Group II metabotropic glutamate (mGlu2/3) receptors
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Besides its essential function in energy metabolism, glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Therefore glutamate is involved in nearly all aspects of brain function including cognition, memory and learning. Consequently, abnormal changes in glutamatergic neurotransmission, in particular excessive glutamate release, can lead to a neuronal dysfunction resulting in a variety of neurological and psychiatric disorders including anxiety and schizophrenia. Therefore, there has been a great deal of interest in developing therapeutic strategies that can influence the function of glutamate receptors.

Before the discovery of metabotropic glutamate (mGlu1-8) receptors, it was thought that glutamate exerts its physiological action through receptors that act directly as ion channels. These ionotropic receptors (iGlu: NMDA, AMPA and kainate) are expressed by nearly all subtypes of neurons and mediate fast excitatory neurotransmission throughout the whole brain. Thus, direct pharmacological manipulation of this group of receptors may produce a global disruption in brain function and produce profound side effects ranging from disruption of movement to impairment of attention and memory.

Unlike iGlu receptors, the mGlu receptors modulates neuronal activity in a manner similar to neuromodulators such as dopamine and serotonin, which have been effective targets of psychoactive drugs for treatment of most psychiatric disorders. In addition, the distribution and function of these receptors is highly diverse and heterogeneous. As a consequence, mGlu receptor ligands might exemplify Ehrlich’s pharmacological ‘silver bullet’ that can modify glutamate neurotransmission in a functionally selective manner.

In the last decade, the members of group II metabotropic glutamate receptors (mGlu2/3) have emerged as potential therapeutic targets. Activation of the mGlu2/3 receptors provide a negative feedback mechanism to prevent excessive
presynaptic glutamate release in limbic regions that have been implicated in pathology of psychiatric disorders. Converging preclinical and recent clinical evidences supported the notion that administration of selective mGlu2/3 receptor agonists, LY354740 and LY379268, represent a novel treatment for schizophrenia and anxiety/stress. Because of some recent conflicting results, the experiments described in this thesis were designed to further evaluate the proposed antipsychotic and anxiolytic properties of these compounds.

**Schizophrenia**

The dopaminergic hypothesis cannot totally explain the neuropathology of schizophrenia and this has led to the search for other errors in neurotransmission and the development of associated models. The psychotomimetic effects of noncompetitive NMDA receptor antagonists such as PCP and ketamine in healthy humans and their ability to exacerbate several psychotic symptoms in schizophrenic patients have prompted a view of schizophrenia as being related to a hypofunctional state of glutamatergic neurotransmission. Attempts to mimic these effects in animals have led to the recognition of parallels with behavioural and cellular abnormalities associated with schizophrenia.

**Chapter 2** describes this NMDA receptor hypofunction model of schizophrenia. Upon single subanasthetic dose of ketamine, the animals exhibit hyperlocomotion, working memory deficits and disruption in sensorimotor gating (PPI). As measured by c-fos expression, these behavioural abnormalities are associated with increased neuronal activity throughout the brain including prefrontal cortex, hippocampus, amygdala, nucleus accumbens and hypothalamus. Previous in vivo microdialysis studies demonstrated that such an increased neuronal activity following administration of NMDA receptor antagonists is linked to increased glutamate release. Thus suppression of this increased glutamate efflux may restore the behavioural deficits evoked by ketamine and other NMDA receptor antagonists.
In fact, acute administration of mGlu2/3 receptor agonists has been demonstrated to prevent hyperlocomotion but not prepulse inhibition (PPI) deficits. Schizophrenia patients are deficient in the normal inhibition of the startle reflex that occurs when the startle stimulus is preceded by a weak prestimulus. The PPI paradigm is thought to be a measure of the deficient sensorimotor gating that underlies sensory flooding and cognitive fragmentation in these patients. Since PPI deficits are one of the key symptoms of schizophrenia resulting in information overflow and thought disorder, the lack of effect of acute pretreatment with LY354740 cast a doubt on the proposed antipsychotic properties of mGlu2/3 receptor agonists. Thus, first we investigated the effects of subchronic pretreatment with LY354740 on ketamine-induced PPI deficits as well as hyperlocomotion and c-fos expression. As described in chapter 3, this approach also failed to restore the ketamine effects on PPI, rather LY354740 appeared to exaggerate the PPI deficit. Furthermore, LY354740 was ineffective to prevent ketamine-evoked hyperlocomotion and c-fos induction.

Interestingly, both LY354740 and ketamine reduced the c-fos expression in the dentate gyrus (DG). This effect of LY354740 and ketamine might contribute to the potentiation of the PPI deficits when the two compounds were applied together: the hippocampus plays an important role in mediation of the PPI disruptive actions of NMDA antagonists and DG is the main hippocampal input with a high mGlu2/3 receptor density. Although speculative, it can be argued that pretreatment with LY354740 suppressed glutamate neurotransmission in DG via activation of presynaptic mGlu2/3 receptors, while ketamine further reduced it by blockade of postsynaptic NMDA receptors. Such a synergistic reduction in glutamate neurotransmission might lead to profound PPI deficits.

As described in chapter 4, acute administration of the more potent mGlu2/3 receptor agonist LY379268 also failed to restore PPI deficits evoked by ketamine. In fact, LY379268 enhanced the ketamine effects on PPI similarly to LY354740. However, LY379268 was able to prevent hyperlocomotion.
Summary

Since we observed synergistic interaction effect on c-fos expression between LY354740 and ketamine only in the DG, in chapter 4 we measured the ex vivo levels of glutamate and monoamines (dopamine, serotonin and noradrenaline) only in the DG following LY379268 and ketamine treatment. We demonstrated here that ketamine reduced the tissue level of glutamate and dopamine and the serotonin turnover. Pretreatment with LY379268 could only prevent the ketamine effect on glutamate without altering the basal glutamate concentration. This is in line with in vivo microdialysis studies which have demonstrated that activation of group II mGlu receptors by LY379268 or LY354740 has no effect on basal glutamate levels in the PFC or nucleus accumbens, even though these drugs can suppress NMDA antagonist-evoked glutamate (but not dopamine) release which was accompanied with blockade of PCP-evoked hyperlocomotion. Accordingly, the present data showed that LY379268 prevents the effects of ketamine on locomotion and glutamate transmission in the DG, but not on PPI and monoamine levels. Therefore, it is conceivable that blockade of ketamine and other NMDA antagonist-induced changes in glutamate neurotransmission can reverse hyperlocomotion, but that it is neither sufficient nor capable in restoring PPI deficits. This latter symptom may rather be linked to changes in monoamine transmission. Previous reports do support such a relationship, as multireceptor-antagonist antipsychotics (mixed 5-HT_2/D_2 antagonists) like clozapine, chlorpromazine, and ziprasidone appeared to be effective in reversing NMDA antagonist-induced PPI deficits. This may help to explain the ineffectiveness of the highly selective mGlu2/3 receptor agonist LY354740 in the present study: the drug did not effectively counter all the wide-ranging neuropharmacological effects of ketamine.
ANXIETY

In general, anxiety- and stress related disorders are thought of as a collection of illnesses that have in common excessive or inappropriate brain excitability within crucial brain region circuits, which leads to the expression of the symptoms. As glutamate is the main excitatory neurotransmitter in the mammalian brain, it is logical that the new approaches for treatment of anxiety/stress disorders could include drugs that modulate glutamatergic functions.

While recent reports strongly suggest a role for mGlu2/3 receptors in the modulation of anxiety and stress-related disorders, all these studies focused on male experimental objects ignoring gender differences in anxiety/stress-related behaviour and in response to psychoactive drugs. Therefore chapter 5 designed to evaluate the anxiolytic properties of LY354740 and LY379268 in both gender. To the end, control and acutely stressed rats (2 days of inescapable footshock [IS] prior to test) were subjected to elevated plus maze test (EPM).

Although none of the compounds showed anxiolytic effects under normal conditions, treatment with LY379268 could restore IS effects on EPM performance in both sexes. LY354740, however, was effective only in stressed females, but not in stressed males. Since the more potent structural analogue LY379268 was able to prevent IS-induced changes in males, the lack of LY354740 effect might be attributable to the dosage (3mg/kg) which is indeed lower than other studies used (10-20 mg/kg). This might also imply that males and females have different pharmacokinetic profiles for these drugs.

The well established anxiolytic effects of mGlu2/3 receptor agonists have been associated with suppressed hippocampal neurotransmission. Furthermore, the hippocampus is particularly sensitive to stress in a gender dependent manner. Studies investigating neurogenesis of the DG have reported that stress reduces hippocampal plasticity in males with either no effect or stimulatory effects seen in females. Thus, we measured the expression of cAMP response-element binding protein (CREB) and its phosphorylated form (pCREB) as a plasticity
marker by using western blot analysis to characterize stress and treatment effect on plasticity in the DG of male and female rats.

Similar to the reversal effects of LY379268 on behaviour, treatment with this compound, but not with LY354740, could normalize the CREB level in stressed males without altering pCREB. In stressed females, however, all three compounds increased pCREB expression but there were no changes in CREB. These findings may indicate that mGlu2/3 receptor agonists have a gender dependent effect regarding regulation of CREB function: in males normalization of CREB level while in females increased activation of CREB appears to be involved in anxiolytic action of these compounds. However, the signaling pathway by which the mGlu2/3 receptors influence CREB function remains to be explored.

**CONCLUSION**

Altogether, our results do not support the proposed antipsychotic properties of mGlu2/3 receptor agonists, LY354740 and LY379268. None of the compound was able to restore sensorimotor gating deficits which are considered as the key symptoms of schizophrenia. However, future studies should examine the effects of these compounds on cognitive dysfunction related to schizophrenia.

On the other hand, our results demonstrated the both LY354740 and LY379268 have anxiolytic properties in elevated plus maze test irrespectively of gender. These findings further suggest that modulation of mGlu2/3 receptor subtypes may indeed represent a new and safe approach for the treatment of anxiety. Since anxiety/stress is the major non-genomic factor that contributes to the expression or exacerbation of in schizophrenic symptoms, recurrence after a period of remission and treatment outcome, mGlu2/3 receptor agonists might be also applied as an additional treatment in combination with antipsychotics to increase the efficiency of the treatment.