PHYSIOPATHOLOGICAL AND THERAPEUTICAL CORRELATIONS IN ALCOHOL DEPENDENCE

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PHYSIOPATHOLOGICAL AND THERAPEUTICAL CORRELATIONS IN ALCOHOL DEPENDENCE (Abstract) No doubt, alcoholism represents nowadays the toxicomany with the highest expansion rate among all population groups, being recognized by the specialists from the medical, social, economic and legal field as a true “toxic pandemy”. Researchers consider ethanol, this small but highly aggressive molecule, to have supremacy if we were to consider the number of pages dedicated to it worldwide on daily bases, in the medical or any other specialty literature. Nonetheless, the large volume of data regarding ethanol toxicity does not seem to simplify things, on the contrary it points out new information about the its negative effects on human body. Ethanol represents a toxic that is rapidly and completely absorbed in the intestinal tract being distributed to most tissues and organs; ethanol is recognized as an enzymatic inductor of its own metabolism but also of the metabolism of numerous therapeutic agents. Keywords: DEPENDENCE, ALCOHOL, TOXICOMANY, WITHDRAWAL, BEHAVIORAL CHANGES

The biological mechanism of alcoholism are still little known reason why it relies especially on hypotheses concerning, mainly, the disruption of neurotransmission after the action of ethanol on cellular membranes and neurotransmitters. The organism’s sensitivity to this action seems to be, to a great extent, determined by genetic factors but, still, behavioral dependence represents the base of alcoholic toxicomany. Its origins indicate an organism predisposed to the consumption of alcohol combined with the availability of alcohol, their good social tolerance and low costs.

Physiopathological factors of physical dependence on alcohol
In general, it is considered to be three key factors involved in the complex phenomenon of the physical dependence on alcohol: intracellular metabolism; biological membranes and neurotransmitters which have constant interactions, especially at the level of neuronal transmission.

The effect of ethanol on intracellular metabolism. The main consequence of the use of alcohol is the considerable increase of the amount of H+ ions in the cells and the increase of the NADH / NAD relation which regulates the intracellular oxidoreduction potential. The result is the alteration of oxygen consumption and of the activity of mitochondria and of the numerous enzymes that intervene in various metabolic processes. For counteracting these effects, progressive metabolic adjustments
appear: increased degradation of ethanol, enzymatic qualitative and quantitative (metabolic tolerance) adjustments (1).

During withdrawal, the fact that the NAHD / NAD relationship decreases brutally disturbs the cellular functioning, mainly that of the nervous cells, case that might influence the beginning of the withdrawal syndrome. Consequently, it is necessary to increase the basis for the brain protein synthesis during chronic alcoholism.

The effect of ethanol on biological membranes. Biological membranes are functionally active entities due to the molecular intra-membranous components. They regulate transmembranous exchanges and also intervene in certain intracellular metabolisms.

The membranous matrix consists of two layers of phospholipids to which numerous molecules are associated: proteins (hormonal receptors, enzymatic systems as the Na+-K+ ATP-asis dependent, membranous transport systems) or glycoprotein (with role in cellular recognition, immune response) (1). For this system to work it is necessary:

- The physical condition of the membrane to be normal which is related to the lipids in the membrane and its mobility and it can be assessed by measuring the membrane fluidity;
- The membrane’s biochemical condition to be normal, which is the distribution, the spatial structure and the activity of the membrane proteins is satisfactory as well as the lipid-protein interaction.

Modifications of the membrane’s physical state

Nowadays it is known that a certain number of substances as detergents and general anaesthetics, including certain alcohols, have physical – chemical effects on the membranes.

Ethanol molecules are liposoluble and they can position themselves between the two layers of membranous phospholipids, conducting to the disorganization of the lipid matrix and the modification of the lipids lateral mobility and increase of membrane fluidity (1).

This effect depends of the dose, the same phenomenon being possible to appear in vivo conditions as well, disrupting the fluidity or the optimal micro-viscosity which is essential to the well functioning of the cells. In case of chronic intoxication there is the possibility of cell adjustment which manifests itself as tolerance to alcohol, with insufficiently identified mechanisms (2). Cells seem to be capable to increase the incorporation of saturated fatty acids and to diminish the ratio of unsaturated fatty acids. Another possibility would be the increase in cholesterol content at the level of the membrane, transmethylation, modification of protein synthesis etc. The result consists in lipid rearrangement which strengthens the structure and increases the stiffness of the membrane which diminishes penetration and the fluidizing action of ethanol (just like of all liposoluble molecules, which partly explains the well known resistance of alcoholic patients to general anaesthetics).

During withdrawal, the break of the new biological balance due to the disappearance of the fluidizing effect of ethanol will be responsible for the apparition of the temporary hyper viscosity of the membrane which will disrupt its functioning until the physiological structure is re-established.

Modifications of the membrane’s biochemical state

Ethanol is also capable of modifying the
functioning of membrane proteins (enzymes, transport proteins, receptors, surface glycoproteins) but it is important to point out that their answer depends to a great extent of: the proteins involved (for ex. Ca^{2+} dependent ATP-ase seems a lot more sensitive to the action of alcohol than Na^{+} - K^{+} ATP-ase dependent; protein localization, the kinetic features of the receptors seem to vary depending of the cerebral area and the genetic factors which have an important role in adjusting and establishing the sensitivity to effects of ethanol.

**Enzymes.** Until now there are few studies dealing with the alteration of biochemical membrane processes under the action of ethanol. Na^{+} - K^{+} ATP-ase dependent is the most studied enzyme as it intervenes in the active transport of sodium and potassium ions and maintaining their transmembranous gradient (1, 2).

In *vitro* studies have shown that after the administration of large doses of ethanol this enzyme is inhibited while after the administration of small doses of ethanol it becomes active.

This effect can explain the behavioural modifications induced by ethanol and it can be caused by the disruption of protein microenvironment consequent to phospholipids alteration accompanied by the modification of both spatial configuration and their activity (3).

**Calcium,** with a key role in membranous stability, in controlling intracellular signals and so in neurotransmission, is also disrupted by ethanol.

In case of chronic intoxication there is an increase of the calcium content in the synaptosomal membranes which seems to be the consequence of long term adjustment to ethanol effects.

**Receptors,** membrane proteins with particular specificity and affinity features for certain molecules can act at membrane level by coupling with an ionic channel or / and through intracellular syntheses, especially enzymatic, acting on the protein synthesis or on certain metabolisms.

The most famous system is the adenyl cyclase functioning unit – AMP cyclic protein kinase which intervenes mainly in the transmission of catecholaminergic signals. Ethanol disrupts the activity of this complex system either by modifying the physical structure of the surrounding environment of proteins, or by acting at the level of the regulating systems: calcium or/and calmodulin (calcium dependent activation protein). During the beginning of ethanol dependence it seems to be an increase in the sensitivity of the receptors at catecholamines. This can be due to the adjustment of the postsynaptic receptors to the activity of the presynaptic systems and to the effects of ethanol at membranous level (1, 3).

During withdrawal it was experimentally demonstrated the diminished answer of adenyl cyclase to the stimulation with dopamine. This decrease of sensitivity can also be pointed out at adrenergic ß receptors. This effect is inconsistent and depends of the brain area taken into consideration: present in the striatulum, it is absent at mesolimbic level because of the different characteristics receptors have in these two regions (4). The effect disappears if the laboratory animals are given sufficient amount of alcohol, which demonstrates that this system has become dependent on alcohol.

Another factor involved in the development of physical dependence on alcohol is represented by neurotransmitters, acetylcholine being the most widespread in the
central nervous system, it is heterogeneously distributed and acts on the muscarinic and nicotinic receptors (1, 4).

Ethanol definitely affects the cholinergic transmission; at very high concentrations of alcohol there will be an increased activity of acetylcholine transferase (which intervenes in the synthesis of acetylcholine) while the activity of acetylcholinesterase (degradation) is diminished. The release of acetylcholine is also disrupted (1, 5).

We must underline that that cholinergic system, especially at the level of the striated system is controlled through the D2 receptor of dopamine, the receptors can be affected by alcohol so that the muscarinic type cholinergic receptors undergo certain modifications during alcohol intoxication (2, 5).

The sensitivity of acetylcholine stands for the adapted answer to the effect of ethanol, but it is difficult to establish the eventual role of the cholinergic system, of the parasympathetic one in particular, during the withdrawal syndrome.

**Noradrenaline** acts through two families of postsynaptic β receptors (β1 and β2) which connect to adenyl cyclase and α, presynaptic (α1) and postsynaptic (α2) which are not connected to adenyl cyclase. Presynaptic receptors in association with neuromodulators (dopamine, GABA, endogenous opioids) regulate the synthesis and the release of noradrenaline. The role of this extraordinarily complex noradrenergic system consists in the modulation of the information arriving at the level of the projection systems.

The action of alcohol on noradrenaline and dopamine has been intensely studied but the results are highly contradictory. It appears that the acute administration of ethanol increases the urinary secretion of catecholamine stimulating thus their release at the level of the suprarenal (stress effect). Some experiments demonstrate that *in vitro*, ethanol diminishes synaptic release, reuptake and the renewal process of catecholamines. Some authors have demonstrated the biphasic structure of this effect: increased release and renewal process in a first phase followed by decrease (according to the behavioural effects of ethanol) (2, 4).

The studies conducted during chronic intoxications are more conclusive. They all underline the increasing renewal percent of noradrenaline and dopamine, as well as their synthesis by stimulation of tyrosine hydroxylase. Their release will be just as intense. All these elements certify the increased functional activity of the neurons which can be due to their adjustment to the depressive effects of ethanol.

**Serotonin** acts through two types of receptors, 5HT1 and 5HT2, with different locations and with different pharmaceutical features. Even if modifications appear, in particular a decrease of brain serotonin that has been invoked in the apparition of tolerance and dependence, it is difficult to point out an effect of alcohol on serotonin, during both acute and chronic phase. Some authors have identified a low level of serotonin during the acute phase and a higher one (due to the adjustment), as a consequence of chronic alcoholism.

There is the possibility that the alteration of serotonin receptors play a role in the appearance of physical dependence. Finally, just like in the case of catecholamine, the disruption of metabolism paths is possible. Thus, the excretion of 5-hydroxytryptophol a compound resulted from the reducing path, increases. Its association with acetilaldehyde will lead,
through various cycles, to the formation of molecules capable of acting at the level of the central nervous system, the β carbolinic acids (3).

β carbolinic acids stimulate the noradrenergic system and they are anxiogenic substances, causing nervousness and favouring the apparition of convulsions, which disappear after the administration of diazepam. The increase of endogenous synthesis of this type of molecules during chronic alcoholism might, consequently, facilitate, if not explain the clinical manifestations recorded during withdrawal.

The gamma-aminobutyric acid (GABA) is widely spread at cerebral level, approximately 30% of brain synapses being GABAergic. Due to its inhibiting feature, it is present mainly in the fast neural circuits, at the level of inhibiting neurons. GABA plays an important role in regulating catecholaminergic systems, inhibiting especially the extrapyramidal dopaminergic and mesolimbic function. Experimentally, an increased level of cerebral GABA diminishes the induced convulsive activities regardless on the induction method.

Various studies dealing with the action of ethanol on the GABA level have contradictory results which seem to be caused by the various protocols used and by the sensitivity differences between the dosing methods (5).

In case of acute intoxication, the GABA level appears to be normal or high, the blocking of Krebs cycle through the excess of H⁺ ions having the tendency to increase the level of glutamic acid and GABA, while in case of chronic intoxication, the GABA level is normal or low (4, 5).

During withdrawal, GABA is frequently low. Moreover, alcoholic patients experiencing convulsions seem to have the GABA level in the cephalorachidian liquid smaller if compared to the witnesses. GABA interacts with a complex system of receptors including at least three different sites: GABA receptor, benzodiazepine receptors (one, connected to the chloride channel, is responsible for the anxiolytic and convulsive action of benzodiazepine, the other, regardless of the chloride channel, induces the other features of benzodiazepine, especially the sedative effect) and the receiving site for picrotoxin, the exciting substance that blocks the GABAergic transmission without modifying the attachment of GABA on its receptor.

The inhibition of the GABAergic transmission explains, at least to some extent, the hyper excitability responsible for the apparition of trembling and convulsions during the withdrawal period, the injection of GABA or agonists attenuating or even suppressing these manifestations while its antagonists emphasize them.

Alcohol can, just as much, determine the disruption of benzodiazepine receptors, phenomenon present only for moderate concentrations and it disappears at large amounts of alcohol. In the clinic it can be underlined the potentiating effect of the association of alcohol and benzodiazepine.

In case of chronic alcoholism, the site of picrotoxin can be equally altered by ethanol decreasing the attachment capacity (3).

Endogenous opioids: are substances from the human body whose physiological action is accomplished through various receptors, determining different pharmacological answers. Thus, peripherally, enkephalins inhibit the release of P substance, transmitter of painful signals, and at central level, numerous aminergic structures, especially dopaminergic, are subjected to the control of opioids. At the level of
the hippocampus, enkephalins have an exciting effect: determine the increase of spontaneous release of pyramidal cells and facilitate the release of neurotransmitters inhibiting GABA through the neighbouring inhibiting interneurons.

Ethanol stimulates the release of endogenous opioids, fact witnessed by their plasmonic and intracerebral level. This answer might be on the other hand related to the stress caused effect which induces the suprarenal and cerebral release of catecholamines and enkephalins and the hypothalamic - hypophysarian secretion of β endorphin and stress hormones, prolactin and especially ACTH. This release might intervene, but only partially, in ethanol dependence.

Much more interesting is the study of the action of ethanol on the functional activity of opioergic systems. It was suggested that the modifications of dopamine metabolism are not due to a direct effect of ethanol but to an action of the enkephalinergic systems. It might be an increase of endogenous enkephalins, at least in some brain regions, but also the action of false neuromediators as tetra isoquinolines on receptors or a modification of the sensitivity of these receptors. It appears that the alteration of the opioidergic systems plays an important role in alcohol tolerance and dependence. In case of chronic administration of alcohol, the adjustment phenomena come into action, interesting the membranes and the secretion of endogenous opioids which will be low (2, 5).

Behavioral dependence. The abusive self administration of a substance relies at the base of the notion of behavioural dependence which can be motivated by a certain emotional condition or motivation; the presence of certain information necessary in triggering the behaviour; the beginning of a learning process; the existence of a voluntary decision to authorize and order the fulfilment of the behaviour.

Two fundamental brain areas intervene in these different phases: the neocortex, the centre of decision and dominating voluntary commands and the paleocortex, which lays at the basis of the state of need and of the learning processes due to the existence of sensorial analysis and memorization systems.

In case of alcohol dependence, the condition is not irreversible as the development of a motivation can allow the reestablishment of the normal control process at the level of the neocortex with the possibility of achieving withdrawal and abstinence.

The consumption of alcohol is induced by external, social-cultural factors and/or for the achievement of pharmacological effects. The perpetuation of this behaviour depends of the continuous presence of external factors, the development of physical dependence and the achievement of pharmacological effects, often being used for overcoming conflict situations which isolated can lead to the development of conjunctural, intermittent alcohol dependence.

CONCLUSIONS

There are numerous neurotoxic substances, alcohol being one of them, capable of determining functional perturbations on the nervous system (functional neurotoxicity) or lesions (structural neurotoxicity).

The word “alcoholism” evokes a particular behavior characterized by the dependence of repeated self-administration of alcohol. This fact requires first of all the existence of an initial impact between a toxic (alcohol) and a predisposed, vulnerable organism. This particular individual
receptivity, possibly genetically determined, expresses itself better if the surrounding environment and the social cultural context are favorable.

REFERENCES

A NOVEL MOUSE MODEL OF SOFT-TISSUE INFECTION USING BIOLUMINESCENCE IMAGING ALLOWS NONINVASIVE, REAL-TIME MONITORING OF BACTERIAL GROWTH

A recent study conducted by Yoshioka K. and collaborators from Japan successfully established a real-time, in vivo, quantitative mouse model of soft-tissue infection in the superficial gluteus muscle (SGM) using bioluminescence imaging. A bioluminescent strain of MRSA was inoculated into the SGM of BALB/c adult male mice, followed by sequential measurement of bacterial photon intensity and serological and histological analyses of the mice. The mean photon intensity in the mice peaked immediately after inoculation and remained stable until day 28. The serum levels of interleukin-6, interleukin-1 and C-reactive protein at 12 hours after inoculation were significantly higher than those prior to inoculation, and the C-reactive protein remained significantly elevated until day 21. Histological analyses showed marked neutrophil infiltration and abscesses containing necrotic and fibrous tissues in the SGM. With this SGM mouse model, they successfully visualized and quantified stable bacterial growth over an extended period of time with bioluminescence imaging, which allowed them to monitor the process of infection without euthanizing the experimental animals. This model is applicable to in vivo evaluations of the long-term efficacy of novel antibiotics or antibacterial implants. (Yoshioka K, Ishii K, Kuramoto T, Nagai S et al. A Novel Mouse Model of Soft-Tissue Infection Using Bioluminescence Imaging Allows Noninvasive, Real-Time Monitoring of Bacterial Growth. PLoS One 2014 Sep 3. PMID: 25184249).

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