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The Loss of Glutamate-GABA Harmony in Anxiety Disorders

Joanna M Wierońska[1], K. Stachowicz[1], G. Nowak[1,2] and A. Pilc[1,3]
[1]*Institute of Pharmacology, Polish Academy of Sciences, Kraków,*
[2]*Department of Pharmacobiology, Medical College,*
Jagiellonian University, Kraków,
[3]*Jagiellonian University, Medical College,*
Faculty of Health Sciences, Kraków,
Poland

1. Introduction

The proper functioning of the central nervous system (CNS) depends on the physiological homeostasis, which is itself maintained and regulated by two opposite forces acting independently to each other, flowing into a natural cycle and always seeking the balance. The thing is about two main amino acid neurotransmitters, glutamate and GABA, creating the opposite excitatory/inhibitory forces in the brain. Together, these two neurotransmitters constitute more than 90% of all neurotransmission, leaving less than 10% for the others. Therefore, to all the possibilities their mutual interaction determinates the proper functioning of the CNS. In Fig. 1, the schematic balance is presented, which mirrors the physiological equilibrium between GABA (represented by the white dots) and glutamate (represented as the black dots).

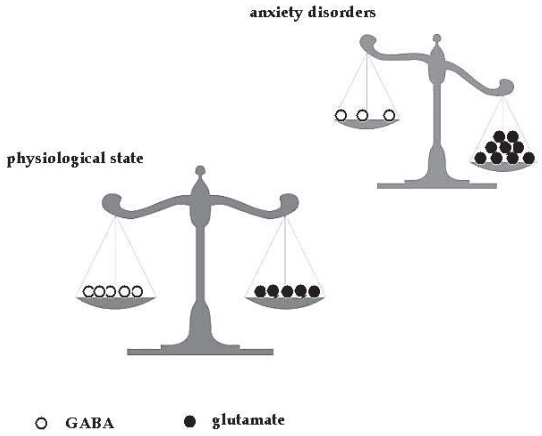

Fig. 1. The schematic balance presenting the equilibrium between GABA and glutamate in the physiological state and its loss (overactivation of glutamatergic system) in the anxiety disorders.

A variety of mechanisms keep the inhibitory/excitatory forces on the physiological level in the CNS. The disruption of the cycle leads, in consequence, to the advantage of one amino acid over another, resulting in psychiatric disorders. In the anxiety disorders that inhibitory/excitatory equilibrium is twisted into increased glutamate level, which will be discussed in Chapter 4. In this short review we will focus on that group of mental diseases in the field of Glu/GABA interactions; the insight into mechanisms of possible therapy will also be presented.

2. Glutamate-GABA turnover

The circle of GABA/Glu transformations is closed in the tripartite synapse, with no beginning and no end, as schematically shown in Fig. 2. In physiological conditions, the GABA vesicle content is in dynamic equilibrium with intraterminal glutamate concentrations (Mathews & Diamond, 2003).

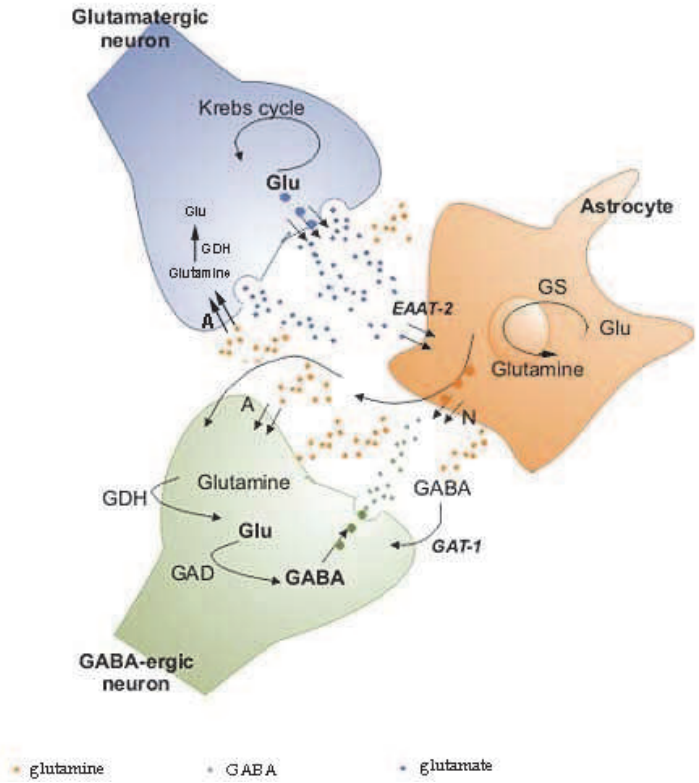

Fig. 2. The schematic presentation of glutamatergic-GABAergic transformation involving the pyramidal neuron, GABAergic interneuron and astrocyte. Glu-glutamate; GABA-γ amino butyric acid; GS-glutamine synthetase; GAD-glutamate decarboxylase; GDH-glutamine dehydrogenase; GAT-1-GABAergic transporter 1; EAAT-2-excitatory amino acid transporter 2; A-system A transporters; N-system N transporters

Glutamate is synthesized in neurons in the tricarboxylic acid circle (Liang et al., 2006) as well as in the glutamate-glutamine (GLX) cycle, which constitutes as an exogenous source of the neurotransmitter. In this cascade of events, glutamate, released from the presynaptic neuronal element, is transported to astrocyte through the EAAT-2 transporter. In the astrocyte, glutamine synthetase converts glutamate into glutamine, which is then transported into extracellular space through system N transporters, and is retrieved by the neuronal system A of amino acid transporters (Chaundhry et al., 2002). In neurons (both GABA and glutamatergic), glutamine is converted to glutamate in a reaction that is catalyzed by phosphate-activated glutamine dehydrogenase. In GABAergic inhibitory neurons glutamate further is converted into GABA by decarboxylation catalyzed with glutamic acid decarboxylase (GAD) (Liang et al., 2006). The inhibitory amino acid is then metabolized by transaminase to succinic semialdehyd and succinic acid, which re-enters the Kreb's cycle and is transformed into glutamate; the glutamate is released and uptaken by the astrocyte, and that closes up the cycle.
In the properly functioning CNS the release of neurotransmitters, and the neurotransmitters' effects evoked on target neuron is mediated by specific receptors.

3. Glutamate and GABA receptors

The neurotransmitter receptors of amino acids are split into several types, most broadly demarcated as ionotropic and metabotropic. Ionotropic receptors constitute as transmembrane ion channels that open or close in response to the binding of a ligand. These receptors convert the chemical signal of a presynaptically released neurotransmitter directly and very quickly into a postsynaptic electrical signal (Olsen & Sieghart, 2008), inducing the inhibitory postsynaptic potentials (IPSPs) or excitatory postsynaptic potentials (EPSPs), thus inhibiting or activating the neuron. Until now, two ionotropic receptors for GABA ($GABA_A$ and $GABA_C$) and three types of ionotropic receptors for glutamate (AMPA, KA, NMDA) have been discovered (Niswender & Conn, 2010; Olsen & Sieghart, 2008). The pharmacology of anxiety has been focused on $GABA_A$ receptors as the main site of action of ligands with anxiolytic activity. Type A of the GABA receptor is composed of five subunits of ligand-gated protein forming a pore selective to Cl^- anions. The subunits of the $GABA_A$ receptor constitute a relatively large family of several classes, including their splice variants (α1-α6; β1-β4; γ1- γ3, δ, ε, Θ, ρ1- ρ3). It enables the formation of a variety of combinations of specific subunits within the receptor, thus making it sensitive, or insensitive, to pharmacological manipulations (Millan, 2003; Olsen & Sieghart, 2008). Generally the receptor is a pentamer consisting of α, β and γ subunits in different combinations, and contains sites for the action of various endogenous and exogenous substances, such as neurosteroids, bicuculine, muscimol, benzodiazepines, ethanol, barbiturates and GABA (Olsen & Sieghart, 2008).
The NMDA receptor, one of the ionotropic receptors for glutamate, is both a ligand-gated and voltage-dependent heterotetrameric ion channel, consisting of two NR1 and two NR2 subunits (Conti et al., 1999). Activation of the receptor results in the opening of the non-selective channel to the cations. The receptor, similarly to $GABA_A$, possesses a variety of binding sites, such as the polyamine modulatory binding site, the Zn^{2+} modulatory binding site, glycine, glutamate, NMDA, MK-801 and phencyclidine binding sites (Danysz & Parsons, 1998). The schematic representation of $GABA_A$ and NMDA receptors with the most important binding sites present on each of them are shown on Figs. 3 and 4.

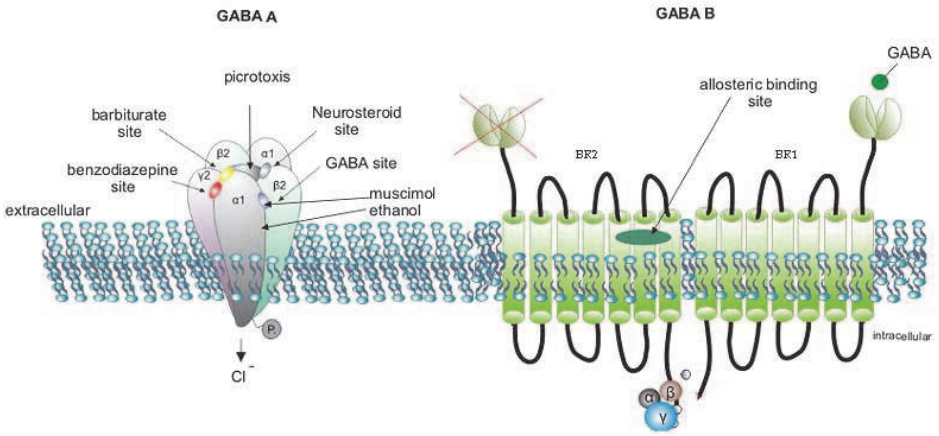

Fig. 3. The schematic representation of ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptors for GABA. The five subunits of $GABA_A$ and their binding sites are shown on the left and the $GABA_B$ heterodimer composed of BR1 (binding a ligand) and BR2 (coupled to G proteins and possessing the allosteric binding site, but not binding the ligand) is shown on the right.

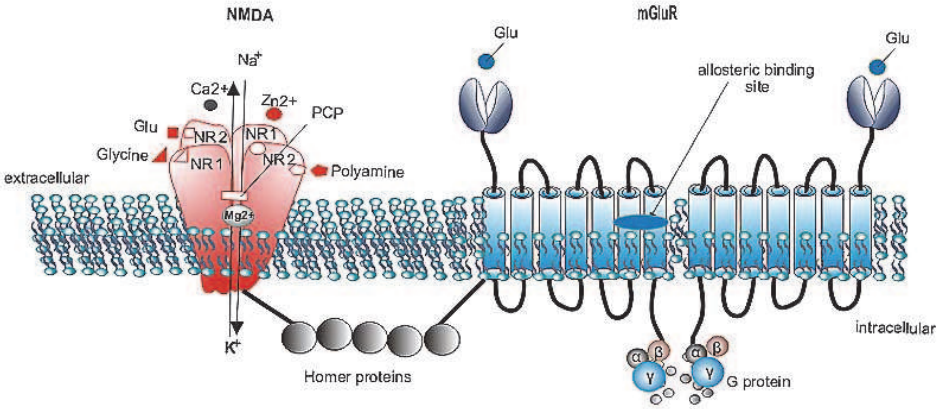

Fig. 4. The schematic representation of ionotropic (NMDA) and metabotropic (mGluR) receptors for glutamate. The four subunits of NMDA (NR1-NR2) and their binding sites are shown on the left and the mGlu homodimer composed of two identical parts of the mGlu receptor is shown on the right. Two orthosteric binding sites must bind a ligand to activate the receptor. An allosteric binding site is present within the 7 transmembrane domain.

By contrast, metabotropic receptors are connected with second messenger systems and exert a rather modulatory role in the CNS (Parmentier et al., 2002). Glutamatergic and GABA metabotropic receptors are linked to the G-proteins system and, opposite to the ionotropic ligand-gated channels, their action is slow and long-lasting (Bockaert et al., 2010). These receptors do not form an ion channel pore, although indirectly they can be linked to ion

channels through signal transduction mechanisms that induces the opening or closing of the channels (Ango et al., 2000). Metabotropic receptors on the presynaptic membrane can inhibit or, more rarely, facilitate neurotransmitter release from the presynaptic neuron (Schmitz et al., 2001). There is one metabotropic receptor for GABA ($GABA_B$ receptor) (Froestl, 2011) and 8 metabotropic receptors for glutamate (mGlu) (Pin and Duvoisin, 1995; Niswender & Conn, 2010). mGlu receptors are divided into three classes according to the sequence homology, pharmacology and the second messenger system they activate. The schematic classification of mGlu receptors is shown in Table 1.

The typical G protein-coupled receptor consists of seven hydrophobic transmembrane domains linked with extra- and intracellular loops, with the N terminus located on the extracellular side of the membrane and C terminus on the intracellular side (Parmentier et al., 2002). Both $GABA_B$ and mGlu receptors belong to the III family of the G protein-coupled receptors (GPCRs). The characteristic feature of this class of receptors is the forming of obligatory, functional dimers, possessing a large extracellular ligand-binding domain which closes up like a venus-flytrap after binding a ligand (Figs. 3 and 4) (Pin Duvoisin, 1995; Niswender & Conn, 2010; Bockaert et al., 2010).

mGluR	Group I positively coupled to phosphatydylo inosytol	mGluR1	a, b, c, e
		mGluR5	a, b
	Group II negatively coupled to adenylyl cyclase activity	mGluR2	
		mGluR3	
	Group III negatively coupled to adenylyl cyclase activity	mGluR4	a, b
		mGluR6	a, b, c
		mGluR7	a, b, c, d, e
		mGluR8	a, b, c

Table 1. The classification of mGlu receptors (Wierońska &Pilc, 2009)

The ligands of the GABAergic or glutamatergic receptors were shown to possess excellent anxiolytic activity (for review see: Pałucha & Pilc, 2007; Froestl, 2011). Below, the hypothesis of the possible mechanisms by which ligands of the receptors for two amino acids restore the lost in the anxiety inhibitory/excitatory balance in the CNS will be presented . But, firstly, a few words on anxiety.

4. Anxiety

The pathophysiology of anxiety disorders is a complex phenomenon and to designate one direct cause of their origin is almost impossible. However, those disorders recently became a serious public problem as a growing percentage of the population is being diagnosed with anxiety every year, and it is the most prevalent mental health problem in Europe and the United States (Wittchen & Jacobi, 2005). According to the classification of psychiatric disorders, the term Anxiety Disorders covers nearly 12 different pathological states (DSM-V), including panic disorder, generalized anxiety disorders, post-traumatic stress disorder, social phobia, specific phobias. These mentally ill people are then mainly excluded from

normal life for months or even years. Therefore, the search for effective and safe medicine is one of the goals of present neuropharmacology. The effectiveness of the drugs depends, largely, on the mechanism of their action. Thus, the important thing is to find out the functionally disrupted pathways in the CNS leading to neuropsychiatric illnesses and to indicate the possible targets for searching new psychotropic drugs. When it comes to anxiety disorders, the involvement of different neurochemical pathways were discussed during the past few decades.

Several neurotransmitters mediate the different components of anxiety, including excitatory amino acids such as Glu and inhibitory such as GABA. Generally, different aspects of anxiety response are mediated by various neurotransmitters in anatomically distinct areas. Important for our consideration dynamic balance between inhibitory/excitatory forces in the brain is thought to be disrupted with increased excitation leading to anxiousness. An increase in the glutamate efflux in the prefrontal cortex and hippocampus was observed after stress (Moghaddam et al., 1993; Bagley & Moghaddam, 1997). Anxiogenic behavior was observed in mice lacking the GAD65, enzyme responsible for converting Glu into GABA (Kash et al., 1999).

5. Present anxiolytics and future perspectives

In the pharmacological treatment of anxiety, drugs with a different mechanism of action are available. These include benzodiazepines, 5-HT1A agonists, and antidepressant medications. They all have their advantages and disadvantages, but as the review concerns GABAergic and glutamatergic neurotransmission, the compounds involving other mechanisms of action will not be discussed here, as they are widely described elsewhere (see: Millan, 2003).

The most efficacious anxiolytic drugs are the positive modulators (PAM) acting at the benzodiazepine binding site on the $GABA_A$ receptor, thus enhancing the affinity of the natural agonist to the receptor, known as benzodiazepines (Sternbach et al., 1974). The number of representatives of the group reaches nearly 80, and diazepam is probably the best known not only as an anxiolytic, but also as a hypnotic drug. Although the drugs have relatively good efficacy, a variety of adverse effects is also described. The most common are: ability to induce tolerance, sedation, myorelaxation, and dependence (Millan, 2003). Moreover, memory impartment and interaction with alcohol can occur. That is supposed to be connected with the activation of the α1 subunit of the $GABA_A$ receptor (Esclapez et al., 1996). The other binding sites of the receptor, such as barbiturates, muscimol or picrotoxin (shown on Fig. 3) are even worse drug targets. Although the anxiolytic-like activity of benzodiazepines is connected with activation of α2-α3 subunits, the majority of drugs activate to some extent the other subunits, too (Gao et al., 1993; Esclapez et al., 1996), thus being responsible for variety of adverse effects that may occur.

The discovery of the metabotropic $GABA_B$ receptor brought new possibilities for searching agents with the mechanism of action based on the enhancement of GABA transmission. Because of the relatively short time since the cloning of the receptor (which was in the year 1997), the clinically effective drug activating the receptor with anxiolytic efficacy are lacking at present; the orthosteric agonist of the receptor, baclofen, introduced in 1977 for the treatment of multiple sclerosis (Sachais, 1977), induces a variety of adverse effects including sedation and miorelaxation, whilst the anxiolysis was not discussed as an asset of the drug. However, in 2000 the first positive modulators of the $GABA_B$ receptor were discovered. The

preclinical trials were promising as all of the compounds possessed anxiolytic activity and were free of adverse side-effects typical for benzodiazepines, such as sedation or miorelaxation (Froestl, 2011). Interestingly, the antagonist of $GABA_B$ receptors are not active as anxiolytics, being rather described as possible antidepressants (Pilc & Nowak, 2005). Table 2 summarizes the main classes of the compounds activating GABA receptors, with special attention to their anxiolytic efficacy.

$GABA_A$ ligands	**benzodiazepine site PAMs** (benzodiazepines)	see: Millan, 2003
	GABA transaminase inhibitors (γ-vinyl GABA, aminooxyacetic acid)	Sherif et al., 1994
	GABA reuptake inhibitors (tiagabine)	Schaller et al., 2004
	GABA agonists (muscimol, THIP)	
	ethanol	Corbett et al., 1991
	neurosteroides	see: Millan 2003
$GABA_B$ ligands	**positive allosteric modulators** (GS39783, CGP7930, CGP13501, NVP-BHF177, (+)-BHFF)	see: Froestl, 2011

Table 2. GABAergic ligands with anxiolytic activity.

Pharmacological investigation of the glutamatergic system had lagged far behind research into the GABA systems because of the limitations connected with the use of ionotropic receptors ligands. Although some of the compounds acting at the NMDA and AMPA receptors were shown to possess anxiolytic activity, the adverse effects after the administration of antagonists of those receptors, such as the psychotomimetic effects and influence on locomotor activity were observed (Danysz & Parsons., 1998). The narrow window between therapeutic doses and doses inducing adverse effects caused a quick end to the therapeutic hopes connected with that receptor. However, it did not shatter the glutamatergic system as a target for anxiolytic drugs. A few clinical trials showed that some commonly used medications were found to exert their therapeutic effect by modulating glutamatergic transmission (*via* the inhibition of voltage-dependent ion channels). Additionally, these compounds were shown to be effective in anxiolytic disorders in randomized, double-blind, placebo-controlled trials (Table 3).

Compound	Anxiolytic activity tested in the clinic
Pregabalin	generalized anxiety disorder
Topiramate	post-traumatic stress disorder, specific phobias
Lamotrigine	post-traumatic stress disorder
Riluzole	generalized anxiety disorder
Tiagabine	generalized anxiety and post-traumatic stress disorder
Valproic acid	panic disorder, social phobia
Phenytoine	post-traumatic stress disorder
Gabapentin	social phobia
Levetiracetam	specific phobias, panic disorder
D-cycloserine	post-traumatic stress disorder, phobia

Table 3. Examples of anxiolytic-like activity of agents modulating glutamatergic activity (Amiel &Mathew, 2007).

The discovery of metabotropic glutamate receptors opened a broad range of possibilities to modulate the glutamatergic system. They became a target for putative anxiolytics, including antagonist, agonist or modulators (depending on the type of receptor they bind). A variety of subtypes involving different second messenger systems, with an expression in all of the brain regions both post- and presynaptically as auto– and heteroreceptors, make those receptors a very attractive therapeutic target. A number of preclinical studies clearly indicated that ligands of those receptors are excellent anxiolytics (Pałucha & Pilc, 2007; Wieronska & Pilc, 2009). Especially interesting agents were found among antagonists of the first group and agonists of the second group of mGlu receptors (Wieronska & Pilc, 2009). The clinical studies, which started in early '90s of the last century with fenobam, a drug with a mechanism of action that was unknown at the time, revealed that the compound was evidently effective as a novel, non-benzodiazepine anxiolytic (Porter et al., 2005). Today, we know that the drug is a negative allosteric modulator of the mGlu5 receptor. Similarly, the mGlu2/3 agonists have undergone positive clinical trials, such as LY354740 and its derivative, LY544344 (Dunayevich et al., 2008).

NMDA ligands	**NMDA channel blockers** *(memantine, MK-801)* **competitive antagonists** *(L-AP4, L-AP7, MDL100453, CGP37849, CGP39551, NPC17742)* **inverse agonists** *(ACPC)* **glycine site antagonists** *(5,7 dichlorokinurenic acid, L 701324)*	see: Danysz &Parsons, 1998
AMPA ligands	**antagonists** *(LY326325, LY382884, LY293558)* **2,3 BZD AMPA site antagonists** *(GYKI52466, GYKI53404, GYKI53655, EGIS8332, EGIS9637, EGIS10608)*	Alt et al., 2007 Kapus et al., 2008
mGlu ligands	**mGluR1 antagonist** *(JNJ16259685, AIDA, LY456236, EMQMCM)* **mGlu5 NAMs** *(MPEP, MTEP)* **mGlu5 antagonist** (fenobam) **mGlu2/3 agonists** *(LY 354740, LY 314582, LY 544344, LY 404039, LY 379268)* **mGlu2 PAMs** *(4-APPES, CBiPES, BINA, LY487379)* **mGlu2/3 antagonists** *(MGS0039, LY341495)* **mGlu4 agonist** *(LSP1-2111, ACPT-I)* **mGlu7 PAM** *(AMN082)*	see: Wierońska &Pilc, 2009 see: Pałucha &Pilc, 2007 Porter et al., 2005 Linden et al., 2005, 2006; Dunayevich et al., 2008 see: Wierońska &Pilc, 2009 Iijima et al., 2007 Stachowicz et al., 2008, 2009, Wierońska et al., 2010

Table 4. Glutamatergic receptors ligands with anxiolytic activity.

The third group of mGlu receptors is the biggest one and has been the least investigated so far, mainly because of the lack of selective and brain-penetrating agents. ACPT-I was the first brain penetrating compound, activating both mGlu4 and mGlu8 receptors. The compound exerted an anxiolytic-like efficacy in rodents (Stachowicz et al., 2009). Later on, a more selective compound, LSP1-2111 was synthesized, and was shown to preferentially activate mGlu4 receptors. Anxiolytic-like activity was described after the administration of relatively low doses (Wieronska et al., 2010). The glutamatergic receptors ligands with anxiolytic activity are listed in Table 4.

Taken all together it appears that, as stated above, both glutamatergic and GABAergic agents may evoke anxiolysis, mainly through agonistic action. Below the mechanism of action of those ligands will be introduced with an indication of a common direction of action leading to inhibition of the excessively active glutamatergic system.

6. Mechanism of action of GABAergic agents

The involvement of the GABAergic system, in particular the action of GABAmimetics stimulating GABA receptors, such as benzodiazepines, is a certainty in the present neuropharmacology of anxiety. The general mechanism of action of those compounds is an enhancement of GABAergic neurotransmission in the brain, which is tantamount to the enhancement of inhibition; however, the point is not in the inhibition per se but, rather, in the cascade of events caused by the inhibition.

As was mentioned above, GABA acts through three different types of receptors. As the pharmacology of the ionotropic $GABA_C$ receptor is the least investigated at present, we will focus on the mechanism of action of the ligands of two others: the ionotropic $GABA_A$ and metabotropic $GABA_B$ receptor (Froestl, 2011). Activation of both receptors causes an inhibition of neuronal excitability. However, what it means exactly depends on the expression of these receptors on the type of neuron.

6.1 $GABA_A$ signaling

Typical anxiolytic drugs, benzodiazepines, act by enhancing the inhibitory effects of GABA at $GABA_A$ receptors containing either an α_1, -2, -3 or -5 subunit. Postsynaptic expression of $GABA_A$ receptors composed of responsible for the anxiolytic-like efficacy α2 - α3subunits was shown mainly on GAD-positive neurons, that is GABAergic interneurons (Gao et al., 1993; Esclapez et al., 1996). Therefore, activation of those $GABA_A$ receptors [see Fig.5 (1)] would inhibit the GABAergic neurotransmission. Moreover, such an inhibition would, in turn, exert anxiolysis only indirectly, possibly through the disinhibition of the GABAergic projection neuronal element, increasing an inhibitory action on pyramidal target neurons. The described mechanism is supposed to be responsible for inhibiting glutamatergic neurons in structures mediating anxiolytic response, such as the lateral amygdala (Rainnie e al., 1991), and medial prefrontal cortex (mPFC) (Gigg et al., 1994).

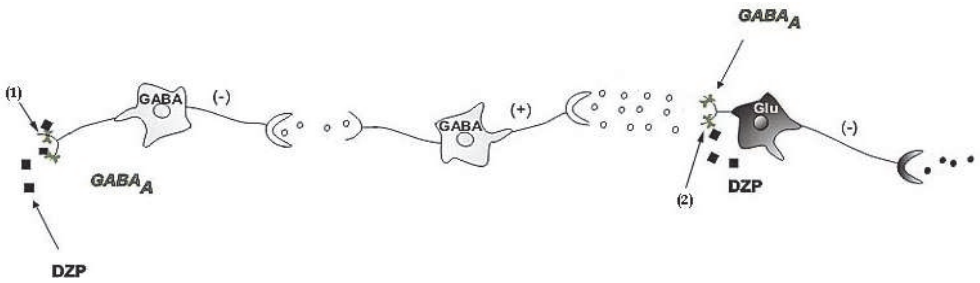

Fig. 5. The schematic neuronal network showing the mechanism of the postsynaptic action of the $GABA_A$ agonist, diazepam (see description in the text). empty dots- GABA; black dots-Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

The GABA released from inhibitory interneurons, and GABAmimetics administered exogenously, may also inhibit the glutamatergic pyramidal neurons *via* $GABA_A$ receptors expressed on the dendrites and soma of pyramidal neurons [see:Fig.5 (2)]. The activation of these receptors would directly lead to the inhibition of excitatory amino acid neurotransmission, as was shown in the electrophysiological studies on IPSPs in the glutamatergic neurons of the basolateral amygdala (BLA) (Chhatwal et al., 2005), as well as in the pyramidal cells of the piriform cortex and hippocampus (Samulack et al., 1993; Kapur et al., 1997).

Although the inhibitory effect of GABA mediated through the $GABA_A$ receptor is commonly considered to be postsynaptic, the presynaptically expressed $GABA_A$ receptors were also described in the variety of neurons in the CNS; however, the pharmacological properties of those receptors are relatively poorly understood. Mossy fibers in the hippocampus representing the axons of granule cells constitute one of the sites of the presynaptic expression of GABA receptors (Jang et al., 2006). Activation of these receptors induces neuron depolarization and facilitates spontaneous glutamate release (Jang et al., 2006). The standard anxiolytic drug, diazepam, was shown to induce an increase in the frequency of EPSPs and the potentiation of muscimol-induced glutamate release (Han et al., 2009). Although at first sight the effect may seem paradoxical, it may fit the theory of diazepam-mediated anxiolysis when considered through a variety of histological and electrophysiological data. The axons of granule cells synapse with a wide variety of inhibitory GABA interneurons in the hilar region of the dentate gyrus before continuing on to innervate pyramidal cells in the CA3 region. Therefore, the increased glutamate release by presynaptically active diazepam [see: Fig.6 (1)] would activate GABAergic interneurons which would then go on to inhibit increased excitation and thus lead to anxiolysis. Therefore, the circle closes up as the excitation leads to inhibition and inhibition inhibits the excitation.

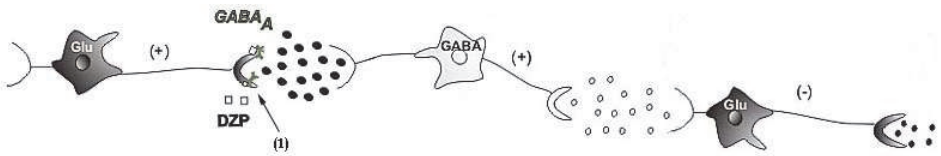

Fig. 6. The schematic neuronal network showing the mechanism of the presynaptic action of the $GABA_A$ agonist, diazepam (see description in the text). empty dots- GABA; black dots-glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

6.2 $GABA_B$ signaling

As an alternative to the ionotropic $GABA_A$ receptor, there is the $GABA_B$ receptor that is capable of exerting the slow and modulatory action of inhibitory neurotransmission, often in close association with the $GABA_A$ pentamer (Kardos et al., 1994). Similar to the above described ionotropic channel, the $GABA_B$ receptor was shown to be expressed both pre- and postsynaptically. Presynaptically expressed heterodimers are generally composed of $GABA_{B1A}$/$GABA_{B2}$ subunits, while postsynaptic neurotransmission is mediated by the $GABA_{B1B}$/$GABA_{B2}$ tandem (Billinton et al., 1999).

The $GABA_B$ receptor has only been cloned relatively recently, so the well documented clinical trials concerning the anxiolytic activity of its ligands are poorly available. However, based on the electrophysiological, histochemical and behavioural studies presenting its ability to balance the excitatory/inhibitory forces in the CNS, it is very likely that it may become a promising target in the search for novel anxiolytics. In the hippocampal slices, the subpopulation of interneurons was selected that inhibits pyramidal cells *via* $GABA_B$ postsynaptic receptors (Samulack et al., 1993; Forti et al., 1997), independently on $GABA_A$ signalling. Therefore, pharmacological stimulation of the $GABA_B$ receptor on pyramidal neurons would exert the inhibitory effect on glutamatergic transmission, thus inducing anxiolytic efficacy [see: Fig. 7 (2)].

Besides the inhibitory influence on pyramidal cells, $GABA_B$–mediated inhibition was also observed on inhibitory interneurons, when measured with whole cell patch-clamp techniques (Mott et al., 1999). Pharmacological stimulation of these receptors would inhibit the inhibition [see: Fig.7 (1)]. As described for $GABA_A$ receptor activation, such action exerts anxiolysis only after the disinhibition of GABAergic network innervating target pyramidal neurons.

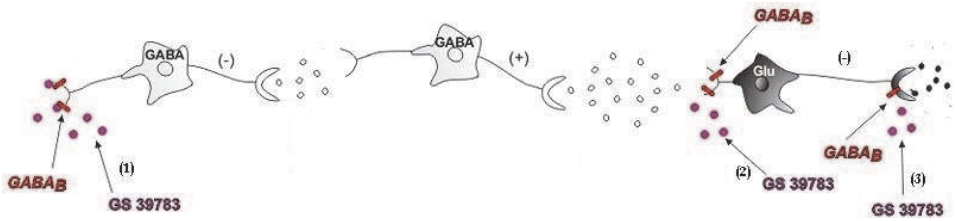

Fig. 7. The schematic neuronal network showing the mechanism of the postsynaptic action of the $GABA_B$ receptor positive allosteric modulator, GS39783 (see description in the text). empty dots- GABA; black dots-Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

The speculation on the mechanism of putative anxiolysis mediated by activation of the $GABA_B$ receptor does not end on the postsynaptic effects. Heterodimers were shown to be localized presynaptically along the extrasynaptic plasma membrane of axon terminals and along the presynaptic active zone in both asymmetrical and, to a lesser extent, symmetrical synapses (Lopez-Bendito, 2004). The fundamental role of these receptors is the inhibition of the release of the neurotransmitter [see: Fig.7 (3)]. The majority of presynaptically expressed $GABA_B$ heteroreceptors was found on the glutamatergic nerve terminals. The activation of these receptors would inhibit the excitatory amino acid release, resulting in anxiolysis.

7. Mechanism of action of glutamatergic agents

The vast diversity of receptors for glutamate creates a variety of possibilities to influence both excitation and inhibition in the brain. The age of glutamate began with the discovery of metabotropic glutamate receptors in 1986, which shortly became a promising alternative to iGlu receptors in a variety of investigations in the field of neuropharmacology (Nicoletti et al., 1986). Presently, the important role of mGlu receptors in anxiety is almost unquestioned and their role as the important anxiolytic drug targets is well established. However, the role of ionotropic receptors, NMDA and to some extent AMPA, is still significant despite the limitations connected with adverse effects induced by their ligands. The expression of the receptor was detected on dendritic terminals of glutamatergic neurons and interneuronal

post-synaptic sites, thus influencing the firing of both inhibitory and excitatory projections (Conti et al., 1999; Standaert et al., 1999; Ratzliff et al., 2001). The variety of different combination of NR1-NR2 subunits results in the existence of the diversity of receptor variants, expressed differently on a subpopulation of neurons and affecting function and selective vulnerability (Landwehrmeyer et al., 1995; Standaert et al., 1999). It creates a potential for altering the balance of inhibition and excitation independently in selected parts of the brain. For example, in the amygdale, the structure known as the responsible for storage of fear memories, NMDA, composed of NR1-NR2B subunits, was shown to be expressed mainly in the synapses of the central nucleus, while in the lateral nucleus the receptor contains both NR2A and NR2B subunits (Sah et al., 2003). It remains open for further investigation whether or not it has some functional meaning.

As was mentioned above, the iGlu receptors will not be discussed here as a putative target for new drugs. However, it is worth mentioning that the blockers of the NMDA receptor were shown to possess anxiolytic-like activity. One of the compounds, memantine, was effective in humans and has undergone successful clinical trials (Aboujaoude et al., 2009). In the preclinical studies, the antagonists of the second iGlu receptor, AMPA, exerted anxiolytic-like efficacy as well, supporting the important role of the iGlu receptors in anxiety (Kapus et al., 2008). The AMPA expression was predominantly found on pyramidal cells and interneurons, among others in the amygdale, known for its role in stress response (Sah et al., 2003). To all the possibilities the action of memantine is mediated *via* the NMDA receptor localized postsynaptically on inhibitory interneurons. Blockade of those receptors by the antagonist [see: Fig.8 (1)] would inhibit the GABAergic tone which would contribute to the stimulatory effect on inhibition followed by the inhibition of excitation.

This experimental data clearly shows that the blockade of the receptors exerts anxiolytic function. In the physiological conditions, the endogenous antagonists are not available or, at least, are not identificable, so the activity of the receptors is regulated predominantly by the glutamate. The level of amino acid regulates both the anxiety state and the anxiolytic response. As the iGlu receptor was shown to be expressed by the postsynaptic membrane of pyramidal neurons, the increased level of glutamate would lead directly to depolarization of the neuron and the activation of glutamatergic network activity, inducing an elevated stress response. Therefore, the anxiolysis could be induced by decreasing the level of endogenously released glutamate. Diminished glutamate release would activate the NMDA receptor to a lesser degree and the excitation of the CNS would remain at a stable level. Such an effect can be achieved by switching on the regulatory machinery of presynaptic glutamate release. mGlu receptors contribute to the effect, being the most important pawns in the circle.

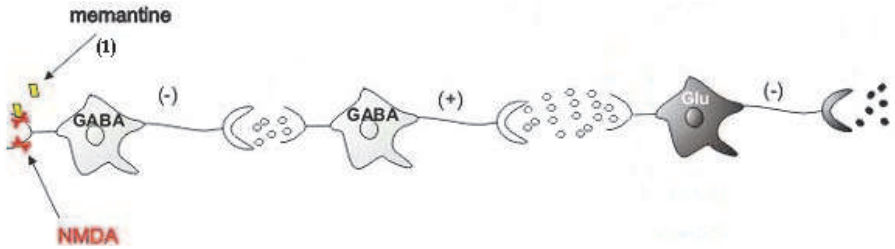

Fig. 8. The schematic neuronal network showing the mechanism of action of the NMDA receptor antagonist, memantine (see description in the text). empty dots- GABA; black dots- Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

Below, step by step, the possible mechanisms of the anxiolytic-like efficacy of metabotropic glutamate receptor ligands will be described, paying special attention to the excellent regulatory action exerted by the II and III group of the receptors both in asymmetrical and symmetrical synapses. The dynamically changing synaptic cleft environment and the amount of available neurotransmitters are dependent on their proper functioning. By contrast to the II and III groups of the receptors, the representatives of the first group tend to mediate the postsynaptic action which itself mediates the slow excitatory current (Pin & Duvoisin, 1995). From this group our deliberations start.

7.1 Group I mGlu receptors

Although the first group of receptors is predominantly distributed on the post-synaptic parts of the neurons, the presynaptic localization was also described.

The ligands of these receptors, especially the antagonists of the mGlu5 subtype, were shown to possess profound anxiolytic activity. A variety of preclinical experiments with negative allosteric modulators of the receptor, MPEP and MTEP, were further confirmed in the clinical studies, when fenobam, a mGlu5 antagonist, was first described as the non-benzodiazepine anxiolytic drug (see: Pałucha&Pilc, 2007; Porter et al., 2005).

The antagonistic action of MPEP, and probably other ligands acting at the mGlu5 receptor, results in the inhibition of stimulated DHPD PI hydrolysis and the neuronal firing in the CA1 area of the hippocampus (Kuhn et al., 2002), generally inducing the inhibition of the target cell. Immunohistochemical studies at the electron microscopy level indicate that mGlu5 receptors form functional oligoheteromers with NMDA receptors, and the group of Homer proteins is responsible for coupling mGlu5 with NMDA (Ango et al., 2000). Electrophysiological and biochemical studies confirm the functional dependence between these two receptors, as the NMDA-mediated current and NMDA-induced increase in the CREB phosphorylation were reduced by MPEP (Lindemeyer et al., 2006). The above findings characterize the inhibitory nature of the mGlu5 antagonists. However, the mechanism of the anxiolytic-like efficacy of the compounds involves the target neuronal elements expressing the receptor. Based on electrophysiological and immunohistochemical data, mGlu5-NMDA complexes are expressed predominantly on the inhibitory interneurons in the hippocampus (Sanon et al., 2010), cortex (Sarihi et al., 2008) or amygdala. As such, it would appear that the MPEP-induced inhibitory action on GABAergic interneurons is responsible for its anxiolytic effect [see: Fig.9 (1)]. This inhibition of the inhibition results in

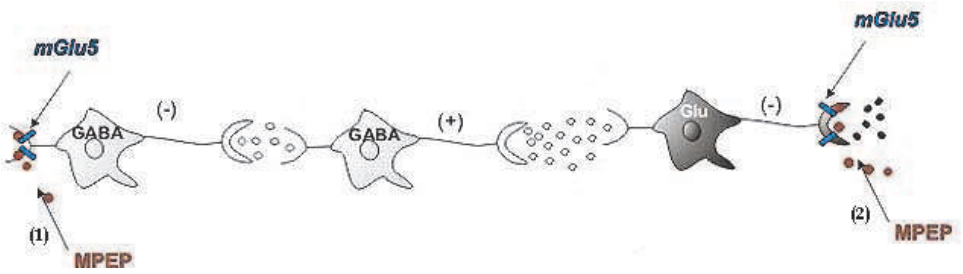

Fig. 9. The schematic neuronal network showing the mechanism of action of the MPEP, mGlu5 receptor antagonist (see description in the text). empty dots- GABA; black dots-Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

a rich repertoire of changes which take place the synapses between interneurons and pyramidal neurons to induce feedback inhibition of increased excitation. Indeed, as microdialysis studies indicate that the level of glutamate, yet not GABA, is decreased after MPEP administration, at least in periagueductal gray, one of the structures involved in anxiety response (de Novellis et al., 2003).
Although it seems that anxiolysis induced by mGlu5 antagonists is mediated mainly through their action at the postsynaptic site, the presynaptic expression of group I mGlu receptors was also described. mGlu5 labelling at axon terminals was not so intensive as on neuronal somata, but was observed in some glutamatergic axonal terminals (Rae et al., 2004). The blockade of these receptors would inhibit the release of glutamate, causing anxiolytic response [see: Fig.9 (2)].

7.2 Group II mGlu receptors

The second group of metabotropic glutamate receptors involves mGlu2 and mGlu3 subtypes, negatively stimulating adenylyl cyclase activity (Niswender & Conn, 2010). The majority of the agonist and positive modulators of these receptors possess excellent anxiolytic efficacy. Among the available ligands it is hard to find one not showing such activity in the preclinical studies. As the agonists mostly activate both subtypes of the receptors, the estimation of the independent participation of each subtype in anxiolysis is difficult. Selective positive modulators of the mGlu2 subtype exert anxiolytic-like efficacy indicating the important role of the receptor in mGlu2/3 agonist-mediated anxiolysis. However, the role of the second subtype is more enigmatic because of the lack of selective, brain penetrating agents acting on the mGlu3 subtype. Some indirect conclusion can be drawn on the basis of the results obtained with the use of mGlu2 knockout mice. The majority of effects observed after administration of mGlu2/3 agonists were lacking in these animals, suggesting the mGlu2-dependent action of ligands (Linden et al., 2006; Woolley et al., 2008). Although some controversial results showing the involvement of the mGlu3 receptor in mGlu2/3-mediated anxiolysis can also be found (Linden et al., 2005), the activation of the mGlu2 receptor seems to be crucial for mGlu2/3 agonists-mediated anxiolysis.
Among all of the mGlu receptor subtypes located in structures connected with fear response, mGlu2 receptor seems to be expressed predominantly on glutamatergic terminals, in pre-terminal rather than terminal portions of the axons (Petralia et al., 1996; Shigemoto et al., 1997). The expression of the mGlu2 receptors, as shown in the diagram, suggests that activation of the receptor occurs during abnormal and elevated glutamate release, allowing the neurotransmitter to regulate its own release. As the receptors are not in close association with glutamatergic synapses and a subpopulation of the receptors not associated with any synaptic junction was identified, the receptor can be probably activated by glutamate of a nonsynaptic origin. The astrocytes constitute the main source of this additional glutamate pool in the CNS [as shown on Fig.10]. The glutamate released by single astrocyte onto adjacent neuronal processes controls simultaneously the excitability of several neighboring pyramidal cells (Angulo et al., 2004), and the mGlu2 receptor could play an important role in this process. Besides this, a growing line of evidence indicates that glutamate is able to escape the synapse from which it is released and diffuse into neighboring junctions to activate receptors there (Diamond, 2002). The occurrence of this type of heterosynaptic inhibition was demonstrated at mossy fibre synapses in the hippocampus (Vogt & Nicoll, 1999), the place where there is rich mGlu2 innervation (Petralia et al., 1996). Exogenously

administrated compounds acting on these receptors would restore the twisted excitatory/inhibitory balance independently on synaptic machinery, playing the supportive role in the self-regulating circle, which can itself be disrupted in a pathological state.

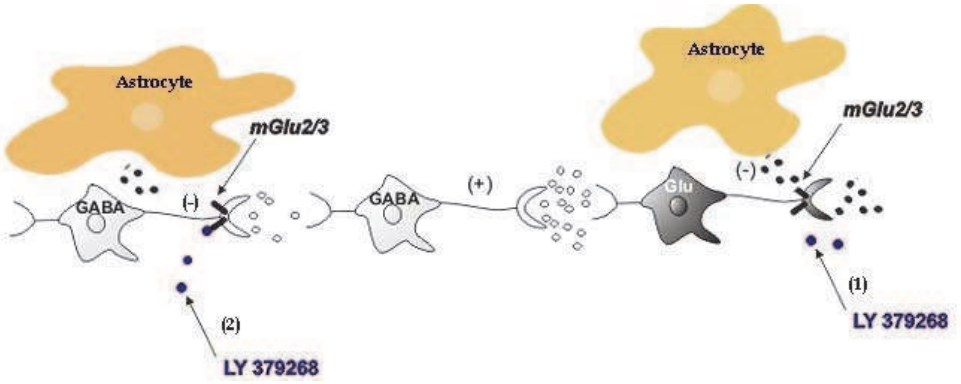

Fig. 10. The schematic neuronal network showing the mechanism of the action of mGlu2/3 receptor agonist, LY379268 (see description in the text). empty dots- GABA; black dots-Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released.

By contrast to group I and group III mGlu receptors (which will be discussed later), mGlu2 agonists directly inhibit glutamatergic neurotransmission, in majority not involving any other neurotransmission systems *via* presynaptic blockade of glutamate release [see: Fig.10 (1)]. In electrophysiological studies, a direct inhibition of projecting basolateral neurons, and the reduction of the excitatory drive were observed after administration of LY354740 and LY379268, mGlu2/3 receptor agonists (Muly et al., 2007). The inhibitory effect on stimulated glutamate release was also observed in microdialysis studies (Xi et al., 2002; Johnson et al., 2005).

However, some studies indicate that the release of GABA in the hippocampus stays under inhibitory control of group II mGlu receptors expressed on GABAergic terminals [see: Fig.10 (2)] (Kogo et al., 1999). If this regulatory action on inhibition contributes to anxiolysis, the intermediary interneuron innervating pyramidal glutamatergic target cells must be disinhibited.

7.3 Group III mGluR

The third group of metabotropic glutamate receptors constitutes the largest family and involves mGlu4, mGlu6, mGlu7 and mGlu8 subtypes. The most important for our consideration are mGlu4 and mGlu7 representatives, as the ligands of these receptors exerted clear anxiolytic action. mGlu6 expression is restricted mainly to the retina (Laurie et al., 1997) and the mGlu8 selective and brain-penetrating ligands have been poorly available so far. The studies concerning the recently synthesized positive allosteric modulator of the receptor, AZ12216052, indicate that stimulation of the receptor could result in anxiolysis (Duvoisin et al., 2010).

Expression of the group III mGlu receptors subtypes is distinct and somewhat complementary throughout the structures involved in anxiolysis. The receptors are, above

all, presynaptic and are usually located close to the center of the synaptic cleft. The receptors show a highly selective expression and subcellular location on nerve terminals modulating neurotransmitter release. Contrary to the other described presynaptic auto- or heteroreceptors, the stimulation of the receptors of the third group decreases not only vesicular and non-vesicular glutamate release (Xi et al., 2003), but also depresses the release of GABA from interneurons (Rusakov et al., 2004).

The mGlu4 receptor can act as autoreceptor expressed by glutamatergic terminals or as the heteroreceptor localized on GABAergic axons, suggesting the role of the mGlu4 receptor in in the regulation of both types of neurotransmitter (Corti et al., 2002). The excitatory input to the hilar-dentate border of the interneurons was depressed after mGlu4 receptor activation (Doherty & Dingledine, 1998). This may result in the disinhibition of GABA-releasing terminals that innervate the principal cells [see: Fig.11 (1)], thus inhibiting glutamatergic network.

The presence of mGlu4 receptors on hippocampal interneuronal terminals projecting from the hilus was also described, and with the use of electrophysiology it was shown, that they innervate the other GABAergic postsynaptic element [see: Fig.11 (2)] (Kogo et al., 2004). The source of excitatory amino acid in this kind of GABA-GABA synapse may come from glutamate spillover, allowing for the heterosynaptic regulation of the functional excitatory/inhibitory network. The disinhibition of the postsynaptic interneuron would regulate the activity of target glutamatergic cells.

Among all of the mGlu group III receptors, mGlu4 receptor revealed a relatively high level of post-synaptic staining, confirmed both in light and electron microscopy studies on pyramidal neurons in the some areas of the hippocampus (Bradley et al., 1996). Whether the anxiolytic-like action of mGlu4 receptor agonists involves the activation of these post-synaptically expressed receptors, however, still remains open for discussion.

The other candidate for regulating the glutamate/GABA level in the CNS is the mGlu7 receptor, widely distributed through the CNS, in the pre-synaptic grid, at the site of the synaptic vesicle fusion (Shigemoto et al., 1996). The axon terminals expressing the mGlu7 receptor were observed to be concentrated densely and specifically on mGluR1α-like immunoreactive GABAergic interneurons [see: Fig.11 (3)] (Shigemoto et al., 1996; Kinoshita et al., 1998). Therefore, the final result of the pre-synaptic action of the activated mGlu7 receptor is modulation of the postsynaptic GABAergic target [see: Fig.11 (3)]. This inhibition would cause the disinhibition of the other interneurons, targeting the glutamatergic network. The pyramidal neurons expressing mGlu7 on their terminals can form synapses with dendrites of the pyramidal cells; however, the expression of the mGlu7 receptor was found to be almost ten-fold higher in these pyramidal axons that innervate the mGluR1 alfa-expressing interneurons (Samogyi et al., 2003). Interestingly, mGlu7 receptors are also expressed on some types of the interneuron population (e.g VIP positive) innervating mGlu1α-somatostatine postsynaptic interneurons [see: Fig.11 (4)] (Dalezios et al., 2002) and creating a kind of GABA-GABA synaptic junction. Similar to the one described for mGlu4, the mechanism of anxiolysis involves inhibition of the GABA release, and in the simplest scenario, the depression of the GABA release could lead to a disinhibition of postsynaptic interneuron and increased GABA release on their terminals, inhibiting the input zone to the pyramidal cells. However, as the affinity of the mGlu7 receptor to glutamate is very low, in these kind of symmetrical GABAergic synapses to all the possibility the receptor is not activated by endogenous glutamate. mGlu7 PAM can possibly sensitise the affinity of the

receptor to glutamate, leading to anxiolysis. In rare cases, glutamate and GABA can be stored and released by the same nerve terminals (Walker et al., 2001), although in a properly functioning brain the glutamate is metabolized to GABA in interneurons. As the impartment in GAD67, the enzyme responsible for catalyzing the reaction was described in mood disorders and it can be speculated that under pathological conditions the glutamate is released by GABAergic interneurons, thus activating presynaptic glutamatergic receptors (Kash et al., 1999).

The distribution of the mGlu8 receptor was observed on the presynaptic active zones of neurotransmitter release on identified GABAergic and putative glutamatergic terminals that create synapses with several types of GABAergic neurons (Ferraguti et al., 2005). The type of the synapse predicts a role in adjusting the activity of interneurons depending on the level of network activity, widely described several times before now. The receptor is often expressed closely to its mGlu7 relative, therefore its action would involve similar mechanisms to those described above.

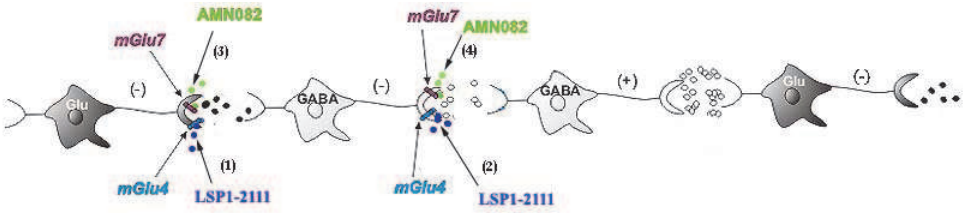

Fig. 11. The schematic neuronal network showing the mechanism of action of the mGlu group III receptor agonist LSP1-2111 (mGlu4 agonist) and AMN082 (mGlu7 agonist) (see description in the text). empty dots- GABA; black dots-Glu; (-)-inhibition; (+)-enhancement; the number of dots indicates the amount of neurotransmitter released

Reassuming the expression and functional consequences of the group III mGlu receptors family it can be concluded, that the receptors are present both on pyramidal and interneuronal terminals. The pyramidal neurons expressing mGlu4/7/8 receptors may contact with both the interneurons and pyramidal cells, but the GABAergic terminals expressing mGlu4, mGlu7 and mGlu8 receptors form the most synapses with the interneurons (predominantly mGlu1α-somatostatin positive), yet not with the pyramidal cells (Kogo et al., 2004), depressing the IPSCs of the inhibitory neuronal elements. Those receptors are activated with the glutamate released by the glia, or heterosynaptically by the glutamate released in the neighboring synapse. Such glutamate spillover enables the synapse to cooperate in regulating the excitatory/inhibitory balance in CNS.

8. Conclusions

The search for new anxiolytic therapy is one of the key areas of modern research, and a hope for the growing number of people affected by anxiety disorder. Besides, the anxiety is commonly comorbid with different psychiatric, and somatic, illnesses, so the proper treatment can constitute a supplementary therapy. There is no better way to improve pharmacological treatment than understanding the complex interaction between excitation/inhibition in the CNS. All that was written in these few pages until now states

that the receptors mediating both fast and slow excitatory or inhibitory currents are present at a broad range of synapses that are postulated to be critical for the maintenance of the correct balance in the brain. The complex interactions between synaptic responses, the releases of the neurotransmitters and receptor trafficking (not discussed here) at the excitatory glutamatergic or inhibitory GABAergic synapses is more complicated than anyone could ever imagine.

More than one type of auto- heteroreceptor can be expressed on one nerve terminal, so the receptors may cooperate with, or antagonize, each other's action. Such a cooperation has already been shown for mGlu7/$GABA_B$ receptors. Besides, each type of pyramidal neuron is likely to be innervated by multiple, functionally distinct GABA cells, which may differ in the mGlu expression. Some other factors, such as variation of the presynaptic receptor level in individual terminals or the state of activation or desensitization of the receptor, may also be important in the final effect of the treatment.

9. Acknowledgements

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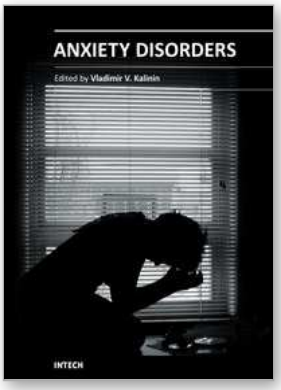

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During the last 2-3 decades drastic research progress in anxiety issues has been achieved. It concerns mostly the study of different subtypes of anxiety and their treatment. Nevertheless, the data on anxiety pathogenesis is less elaborated, although here a multidimensional approach exists. It includes neurochemistry, pathophysiology, endocrinology and psychopharmacology. Again, we are able to recognize the multifarious sense of anxiety, and the present collective monograph composed of 16 separate chapters depicting the different aspects of anxiety. Moreover, a great part of book includes chapters on neurochemistry, physiology and pharmacology of anxiety. The novel data on psychopathology and clinical signs of anxiety and its relationship with other psychopathological phenomena is also presented. The current monograph may represent an interest and be of practical use not only for clinicians but for a broad range of specialists, including biochemists, physiologists, pharmacologists and specialists in veterinary.

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Phone: +86-21-62489820
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