THE GABA_A RECEPTOR AND THE NEUROPHARMACOLOGICAL PROPERTIES OF 1,5-BENZODIAZEPINES OF MEDICINAL INTEREST: A REVIEW

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ABSTRACT

This article reviews how GABA_A receptors act within the neuronal system, focusing on the neuropharmacological properties of some 1,5-benzodiazepines. They are generally involved in various physiological processes, such as brain excitability control, anxiety modulation, thirst, circadian rhythm, cognition, alertness, memory and learning. The 1,5-benzodiazepines of medical interest differ from others benzodiazepines, as 1,4-benzodiazepines, traditionally used in clinical psychiatry, in nitrogen positions in aromatic ring. They are commonly obtained by condensation reactions between ortho-diaminoerenes and 1,3-diketones, with sulphuric acid as reaction catalyst. Several of these drugs have shown promising results and have the potential to either replace or act as alternatives to 1,4-benzodiazepines.

Keywords: Clozapine, Olanzapine, Clobazam, Medicinal chemistry.

Introduction

The 1,4-aminobutanoic or γ-aminobutiric acid (GABA) was discovered in 1950, however its Central Nervous System (CNS) depressor activities were evidenced years later (Bloom, 2005).

It’s a neurotransmitter amino acid (Figure 1) (Bloom, 2005; Forman et al., 2009) found abundantly in mammals CNS (Forman et al., 2009; Chebib & Johnston, 2000; Martin & Dunn, 2002; Sieghart & Sperk, 2002; Davis & Murray, 2006), astrocytes (Forman et al., 2009), spinal cord, peripheral nerves liked to liver, spleen and heart (Davis & Murray, 2006), and it’s present in approximately 40% of neurons (Martin & Dunn, 2002).

GABA is responsible for inhibitory neuronal action, induced by membrane hyperpolarization (Forman et al., 2009; Chebib & Johnston, 2000; Martin & Dunn, 2002; Sieghart & Speck, 2002; Davis & Murray, 2006), and resistance decreasing of this membrane by Cl⁻ influx increasing (direct effect). Nevertheless, the inhibitory action may be obtained by the potassium (K⁺) efflux increasing and closing Ca²⁺ channels (indirect effect) (Forman et al., 2009). GLUT will depolarize membrane by sodium (Na⁺) and Ca²⁺ influx increasing; causing excitement (direct effect), although will also occur excitement if only Ca²⁺ influx (indirect effect) (Forman et al., 2009; Chebib & Johnston, 2000; Martin & Dunn, 2002; Sieghart & Sperk, 2002; Davis & Murray, 2006).

GABA Subunits

GABA is divided into subunits (isoforms), which are pharmacologically classified as: GABA_A, GABA_B and GABA_C (Chebib & Johnston, 2000). GABA_A and GABA_C receptors are coupled to intrinsic chloride channels, while GABA_B receptor is related to G protein (Bloom, 2005; Forman et al., 2009; Chebib & Johnston, 2002). In this review, only GABA_A receptor is discussed.

GABA_A Receptor

GABA_A receptor is a glycoprotein that possesses the most rapid inhibitory activity in synaptic transmission (Jasen et
al., 2008) and it develops its function in neurons and glial cells (Alfaro, 2010). It shows pentameric structure compounded by, at least, 16 subunits (Forman et al., 2009; Alhambra et al., 2011), which presents a total length of 8nm inside the pentamer (Martin et al., 2009). These polypeptide subunits are divided into: α, β, γ, δ, ε, ρ (Forman et al., 2009; Sieghart & Sperk, 2002; Davis & Murray, 2006; Alfaro, 2010), π, and θ (Forman et al., 2009; Sieghart & Sperk, 2002; Davis & Murray, 2006), which also have some variants: α1-6, β1-4, γ1-4, γ1, ε1, and ρ1-3 (Davis & Murray, 2006; Alfaro, 2010). GABA_A receptor has a complex heteromeric structure (Sieghart & Sperk, 2002) containing the GABAergic receptor itself, the benzodiazepines binding site (BZDs) and chloride dependent-channel, which act as inhibitory neurotransmitters (Forman et al., 2009; Sieghart & Sperk, 2002; Alfaro, 2010). Some studies suggest that these subunits can rearrange within each other in 500 different forms (Sieghart & Sperk, 2002).

In general, GABA_A receptor is involved in many physiological processes due to its variety of subunits rearrangement, for example: brain excitability control, anxiety modulation, hunger and thirst sensations, circadian rhythms, cognition, alertness, memory and learning (Sieghart & Sperk, 2002). Concerning to pharmacological effects modulation, GABA_A has the receptor site to BDZs that consists of a rearrangement from subunits; α1, α2, α3 or α5 combined whit β and γ subunits. This rearrangement allows the emergence of an interface α-γ (Martin & Dunn, 2002; Sieghart & Sperk, 2002; Davis & Murray, 2006; Jasen et al., 2008; Alhmabra et al., 2011), forming a recognizing site to benzodiazepine drugs (Figure 2) (Martin & Dunn, 2002; Sieghart & Sperk, 2002; Martin et al., 2009).

The GABA_A receptors are organized in areas called domains. These GABA_A domains are better observed when axis plane is analyzed perpendicularly (Figure 3) (Martin et al., 2011).

Therefore, chemical substances that link to GABA_A receptors may present three different activities in organism: agonist action (called Positive Allosteric Modulation - PAM), and consequently, an increase of Cl⁻ influx in chloride dependent channels;

antagonist action (called Negative Allosteric Modulation - NAM), causing a decrease of Cl⁻ influx in chloride dependent channels; neutral antagonism (NA), without effects on chloride dependent channels (Alhambra et al., 2011).

All pharmacological effects from BDZs, as sedation, anxiolytic effect, anticonvulsant effect and muscle relaxation, are produced by link with this isoform (Martin & Dunn, 2002; Davis & Murray, 2006; Martin et al., 2009). Therefore, depending on the chemical compound linked to the BDZs site, it will have several action mechanisms and any of these will produce several pharmacological effects. For example, α₁ agonists (PAMs) will produce sedation, as primary activity (sedative/hypnotic effect) (Jasen et al., 2008; Alhambra et al., 2011) and, secondarily, classical adverse effects (ataxia, alcohol potentiating, tolerance and dependence) (Alhambra et al., 2011), in α₂ and α₃ subunits they will produce, generally, anxiolytic activity (Jasen et al., 2008; Alhambra et al., 2011); antagonists (NAMs) will be anxiogenic in these subunits (Alhambra et al., 2011). BDZs show equipotent activities to α₁, α₂, α₃ and α₅ subunits of GABAₐ receptor. If the binding site doesn't have γ₂, has α₄ or α₆, this site will be insensible to PAMs action (Jasen et al., 2008; Martin et al., 2009).

Since 1996, the World Health Organization (WHO) suggests the implementation of benzodiazepines in pharmacological therapies for generalized anxiety disorders (apprehension, motor tension or autonomic overactivity); phobic symptoms and panic attacks; psychoses (acute and chronic organic brain syndrome) and schizophrenia and related disorders; mood disorders (depression and mania); personality disorder; suicidal patients; seizure disorders; tardive dyskinesia and akathisia; somatic presentations; muscle spasm; and sleep disorders. Furthermore, benzodiazepines are used as pre-operative sedatives, anesthetics and unpleasant investigation procedures. GABAₐ receptor has a great scientific value, consequently, many researchers all over the world have studied it, because it's related to action of many important substances, as BDZs, barbiturates, ethanol, anesthetics, etc. (Forman et al., 2009; Chebid & Johnston, 2000; Martin & Dunn, 2002; Sieghart & Sperk, 2002).

1.5 - Benzodiazepines

Several pharmaceutical companies are developing a large number of substances with similar structure to diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) (Kuch, 1979) which was synthesized by Sternbach in 1961 (Figure 4), whose commercialization started in 1963 as Valium® (Essman, 1973). 1,4-benzodiazepinics derivatives are known by the presence of four classes of drugs: anticonvulsants, anxiolytics, skeletal muscle relaxants, and sedative/hypnotics (Guzman et al., 1984).

Several modifications were realized in positions 1, 2', 4' and 7 from diazepam structure aiming to elucidate structure-activity relationship (SAR) of this drug (Sternbach, 1979). These modifications contributed to results showed in table 1.

**Figure 4.** Synthesis of diazepam developed by Sternbach.
Table 1. Structure-activity relationship (SAR) of diazepam.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Activity</th>
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<tbody>
<tr>
<td>A 7 -NO₂, -Cl, -F, -CF₃</td>
<td>Very high</td>
</tr>
<tr>
<td>A 7 -CH₃, -OCH₃</td>
<td>Low</td>
</tr>
<tr>
<td>B 1 -CH₃ tert-butyl</td>
<td>High Complete inactivation</td>
</tr>
<tr>
<td>C 2' -Cl, -F any substituent</td>
<td>High Very low</td>
</tr>
</tbody>
</table>

The 1,4-benzodiazepines drugs are considered safe and effective to many types of epilepsies, acting also as coadjuvant in treatment of refractory epilepsy. However, the use of the therapeutic class is limited, mainly because it causes psychomotor prejudices and tolerance to patients (Tietz et al., 1989). Regarding sleep therapy induction, the responsible agents for this activity are diazepam-metabolite products (Nicholson et al., 1977), by Cytochrome P450 (CYP450) enzymatic system, involving CYP2C19 and CYP3A4 (Saari et al., 2011) (Figure 5), known as Temazepam (3-hydroxydiazepam) (Nicholson et al., 1977), and oxazepam (3-hydroxy-N-demethyl diazepam) (Nicholson, 1979). The main disadvantage that these exhibit is the remaining sleep after treatment, an effect commonly known as latent sleeping or residual hypnotic effect (Nicholson et al., 1977), causing also a loss in reaction time of patients (Kesson, 1978).

Figure 5. Diazepam metabolism pathway by CYP450.

Other research projects led to a new class of benzodiazepines, 1,5-benzodiazepines, containing nitrogen in different positions in the seven members ring, as showed in figure 6.
1,4-Benzodiazepine

1,5-Benzodiazepine

**Figure 6.** Benzodiazepine ring structures differences.

The transference of an atom of nitrogen, from position 4 to 5, confers to these derivatives the capacity of reduce the psychomotor prejudice observed in patients who used 1,4-benzodiazepine derivatives (Tietz et al., 1989). There are numerous methods, conditions, different catalysts and synthetic routes, used to obtain these compounds (Al-Said & Ishtaiwi, 2005). They are usually obtained by condensation reactions between *ortho*-diaminoarenes with 1,3-diketones, where, initially attacks to carbonyl carbons occur, and then water elimination (Eicher & Hauptmann, 1995) (figure 7).

**Figure 7.** Standard synthesis for 1,5-benzodiazepines.

1,5-Benzodiazepine Drugs Of Medicinal Interest

**Clobazam (Frisium®)** - Clobazam (7-chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4-dione) was the first anxiolytic from this class synthesized by Rossi et al. in Maestretti laboratories (Italy) in 1961. This drug was obtained by reaction of 2-nitrodiarylamines with malonic acid, with a subsequently reductive ring closure (Kuch, 1979) (Figure 8).

In 1,4-benzodiazepines imine bond is susceptible to oxidation, this peculiarity provides to diazepam and structurally similar drugs their biological activity. But in 1,5-benzodiazepines the existence of imine is not essential to this activity. Diazepine ring, in Clobazam, doesn’t suffer any transformation after metabolism (Kuch, 1979).

Some information about Clobazam clinical applications and in vivo and in vitro biological characteristics could be cited: Clobazam shows smaller residual hypnotic effect in patients then diazepam (Nicholson et al., 1977) and chlordiazepoxide (Rickels et al., 1981); it doesn’t increase total sleep time (Nicholson et al., 1977); it doesn’t modify reaction time during treatment (Nicholson, 1979); efficient anxiolytic effect; it doesn’t present lethargic effect post-treatment (Nicholson, 1979; Rickels et al., 1981); effective against several epilepsies (Corman & Guberman, 1998; Wang & Hug, 1993; Heller & Ring, 1988; Shimizu & Abe, 1982; Wildin & Pleuvry, 1992; Bardy & Sepala, 1991); effective against Lennox-Gastaut syndrome, a type of childhood-onset epilepsy (Hancock & Cross, 2013).

Clozapine (Leponex®) - (3-cloro-6-(4-methylpiperazin-1-yl)-5H-benzo[c][1,5]benzodiazepine, figure 9) was discovered twice, first in Europe, 1960, when German and Austrian clinicians investigated its antipsychotic potential. The second discovery occurred in the United States in 1980, when it was successfully used as treatment against schizophrenia. However the specific date of clozapine origin is not known, this situation was known as "clozapine paradox" (Hippius, 1989). Clozapine is considered a neuroleptic agent clinically effective in schizophrenia having, as its main advantage, minimal Extrapyramidal Symptoms (EPS) (Chakrabarti et al., 1989; Morimoto et al., 2002; Gaszner & Makkos, 2004). It is worth mentioning that it is not associated to tardive dyskinesia and to hyperprolactinemia. The mechanism of action of Clozapine is not completely elucidated (Gaszner & Makkos, 2004).

In addition to this agent being a potent dopaminergic antagonist of D₁ (Llorca & Pere, 2004), D₂ (Chakrabarti et al., 1989; Llorca & Pere, 2004), D₃, D₄ (Gaszner & Makkos, 2004; Llorca & Pere, 2004), and D₅ (Llorca & Pere, 2004) receptors, clozapine is able of showing effects in other receptors, for example, muscarinic, cholinergic (Chakrabarti et al., 1989; Gaszner & Makkos, 2004), serotoninergic (5-HT₃,4,6,7), histamine receptor (H₁) and α₁,₂ adrenergic (Gaszner & Makkos, 2004). Thereby, the linkage of this drug with any of these receptors can be responsible for the EPS reduction (Chakrabarti et al., 1989). Clozapine has some limitations as weight gain, excessive salivation, sedation, cardiovascular system impairment, seizure threshold decrease (Morimoto et al., 2002; Gaszner & Makkos, 2004; Bitter et al., 2004), muscular relaxation, lack of motor coordination (Chakrabarti et al., 1989), and in other cases,
it can conduce to granulocytopenia and agranulocytosis in some patients, especially those who have advanced age (Llorca & Pere, 2004), being lethal in 0.5-2.0% cases (Bitter et al., 2004). Clinically, clozapine is used to treat schizophrenic patients whose conventional therapy, as typical and atypical antipsychotic (neuroleptic), is not effective (Gaszner & Makkos, 2004; Llorca & Pere, 2004; Bitter et al., 2004; Husseneather, 2004). Clozapine therapeutic introduction to these patients has as objective to restore cognitive, interpersonal and occupational functions, besides reducing positive and negative symptoms in psychosis. Its efficacy is similar to many atypical antipsychotics (Nardone et al., 2004; Llorca & Pere, 2004), being lethal in 0.5-2.0% cases (Bitter et al., 2004).

Clozapine was used as a leader compound for synthesis of several potentially active molecules. Chakrabarti et al. synthesized a derivative molecule of clozapine, Flumezapine (Thieno-[2,3-b][1,5]-benzodiazepine) (figure 10). It had a significant neuroleptic in vitro activity, similar to clozapine. In their study some SAR characteristics were elucidated: halogen substituents in position 7 give to the molecule significant neuroleptic activity, but a dichloride substituent reduces significantly its activity.

Another derivative with medicinal importance, obtained from clozapine, was synthesized by Morimoto et al. This derivative, 8-fluoro-1,2,4-methylpiperazin-1-yl]-6H-[1]benzothieno[2,3-b][1,5]benzodiazepine maleate, was named as Y-931 (figure 11). In vivo biological assays were conducted and it showed potent atypical antipsychotic activity. Bitter et al., showed, in his research, that there is no difference between clozapine and Olanzapine in their efficacies and both neuroleptic agents exhibit similar risks of develop EPS.

Clozapine (Zyprexa®) (2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine) (figure 12) is an antipsychotic agent with high affinity to serotoninergic (5-HT2A,2C), dopaminergic (D1,2,3,4), muscarinic (M1,5) histaminergic (H1) and α1-adrenergic receptors. Its use in patient medical therapeutic has as objective to treat positive and negative schizophrenia symptoms (Elian, 1998). Olanzapine is more effective than haloperidol in EPS reduction (De Sarro et al., 1992).

**Figure 10.** Flumezapine structure.

**Figure 11.** Y-931 structure.

**Figure 12.** Olanzapine structure.

**Figure 13.** Derivatives of 2,3-dihydro-1,5-benzodiazepine (a) and [1,2,4]oxadizolo[4,5-a][1,5]benzodiazepine (b).

In 1992, De Sarro et al. synthesized derivatives from two leader compounds, first one from 2,3-dihydro-1,5-benzodiazepine (a) (standard molecule, figure 7), Second one derived from [1,2,4]oxadizolo[4,5-a][1,5]benzodiazepine (b), these derivatives were administrated in rats and their anticonvulsant activities in rats brains were evaluated. After several modifications in their substituents (figure 13), they showed that: a more potent then b and clozapam; high dose of a conduce to hypothermia; derivative from a containing oxygen in R3 and phenyl in R4 showed a significant activity reduction when the rats were treated with flumazenil (2.5 mg/kg), this drug is used as antidote to benzodiazepine intoxication. These results suggested that the mechanism of action of this derivative is similar to classical 1,4-benzodiazepines (De Sarro et al., 1992).

In 2010, Ha et al. synthesized several derivatives from 2,3-dihydro-1,5-benzodiazepine, by reaction of 1,2-phenylenediamine and ketone (2.5 mmol), using sulfuric acid as catalyst (0.1 mmol) in methanol (figure 14). Their objective was to obtain compounds with potential anti-inflammatory activity in microglial cells (Ha et al., 2010).
One of these derivatives, 2,4(2-thiphenyl)-2,3-dihydro-1,5-benzodiazepin-2-yl (figure 15), has shown efficient inhibitory activity of nitric oxide (NO) production, acting by enzyme Inducible Nitric Oxide Synthase (iNOS) suppress in microglial cells, suggesting a new therapy to neuro-inflammatory and neuro-degenerative diseases (Ha et al., 2010).

Ben-Cherif et al. in 2010, synthesized two compounds, 4-(2-hydroxyphenyl)1,3-dihydro-1,5-benzodiazepin-2-ones and benzopyra[n4,3-c]-1,5-benzodiazepinone (RG0501 and RG0502, respectively) (figure 16). These compounds showed hypnotic activity and capacity to antagonize convulsions (Ben-Cherif et al., 2010).

Conclusion

Considering the foregoing, it is possible to observe the importance of GABA<sub>A</sub> receptor in modulation of several neuropharmacological activities performed by linkage of GABA<sub>A</sub> receptor with BDZ, barbiturates, ethanol, and general anesthetics. These chemical substances can initiate positive allosteric modulation, negative allosteric modulation or neutral antagonism. Regarding drugs of BDZ class, it is obvious that the interaction of these with subunit α<sub>1</sub> and γ<sub>2</sub> interfaces occurs the modulation of pharmacologic activity: anxiolytic, anticonvulsant, hypnotic, sedative, muscular relaxant, antipsychotic, and others. 1,4-benzodiazepines can produce activity in several epilepsies, although their main disadvantages in this treatment are the development of tolerance and psychomotor prejudices. About 1,5-benzodiazepines, it was observed that transference of nitrogen atom from position 4 to position 5, in diazepine ring,
the molecule shows less psychomotor prejudices to patient and it also allows that metabolism by CYP450 do not open this ring. Clobazam, clozapine and olanzapine are set in treatment of patients whose traditional drugs used (1,4-benzodiazepines, for example) are not able to cause an effect. It is possible that due to this pharmacologic class shows less residual hypnotic effect and reduced EPS, does not alter reaction time of patients, it is also able to treat positive and negative schizophrenia symptoms. Several researches show that 1,5-benzodiazepines are extremely versatile about their neuropharmacological activities in central nervous system, from anxiolytic to neuroantinflammatory. Therefore other researchers are needed to elucidate regarding properties and pharmacologic mechanisms of these drugs. It is necessary to investigate new derivatives that could be used therapeutically as safe and efficient drugs in treatment of patients with various types of central nervous system disorders.

REFERENCES


