REVIEW ARTICLE

Significance of the plasma membrane for the nerve cell function, development and plasticity

Jindrich Mourek [1,2], Milos Langmeier [1], Jaroslav Pokorny [1,3]

1 Charles University in Prague, First Faculty of Medicine, Institute of Physiology
2 Faculty of Health and Social Studies, South Bohemian University, České Budějovice
3 Institute of Health Studies, Technical University of Liberec.

Correspondence to: Prof. Jaroslav Pokorny, MD., DSc.,
Institute of Physiology, First Faculty of Medicine,
Charles University in Prague,
Albertov 5, 128 00, Prague 2, Czech Republic.
PHONE: +420 224968416; FAX: +420 224918816; E-MAIL: pokorny@lf1.cuni.cz

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Abstract

Lipoid character of plasma membrane namely the presence of polyenic fatty acids enables to interact with membrane proteins and in certain extent also to modulate their function. During the development, molecules of membrane fatty acids become more and more complex, and the ratio of polyenic fatty acids/ saturated fatty acids in the brain rises, while the concentration of monoenic fatty acids remained relatively stable. This phenomenon is apparent also in the ratio of unsaturated fatty acids OMEGA-3 in plasma of newborns which correlates with the birth weight.

Plasma membrane reflects local specializations of nerve cells. Its composition varies in functionally specialized regions called domains. Specialized domains of nerve cells determine the function of dendrites, soma, axon, axon hillock ect. Premature weaning of laboratory rats results in structural changes and in the increase of excitability of neuronal circuits in hypothalamus, septum and hippocampus which indicate the possibility of membrane composition changes.

In synapses, transport proteins of synaptic vesicles, act together with the specific proteins of the presynaptic membrane. Membrane proteins determine the release of neurotransmitter at different conditions of synaptic activity, and they can contribute to the recovery of neurotransmitter content after the repeated hyperactivity. In the model of experimental kindling, repeated seizures bring about decreases and distribution changes of synaptic vesicles.

Abbreviations :

CNS	- Central Nervous System
DHA	- Docosahexaenoic Acid
MMP	- Matrix-metalloproteinase
NGF	- Nerve Growth Factor
PUFA	- Polyunsatureated Fatty Acid
SNAP-25	- Synaptosomal Associated Protein 25
SNARE	- Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptors
TIMP	- Tissue Metalloproteinase Inhibitor
VGCC	- Voltage-Gated Calcium Channel

INTRODUCTION

The vast majority of works published in last decades and dedicated to interpretations and clinics of psychiatric diseases deals mostly with issues of neurotransmitters, their production, reuptake, metabolism etc. It corresponds with the boom of publications about receptor fields, immunity issues, stress etc. Only marginal part in these relations is dedicated to problems and status or changes of cell membranes of neural elements.

In series of symposia which took place at the occasion of Psycho-pharmaceutical congresses in Jeseník (2004–2009), (Mourek & Pokorný, 2005; Mourek *et al.* 2006a; Mourek *et al.* 2006b; Mourek *et al.* 2007) authors of this paper tried to correct such distinct disbalance focusing at various scientific aspects of the cell membrane.

Lipoid character of plasma membrane and its hydrophobic properties enable to form indefinite number of interaction with membrane proteins and in certain extent to modulate their function. The membrane is at the same time a dynamic and plastic structure. It makes the membrane vulnerable to many factors of the internal and external environment.

Plasma membrane reflects local specialization of nerve cells. Its composition varies in functionally specialized regions called domains. Specialized domains of nerve cells determine the function of dendrites, soma, axon, axon hillock etc.

In synapses, transport proteins of synaptic vesicles, act together with the specific proteins of the presynaptic membrane. They determine the release of neurotransmitter at different conditions of synaptic activity, and they can contribute to the recovery of neurotransmitter content after the repeated hyperactivity. In the model of experimental kindling, repeated seizures bring about decreases and distribution changes of synaptic vesicles.

THE ROLE OF OPTIMAL COMPOSITION OF LIPIDS IN PLASMA MEMBRANE FOR THE BODY DEVELOPMENT AND FUNCTION

The developmental studies the cell membrane composition and function brought several new impulses. In postnatal development of mammalian central nervous system (CNS) a steady increase in ratio of polyenic fatty acids/saturated fatty acids was identified while the concentration of monoenic fatty acids remained relatively stable. At the same time apparent differences in the maturation rate between individual regions of CNS were observed. The increase of PUFA OMEGA-3 (mainly docosahexaenoic acid) in the grey matter of the cortex represented approximately 20% of all present fatty acids. This applies both for laboratory rats and for humans. The supply of these acids in the blood plasma (their plasma levels) increased during the development in a similar proportion. More than that, we demonstrated highly positive correlation between newborn birth weight and serum concentration of PUFA OMEGA-3 (Mourek & Dohnalová, 1996) along with a significant lower levels of serum PUFA OMEGA-3 in the risk newborns (hypotrophy, gestational diabetes, immaturity, low birth weight). It is interesting, that the group of PUFA OMEGA-6 was in the risk newborns only slightly afflicted. At the same time, retardation or alternation of physiological maturation process was accompanied with the higher concentration of saturated fatty acids with shorter chain.

Another factor that markedly influences fatty acid composition of plasma membrane or the of pool fatty acids in the body is the activity of various stressors: Stress is related to the higher production of free oxygen radicals and such radicals preferably interact with polyunsaturated fatty acids. We described in experimental models of the stress a decrease of mainly PUFA OMEGA-3 in the brain, (Mourek *et al.* 1995) but the same applies for women in childbed and their newborns if a stress factor (adrenergic – pain) is studied (Partusisten, Mydlilova *et al.* 1991). Similar findings were described in animal experiments after the administration of adrenaline or isoprenaline (Mourek & Koudelová, 1997). Action of stressors (especially durable or inadequate) seems to bring about significant changes in the composition of plasma membrane in nerve cells. This occurs probably by destruction of PUFA (preferable by attacking PUFA-OMEGA-3). We consider it very important that various changes in homeostasis (hyperglycemia, hyperlipidemia, abundance of saturated fatty acids, disbalance and disproportion of PUFA-OMEGA 3/6, derangement of e.g. Mg^{2+} etc.) can cause a depletion of unsaturated fatty acids with long chain even in conditions of a sufficient supply of essential fatty acids (linoleic and linolenic acid). Such homeostatic changes have a negative effect on the activity of involved enzymes – desaturases and elongases.

Another factor that influences the fatty acids composition in plasma membranes is the nutrition. One of the most discussed questions is a disproportion between high PUFA-OMEGA-6 intake and insufficient PUFA-OMEGA-3 intake (Simopoulos, 2008). Given that ratio higher than 5:1 was acceptable some years ago, lowering (4:1) of this ratio is recommended nowadays. The abundant intake of saturated fatty acids can negatively influence the optimal composition of plasmatic membranes as well (negative effect on fluidity).

Additional interesting issue in the nutritional effects on the optimal development and composition of plasma membranes is the availability of breast milk. Balanced content of PUFA-OMEGA-3 in the breast milk as well as surprisingly high amount of docosahexaenoic and arachidonic acid in colostrum (Mydlilova *et al.* 1992) together with high antioxidant capacity of the milk are an indirect proof of the importance of these components for the physiological development. Unique structural and functional properties of docosahexaenoic

acid enable the function of various membrane proteins (channels, receptors, enzymes etc.). The long chain of this acid (22 carbons) and six double bonds allow the molecule to form hundreds of millions of stereometric variants (rotation and folds). The docosahexaenoic acid represents a structural-functional element which enables the optimal localization or molecule orientation of above mentioned proteins (Mourek, 2005; Maes *et al.* 2007).

Along with the maturation of body functions, undergoes maturation of plasma membrane of nervous elements (Tilney & Rosett, 1931). It becomes an optimal vehiculum and environment for implantation of functional proteins. The membrane is at the same time a dynamic and plastic structure which makes it also vulnerable to many factors of the internal and external environment. Alterations of the membrane composition, including changes in distribution of particular fatty acids (and probably also particular phospholipids) might represent a serious handicap for mentioned activities. We point out the fact, that as in cardiovascular diseases, psychiatric disorders and also in some autoimmune diseases – including diabetes mellitus – the depletion of PUFA-OMEGA-3 is detected – and again, supplementation with these acids (DHA and eikosapentaenic acid above all) can improve the patient's clinical and laboratory status (Mourek 2008; Mincke *et al*. 2006; Moura *et al.* 2008).

LOCAL SPECIALIZATION AND DISTINCTION OF NEURONAL MEMBRANE

Beside the semifluid phospholipids bilayer and molecules of cholesterol, membrane contains also glycolipids and proteins. Membrane glycolipids tend to aggregate at a defined region of the cell membrane (e.g. at the apical membrane of enterocytes) where they have a protective function and their antigenic properties enable cell recognition. Each protein has a specific function and to provide complex functions they aggregate within specialized regions of the neuronal membrane forming functional domains (e.g. receptor proteins combine with ion channels and interact with cytoskeleton in the postsynaptic membrane) (e.g. the axonal and dendritic membrane) (Lai & Jan, 2006). Some domains are very small (e.g. the head of a dendritic spine), and they are known as microdomains (Kado, 1994; An & Zenisek, 2004).

The typical example of a domain is the region of a synaptic contact, both at the presynaptic and postsynaptic side. Such domain contains receptor molecules for the interaction with neurotransmitter, ion channels and an anchoring them to the cytoskeleton, to the matrix of the synaptic cleft and partly also to the presynaptic element. Synapses can changes the effectiveness of signal transmission (the weight of a synapse) and therefore represent the place of a memory trace.

Interaction of membrane proteins with cytoskeleton determines various cell functions. The best known is the coupling of membrane excitation with interaction of contractile elements in the skeletal muscle during contraction or similar events in the smooth muscle or in several non-muscle cells, including neurons (Calderon-Velez & Figueroa-Gordon, 2009; Lee *et al.* 2009).

Interaction between plasma membrane domains and the cytoskeleton enables the function of some sensory cells. In the hair cells of the inner ear the cytoskeleton fibres operate the gate of mechanically gated ion channels and thus generate the receptor potential. In other cells cytoskeleton can transmit signals received by cell membrane to the nucleus in the processes of transcription control (Uribe & Jay, 2009).

Interaction of membrane proteins with surrounding cells and elements of the extracellular matrix is based namely on the activity of cell adhesion molecules. In the nervous tissue they are called N-CAM (neuronal cell adhesion molecules) (Panicker, 2003). They are member of a big family of substances structurally linked to immunoglobulines. N-CAM are primarily aimed to form mechanical connections between cells. Cell adhesion proteins unable also cell recognition and therefore they determine the cell migration, dendritic and axonal growth and synapse formation.

Important aims of the adhesive molecules during cell interaction are elements of the extracellular matrix which are the components of the nerve tissue microenvironment. Extracellular matrix is composed from a porous hydrated gel of polysaccharides with various fibrous structural proteins (collagen and elastin) and adhesive proteins (fibronectin, laminin). It provides not only a mechanical support to the nerve cells, but it also represents a communication medium for the flow of signalling molecules. It also enables elementary homeostatic functions (diffusion of nutrients, metabolites, respiratory gasses) and it gives room to neuroplastic processes (Bonneh-Barkay & Willey, 2009).

Membrane microdomains represent a dynamic system which determines the plasticity of neuronal circuits. Neuroplastic mechanisms are based on the possibility to modulate interactions of microdomains with the neighbouring elements. It requires a controlled release and renewal of bonds formed by adhesive protein molecules, which is the function of metalloproteinases.

Matrix-metalloproteinases are zinc-dependent endopeptidases and their proteolytic activity can be highly selective (focal) and time limited. More than 20 matrix-proteinases (MMPs) has been described and four of their endogenous tissue inhibitors (TIMPs). The common denominator of their function, the release of membrane microdomain interactions, enables migration, dendritic and axonal growth, synaptogenesis, and myelination during the development – the developmental plasticity (Jaworski & Fager, 2000). MMPs also control adaptation of neuronal circuits with the

functional load, changes of "synaptic weight" during the long-term potentiation and other forms of learning. Similar mechanisms are responsible for remodelling and synapse formation during recovery of neuronal circuits after impairment.

Regulation of the genetic expression for the formation of MMPs is controlled by growth factors, cytokines and some hormones (thyroxin, NGF). The newly synthesised proteins are activated by an enzymatic cascade with enzymes like plasmin, urokinase and MMPs in the mechanism of positive feedback. Important component in the control of MMPs activity are their tissue inhibitors (TIMPs) (Jaworski & Fager, 2000). They represent multifunctional proteins which also participate in the control of proliferation, migration, differentiation and apoptosis (Jourquin, 2005).

Metalloproteinases were also found to participate in various pathogenetic mechanisms of the nervous system. Due their role in the remodelling of neuronal circuits they participate in the development of epilepsy (Michaluk & Kaczmarek, 2007) during the progress of neurodegenerative disorders (e.g. Alzheimer disease, dementia) (Yong *et al.* 1998; Helbecquea *et al.* 2007; Bignami *et al.* 1994; Shibataa *et al.* 2005), or at some psychiatric disorders (Slotkin *et al.* 2007). MMPs play a role in permeability changes (Rosenberg, 1995) of the blood brain barrier and thus in the development of acute brain injury. MMPs promote neuronal death by disrupting cell-matrix interactions (Lee *et al.* 2004) and they are therefore expected to participate in the mechanism of at hypoxic-reperfusion syndrome (Waseem *et al.* 2005) or excitotoxic cell death (Tana *et al.* 2003; Riljak *et al.* 2007). In the brain and elsewhere in the body they are involved in the development of atherosclerosis and in the invasive growth of tumours (Lampert, 1998).

Effects of nutrition on the development of plasma membranes (Mourek & Dohnalová, 1996), discussed in the first part of the article, manifest also in global functions of neuronal circuits. Malnutrition during early development brings about impairment of the structure as well as of the integrative function of the brain (Cravioto, 1966). Experiments revealed that the premature weaning of laboratory rats at the 15th day of life which includes two days of fasting with subsequent switch from the high-fat maternal milk to standard high-saccharide diet, results in structural changes and in the increase of excitability of neuronal circuits in hypothalamus, septum and hippocampus (Nováková *et al.* 1972).

These results indirectly indicate the possibility of membrane composition changes after the premature weaning. However, the increase in excitability can also develop on the basis of changes in the mechanisms controlling the growth of neuronal processes and synaptic formation. It would result from altered activity of adhesion proteins and namely the enzymes which are responsible for cell contact remodelling (e.g., metalloproteinases).

SYNAPTIC MEMBRANE AND ASSOCIATED PROTEINS

Presynaptic apparatus is a dynamic structure namely in relation to the functional cycle of synaptic vesicles and to the related regulation of permeability in voltage-gated calcium channels (VGCC). Action potentials spreading along the presynaptic membrane open VGCCs which increase permeability for calcium. The influx of calcium into the presynaptic element triggers transport of synaptic vesicles toward the presynaptic membrane, and induces exocytosis and the neurotransmitter release into the synaptic cleft (Kumashiro *et al.* 2005).

At resting conditions, majority of the synaptic vesicles is anchored by the binding proteins of the actin type within the active zone and only a small part is in the direct contact with the presynaptic membrane. Elevation of the calcium level can induce relaxation of the connections among vesicles and at the same time activate transport proteins in their membrane.

Transport proteins, act together with the specific proteins of the presynaptic membrane forming so called "SNARE" complex (Chen *et al.* 2005; Stein & Jahn, 2009). Among the proteins of plasma membrane "Syntaxin" and "SNAP-25", among the vesicular proteins namely "Synaptobrevin" have essential role. To release synaptic vesicles from the plasma membrane and for the termination of the neurotransmitter release "Clathrin" and "Dynamin" are employed (Takei *et al.* 2005; Ellena *et al.* 2009; Clayton & Cousin, 2009; Clayton *et al.* 2009).

Interaction of vesicles with the plasma membrane follows two basic patterns. When the concentration of calcium in the presynaptic terminal is comparatively low, synaptic vesicles fuse with the membrane for a long interval (about 20 seconds) – mechanism "Kiss and stay" is employed (Fesce & Meldolesi 2005). At high levels of calcium, vesicle is released immediately after its interaction with membrane (< 1 ms), neurotransmitter is released by the process "Kiss and run" (Granseth *et al.* 2009; Wu & Wu, 2009).

Close relation between the distribution of synaptic vesicles and the activity of synapses was demonstrated in the study of time related changes in the distribution of vesicles in presynaptic terminals in the model of experimental kindling. One minute after the third repetition of a seizure, number of vesicles markedly decreases. The total number of synaptic vesicles drops to 34.2% of that in controls; the number of vesicles in the vicinity of the presynaptic membrane (the active zone) was only 33.3% of controls (p<0.001) (Langmeier *et al.* 1983).

Ten minutes after the third repetition of a seizure, number of synaptic vesicles markedly rose. The total number reached 195.4% and the number of vesicles in the active zone was 169.1% of that in controls (p<0.001) (Langmeier *et al.* 1980).

One hour after the seizure, number of synaptic vesicles was found to be the same as in controls. The total number reached 96.1% and the number of vesicles in the active zone was 93.2% of that in controls ($p<0.001$) (Langmeier & Mareš, 1985).

The initial seizure-induced decrease in the number of vesicles probably results form the exhaustion of the neurotransmitter pool after the repeated hyperactivity. Elevated number of vesicles found in interval of three minutes after the seizures can be the manifestation of restoration mechanism and gradual adaptation to the elevated functional demands. Effectives of the compensation show the normal ultrastructural image of synapses ten minutes after the seizures.

CONCLUSION

The reviewed data allow authors to draw a general conclusion and remarks:

As the extent of this article is limited, authors paid attention exclusively to – from their point of view – most important problems. They are aware of existence of several other issues and questions.

Authors mostly drew from their own experience and work and the article is not a full review but rather a set of their own findings.

The molecular aspect of plasma membrane composition is *de facto* a new problem and therefore more experience and experiments will be necessary to develop this concept.

Authors are fully aware that contemporary techniques and methods are not yet fully sufficient for the studying such problems (it is not known yet e.g. if the membrane composition of oligodendroglia or astrocytes is similar to plasmatic membrane of neurons; the differences in plasma membrane composition in different brain regions can be hypothesized etc., etc.)

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