norepinephrine and dopamine – to a lesser extent adrenaline – are critical and essential molecules for functioning and behavior was first stated in 1965 (Kaufman and Friedman, 1965). The results of our first measurement are presented in Figure 1.

Throughout postnatal life, female Wistar rats showed a significantly higher concentration of dopamine beta-hydroxylase in plasma than males. This applies to the early postnatal period, to the period of the so-called "weaning" (around the

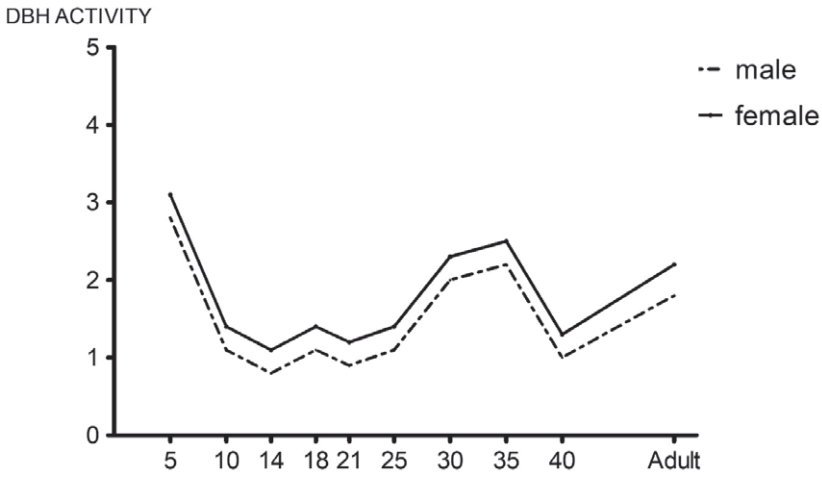

Figure 1 – Changes in dopamine beta hydroxylase in the plasma of female and male rats (Wistar). The measurements took place at intervals from the 5th day of postnatal life to adulthood (age 2 months). Enzyme activity is expressed in nmol/min/ml. Results in females are connected with a solid line, dashed in males.

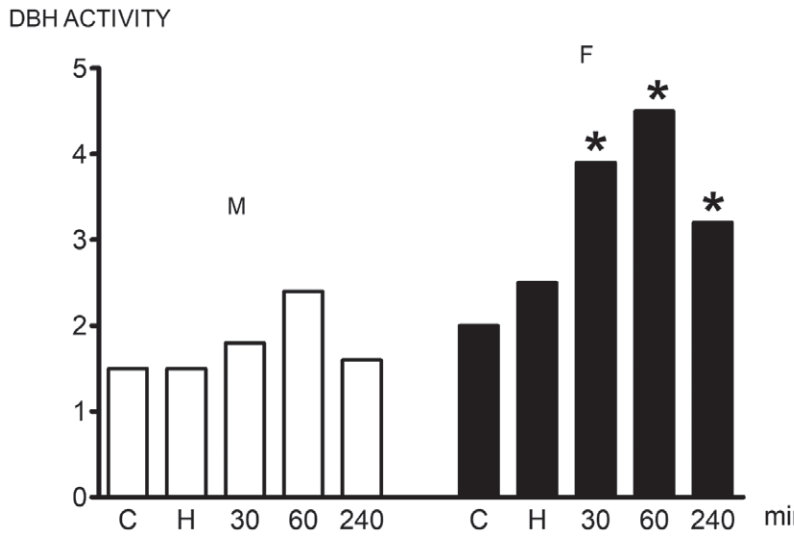

Figure 2 – The effect of stress, i.e. the effect of aerogenic altitude hypoxia on dopamine beta hydroxylase activity in the plasma of adult males (M) and females (F). The hypoxia corresponded to 7,000 m and lasted 20 minutes under euthermic conditions. C – control value, H – measurement immediately after the end of hypoxia, subsequent measurement after the end of hypoxia in time periods given in minutes. Activity expressed in nmol/min/ml. The asterisk indicates the statistical significant difference to control values (P<0.05).

21st day of postnatal life) and to the period of completed puberty (the 35th day of postnatal life). We exposed adult animals to 20 minutes of experimental stress. i.e. to so-called short-term altitude hypoxia, corresponding to 7,000 m, (pO_2 = 8.6 kPa) in a euthermic environment (Koudelová and Mourek, 1990). The results are presented in Figure 2.

We found highly statistically significant differences (much higher values of DBH) in females 30, 60 and 240 minutes after the stress. This higher response in females means a lot: it is accompanied with demonstrable benefits for the body = norepinephrine effects of on metabolism, cardiovascular system, etc. This can finally bring a more successful managing the previous stressful situation.

Table 1 – Ascorbic acid (ascorbate) content in cortex, mesencephalon and cerebellum in adult females and male rats (Wistar)

	Cortex	Mesencephalon	Cerebellum
Females	4.06 ± 0.09	2.50 ± 0.91	4.28 ± 0.32
Males	2.59 ± 0.04	1.57 ± 0.04	1.64 ± 0.05

Values are given in mmol/kg w.w. tissues (w.w. – wet tissues, immediately after dissection); averages ± SD (standard deviation)

Ascorbic acid

The synthesis of norepinephrine has one *sine qua non* condition for the effectiveness of DBH – this condition (or cofactor) is the presence of ascorbic acid as a reducing agent. Therefore, if DBH has consistently higher levels in females, and if these higher levels are to be able to increase activities at the same time, then a higher quantum of ascorbic acid must be available in the female. Therefore, we performed experiments to monitor the content of ascorbic acid in individual regions of the CNS (Koudelová and Mourek, 1991), including an experiment with stress. Results (on adult females and males [Wistar]) – in a simplified form presents Table 1.

Both in the cortex (grey matter) and in the subcortical areas of the mesencephalon or in the cerebellum, the ascorbic acid content was significantly higher in females than in males. It can be explained that DBH activity in females has a significantly higher capacity than in males. In another experiment, we measured ascorbic acid in the CNS (cortex and brain stem) after the exposure to a stressful situation (hypoxia corresponding to 9,000 m and lasting 20 minutes under euthermic conditions, pO_2 = 6.4 kPa). The results are given in Figure 3.

In both the cortex and medulla oblongata, stress-induced increase of ascorbate levels was always significantly higher in females – compared to males. These results correspond to our results with DBH. In reality, this means a greater capacity for the production of noradrenaline in the CNS, with larger (more adequate) functional

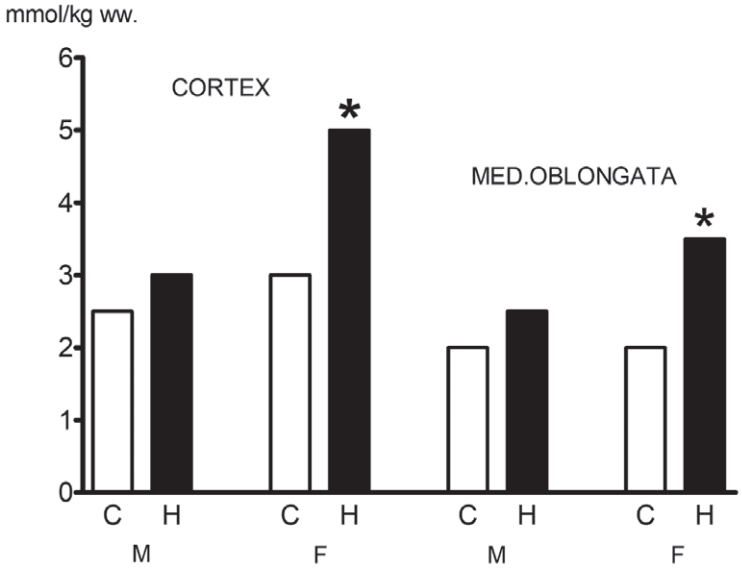

Figure 3 – The content of ascorbic acid (ascorbate) in the cerebral cortex and in the medulla oblongata of 21-day-old laboratory rats. C – control value, H – value after altitude hypoxia (9,000 m for 20 minutes). F – females, M – males. The asterisk indicates the statistical significance to control values (P<0.01). The ascorbate content is expressed in mmol/kg w.w.

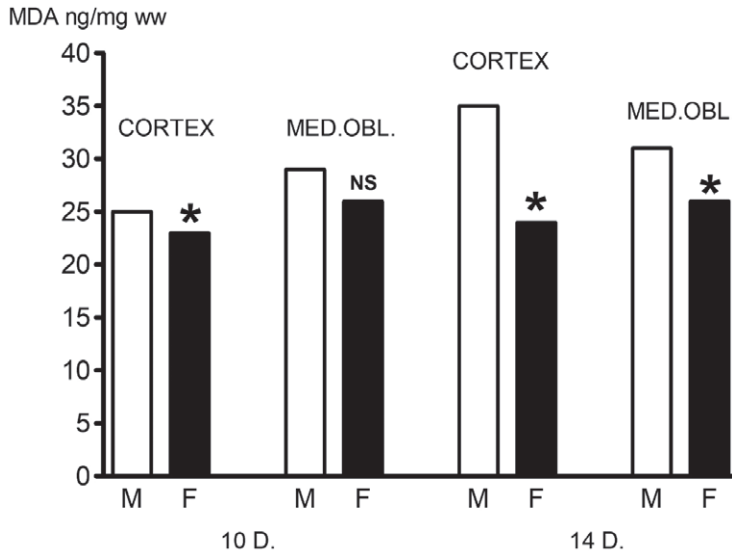

Figure 4 – Influence of altitude hypoxia (7,000 m, 20 minutes) on lipoperoxidation processes (production of malonyldialdehyde [MDA] in the cerebral cortex and spinal cord of 10- and 14-day-old laboratory rats [Wistar]). F – females, M – males. The asterisk indicates the statistical significant difference between males and females (P<0.01). MDA production in ng/mg w.w.

capacity. Indeed, catecholamines significantly increase membrane Na-K ATPase activity (E.C.6.1.3.) both *in vivo* and *in vitro* experiments (Mourek, 1979, 1985, 1987). This enzyme is an important factor contributing to the level of the resting membrane potential. An increased value of the resting membrane potential means a lower level of the neuronal excitability (the threshold for the activation is increased). Thus, in terms of today's ADHD therapy, females are significantly better provided than males.

In the introduction, we cited the authors (Kul et al., 2015; Sezen et al., 2016) who consistently report increased production of oxygen radicals (ROS) in individuals with ADHD. ROS attack various molecular structures of the mammalian cells, but they still have a certain "preference". These may be unsaturated fatty acids (especially localized in cell membranes) that in the oxidative milieu may undergo a disintegration process. Already in 2005 (Mourek et al., 2005) we carried out an experiment using laboratory rats at the age of 10 and 14 days of postnatal life. Both male and female animals were-repeatedly exposed to the stressor, represented by a 20-minute stay in simulated conditions of altitude hypoxia (corresponding to 7,000 m, pO_2 = 8.6 kPa). Subsequently, production of malonylaldehyde was measured in the cortex and in the spinal cord. The results are documented in Figure 4.

In all 4 groups, female rats showed lower malonylaldehyde production than males. Three of those differences were significant.

As already mentioned, since the very beginning of their existence, individuals with XY chromosomal combination have higher morbidity and mortality. Perinatal morbidity and/or exposition to risk conditions in boys represent a putative error factor for the developmental cascades, especially for such a sensitive and at the same time energy and substrate demanding development process as is the formation of CNS microstructure.

Conclusion

The presented results of experiments on laboratory rats represent a *de facto* hypothesis that can be applied to humans. Considering all its critical issues, we presume a certain validity of our findings to the possible therapeutic procedures. We consider the question whether the sufficient (= physiological) release of noradrenaline (and dopamine, to a lesser extent also adrenaline) in the CNS catecholaminergic neuronal circuits represents the basic regulatory element for the level of neuronal membrane Na-K ATPase activity in the regulation of neuronal excitability. The state, value and variability of the resting membrane potential of neurons is directly linked to their functional activity in the sense of a binary response (+ or –). We are fully aware that beside our hypothesis, there can be (and probably are) other "players" in the game.

We have demonstrated in three series of experiments that females carrying XX chromosomes are biologically favoured. This leads us to the final idea that the disparity between boys and girls in the incidence of ADHD symptoms is an expression of the basic biological axiom about the advantage (protection) of XX carriers. They are more important for the survival of the species.

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