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Calcium channels and heavy metals involved in autism

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A B S T R A C T

Autism spectrum disorders (ASDs) are a group of developmental disabilities that can cause significant social, communication and behavioral challenges, Autism is also a neurodevelopmental disorders characterized by impairments in communication and social behavior (Cermak SA 2010). The role of calcium channels in autism is an important part of research and new data will be developed in this review. Interestingly, the results show that many children with autism have a mutation in ionic channels and especially in the $Ca_{v1.2}$ and $Ca_{v3.2}$ calcium channels which are very sensitive to metals traces (Grabrucker AM. 2012). Other studies have shown that autism and behavioral disorders are closely related to poisoning by toxic trace metals or xenobiotics and also show that these toxins generate biochemical disturbances that may lead to physical and / or psychological disorders in children, with repercussions in adolescence and into their adult lives. The aim of this paper is to describe the impact of calcium channel activity on the autistic individual, and also to develop the results concerning levels of trace metals found in blood and urine of children with autism; then we will see the able link between calcium channel, heavy metals and autism and provide a study that can confirm or cancel this hypothesis of this triple interaction.

Introduction

Autism Spectrum Disorders (ASD), is a group of neurodevelopmental disorders appearing at an early age and are linked to impairments in communication, imagination and social interaction. Otherwise, individuals with this disease may have other impairments such as motor function and coordination, abnormalities in visual and auditory processing, also various

gastrointestinal symptoms, and immune dysfunction.

Recent studies have shown various biological abnormalities in autism, such as irregularities in cholesterol metabolism, neurotransmitter systems, mitochondrial enzyme activities, decreased cerebral blood flow and levels secretion rhythms of

hormones; and increased cerebral water content (N. BS Lozac 2007).

Autism has always been linked to several mutations, but we do not know how these mutations produce these specific deficits in cognitive and social behavior.

Studies have identified many such genes that either directly or indirectly control intracellular Ca^{2+} levels or are regulated by elevations in neuronal Ca^{2+} levels. These genes encode ion channels, neurotransmitter receptors and Ca^{2+} regulated signaling proteins that are crucial for development of the central nervous system (Krey JF 2007)

A review of recent studies shows that functional mutations on genes encoding voltage-gated calcium channels can lead to ASD.

One of these study reports the existence of Timothy syndrome, which is due to a recursive de novo mutation of calcium channel CACNA1C, in other words a mutation that prevents the inactivation of the channel and leads to Ca^{2+} currents prolonged inward; In addition to the CACNA1C channel, a CACNA1H voltage-gated Ca^{2+} channel has also been implicated in ASDs. The function of T-type channels in the brain is not completely understood, but they are known to regulate the oscillatory behavior of neurons in the cortex and thalamus.

Even if autism is often linked to the genetic component, it should be noted some discordance in diagnosis between some monozygotic twins, and suggests that autism is linked to environment causes associated with heavy metals and xenobiotic.

In this paper, we discuss these genetic aspect that support the hypothesis of the defects associated with calcium channels, we will

also talk about the link between environmental factors and Ca^{2+} signaling pathways in neurons.

Finally, we will propose some studies that can radically confirm or deny this triple interaction.

Calcium channels

Calcium is one of the major second messenger molecules most used by living cells, signaling carried by calcium ions play an essential role in many cellular processes and due to its universal nature of disturbance of calcium homeostasis in disease conditions may have many consequences (N. BS Lozac 2007)

Calcium enters the cell traversing the extracellular medium and runs through the plasma membrane and penetrates inside the cell and is regulated either by the pumps, or by protein buffers but we are more interested on the voltage-gated calcium channels located on the plasma membrane, this entry defines an important physiological event and the proper functioning of these channels is crucial in many cellular processes (N. BS Lozac 2007).

Voltage-gated calcium channels are in diverse cells of the human body and their pharmacological properties are independent of cell type where they reside (N. BS Lozac 2007)

L-type voltage-gated Ca^{2+} channel $Ca_{v1.2}$

L-type calcium channel are omnipresent in cells of the central nervous system, immune system and in the gastrointestinal tract.

The LTCC are vastly expressed in the brain, and their optimal activity is absolutely needed in many aspects of brain

development in the embryonic and postnatal level, also in migration and the structural organization of developing neurons.

Therefore the secretion of neurotransmitters and hormones is closely related to the calcium entry through these channels and also plays a definite role in the development of motor coordination and sensory processing. A mutation in a gene which encodes the calcium channel $Ca_{v1.2}$ leads to a multisystem disorder called Timothy syndrome. Disorder that involves a loss of a channel inactivation and calcium overload in several cell types, these disturbances prove the multitude of dysfunctions related to the disease such as: immune deficiency, congenital heart disease, hypoglycemia, cognitive abnormalities, irregular sleep patterns, and autism. The calcium channels $Ca_{v1.2}$ are essentially present in the cell bodies of mature neurons and dendrites, this channel is also able to regulate the properties of neurons by activating signaling pathways allowing the transcription of some genes. It has been described both direct and indirect mechanisms for transcription of certain genes.

The mutations associated with Timothy syndrome prevent voltage-dependent inactivation of $Ca_{v1.2}$, which causes the channels to have longer open periods and carry more Ca^{2+} than wild type channels. However this does not mean that the mutation prompted systematically on $Ca_{v1.2}$ channels induced consequences in neurons development and autism, which is why a hypothesis of environmental predisposition study is to investigate.

T-type voltage-gated Ca^{2+} channel $Ca_{v3.2}$

T-type channels have distinct functional properties compared to L-type channels, faster inactivation kinetics and activation

and slower deactivation kinetics, and since T channels are able to activate by low depolarization near the resting potential of the membrane, their functions are related to the control of neuronal excitability, so the studies announced for the most that T channels are involved in the treatment of neural signals.

A study of 461 individuals with autism showed that 6 of them expressed a CACNA1H ($Ca_{v3.2}$) mutation (Krey JF 2007), it was clear that the mutation was not the only factor, since other member of the same non-autistic family had the same missense mutation, the study we are starting to prove that in addition to genetic abnormalities, there may be environmental factors at low doses affect the start of this disease.

Environmental influences

We often approach the genetic component of autism but it should not be forgotten that several studies have shown that autistic individuals have a blood test with high percentages of heavy metals such as lead, iron, and zinc, and copper.

Environmental factors were long related to a wide range of neurodevelopmental deficits.

Prenatal exposure to environmental toxins leads to cognitive, motor, behavioral and sensory disorders; also as it has been proved that encephalopathy, convulsions or paralysis cerebral are associated with exposure of children perinatal at high doses of heavy metals.

Ion channels are a target of a many environmental toxins that are able of altering their activity. Numerous environmental agents, affect functioning of membrane as well as intracellular calcium channels, thus

affecting cellular calcium homeostasis (N. BS Lozac 2007) , in many types of cells, including neurons. These effects that we will try to develop on our laboratory, by experiments some heavy metals (Lead, iron, copper and zinc).

Lead

Lead is a heavy metal widely in nature since prehistoric times, as it is used in several sectors including the medicines, paintings, pipes, ammunition, vitrified ceramic, and, in more recent times, in alloys for welding, and also in some product dyes, jewelry, plastic bibs and in sets of colors.

All this, means that the concentration of this heavy metal in the environment has increased significantly in recent years and our exposure to lead is tangible everywhere.

The determination of the level of lead in the blood of autistic is complete and extremely easy to do but we note that the half-life of lead, mercury, and other toxic metals in the blood is weeks to months, The inability of the organism to manage and eliminate lead effectively causes this metal to accumulate in the body. Though the half-life of the metal in blood is only 35 days, in the brain it is about 2 years, and in bone it can last for decades so those metals rapidly leave the blood and accumulate in tissue and/or bone (Garza A 2006)

Lead intoxication can result in disruption of certain cellular signaling processing, the generation of action potentials in certain nerve cells, and the function of various proteins and enzymes.

Much of the damage caused by lead in cellular physiology is caused by its capacity to substitute for various polyvalent cations (calcium, zinc and magnesium) in their

binding sites, these interactions allow lead to affect various important biological processes, there including transport of metal, energy metabolism, apoptosis, ionic conduction, cell adhesion, and inter intracellular signaling, various enzymatic processes, protein maturation, and gene regulation (Garza A 2006).

Iron

Iron is used in living organisms mainly to transport oxygen, or catalyze electron transfer reactions, nitrogen fixation or DNA synthesis. In solution, the iron may exist in two oxidation states, the ferrous Fe (II) and ferric iron Fe (III). It is slightly soluble at physiological pH, especially when it is in the oxidized form of Fe (III) and to prevent it rushes, living organisms produce many proteins that are used to transport or store it in the cells.

Iron is essential for early brain development, because it contributes to production of neurotransmitters, myelination (development of a sheath around nerve cells) and immune function, said Dr. Rebecca Schmidt. All three of these functions have been associated with autism in other studies, but excessive levels can cause damage like disorder of calcium homeostasis and oxidative stress.

Zinc and Copper

Zinc is essential for all mammals because it has various actions on nerve cells, and it was often mentioned that it is very toxic to neurons in small quantities.

At high concentrations, zinc (Zn^{2+}) is naturally attached to cell membranes, so it is considered a reversible inhibitor of calcium, it is also involved in the kinetics of sodium and potassium currents.

Zinc is essential for many functions. Low zinc is associated with emotional instability, developmentally delayed slow growing digestive disturbances and alteration of the synthesis of proteins.

Copper (Cu), is also an essential element for living cells.

Its role is important in the redox reaction because it is easily converted from Cu^+ to Cu^{2+} , it is transported by the particular ceruloplasmin, antioxidant binding protein copper, which is synthesized in many tissues including the brain. High copper concentrations are linked to infection, inflammation, trauma, excessive food intake, systemic lupus erythematosus, and autism.

These low levels of zinc and high of copper have arrested several researchers who have begun to study the balance Cu/Zn which can be a path to new discoveries (Russo AJ.2011).

The aim of this review as stated before, is the highlight of some heavy metal imbalances that may be associated with calcium channel activity under consideration, these theoretical items allow us to begin an electrophysiological study to explore these aspects.

Calcium channels and heavy metals

Several ion channels are sensitive to the action of lead, as the basis for cellular excitability, this channels are the most important target for lead.

The voltage-dependent calcium channels allow the flow of a large number of monovalent and polyvalent cation, such as lead. The pore diameter and the length of the

ion channel involves a wide range of permeability, so that is why the channel is in a position to host in its interior more than one ion simultaneously (Fig. 2) (Garza A 2006)

When the affinity of cation is high in the EEEE locus, the input channel pore cation is faster. Even the interaction with the EEEE locus are competitive, it is usually the cation with the highest affinity that binds to the selectivity filter and displaces other ions from the channel pore (Garza A 2006)

All this explains why calcium channels are selective, their pore forming region has a higher affinity for calcium than sodium or potassium, for example, which are obviously more abundant.

But as it was shown by other foreign cation from the body, such as lead that have an even greater affinity for EEEE than calcium.

This allows cation such as lead, to flow slowly through the pore thereby acting as inhibitors of the channels, and involves the entrance of trace metals inside the cell.

While several types of ion channels are affected by lead, L-type calcium channels look like particularly sensitive. It is interesting noting that, whereas the main action of Pb is blockade of those channels and inhibition of calcium traffic, several in vitro studies also observed certain unexpected effects, such as improving the flow of calcium into in some cells or the sudden unblock of channels following strong depolarization (N. BS Lozac 2007)

This difference in reacting may be due to mutations in the calcium channel, a topic on which our team is interested. Iron has multiple roles in maintaining normal cellular function in the brain and in the other parts of

body. It is an essential cofactor in many important biological pathways, including neurotransmitter synthesis.

Iron and calcium are essential for neuronal function, but when they are in an excessive level, they induce neuronal injury and can even cause neuronal death. Some studies suggest that the closed voltage calcium channels (VGCCs) are an alternate route for iron entry into neuronal cell lines under conditions of iron overload (N. BS Lozac 2007).

Our next studies will discuss the action of iron (Fe^{2+} and Fe^{3+}) and the dose-response of this heavy metal on different types of calcium channels.

Copper and zinc are trace elements that play essential roles in several cell functions. They are present at high levels in the brain, particularly in regions such as the olfactory bulb, hypothalamus, and hippocampus (Donaldson et al., 1973; Ono and Cherian, 1999; Frederickson et al., 2000). These metal ions accumulate in synaptic vesicles, especially in some glutamatergic neurons (F. Aedo 2007).

Copper and zinc activate the spontaneous firing rate of olfactory epithelial cells when added in the nM and low μ M range, respectively. In contrast, at higher concentrations, they inhibit firing (F. Aedo 2007). The effects of copper and zinc are important for neuronal physiology because both ions are liberated to the synaptic space during normal neuronal activity.

It is very important to assess the effect of zinc and copper on the different calcium channels to evaluate the importance of this ratio in individuals with autism.

Conclusion

The interplay between calcium channels and autism is present and has been studied by various researchers, it implies that there are many mutations on calcium channels that can lead to autism, particularly in Cav1.2 and Cav3.2 calcium channels and have inhibitory effects, really like heavy metals, the idea now is to add different factors and compare them, this study that will explain that the dose response is not similar in all individuals.

Unfortunately, Heavy metals and calcium channels mutation does not represent the only causes of autism. Technological progress means that new chemical agents of uncertain pathogenic potential are entering the environment, various other environmental toxins are capable of modulating the functioning of calcium channels, including different organophosphates, phthalates and other industry compounds employed as solvents, flame retardants and pesticides.

Animal and in vitro and studies have demonstrated that various calcium antagonists, are able to attenuate negative effects of these agents on cell function and survival.

Even though potential adverse effects of heavy metals are usually known, limited reports are available regarding the investigation the relation between these elements and calcium channels and autism.

The impact of heavy metals on calcium channels will maybe never be the same, for a case with a mutation and wild type, which is the subject of my current electrophysiological research.

Fig.1 Predicted topology of CACNA1H showing the location of each mutation (Igor Splawski et al. 2006)

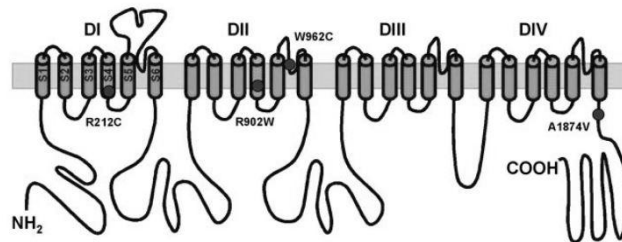
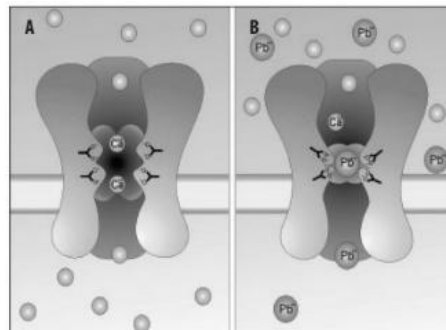


Fig.2 Model proposed for the function of the selectivity filter and its interaction with lead ions



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