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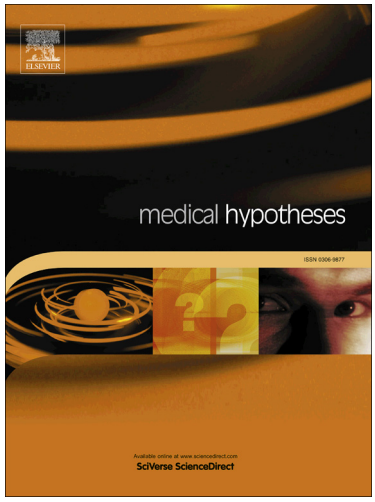

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Could ethanol-induced alterations in the expression of glutamate transporters in testes contribute to the effect of paternal drinking on the risk of abnormalities in the offspring?

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Abstract

It has been known that a preconception paternal alcoholism impacts adversely on the offspring but the mechanism of the effect is uncertain. Several findings suggest that there are signalling systems in testis that are analogous to those known to be altered by alcoholism in brain. We propose that chronic alcohol affects these systems in a manner similar to that in brain. Specifically, we hypothesise that excessive alcohol may disturb glutamatergic-like signalling in testis by increasing expression of the glutamate transporter GLAST (EAAT1). We discuss ways how to test the hypothesis as well as potential significance of some of the tests as tools in the diagnostics of chronic alcoholism.

Background

Presence of apparent cognitive deficits in children fathered by heavily drinking men has been known since antiquity (as noted in [1]). The mechanism(s) by which paternal alcohol-drinking inflicts damage on the offspring remain(s), however, obscure. It has been suggested that the DNA in male gametes affected by alcohol is altered epigenetically, e.g. by an interference in the methylation of DNA cytosine but the evidence, particularly when obtained in humans, is scarce and may be contradictory. Some groups have reported a decrease in the expression and activity of a DNA methyltransferases [1,2] resulting in a failure of orderly suppression of specific DNA sites in male-contributed alleles [1]. In contrast, results of other studies, using male mice exposed to ethanol, implied that an increased methylation of specific DNA loci, e.g. cytosine-rich sequences ("CpG islands") in the promoter region of the *DAT* gene encoding a dopamine transporter, could be at fault [3]. Disturbed expression of *DAT* has, indeed, been causally linked to an attention-deficit/hyperactivity disorder (ADHD)-like behaviour similar to that observed in the offspring of the ethanol-exposed male mice [3]; (cf. review of animal models of ADHD [4] and altered expression of *DAT1/SLC6A3* in human ADHD [5]). Here we propose an alternative hypothesis which, in our view, fits better the broad spectrum of deficits encountered in the offspring of alcoholic males. It is based on the probable presence in the testis of signalling mechanisms similar to those known to exist in the central nervous system.

The main synaptic transmitters in brain are L-glutamate (excitatory) and GABA (inhibitory); (for a historical review see [6]). Both glutamatergic and GABAergic synapses are thought to be strongly involved in mediating the effects of alcohol [7], particularly via the NMDA-type of glutamate receptor and the alpha4-subunit containing GABA(A) receptor [8,9,10]. Both GABAergic and glutamatergic signalling systems, including the two types of receptors mentioned above, are altered in alcoholism [11,12,13,14]. Interestingly, it is not only the synaptic receptors which are affected by alcohol but also the neurotransmitter-inactivating mechanisms that are changed in alcoholic brains, particularly in the case of glutamatergic neurotransmission.

In the central nervous system (CNS), synaptically released L-glutamate is inactivated by several specific transporters located mainly but not exclusively in the plasma membrane of surrounding astrocytes. There are five genes coding for the transporters (reviews: [15,16]) but protein products of two of them predominate: GLAST transporter encoded by *SLC1A3* and GLT1 transporter encoded by *SLC1A2*. GLT1 and GLAST are also referred to as EAAT1 and EAAT2, respectively, particularly when discussing human brains. Both transporters require Na^+ and K^+ transmembrane gradients as the driving force to transport L-glutamate from the extracellular space (reviews: [15,16]). In addition, GLAST acts as a chloride-selective ligand- (L-glutamate-) gated channel and thus has a capability to

hyperpolarise GLAST-expressing cells in the presence of L-glutamate [review: 16]. Both GLAST and GLT are mostly expressed in the central nervous system [15,16]; the only other tissue which expresses them in significant quantities is testis [17,18,19,20,21].

In the testes, GLT1 (EAAT2) has been detected in the interstitial cells but it is located mainly in the seminiferous tubules, both in the Sertoli cells and in the sperm. GLAST is found, apart from the Sertoli and interstitial cells, in the sperm, apparently in its anterior part, possibly concentrated in the acrosome [19,21]. The other glutamate transporters that have been detected in the testes are EAAT5 which acts mostly as an L-glutamate-gated chloride channel [22,23] and EAAT3 [19,21].

The precise role of glutamate transporters in the testes is not known. However, L-glutamate is the most abundant free amino acid in testis [24] and is present inside the seminiferous tubules. Moreover, the compartment is separated from the blood stream by a very tight blood-testis barrier formed by tight junctions in the Sertoli epithelium. The presence of L-glutamate (together with several "synaptic" proteins; cf. [19] and receptors [20,21]) sequestered behind such barrier would seem to imply that a signalling apparatus, possibly involving germ cells and sperm, both in their mature and immature forms either dormant or active and mediated by L-glutamate, exists and functions in testes. Glutamate transporters would then provide a regulating ("inactivating") mechanism analogous to that functioning at brain synapses (reviews: [12,13). Alternatively, L-glutamate transport, mainly by GLAST (EAAT1) and EAAT5 could trigger chloride influx thus hyperpolarizing and activating the sperm [21].

The Hypothesis

It has been reported that chronic exposure to large doses of alcohol is associated with significant (several-fold) increases in the expression of GLAST (EAAT1), both in mice and men [25,26]. Should a similar overexpression of GLAST (EAAT1) occur in testes under similar circumstances (chronic severe alcoholism), sperm would become much more susceptible to the activation than the sperm of non-alcoholic males possibly leading to a formation of active immature sperm. Subsequent fertilization by such immature sperm would significantly increase the risk of developmental defects in the offspring [27].

Evaluation

It should be understood that the developmental defects caused by the paternal alcoholism are distinct from the better known Foetal Alcohol Spectrum Disorder (FASD) and would be entirely independent of the alcoholic status of the pregnant female. The hypothesis does not negate the role of epigenetics as a mediator of the effect of paternal alcohol consumption on the offspring; there is ample evidence for such mechanisms from rodent studies (for a review see [28]). The present hypothesis adds, however, an extra element to the picture and could help to explain the complexity of the phenomenon [28].

Testing the hypothesis would present several significant challenges. Firstly, glutamate transporters are expressed in a number of splice variants and the splicing pattern in the testes appears very different from that in the CNS [23]. Use of single antibodies against GLAST (EAAT1) or GLT1 (EAAT2) in the testicular tissue could, therefore, easily miss or underestimate the full extent of the changes in GLAST (EAAT1) expression putatively caused by chronic alcoholism. Suitable antibodies against a range of splice variants are available [29,30] though not yet on commercial basis. The glutamate

transporter immunohistochemistry would have to be complemented by in situ hybridization using judiciously selected antisense oligonucleotides to reveal the full extent of the changes and their loci. Secondly, not just the presence but also the function of GLAST (EAAT1), GLT1 (EAAT2) and EAAT5 both in the seminiferous tubules and in the sperm would have to be investigated. Initial approach encountered significant methodological hurdles [21] but these can be overcome. Given the proposed hypothetical importance of the transporter molecules in the sperm activation, samples of sperm should perhaps be used directly in this type of studies. Thirdly, a link between the proposed overexpression of GLAST in the testes and/or in the sperm following chronic exposure to alcohol and observed deficits in the offspring would have to be established. This experimental component seems crucial in testing the validity of the hypothesis but may also be the most difficult, probably requiring a large (inter-disciplinary) animal-based approach. In the meantime, GLAST (EAAT1) expression in the sperm of alcoholic men could be looked at and perhaps evaluated as a possible diagnostic test for the severity of chronic alcoholism.

Conclusion and Significance of the Hypothesis

We propose that the mechanism of the effect of paternal alcoholism on inborn deficits in the offspring can be at least in part explained by a changed pattern of the expression of glutamate transporters in the testes of alcoholic fathers. This would involve, in particular, overexpression of GLAST (EAAT1) glutamate transporter. Testing the hypothesis presents significant methodological challenges but none of them seem in principle unsurmountable. Additionally, the tests could yield an important diagnostic tool potentially useful in assessment of chronic alcoholism in men. Should the hyperactivity of glutamate transporter GLAST be an important part of the mechanism, there is extensive pharmacological information on structural requirements of glutamate transporters that could greatly facilitate design and synthesis of specific inhibitors [31,32].

Conflict of Interest Statement

None of the authors has any competing interests

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List of abbreviations

ADHD, Attention Deficit Hyperactivity Disorder; CpG, Cytosine and Guanine separated by phosphate (the target of DNA methyltransferases); DAT, Dopamine Transporter; EAAT, Excitatory Amino Acid Transporter; FASD, Foetal Alcohol Spectrum Disorder; GABA, γ-aminobutyric acid; GLAST, Glutamate and Aspartate Transporter; GLT, Glutamate Transporter; SLC, Solute Carrier family of membrane transporter proteins.

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