Expression of metabotropic glutamate receptor subunits 2/3 and 5 in the rat locus coeruleus

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Abstract

Background: Research has implicated glutamatergic dysfunction in the pathophysiology of schizophrenia. The metabotropic group of glutamate receptors, in particular mGluR2/3, has been found to reverse effects of phencyclidine in rats, hence making mGluR2/3 a potential target for atypical antipsychotics. The locus coeruleus (LC) is the central noradrenergic pathway in the central nervous system, and recent findings suggest that the presence of mGluR2/3 in the LC might give more insight into the mechanisms of action of antipsychotics.

Materials: Sections of the rat brain containing the LC were immunostained with fluorescent antibodies. Pictures of stained LC were then taken using confocal laser scanning microscopy, and subsequently analysed.

Results: Merged pictures showed staining of TH with mGluR2/3 and mGluR5 respectively in the same location- the LC neurons.

Discussion: The results show that mGluR2/3 and mGluR5 are co-localised with TH, meaning that they are present in the LC neurons. Previous research on the influence of atypical antipsychotics on the LC suggests that mGlu receptors could be a part of their mechanisms of actions. There is a new possibility of mGluR5 in the LC as a possible target for antipsychotics.

Conclusion: This study provides significant evidence towards the hypothesis that atypical antipsychotics act through noradrenergic pathways.

Introduction

Research has implicated glutamatergic dysfunction in the pathophysiology of schizophrenia. For example, it has long been recognised that phencyclidine (PCP or “angel dust”), a potent blocker of ionotropic NMDA receptors, produces effects in rats and humans which are similar to symptoms of schizophrenia (Javitts and Zukin 1991). This has led to NMDA receptor hypofunction as a possible cause of schizophrenia. However, current interest has shifted to the activity of the metabotropic group, in particular the mGlu2/3 (Group II) receptors. mGluR 2/3 agonists have been found to reverse specific PCP-evoked behaviours (Moghaddam and Adams 1998), hence mimicking some of the actions of atypical antipsychotics such as clozapine (Cartmell et al. 2000) and risperidone (Cartmell et al. 2001). This suggests that the mechanism of antipsychotics might be related to mGlu2/3 receptor activity.

The locus coeruleus(LC) is the most prominent noradrenergic nucleus of the mammalian CNS. It has widespread efferent innervations to regions such as the prefrontal cortex, which shows decreased glutamate uptake in patients with schizophrenia. Using antipsychotics
such as olanzapine to target the LC neurons might play a part in ameliorating the negative symptoms of schizophrenia (Dawe et al. 2001).

This study builds upon previous work, which showed the presence of mGlu2/3 receptor subunits in the LC (See et al. 2001). A Group I metabotropic receptor, mGluR5, on which little research has been carried out in relation to the LC, will also be studied. The objective here is to use fluorescent double-immunolabelling and confocal laser scanning microscopy to confirm co-localisation of mGlu2/3 and mGlu5 receptor subunits with TH in LC neurons, and hence the possibility of antipsychotics working through noradrenergic pathways.

**Materials and methods**

**Immunocytochemistry**

The rat brain sections have already been paraffin-embedded and sectioned. The optimal staining conditions for the tyrosine hydroxylase primary antibody (an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy-terminus of tyrosine hydroxylase of human origin, Santa Cruz Biotechnology) were found to be 1:50 dilution with incubation at 4°C for 24 hours. The primary antibody for mglu 2/3 receptor (affinity purified polyclonal rabbit antibody, Chemicon Int’l) was used at 1:400 dilution with 4°C incubation for 2 days initially, but was changed to 1:100 when fluorescent labeling was found to be inadequate. The primary antibody for mGlu5 receptor (affinity purified polyclonal rabbit antibody, Chemicon Int’l) was used at 1:100 dilution at 4°C for 2 days.

The secondary antibodies used were fluorescent donkey anti-goat (Alexa Fluor 594 donkey anti-goat) for labeling tyrosine hydroxylase and FITC (for donkey anti-rabbit IgG-FITC, sc-2090, Santa Cruz Biotechnology) for mGluR2/3 and 5 initially, but was changed to a fluorescent amplification agent in order to amplify the signal. The concentration used for Alexa Fluor was 1:400, and incubation at room temperature for one hour. This step replaced the step for the biotinylated secondary antibody in the ABC staining protocol. The fluorescent amplification agent (TSA™-Plus Fluorescence Palette System, Perkin Elmer Life Sciences) was used at 1:60 concentration for five minutes at room temperature. Tween 20 was added to the incubation medium for both primary and secondary antibodies, in order to increase the penetration of the antibodies.

**Results**

The locus coeruleus is identified by its orientation with respect to certain landmarks which are more easily identifiable, such as the IVth ventricle (IV) which is dorsal to the LC and the large cells of the mesencephalic nucleus of the Vth nerve (ME5), which is lateral to the LC. The pictures below contain brain sections of the locus coeruleus.
Figure 1 shows the merged staining and co-localisation of mGluR2/3 and TH, while Figure 2 shows the merged staining and co-localisation of mGluR5 and TH. Both metabotropic receptors are shown to be present in the LC.

Besides the LC, the cerebellum area also had rather vivid staining for mGluR2/3, with the surrounding of the granulose cells being stained a brighter green than the interior of the cell. The general U-shape of the green staining follows the folds of the cerebellum. It is observed that the pattern of staining for mGluR5 is quite different from mGluR2/3, with the green sections forming dotted circular regions on the cell surfaces, rather than fully staining the outer part of the cell, which is the case for mGluR2/3.

Discussion

The pictures in Fig 1 and 2 clearly show that mGlu2/3 receptors and mGlu5 receptors are indeed present in the locus coeruleus (See et al.). The pictures of the cerebellum served to show that the staining for both mGlu receptors were specific. Several modifications had to be made to the initial ABC staining protocol so that the fluorescent staining would be as bright as possible.

The merged pictures were not very clear in confirming co-localisation of the metabotropic receptors and TH. This is primarily due to the low level of penetration of the TH primary antibody, since confocal microscopy revealed that the Alexa fluorescent antibodies appeared to only stain the surface of the section. It also did not show very specific staining, as large areas of background were also detected, which is apparent in Fig 1 and 2. Even though the co-localisation is not very evident, the fluorescent pictures for mGluR 2/3 is sufficient to show that they are definitely present in the LC neurons, judging by the position and orientation of the stained cells.

Vandergriff et al. (1999) found that the mGlu2/3 receptor agonist LY354740 attenuates morphine-withdrawal induced activation of locus coeruleus (LC) neurons, and made an important suggestion that post-synaptic mGlu2/3 receptors present in the LC might be partly responsible for the inhibition of LC neurons. This study has shown that mGlu2/3 receptors are indeed present in the LC, hence it is highly possible that atypical

IVth ventricle

IVth ventricle
antipsychotics, which activate the LC (Dawe et al. 2001), acts through mGlu receptors in the LC neurons. The mechanisms of action on the LC are still unclear, especially since it has limited afferent projections. A possible site of interest is the nucleus paragigantocellularis (PGi), which sends a major glutamatergic afferent to the LC. It has been hypothesized that since lesions of the PGi reduce morphine withdrawal symptoms, activation of mGluR2/3 in the PGi may play a part in reducing activation of LC neurons (Vandergriff et al. 1999).

Previously, a selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) has been found to reverse the akinetic deficits in a rat model of parkinsonism (Breysse et al. 2002), and the mGlu5 receptor subunit is currently being investigated as a potential therapeutic target for treatment of Parkinson’s Disease. The localization of mGluR5 in the locus coeruleus opens up a new possibility for research into the Group I metabotropic glutamate receptors, and their possible involvement in the mechanisms of atypical antipsychotics.

**Conclusion**

Analysis of the fluorescent pictures taken with confocal microscopy confirm that mGluR2/3 and mGluR5 are present in the locus coeruleus. This provides significant evidence towards the hypothesis that atypical antipsychotics act through noradrenergic pathways (i.e. LC). This has significant implications for understanding the mechanisms of atypical antipsychotic drugs. A good point of continuation from this study would be to examine and compare the effect of chronic treatment of various atypical antipsychotics on the level of the mGluR5 and mGluR2/3 in the locus coeruleus and the prefrontal cortex, since previous work on acute treatment (See et al. 2002) actually showed a downregulation of mGluR2/3 by atypical antipsychotics haloperidol and clozapine.

**References**


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