USING ANXIOLYTICS IN EPILEPSY: NEUROBIOLOGICAL, NEUROPHARMACOLOGICAL AND CLINICAL ASPECTS

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ABSTRACT

Anxiety disorders represent a common psychiatric comorbidity in patients with epilepsy affecting prognosis and quality of life. However, they are still underdiagnosed and undertreated. In clinical practice, a number of compounds are currently used as anxiolytics, with benzodiazepines being the most popular ones. However, other drug classes, especially antiepileptic drugs, are increasingly prescribed for the treatment of anxiety. This paper discusses the neurobiological targets and basic neuropharmacological aspects of anxiolytics in order to give to the reader a clear insight of their activity and mechanism of action. Clinical data regarding the treatment of anxiety in both adults and children with epilepsy is also summarised, emphasising the need for further studies.

INTRODUCTION

Despite the increasing interest in psychiatric problems in epilepsy, rather limited literature is available on anxiety disorders (Hamid et al. 2011) and this is probably due to the high comorbidity rates with mood disorders which obscure the distinctive features of anxiety (Jones et al. 2005). However, it seems rather evident that anxiety disorders are frequently present in patients with epilepsy (Brandt et al. 2010), having a significant impact on quality of life (Jacoby et al. 2015) and playing probably a key role on suicidality among depressed patients (Placidi et al. 2000).

From an evolutionary perspective, anxiety represents a normal adaptive response to threat or stress and is characterised by a set of preformed behavioural patterns in response to visual, auditory, olfactory, or somatosensory stimuli (Price 2003). In addition, anxiety may also be the result of cognitive processes mediating the anticipation, interpretation, or recollection of perceived stressors and threats. From a neurobiological perspective, the Pavlovian-fear conditioning and fear-potentiated startle response, are well known models of anxiety and they have been extensively used to study anxiety largely because of their amenability to experimental manipulation (Martin et al. 2009). These neurobiological models have clarified an extended anatomic brain network which centres on the amygdala and a number of connected structures including mesiotemporal cortical structures, the sensory thalamus and cortices, the orbital and medial prefrontal cortex, the anterior insula, the hypothalamus, and multiple brainstem nuclei (Martin et al. 2009). The amygdala is determinant in the experience of fear and its autonomic and endocrine response (through the output to the hypothalamus). The output to the periaqueductal grey nuclei is implicated in avoidance behaviour while the hippocampi play a role in the re-experiencing of fear (Nees and Pohlack 2014; Cacciaglia et al. 2015). Spontaneous activation of fear circuits is the major hypothesis for explaining symptoms in anxiety disorders and the reduction of such an excessive output from these neurons

represents the main target of anti-anxiety treatments. In this regard, it is interesting to note that such a mechanism has a number of similarities with the excessive outburst typical of epileptic neurons and easily explain why some of the agents used in the treatment of epilepsy are also effective in anxiety and vice versa (Mula et al. 2007).

The term anxiolytics refers to a class of compounds that are used to treat anxiety symptoms (Stahl 2008). They are often discussed together with hypnotics as most of them are also used to induce sleep (**Table 1**). Anxiolytics were traditionally named "minor tranquilizers" to distinguish them from neuroleptics or antipsychotics that were defined "major tranquilizers". Traditionally, anxiolytics were thought to interact only with the GABA-A receptor complex but, over time, neuropharmacological studies have shown a number of other neurochemical pathways which can improve anxiety symptoms either directly or indirectly through a modulation of GABAergic neurotransmission (i.e. voltage gated calcium channels, alpha and beta adrenergic receptors, serotonin neurotransmission). This paper is aimed at discussing the neurobiological and neuropharmacological aspects of anxiolytic medications with special attention to epilepsy.

MOLECULAR TARGETS

GABA-A receptors

GABA is the principal inhibitory neurotransmitter of the brain and, along with serotonin and noradrenaline, is one of key targets for anxiolytics (Mula et al. 2007). The GABA-A receptor has a heteropentameric structure surrounding a central pore and is made up of different subunits (Möhler et al. 2001; Mula 2011) (**Figure 1**). GABA-A receptors can be categorised into three groups based on their alpha isoform content: alpha 1-containing receptors have greatest sensitivity towards BZs (type I); alpha 2, 3 and 5-containing receptors

have similar but distinguishable properties (type II); and alpha 4- and 6-containing assemblies have very low BZ affinity (Möhler et al. 2001). GABA-A receptors containing the delta subunit instead of the gamma are considered BDZ insensitive. In terms of brain localisation, for example, GABA-A receptors containing the alpha 1 subunit are densely represented in the cerebral neocortex (throughout), the hippocampus (DG, CA1 and CA2) and cerebellum, while the alpha 4 and 6 subunits are typical of cerebellar granule cells (Saari et al. 2011). Mutations or genetic variations of the genes encoding the alpha 1, alpha 6, beta 2, beta 3, gamma 2, or delta subunits (GABRA1, GABRA6, GABRB2, GABRB3, GABRG2, and GABRD, respectively) have been associated with a number of epileptic syndromes from genetic (idiopathic) generalized epilepsies (e.g., juvenile myoclonic epilepsy, childhood absence epilepsy) to severe epileptic encephalopathies like Dravet syndrome or Lennox–Gastaut syndrome (Hirose 2014).

The neurobiology of GABA-A receptors also explains why different compounds have a different spectrum of activity. For example, the sedative-hypnotic effect and partially the anti-seizure effect are due to the allosteric positive modulation of GABA-A receptors containing the alpha1 isoform, while the anxiolytic effect seems to be related to those containing the alpha2 isoform (Mula 2011). Non-benzodiazepine compounds, such as zolpidem, are often selective for the alpha1 and alpha5 isoforms and are, therefore, sedative-hypnotic with partial anti-seizure properties (Mula 2011). However, it is important to emphasise that all these subunits are subjected to gene variations and different polymorphisms may be responsible for different anticonvulsant responses to benzodiazepines.

In addition to the biochemical structure of GABA-A receptors, GABA-mediated inhibitory neurotransmission can be classified into "phasic" or "tonic" (Mula 2011). Phasic inhibition is a short-lasting inhibition typically generated by the activation of GABA-A receptors containing the gamma subunit and following action potentials in a presynaptic interneuron. Tonic inhibition is represented by the GABA-A conductance activated by ambient GABA in the

extracellular space (Farrant and Nusser 2005) and is mediated by molecularly and functionally specialized GABA-A receptors containing the delta subunit. Tonic inhibition is a long-lasting form of inhibition and it doesn't seem to be affected by the tolerance phenomenon typical of GABA-A receptor stimulation (Farrant and Nusser 2005; Mula 2011). The concept of tonic inhibition has received increasing attention in current literature. Neurosteroids target GABA-A receptors containing the alpha4 and alpha6 isoforms, which tend to co-localize with the delta subunit, and are therefore benzodiazepine insensitive (Bianchi et al. 2002). For this reason, neurosteroids have a reduced liability to tolerance and their potential usefulness in both epilepsy and anxiety is receiving increasing attention (Mula 2011). Interestingly enough, other well-known antiepileptic drugs (AEDs) seem to increase tonic GABA-ergic neurotransmission, namely phenytoin (Wong and Teo 1986) and lamotrigine (Wang et al. 2002).

Finally, for completeness, it is worth mentioning GABA-B and GABA-C receptors although they don't seem to play a role in anxiety. GABA-B receptors are expressed presynaptically at GABAergic and glutamatergic synapses and they decrease neurotransmitter release by reducing calcium influx. Baclofen is a classic GABA-B receptor agonist because it is selective for GABA-B receptors and does not activate GABA-A receptors. GABA-B selective agonists promote spike-wave discharges while antagonists (i.e. phaclofen) suppress them in rodent models of absence epilepsy (Manning et al. 2003).

GABA-C receptors are characterised by the rho subunit and play a unique functional role in retinal signal processing (Zhang et al. 2001).

Noradrenaline and serotonin neurotransmission

Both noradrenaline and serotonin neurotransmissions play an important adaptive function in responding to threat or stress.

Noradrenaline increases vigilance, modulates memory, mobilizes energy stores, and elevates cardiovascular function. Nevertheless, these biological responses to threat and stress can become maladaptive if they are chronically or inappropriately activated. Exposure to stressful stimuli of various types increases central noradrenergic (NE) function especially in the Locus Coeruleus (LC), the hypothalamus, the hippocampus, the amygdala, and the cerebral cortex (Samuels and Szabadi 2008). The firing activity of LC neurons also increases during exposure to fear-conditioned stimuli and other stressors or threats (Steckler et al. 2005). The recurrent symptoms of anxiety disorders, such as panic attacks, insomnia, exaggerated startle, and chronic sympathetic autonomic arousal, may conceivably reflect elevated NE function. Patients with post-traumatic stress disorder (PTSD) and panic disorder (PD) show evidence of heightened peripheral sympathetic nervous system arousal that, because of the correlation between peripheral sympathetic activity and central noradrenergic function, is compatible with the hypothesis of increased central NE activity in these disorders (Blechert et al. 2007). BDZs decrease LC neuronal firing activity but other agents, specifically targeting noradrenergic receptors such as beta or alpha blockers, are successfully used in the treatment of anxiety symptoms.

On the contrary, the role of serotonin (5-HT) neurotransmission in anxiety disorders is still matter of debate with an equal number of studies identifying normal or altered 5-HT receptors and/or serotonin transporter (SERT) function (Maron et al. 2004a, 2004b; Freitas-Ferrari et al. 2010). During exposure to fear-conditioned stimuli the 5-HT turnover in the medial prefrontal cortex correlates with the severity of stress and stimulates both anxiogenic and anxiolytic pathways within the forebrain, depending on the region involved and the 5-HT receptor subtype that is predominantly stimulated. A well-known and influential hypothesis about the involvement of the serotoninergic system in anxiety postulates that 5-TH2A receptors of the amygdala mediate the anxiogenic effects while 5-HT1A receptors in the

hippocampi provide resilience to aversive stimuli (Graeff et al. 1993). This is confirmed by the 5-HT1A receptor knock out animal model exhibiting anxiety behaviour and the anxiolytic effect of 5-HT1A receptor agonists (Ramboz et al. 1998). In this regard, it is important to note that glucocorticoids modulate the genetic expression of both 5-HT1A and 5-HT2A receptors (Watanabe et al. 1993; López et al. 1998). In fact, gene expression of post-synaptic 5-HT1A receptors in the hippocampi is down-regulated by corticosteroids whereas 5-HT2A receptors seem to be up-regulated (Watanabe et al. 1993; López et al. 1998). This mechanism would explain the number of plastic changes and brain network abnormalities in patients with anxiety disorders and the role of acute and chronic stress in such changes. Still, this would also explain why SSRIs or other serotoninergic antidepressants are successful for the long term treatment of anxiety disorders rather than being anxiolytics per se.

Voltage-gated ion channels

Voltage-gated ion channels have always been popular in psychiatric literature (Gargus 2006). Their types, primarily recognized as Na+, K+, Ca2+, Cl-, have been basically associated with neuronal firing and processes. Therefore, any drug targeting ion channels can influence all systems related with neuronal activity. In the context of anxiety, calcium channels are those that have received increasing attention as they showed to improve anxiety symptoms in animal models of anxiety (Mula et al. 2007; Zamponi 2016).

Calcium channels consist of two families: High Voltage-Activated (HVA) and Low Voltage-Activated (LVA). The HVA family comprises L-type (generating a long-lasting current) and N-, P-, Q-, and R-type channels (expressed in nerve terminals and responsible for the calcium entry that triggers neurotransmitter release) (Mula 2009). They are heterotrimeric structures consisting of three subunits: alpha, beta, and alpha2-delta. The alpha subunit forms a pore

with the ancillary subunit beta while the alpha2-delta subunit forms a functional pore by linking with the subunit alpha. The LVA family consists only of T-type channels which are monomers and are composed only by the alpha subunit.

LVA and HVA differ in function, localisation and electrophysiological activity. Although this represents an oversimplification, LVA T-type channels generate transient currents, have a somatodendritic localization and are critical to pacemaker activity and some patterns of burst firing, while HVA channels are more likely to be implicated in neurotransmitter release. There is some evidence that calcium channels, particularly HVA channels, may be implicated in the pathophysiology of mood disorders (Lodge and Li 2008). Genetic variation in CACNA1C, a gene encoding the alpha 1C subunit of the L-type voltage-gated calcium channel, has been associated with bipolar disorder, depression and schizophrenia (Bhat et al. 2012). It is also well known that calcium channels modulators can have either depressogenic or antidepressant properties (Perucca and Mula 2013). Drugs targeting the alpha2delta subunit like Gabapentin and Pregabalin seem to have anxiolytic properties (Joshi and Taylor 2006) but the reason for that still remains unexplained. One possibility is that the blockade of HVA calcium channels translates into a reduction in glutamate release (Farber et al. 2002), which may be ultimately responsible for positive effects on mood and some antianxiety properties. However, further studies are needed. At any rate, there is enough clinical evidence (see below) to support their use in anxiety disorders.

SPECIFIC ANXIOLYTIC AGENTS

Benzodiazepines

Benzodiazepines (BDZs) are a class of drugs chemically characterised by a benzene and a diazepine ring fused together plus a third benzene ring (**Figure 2**). Chlordiazepoxide was the

first synthetized BDZ and was accidently discovered by Leo Sternbach in 1955. It was also the first BDZ introduced into clinical practice and made available by La-Roche in 1960. After that, numerous different BDZs were synthetized and approximately 30 of them are currently available in clinical practice being important compounds not only as anxiolytics or hypnotics but, most importantly, in the treatment of status epilepticus, epileptic seizures, and in general anaesthesia (Saari et al. 2011).

Clinicians are often unaware of the wide range of BDZs available in the market and how they differ in terms of activity and clinical effects (Stahl 2008; Schatzberg and Nemeroff 2009). BDZs can be divided into a number of subgroups according to different chemical and pharmacological parameters **(Figure 2)**. .

The majority of compounds which are well-known to clinicians belong to the 1,4 group while Clobazam is the only 1,5 benzodiazepine. The difference in the chemical structure between Diazapam (1,4 BDZ) and Clobazam (1,5 BDZ) is shown in **Figure 2**. The main difference between 1,4 and 1,5 BDZs is in the hypnotic effect (Nicholson 1979). In fact, while all 1,4 BDZ have more or less a significant hypnotic effect, 1,5 BDZs lack in such an effect, explaining why clobazam has a different impact on cognitive functions as compared to diazepam (Bawden et al. 1999; 2001). As a note, both Clozapine and Olanzapine are also 1,5 benzodiazepine but their chemical structure is anyway completely different from the group of compounds that are named BDZ. Another important BDZ subgroup is that of Imidazo-BDZ and Midazolam is an example (Figure 2). Their distinctive feature is mainly from a chemical point of view because they possess a pH-dependent water-solubility. In fact, below pH 4 they are freely water-soluble, while at physiological pH of plasma the ring closes and the drug becomes lipid-soluble and rapidly penetrates the blood brain barrier to exert its action. They are, therefore, more water soluble/stable than other BDZs.

Finally, BDZs can be also classified as short-, intermediate- or slow-acting according to pharmacokinetic and pharmacodynamic parameters (**Table 2**) (Stahl 2008). Short-acting BDZs are hypnotics and can be used to induce general anaesthesia while slow-acting benzodiazepines are recommended for the treatment of anxiety. In this context, it has to be acknowledged that some BDZs have a complex and extensive metabolism leading to a number of metabolites that, in some cases, are active and contribute to the final pharmacological effect. For example, BDZs like flurazepam present a mixed profile being short-acting in terms of a rapid hypnotic effect but the pharmacological activity is quite sustained due to the long half-life of the active metabolite (**Table 2**). It is beyond the aim of this paper an extensive discussion on pharmacokinetics of BDZ but the reader should bear in mind that individual differences in the metabolism of BDZ may be responsible for inter-individual differences in the magnitude of the effect and the onset of side effects. In fact, the elimination half-life of diazepam, as well as other long half-life BDZ, is twice as long in the elderly compared to younger individuals and doctors should always adjust the dosage to the age.

Antiepileptic drugs

During the last 15 years, clinical researchers became increasingly interested in the potential for AEDs to improve or control anxiety symptoms. This was due to the limitations connected with the long term use of BDZ (see below) and the number of patients still refractory to first line treatment. In addition, as already briefly discussed, the spontaneous activation of fear circuits has a number of commonalities with the spontaneous activation of brain networks described in epilepsy. For all these reasons, a number of AEDs have been trialled in anxiety disorders (Mula et al. 2007). Despite a considerable number of published studies, the majority of them have several methodological limitations: inadequate sample size;

lack of a placebo control; use of unspecific outcome measures (i.e. the clinical global impression scales); lack of controlling for concomitant bias (i.e. comorbidities, diagnostic subtypes, concomitant medications). These factors may help explaining why AEDs have yielded inconsistent results in the treatment of anxiety disorders. At present, the strongest evidence is for Pregabalin (at dosages between 300 mg and 600 mg) in patients with generalized anxiety disorder with and without comorbid depression (Feltner et al. 2003; Pande et al. 2003, 2004; Montgomery et al. 2008; Diaper et al. 2013). Pregabalin (Pande et al. 2004; Kawalec et al. 2015) and Gabapentin (Pande et al. 1999) showed also promising results in social phobia but further studies are needed. For the remaining AEDs results are still preliminary with not many randomised controlled trials (Mula et al. 2007).

Antidepressants

Antidepressants are increasingly used in the treatment for anxiety disorders (Bandelow et al. 2008) and this is due to the number of limitations associated with BDZ such as tolerance, dependence and the risk of withdrawal (see below). Data is available mainly for selective serotonin re-uptake inhibitors (SSRIs) and serotonin and noradrenaline re-uptake inhibitors (SNRIs) and for the long-term treatment. It is still controversial why antidepressants are effective in anxiety but this seems to be related to the number of plastic changes to the noradrenergic and serotoninergic neurotransmission. In panic attack disorder SSRIs showed to be as effective as tricyclics but better tolerated (Bakker et al. 2002). Data on generalised anxiety disorder focused mainly on venlafaxine, paroxetine and imipramine and all of them showed good efficacy but overall they seem to be as effective as lorazepam or pregabalin (Mula and Strigaro 2010). In social anxiety disorder and post-traumatic stress disorder data is

still limited with promising evidence for sertraline and paroxetine and further studies are needed (Mula and Strigaro 2010).

EVIDENCE FROM CLINICAL STUDIES

Anxiety disorders are chronic conditions with a clear relapsing/remitting course and this is demonstrated by a number of cross-sectional and prospective studies (Maser 1990). This concept is of great relevance as, even nowadays, clinicians are focused almost entirely on the acute control of anxiety symptoms and only secondarily acknowledge relapse prevention. In addition, the natural history of anxiety disorders is frequently complicated by Axis I (e.g. major depression, bipolar disorder, psychoses etc) and Axis II (i.e. personality disorders) comorbidities which have a major impact on response to treatment. For example, 73% of patients with panic attacks have other comorbid conditions, ranging from major depression to substance abuse to personality disorders (Maser 1990) that need to be taken into account in any long-term anxiolytic treatment. BDZs have been historically considered first line treatment for the acute management of anxiety but their long-term use should be avoided as BDZs may lead to complications, such as abuse liability, dependence, and withdrawal syndrome. Long-term used is usually defined by a period of daily use of at least three months (Voshaar et al. 2006) because this seems to be long enough to cause neural adaptation changes that counteract the drug's effects (tolerance phenomenon). In these subjects even dose reductions may cause rebound symptoms that are almost identical to those for which the drug was initially taken (e.g. insomnia, agitation, panic attacks). In epilepsy, tolerance and dependence are even more relevant as seizures are common manifestations of a withdrawal reaction. In general terms, long half-life BDZ should be preferred as compared to short acting compounds because less associated to rebound symptoms but the management of withdrawal should be

planned on a case by case basis depending on age, concomitant comorbidities and seizure risk. Although less evident than BDZ, the potential risk of dependence with pregabalin should be considered (Caster et al. 2011).

Data on treatment of anxiety disorders in epilepsy is still limited and relies heavily on clinical experience (Kerr et al. 2011). However, as already mentioned, it is evident that anxiety disorders represent a frequent comorbidity in patients with epilepsy (Brandt et al. 2010). The Commission on Neuropsychiatry of the International League Against Epilepsy published a collection of papers about treatment strategies in adults with epilepsy and psychiatric disorders (Mula and Kanner 2013) and one paper was dedicated to the treatment of anxiety disorders (Mula 2013b). Evidenced-based therapeutic strategies in patients with anxiety disorders without epilepsy can be easily adapted to patients with epilepsy considering specific needs **(Table 3**). In panic disorder, a combined approach, namely SSRIs and cognitive behavioural therapy (CBT) is recommended for the acute phase while long-term maintenance treatment can be combined or based on CBT alone depending on the individual patient. In generalized anxiety disorder (GAD), pregabalin can be reasonably considered first choice for the acute and long-term maintenance treatment as it is licensed, although not everywhere, in both conditions. In social anxiety disorder and post-traumatic stress disorder, SSRIs, in particular sertraline and paroxetine, should be preferred for their low risk of interactions and the favourable tolerability. For obsessive compulsive disorder (OCD) CBT should always be considered first line treatment. When drug treatment is needed, SSRIs, in particular sertraline 100 mg, have to be preferred. Although it is reasonable to embrace standardized protocols of treatment developed for people with anxiety without epilepsy, it is also evident that psychiatric disorders of epilepsy present, more often not, with atypical features (Mula 2013a)

that may require individualised approaches. For this reason, studies in patients with epilepsy are anyway urgently needed.

Regarding children with epilepsy, data is even more limited than in adults. This is quite surprising if we consider that, in the general population, anxiety disorders are much more common in children than in adults (Costello et al. 2005; Franz et al. 2013). In addition, children with anxiety disorders seem to be at increased risk of further psychiatric comorbidities such as ADHD or conduct disorder (Kendall et al. 2010). Finally, it seems now established that half of adults with anxiety or depression had a history of anxiety onset before age 15 (Kim-Cohen et al. 2003). For all these reasons, The American Academy of Child and Adolescent Psychiatry has recommended that children and adolescents are routinely screened for symptoms of anxiety (Connolly et al. 2007). It seems, thus, evident that both a careful assessment and a prompt treatment in children with epilepsy would probably reduce the development of major problems during adulthood. Jones has recently reviewed management and treatment of anxiety disorders in children and adolescents with epilepsy (Jones 2014). SSRIs remain the first line treatment especially for OCD and there is promising data about venlafaxine in GAD and separation anxiety disorder. Again, PGB represents a good therapeutic option but there are no studies about PGB in children with epilepsy and anxiety disorders.

Finally, it is important to mention that children may present paradoxical reactions to short acting-BDZs (Jackson et al. 2015). This is a well-known phenomenon, for example, with midazolam when used in inducing mild sedation for elective surgical/invasive procedures (McKenzie and Rosenberg 2010). Patients with intellectual disabilities have the same liability of children and may develop paradoxical agitation and aggressive behaviour with short-acting BDZs (Barron and Sandman 1985). For this reason, short-acting BDZs should be avoided or carefully used in these patients.

CONCLUSIONS

Anxiolytic treatment is often perceived by clinicians as safe and easy. However, a multitude of different compounds with different mechanisms of action and peculiarities are available. In the context of epilepsy this is even more relevant as the main target of most of these medications is exactly the same of antiepileptic drugs. Controlled studies in both children and adults with epilepsy are urgently needed in order to develop tailored treatment strategies for anxiety disorders in this specific subgroup of patients.

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Figure legends

Figure 1. Illustration of GABA-A receptor. Eight types of receptor subunits have been cloned, with multiple subtypes within some classes: alpha 1-6, beta 1-4, gamma 1-4, delta, epsilon, pi, rho 1-3 and theta but the majority of GABA-A receptors consist of alpha, beta and gamma subunits with a stoichiometry of 2:2:1 (Möhler et al. 2001; Rudolph et al. 2001). In some cases the epsilon or delta subunit may replace the gamma subunit. The combination of different subunit isoforms characterises GABA-A receptors expressed in specific areas of the brain and modulating different functions. The benzodiazepine (BDZ) binding site is located at the interface of adjacent alpha and gamma subunits; therefore, the presence of the gamma isoform is instrumental in determining BDZ sensitivity while the specific alpha isoform contributes to a different selectivity and sensitivity.

Figure 2. Benzodiazepine structure and examples. Up left: The three rings are required for BDZ-receptor binding activity. Substituents in position 1,2 and partially 7, influence pharmacokinetics and half-life but not the affinity and activity of the individual drug on the BDZ-receptor site. BDZs can be also classified according to the relative position of the nitrogen atom in the heterocyclic ring as 1,2; 1,3; 1,4; 1,5 and 2,4. **Up right:** Position R2' may be unsubstituted or contain a halogen atom (F or Cl), a process called halogenation. Halogenation generally increases BDZ activity as it happens for example with Triazolam and Lorazepam which represent the Cl-substituted version of Alprazolam and Oxazepam, respectively. **Low left:** The amide group in position 2 in the diazepine ring can be replaced by a heterocycle ring, such as imidazole or triazole, generating two distinct subgroups of heterocyclic benzodiazepines named Imidazo- and Triazolo-benzodiazepines. Midazolam is an example of Imidazo-benzodiazepines while alprazolam and triazolam (up roght) are examples of Triazolo-benzodiazepines. i are both examples of Triazolo-BDZs**)**. **Low right:** Clobazam and Diazepam as examples of 1,4 and 1,5 BDZ with similar structure.

SHORT QUESTIONS WITH ANSWERS:

1. **What's the difference between 1,4 and 1,5 benzodiazepines?**
    a. They have different half-life and metabolism
    b. 1,5 benzodiazepine do not bind the GABA-A receptor complex
    c. 1,5 benzodiazepines do not have a significant hypnotic effect
    d. 1,4 do not have muscle relaxant properties

Correct answer c: while all 1,4 BDZ have more or less a significant hypnotic effect, 1,5 BDZ lack in such an effect, explaining why clobazam has a different impact on cognitive functions as compared to diazepam.

2. **Which of the following statements is correct regarding the use of anxiolytics in children?**
    a. Short acting benzodiazepines represent first line treatment in children
    b. Short acting benzodiazepines should be avoided or carefully used in children
    c. 1,4 benzodiazepines are contraindicated in children
    d. 1,5 benzodiazepines are contraindicated in children

Correct answer b: Children may present paradoxical reactions to short acting-BDZs. This is a well-known phenomenon, for example, with midazolam when used in inducing mild sedation for elective surgical/invasive procedures. Adults patients with intellectual disabilities have the same liability of children and may develop paradoxical agitation and aggressive behaviour with short-acting BDZs. For this reason, this specific subcategory should be avoided or carefully used in these patients.

3. **What can be considered first line treatment in patients with epilepsy and generalised anxiety disorder?**
    a. 1,5 benzodiazepines
    b. Imidazobenzodiazepines

c. Levetiracetam

d. Pregabalin

Correct answer d: In generalized anxiety disorder, Pregabalin can be considered first line treatment for the acute and long-term maintenance treatment as it is licensed, although not everywhere, in both conditions.