The α_5 subunit-containing $GABA_A$ receptor: a target for the treatment of cognitive defects

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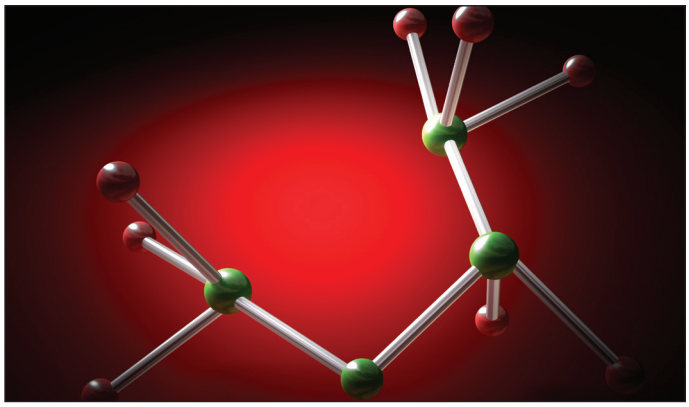
Amnesic effects of benzodiazepines are in part the result of the activity of α_5-subunit containing $GABA_A$ receptors (GABRA5). Negative modulators at this receptor could improve cognition. In order to explore this beneficial effect, this article reviews the evidence on the effects of GABRA5 negative modulators and searches potential uses for such drugs. A literature search found a number of GABRA5 negative modulators. These drugs generally improve hippocampal-dependant learning via an increase in long-term potentiation (LTP) in the hippocampus. Passive avoidance learning was also improved. In addition, the compounds examined demonstrated minimal side effects partly due to lack of binding to different alpha subunit-containing $GABA_A$ types. Due to its beneficial properties, there is potential for such a drug in treating Alzheimer's, alcohol-related amnesia and Down syndrome. Despite the myriad animal studies that utilised GABRA5 negative modulators, only three human studies were found. Due to its cognitive enhancing properties and minimal side effects, further human trials should be conducted in order to ascertain the potential of such drugs in treating cognitive deficits.

Introduction

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain and is responsible for regulating neuronal excitability. There are at least three different receptors that it targets — $GABA_A$, $GABA_B$ and $GABA_C$. [1] The $GABA_A$ receptor is the main target for the popular class of drugs, the benzodiazepines. This receptor is an ionotropic membrane receptor, which facilitates the movement of chloride into cells. In neurons, this increases the threshold needed to excite them. [2] Benzodiazepines are positive modulators at this receptor and exert their effects by binding to the interface between the γ_2 and α subunits on the $GABA_A$ receptor. [3] Since they are modulators and not agonists, they do not work in the absence of GABA. [4] They only bind to the receptors containing the α_5, α_3, α_2, and α_1 subunits. [5] Each of these subunits mediates different effects. In knockout mice, the α_1 subunit has shown hypnotic/sedative effects, whilst the α_3, α_2 and α_5 subunits have anti-anxiolytic effects and these have been exploited for therapeutic use. [6]

Despite their uses in treating various conditions, traditional benzodiazepines have numerous side effects. The main side effects associated with therapeutic use are amnesia, confusion, impaired coordination and dizziness. There may be tolerance due to rapid escalation in the dose needed to provide the required effect. There are also long-term issues with dependence. In acute overdose the most life-threatening effect is respiratory depression, especially when combined with alcohol. [7]

Interestingly, it is thought that positive modulators at the α_5 subunit-containing $GABA_A$ receptors (GABRA5) produce the anterograde amnesia associated with benzodiazepine use. [7] Most of these receptors are found in the hippocampus (a brain region associated with memory) and provide tonic inhibition in this region. [8] The exploitation of this receptor has led to the increasing use of the infamous 'date rape' drug flunitrazepam, which is a positive modulator at the GABRA5 in addition to its other functions. Because of its amnestic effects, victims are unable to recall events following intoxication and this provides a major challenge for prosecutors. [7] Despite these negative properties, by using a GABRA5 negative modulator the opposite effect might



be achieved and cognition improved. The use of such a drug could potentially improve the quality of life for those living with cognitive defects and could also counteract drug-induced amnesia (for example, alcoholic 'blackout'). However, this must be balanced with the inverse activity of non-selective negative modulators which could produce convulsant or anxiogenic effects. [4]

Based on the premise that a GABRA5 negative modulator could improve cognition, the aim of this literature review was to review the evidence on (1) the effects of GABRA5 negative modulators on cognition; and (2) investigate the potential of GABRA5 negative modulators in managing conditions involving cognitive defects.

Effects of GABRA5 selective negative modulators

A number of GABRA5 negative modulator compounds were examined. Many of the studies used animals as subjects. Studies in animals provide a solid starting platform for understanding the various physiological changes that a drug induces. [9] Of particular importance is the avoidance of potential side effects as a result of non-selective actions at other $GABA_A$ receptors. Side effects could include an increase in anxiety, aggressiveness, motor impairment, inability to sleep and proconvulsant effects. [7] Ultimately, the knowledge gained from animal experimentation can be used to conduct safe and effective clinical trials.

Dawson *et al.* [10] examined a compound named α51A (3-(5-Methylisoxazol-3-yl)-6-[(1-methyl-1,2,3-triazol-4-yl)methyloxy]-1,2,4-triazolo[3,4-*a*]phthalazine), which has selective negative modulator effects at GABRA5. The authors found that α51A reversed the inhibiting effects of GABRA5 in the hippocampus in rats and mice. This resulted in an increase in performance in a memory test named the "delayed matching-to-position version of the Morris water maze", which is a hippocampus-dependant cognitive test. [11] In addition, 'long-term potentiation' (LTP), which is thought to underlie the synaptic changes that take place during memory formation, was found to be enhanced in the hippocampus. [11,12] Benzodiazepine (agonist) effects and non-selective $GABA_A$ negative modulator effects were also examined. The authors found no anxiogenic, convulsant, withdrawal or motor-impairing effects from the drug. [10]

Only certain components of memory have shown to be improved by GABRA5 negative modulators. Collinson *et al.* [13] extrapolated on the results obtained by Dawson *et al.* [10] and looked at the effect of a modified version of α51A, α51A-II. They separated memory into three components — encoding, consolidation (conversion into long-term

memory) and recall. Results were obtained by measuring performance in the delayed matching-to-position (DMTP) version of the Morris water maze in rats. The authors found that the compound improved encoding and recall but not consolidation in this hippocampal-dependant memory test. [13]

The effect on cognition by another selective GABRA5 negative modulator was examined by Ballard *et al.* [14] This was done by the use of an imidazo-triazolo-benzodiazepine compound named RO4938581. The effect of this compound on cognition was examined in rats. [15] This compound demonstrated similar effects to those found by Dawson *et al.* [10] in that they found no convulsant or anxiogenic effects. In addition, similar to Dawson *et al.* [10], there was an increase in hippocampal LTP. The authors also found that working memory was enhanced since RO493881 reversed scopolamine-induced working memory impairment. [14] This was shown by an increase in performance in the DMTP task, which is used to assess spatial working memory. [14,15] It also reversed diazepam-induced spatial impairment. This was demonstrated by an increase in performance in the Morris water maze task. [14]

'Moderate' GABRA5 negative modulators improve passive avoidance learning but generally have no effect on active learning. [16] This was shown by an experiment conducted by Savic *et al.* [16] in which they examined effects of PWZ-029 (a 'moderate' GABRA5 negative modulator) on passive and active learning avoidance in rats. The result was obtained through various shuttle-box based behavioural experiments. This experiment proved that even at 'moderate' efficacy a GABRA5 negative modulator can induce memory formation. The compound also had no effect on muscle tension and anxiety (non-selective side effects). [16] Although promising, this study was limited by the fact that the compound only had 'moderate' negative modulator activity at GABRA5 so using a more efficient compound may display different effects on avoidance learning. Despite this limitation, this shows that the use of a 'moderate' GABRA5 negative modulator would be beneficial in the treatment of disease due to its limited side effects and its memory-enhancing properties. [16]

Application in management

The compounds examined in this review show that selective GABRA5 negative modulators have nootropic effects without any serious side effects, which are seen in non-selective negative modulators at the alpha subunit of the $GABA_A$ receptor. [17] Thus, there is strong potential for the use of GABRA5 negative modulators in healthcare settings. One major limitation is that most of the data obtained for this review was from animals. Further human trials need to be conducted to ascertain the potential of this drug. Drawing on the literature, possible future uses for a GABRA5 negative modulator are detailed below.

GABRA5 negative modulators could be used to treat Alzheimer's disease since GABRA5 is preserved in Alzheimer's disease patients. [18] Alzheimer's disease is commonly characterised by the gradual worsening of ability to remember new information. [19] Administration of a GABRA5 negative modulator could help with the 'encoding' and 'recall' of this information. [8] It could also be used to treat mild cognitive impairment (MCI), which is a risk factor for later developing the disease. [20] Administration of such a drug to patients may provide relief to older caregivers, who often show signs of sleep detriment. [21]

A review by Attack [22] in 2010 found two human trials on the GABRA5 negative modulator α5Ia and one trial on MRK-016. Since then no human trials were found and this could be an area of future research. The first study found that a potential application of GABRA5 negative modulators is the treatment of alcohol-induced amnesia. Nutt *et al.* [23] found that pre-treatment reduces alcohol's amnestic effects in humans. This was measured by word list learning which is linked to hippocampal processing. Alcohol-induced amnesia has been shown to predict future alcohol-related injury. [24] Therefore, the use of a GABRA5 negative modulator may help in reducing this risk. In addition, it may also reduce alcohol-related stress since it has been found that amnestic episodes related to alcohol have resulted in moderate psychological stress. [25]

Unfortunately, GABRA5 does not improve age-related cognitive defects. In fact it has been found that α5IA significantly impairs cognition in the elderly despite having positive effects on the young. Attack [22] found that young subjects (mean age 22 years) performed much better than older subjects (mean age 72 years) on the paired associates learning test, which is sensitive to age-related cognitive decline. Therefore, this trial showed no potential in reversing age-related cognitive decline. This demonstrates that careful consideration based on age should be taken in to account when using this drug.

MRK-016 (3-tert-butyl-7-(5-methylisoxazol-3-yl)-2-(1-methyl-1H-1,2,4-triazol-5-ylmethoxy)-pyrazolo[1,5-d]-[1,2,4]triazine) is another negative modulator and showed greater LTP in rat hippocampal slices than α51A. It also enhanced performance in the DMTP and Morris water maze tasks, which are used to test spatial memory. In humans, it was well tolerated in young adults with a maximum tolerated dose of 5 mg with 75% occupancy. In elderly subjects, however, it was poorly tolerated even at 10% of the maximum dosage in young adult males. Therefore, this particular drug has been precluded for development. [26]

Recent trials of GABRA5 agonists, in particular L-655,708 and MRK-016, have focused on restoring post-anaesthetic cognitive deficits. Lecker *et al.* [27] found that L-655,708 and MRK-016 reduced the potentiation of GABRA5 post-inhalation of isoflurane and sevoflurane. A further study by Zureck *et al.* [28] found that short-term memory assessed by the novel object recognition task was fully reversed by L-655,708 after isoflurane anaesthesia. This demonstrates the potential use of L-655,708 in reducing post-anaesthetic amnesia. However, further studies which include those performed on humans are needed to validate the potential of MRK-016 and other GABRA5 negative modulators in reducing post-anaesthetic amnesia.

The use of GABRA5 negative modulators could help with treating cognitive deficits related to Down syndrome. A recent review by Martínez-Cué *et al.* [29] investigated this specific application. It identified two studies that examined the effects of a GABRA5 inverse modulator on a Down syndrome mouse model (Ts65Dn). Braudeau *et al.* [30] found that acute treatment with the GABRA negative modulator α5IA improved learning deficits in the Morris water maze task. The second study also showed that chronic administration of a similar drug, RO4938581 has also been shown to have memory-promoting effects in the Morris water maze task on Ts65Dn mice. [31] In a practical sense, administration of such a drug could improve performance in learning a wide range of functional skills in those living with Down syndrome. For example, in children this may include learning how to use the toilet and administering self-care. [32]

Conclusion

The literature supporting the use of a GABRA5 negative modulator in the treatment of cognitive deficits is promising. GABRA5 negative modulators exert their actions by enhancing hippocampal dependant memory formation. There are minimal side effects as no withdrawal symptoms, convulsant, anxiogenic or motor-impairing effects were found. There is great potential for the use of GABRA5 negative modulators as they have been shown to reduce alcohol-related amnesia and may have potential in the treatment of Alzheimer's disease. They could also treat cognitive deficits in Down syndrome patients, increasing the speed at which they learn functional skills. Due to these favourable findings, there is an increased need for human clinical trials in order to validate the potential for this important receptor target.

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Conflict of interest

None declared.

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