Chapter 2

Benzodiazepines and Anxiety Disorders: From Laboratory to Clinic

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Additional information is available at the end of the chapter

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Abstract

Benzodiazepines (BDZs), which are among the most widely prescribed drugs in current psychiatric practice, act as positive modulators of GABAergic neurotransmission. They are used to treat a wide range of disorders, from anxiety, affective disorders and insomnia to epilepsy, alcohol withdrawal and muscle spasms. However, the development of tolerance and dependence after long-term BDZ treatment, as well as the abuse potential, limit their use. Although some other classes of drugs are currently considered as a better choice for long-term treatment, BDZs to date still remain indispensable drugs. They are widely prescribed for anxiety disorders, with high levels of evidence existing for the short-term BDZ use in panic disorder and generalized anxiety disorder, intermediate for social anxiety and poor in post-traumatic stress disorder and obsessive-compulsive disorder. Future studies are intending to develop the new selective drugs that act via BDZ receptors, but with novel, narrow profile of action. Furthermore, the research on alternative therapeutic approaches of psychiatric disorders has shifted the focus onto therapeutic potential of natural BDZ ligands.

Keywords: GABA, benzodiazepines, anxiety disorders, pharmacology, behaviour

1. Introduction

It was midst of twentieth century when the pharmaceutical industry recognized the growing need of modern society for various kinds of tranquilizers. The first real breakthrough was achieved with a group of drugs called barbiturates, which were initially considered to be almost omnipotent and safe [1]. However, with the growth of their use, the adverse effects of barbiturates have been painfully discovered—the development of drug addiction, a risk of overdose and increased number of suicides. Therefore, the intensive search for a better sedative

has been continued. In the mid-fifties Dr. Leo Sternbach and his team from the company Hoffman-La Roche came into the focus of scientific publicity [2]. As it often happens in science, significant breakthroughs occur as a result of dedicated professional work and partly due to accidental discoveries. Working on a research of various molecules with possible sedative effects, Dr. Sternbach decided to re-examine benzheptoxidiazines, the group of substances that he worked on in Poland 20 years ago. Nearly 2 years later, while cleaning the warehouse, one of his assistants came across the substance left in storage for possible later testing, and Dr. Sternbach decided to examine it, just in case. It was the first 1,4-benzodiazepine, called chlordiazepoxide, demonstrating anxiolytic, hypnotic and muscle relaxant properties and only minor side effects, which has been marketed in a pharmaceutical form as Librium. Encouraged by the success of chlordiazepoxide, Hoffman-La Roche continued the research on related substances, which resulted in the synthesis of diazepam, an anxiolytic 3–10 times more potent than its precursor. Over 40 different benzodiazepine derivatives were synthesized in the following decades.

The next great success was the synthesis of triazolo-benzodiazepine analogues, such as alprazolam. Alprazolam is a specific anxiolytic in many ways—primarily because of its complex chemical structure, but also for its combined anxiolytic and antidepressant effects, which makes it useful for the specific cases of neuroses accompanied by depressive behaviour. However, in spite of extensive research, it took many years for researchers to associate benzodiazepines (BDZs) and their effects on γ-aminobutyric acid (GABA) and to propose their mechanism of action [3]. The mechanism of action of BDZ was established only 15 years after their introduction into clinical practice, during sixties [4], whereas 20 years passed until complete revelation of complex architecture of BDZ receptors [5]. Discovery of β-CCE, the first BDZ inverse agonist, in 1980, proved to be crucial for the future development of the benzodiazepine pharmacology, because it supported the idea of the existence of BDZ binding site subtypes in specific brain regions, which could have different physiological functions [6]. In contrast to other sedative-hypnotic drugs such as barbiturates, there is a specific antagonist for BDZ—flumazenil, which acts as a competitive antagonist in the presence of BDZ agonist compounds [7].

2. GABA and the brain

The inhibition and excitation of neural networks form the basis of information transfer in mammalian central nervous system (CNS). In an adequate balance between inhibitory and excitatory actions of neurotransmitters lies the key of normal functioning of the most complex processes in the brain [8]. The mutual coordination of main inhibitory neurotransmitter, GABA, and the major excitatory neurotransmitter, glutamate, is responsible for an adequate rhythmic activity, of both single as well as of group of neurons, thus altering synaptic plasticity and ensuring the normal functioning of CNS [9]. Decreased or increased activity of one or the other system is associated with number of neurological and psychiatric diseases [10, 11]. In addition, it has been suggested that the activity of inhibitory interneurons, most of which are GABAergic, defines the spatiotemporal framework, necessary for the different patterns of neural oscillations, which are essential for information processing in different brain structures

[12–14]. GABA was discovered in the 1950 by two independent research groups in the brain of mice, and it took additional 20 years to be officially proclaimed as a neurotransmitter [15–17]. The importance of GABA in neurotransmission is depicted by the fact that every third chemical synapse in the brain uses GABA as a neurotransmitter [18]. GABAergic synapse is the site of action of several different classes of drugs that modulate inhibitory neurotransmission [19]. Among these drugs are BDZs that have a wide spectrum of indications and represent a gold standard in treatment of anxiety disorders [20–22]. In the mid-seventies, the relationship between GABAergic system and anxiolytic action of BDZ was revealed, demonstrating that BDZ facilitates GABA neurotransmission. As positive modulators of GABAergic neurotransmission, BDZs have been described and examined in detail.

GABA achieves its effects by acting via two types of receptors: ionotropic GABA-A and metabotropic GABA-B receptors [23]. Although the third type for GABA receptor has been identified, and has its pharmacological specificities, the term GABA-C has not received broad consensus among experts, and IUPHAR (The International Union of Basic and Clinical Pharmacology) has classified it within GABA-A receptors [24]. GABA receptors, which are mainly coupled to the chloride channel but in varying degrees, can also couple to calcium, sodium and potassium channels. GABA-A receptors mediate the majority of GABA inhibitory actions in the CNS [10]. GABA-B receptors are localized pre- and post-synaptically, and negatively modulate adenylyl cyclase and inositol triphosphate synthesis, with final effect of potassium activation and/or inhibition of voltage-dependent calcium channels. Depending on the localization of GABA-B receptors, GABA-mediated inhibitory influences can be potentiated (post-synaptic receptors, presynaptic heteroreceptors on glutamergic endings) or reduced (autoreceptors) [24, 25].

GABA-A receptor is a pentameric complex made of transmembrane proteins that form ion channel, selectively permeable to chloride anion. These ligand-gated receptors are assembled from various (α1–6, β1–3, γ1–3, δ, ε, π, θ) subunits and their functional and pharmacological properties depend on their subunit composition [19, 26], GABA-A receptors are mainly localized in synapses, on post-synaptic membrane. In various regions, they are also localized extrasynaptically, especially when it comes to GABA-A receptor complex containing α4, α5 or α6 subunit [27, 28]. GABA-A receptors are also present on glial cells, and they could play an important role in adaptation of these cells to the needs of surrounding neurons [29]. GABAergic mechanisms are also involved in metabolic processes [19]. Negative correlation between the intensity of GABAergic neurotransmission and metabolic processes in cerebral tissue has been established [30, 31]. Activation of GABA-A receptors leads to a change in conformational state of associated ion channel, which results in an increased permeability to chloride ions [19, 24]. There are 14 different, structurally specific binding sites determined at the GABA-A receptor complex (**Figure 1**).

In addition to benzodiazepine binding site, i.e. BDZ receptor, at least 13 different, structurally specific sites on GABA-A receptor complex have been identified: (1) GABA and other agonists binding site, as well competitive antagonists; (2) picrotoxin site close to ion channel; (3) barbiturates binding site; (4) neuroactive steroids binding site; (5) ethanol binding site; (6) inhalation anaesthetics stereoselective binding sites; (7) furosemide diuretic binding site; (8)

Zn^{2+} ion binding site; (9) other divalent cations binding site; (10) La^{3+} ions site; (11) phosphorylation of specific protein kinases sites; (12) phospholipids binding sites; (13) sites involved in interaction of GABA-A receptor and microtubules, which take part in receptor grouping on post-synaptic membranes [32]. Via these binding sites, different modulators of GABA-A receptor complex exert their effects, acting as positive allosteric modulators, that potentiate the GABAergic effects, as negative modulators, that reduce the effects of GABA, and as neutral allosteric modulators, that competitively block the effects of these two types of agonists—antagonists [33]. Partial agonists and partial inverse agonists do not show full positive or negative modulation, even in the highest concentrations.

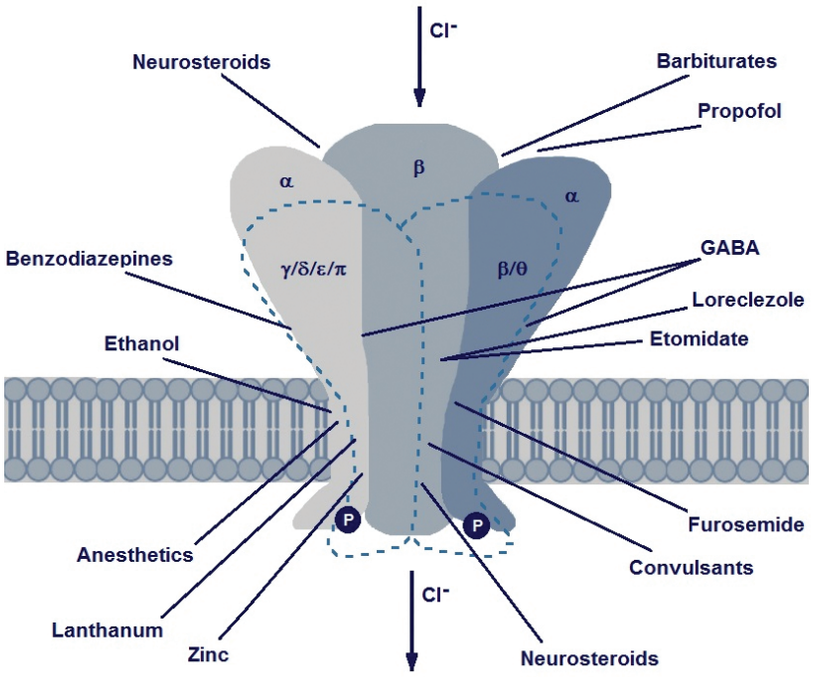

Figure 1. Schematic representation of GABA-A receptor complex and its subunits.

3. GABA-A receptor complex: a therapeutic target for BDZs

BDZ and other non-benzodiazepine analogues that bind to BDZ regulatory site (BDZ receptor), placed at interface between α and γ subunits, allosterically increase GABA receptor affinity. The result of this modulatory influence is increased opening frequency of ion channel in the presence of given neurotransmitter concentration, i.e. increased efficiency of GABAergic neurotransmission [34]. Although the $\gamma 2$ subunit presence is necessary, the selectivity by which BDZ ligands bind to GABA-A receptors of different structure primarily depends on six different α subunits. The functional and pharmacological properties of GABA-A receptors depend on their subunit composition [19]. Thus, $\alpha 1$ GABA-A receptors mediate sedative, amnesic and partly anticonvulsant, but not anxiolytic action of diazepam, in which $\alpha 2$ subunit

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plays the major role [35]. That is how zolpidem has high binding affinity to GABA-A receptors containing α1 subunit [35, 36]. Today, GABA-A receptors containing α1 subunit are mainly marked as GABA-A1 receptors. 1,4-benzodiazepines, such as diazepam, with nearly same affinity bind to GABA-A1 receptors as to GABA-A receptors containing α2, α3 and α5 subunits, marked as GABA-A2, GABA-A3 and GABA-A5 receptors. Therefore, diazepam shows comparable affinity to all BDZ-sensitive receptors (GABA-A receptors containing γ2, β, α1, α2, α3 or α5 subunit), while it does not bind to BDZ-insensitive receptors (receptors containing α4 or α6 subunit). On one GABA-A receptor complex, there is only one BDZ recognition site; although by rule this protein complex contains two α subunits [37]. Namely, γ subunit via specific amino acid residues takes part in BDZ receptor formation and is most probably placed in the presence of only one α subunit, so that the possibility of another BDZ molecule binding via another α subunit remains unaccomplished. On the other hand, GABA binds to receptor at the interface between α and β subunits, which are present with two pairs of neighbouring macromolecules, so that two GABA molecules can bind to one receptor complex.

Electrophysiological researches indicate that BDZ, applied at nanomolar concentrations, leads to conformational changes of one GABA binding site, most probably the one whose forming includes α subunit that was also involved in BDZ binding. Therefore, thanks to conformational change transfer from the α-γ interface (BDZ receptor) to the neighbouring α-β interface (GABA binding site), BDZ increases the binding affinity of one GABA molecule. Conformational changes do not transfer to other α subunit, i.e. to another GABA molecule binding site, which could explain the fact that BDZ receptors cannot by themselves open receptor ion channel, and that, unlike barbiturates, they cannot act in the absence of GABA [37].

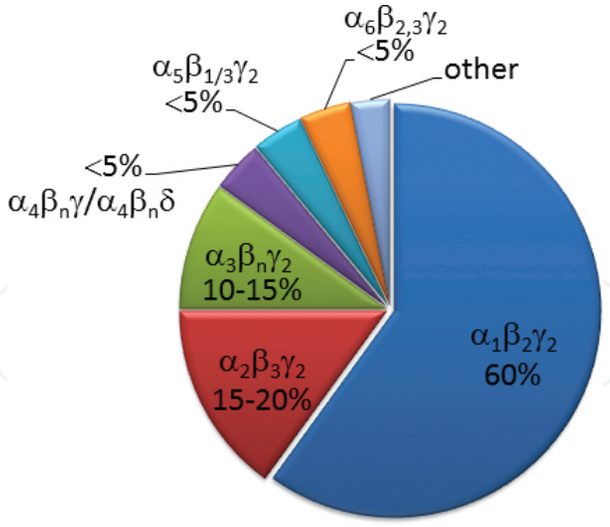

Figure 2. The distribution of different GABA-A receptor subtypes in the brain.

Receptors containing α1, α2, α3 or α5 subunit, in combination with γ2 or some of the β subunits, represent dominant fraction of GABA-A receptors and α1β2γ2S is the most common combination found in the brain (**Figure 2**) [24, 36]. Around 60% of all GABA-A receptors contain α1 subunit. Regions in which these receptors are absent are rare; an example are motor

neurons in spinal cord, but the distribution in certain regions is very unequal [36, 38]. In amygdaloid complex, for example, α1 subunit is dominantly present in basolateral nucleus, and rarely found in the central nucleus [39]. The GABA-A receptors containing α2 subunit represent 15–20% of entire population [36]. In small number of structures, this subunit is completely absent, and it is in general less expressed than α1 subunit. One of the exceptions is central nucleus of amygdala, in which α2 subunit is dominant [39]. Receptors containing this subunit are densely distributed at axon initial segments of cerebral cortex and hippocampus projection cells [35, 40]. The receptors with α3 subunit are found in about 10–15% of the whole GABA-A receptor number [36]. The distribution is unequal, and in a number of regions these receptors are absent [38]. Less than 5% of GABA-A receptors contains α5 subunit. In certain structures, these receptors are more expressed (certain regions of cerebral cortex, olfactory system, hypothalamus, basal ganglia) [38], while in hippocampus α5 subunit is present in about 15–20% of all BDZ-sensitive GABA-A receptors [36, 41–43].

4. BDZs in clinical practice

BDZs are nowadays among the most widely prescribed drugs in current psychiatric practice and have relatively favourable pharmacokinetics and pharmacodynamic profile. Pharmacological profile of BDZ includes sedation, anxiolysis, hypnotic effect, anterograde amnesia, muscle relaxation and anti-convulsant properties. Different BDZs are available in practice, and basic indication of each BDZ preparation is declared by its most manifested effect. According to the guidelines and recommendations, systematic reviews and meta-analyses, there is a spectrum of use of BDZ in psychiatric clinical practice: anxiety and affective disorders, alcohol withdrawal, sleep disorders, delirium, aggressive behaviour in psychoses and neuroleptic-induced disorders (**Figure 3**) [2, 44, 45].

The main advantage of BDZs is their quick action, which can be seen soon after the first taking of the drug. BDZs are usually taken orally or may be administered intravenously. Due to well absorbance, they usually reach their maximum of concentration in plasma for about 1 h. They bind strongly to plasma proteins, and their high liposolubility causes many of them to accumulate gradually in the fatty tissue. BDZs are generally metabolized and excreted as glucuronides in urine [46]. According to their pharmacokinetics properties, BDZs are divided into three groups: short-acting (triazolam and midazolam), intermediate-acting (alprazolam, clonazepam, lorazepam, nitrazepam) and long-acting (diazepam, chlordiazepoxide, flurazepam).

They are mostly non-toxic and due to their large therapeutic range, BDZs are considered to be safer drugs than other medicaments of similar function (e.g. barbiturates). However, BDZ should be avoided or administered at lower dosages in the elderly, used sparingly in children, and applied with caution in the first and third trimesters of pregnancy and while breastfeeding [47]. Taken in excessive doses, they may lead to death very rarely, but we should bear in mind that the risk of toxic effects is increased in the presence of other CNS depressants, especially alcohol. The main side effects are drowsiness, confusion, anterograde amnesia and

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impaired motor function. Tolerance occurs following prolonged treatment with all benzodiazepines [48, 49], as well as an addiction which is their main drawback. Tolerance to the sedative effects develops already after relatively short period of exposure to BDZ, while the development of tolerance to the anxiolytic effects of these drugs require prolonged treatments. Tolerance to the BDZ anticonvulsant activity develops at medium speed. The speed of development of tolerance also depends on the BDZ dose administered. According to the efficacy of these drugs, tolerance and dependence develop to a lesser extent following treatment with partial BDZ agonist than after administration of full benzodiazepine agonists. Because of the possible development of addiction and withdrawal syndrome, it is advisable to limit BDZ therapy to a maximum of 4 weeks continuously, and then implement a gradual dose reduction [50]. The risk-benefit ratio remains positive in most patients in the short term, but is un-established beyond that time [51]. It should be also noted that occasionally they can cause a paradoxical effect, such as increased anxiety, excitement, irritability and aggressive behaviour.

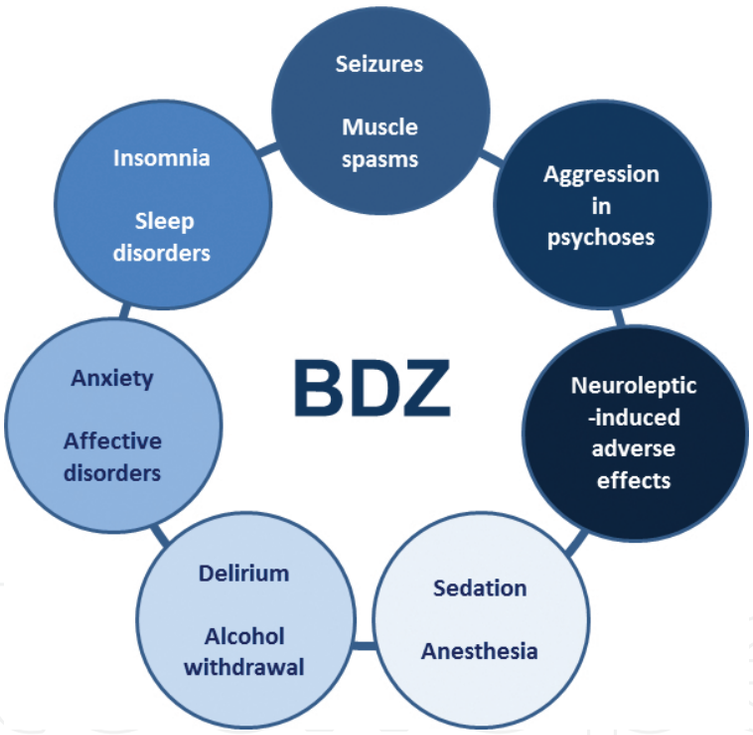

Figure 3. The use of BDZs in psychiatric clinical practice.

4.1. BDZs and anxiety disorders

According to the international guidelines, selective serotonin reuptake inhibitors (SSRIs) and serotonin/norepinephrine reuptake inhibitors (SNRIs) are currently recommended as the drugs of first choice for treatment of anxiety disorders, suggesting BDZ for the second-line or follow-up therapy [44, 45, 52]. Nevertheless, some recent studies have demonstrated that long-term use of BDZ can be effective and safe and that BDZ can be combined with antidepressants

and psychological therapy to produce optimal outcomes [53]. Such findings have given an incentive to reconsider the role of BDZ for therapy of anxiety disorders. Due to rapid onset, BDZs are effective especially in the acute phase of anxiety. However, in addition to the side effects, one of the main disadvantages is that BDZs have no confirmed antidepressant activity, since comorbidity between anxiety and depression becomes the rule rather than the exception [44, 45, 52]. Although, alprazolam is internationally registered for the treatment of anxiety associated with depression, evidence of its specific antidepressant effect as a single treatment is inconclusive. In an extensive intervention review by van Marwijk et al. [54], alprazolam appears to reduce depressive symptoms more effectively than placebo and as effectively as tricyclic antidepressants. However, the studies included in this review were heterogeneous, of poor quality and only addressed short-term effects, thus limiting confidence in these findings. Here, we summarize the current available information related to the BDZ use for the most common anxiety disorders: generalized anxiety disorder, social anxiety disorder, panic and post-traumatic stress disorder.

4.1.1. Generalized anxiety disorder

Due to slower onset of action of SSRIs and SNRIs, BDZs are still recommended for the acute treatment of generalized anxiety disorders (GAD), either concomitantly until the effects of the antidepressant become apparent, or as a short-term measure for increased anxiety [47]. The efficacy and safety of BDZs in GAD, particularly for alprazolam and diazepam, were assessed in numerous randomized controlled trials (RCT) [55–67]. Bandelow et al. [44] recommended alprazolam in a dose between 1.5 and 6 mg when used adjunctively with an antidepressant and World Federation of Societies of Biological Psychiatry (WFSBP) guidelines also included the use of diazepam and lorazepam for the treatment of GAD in adult daily doses of 5–15 and 2–8 mg, respectively. WFSBP guidelines have rated both compounds with a category of evidence A for GAD treatment. However, the overall recommendation grade is lower, since the long-term treatment studies with BDZs in GAD are lacking and these compounds should only be used when other drugs or cognitive behavioural therapy (CBT) have failed [44]. Other BDZs, such as lorazepam and bromazepam, have been also studied and reviewed, but with less evidence [68, 69]. A meta-analysis by Baldwin et al. [70] reviewed 27 RCTs of drug treatments for GAD, and lorazepam was the only BDZ included. Fluoxetine has been ranked first drug of choice according to response and remission and pregabalin for tolerability, while lorazepam efficacy has been found low but with a limited data.

4.1.2. Social anxiety disorder

Effective treatment of social anxiety disorder (SAD) includes CBT and a spectrum of medications including some antidepressants, BDZs and anticonvulsants [71]. Many trials have been conducted with BDZs in the treatment of SAD. Munjack et al. [72] showed superior clinical efficacy of clonazepam for the patients with SAD, while Gelernter et al. [73] demonstrated a modest effect for alprazolam. Subsequently, Davidson et al. [74] demonstrated clinical benefits of clonazepam treatment for SAD, fear and phobic avoidance, inter-personal sensitivity, and on disability measures. Otto et al. [75] found that clonazepam or CBT were equally effective

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in acute treatment, while some authors found mixed results [2]. Anyhow, the reference data indicate BDZs to be efficacious treatments in SAD, well tolerated and with rapid effect; however, not as first-line treatment because of their potential withdrawal difficulties and limited spectrum of action. Thus, for SAD therapy, BDZs are rated with a category of evidence B by the WFSBP guidelines [44].

4.1.3. Panic disorder

Over the past 30 years, BDZs have been successfully used to treat the core symptoms of panic disorder (PD). Namely, the most robust evidence of BDZ efficacy in the treatment of anxiety was determined in panic disorder. BDZs are generally shown to be effective for a broad range of PD symptoms, with their rapid and maintained effect over a 7- to 8-month period [76]. The RCTs have demonstrated that alprazolam, diazepam, clonazepam and lorazepam are all clinically effective in PD. One of the first RCTs confirming the effect of BDZs in PD was performed with patients who were randomized to placebo group or alprazolam treatment for 8 weeks [77]. The efficacy of alprazolam was clearly demonstrated in patients usually consequently treated with tricyclic antidepressants or MAO inhibitors. In a following study, results indicated that alprazolam and diazepam appeared equally effective in patients with panic attacks and generalized anxiety compared to placebo [78]. Many trials subsequently attested the short-term efficacy of BDZ in relieving the core symptoms of PD. Thus, BDZs are rated for PD treatment with category of evidence A by the WFSBP guidelines, and this rate is consistent with their acute efficacy [44]. However, sufficient efficacy in PD is still lacking for the long-term BDZ therapy.

4.1.4. Post-traumatic stress disorder

The use of BDZ in post-traumatic stress disorder (PTSD) is poorly supported by current literature, and only a few available studies demonstrate mixed results. In the study by Braun et al. [79], 5 weeks of alprazolam treatment, showed only minimal improvement in the core symptoms of PTSD. In a following study, results indicated that alprazolam and clonazepam, after 6 months of treatment, appeared equally effective in patients with PTSD compared with control subject [80]. Moreover, some preclinical studies showed even increased vulnerability to stress after alprazolam single application in the animal model [81]. Thus, use of BDZ as a monotherapy in PTSD is not currently supported (category of evidence F by the WFSBP guidelines) [44]; however, potential benefits of combined treatment remain to be further elucidated. Similarly, mixed/negative results were found for efficacy of BDZ treatment in obsessive-compulsive disorder (OCD) [2, 82].

5. The perspective of BDZ research and development

It has been clearly shown that classic benzodiazepines, such as diazepam, achieve their effects by enhancing GABA neurotransmitter activity at the number of GABA-A receptors (BDZ-sensitive receptors), while they do not bind to the rest of receptor population [11]. At the

beginning of the twenty-first century, using technology of genetic engineering, the conditions were created for establishing the correlation between certain effects of BDZ receptor ligands and their specific molecular and neural substrate of action [83]. Nowadays, these highly specific studies will probably result in the development of selective drugs that act via BDZ receptor with novel, narrow profile of action—therapeutic as well as adverse. Furthermore, the discovery and synthesis of a number of natural BDZ ligands have been initiated.

5.1. Pharmacokinetic and pharmacodynamic modifications

Pharmacokinetic modifications represent favourable approach since the substances with similar pharmacological activity are adapted to different therapeutic indications. The application of different BDZ as anxiolytics, hypnotics or myorelaxants, is primarily determined by their pharmacokinetic properties, such as dosage form, administration route and the presence of active metabolites. By reducing time of their action, great success has been achieved in the treatment of insomnia, thereby avoiding the morning drowsiness, and enabling the motor vehicle operation [84]. However, this type of modifications could not solve problems such as cognitive impairments and loss of coordination, especially in elderly. Since pharmacokinetic modifications did not lead to separation of desired and unwanted effects of BDZ, new approaches applied to the synthesis of a number of substances with different pharmacological profiles.

Along with pharmacokinetic modifications, a new concept emerged with the assumption that BDZ with lower efficiency at GABA-A receptors could maintain the useful anxiolytic effect of standard BDZ, but without unwanted side effects [85]. By using this concept, bretazenil was developed, which has not shown *in vitro* selectivity for certain subtypes of GABA-A receptor [86, 87]. Although acting as partial agonist of BDZ receptors, bretazenil has been shown to be potent anxiolytic and anticonvulsant in animal models [86, 88, 89]. Despite of bretazenil anxiolytic effect, noticed in the human studies, clinical studies were discontinued in the first phase due to deep sedation, whereby the sedative effect of bretazenil even at lower doses could be compared to the effects of diazepam and ethanol combination. Sedative effect of bretazenil was so pronounced that certain responders fell asleep while performing routine tests [90]. Further research in the field of non-selective partial agonists of BDZ receptors have led to the synthesis of imidazenil, a substance with 30–50% lower efficiency than diazepam [91]. Although imidazenil is more potent than bretazenil and practically without any potential for inducing sedation in preclinical models, studies on humans have not confirmed the expected results [92, 93]. It is important to mention that imidazenil showed a lower efficiency at $\alpha 1$ GABA-A receptors in relation to diazepam, which could be one of the reasons of such favourable pharmacological profile, but also indicated new possibilities in the search for ideal anxiolytic drug [94]. However, after several unsuccessful attempts, the idea of partial agonist development was not so attractive any more, especially in the light of discovery that there are several possible subtypes of GABA-A receptor complex. As a result, the interest in the new drug development has been directed towards selective ligands [36].

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5.2. The development of BDZ ligands selective for certain receptor subtypes

Diazepam, synthesized in 1963, for many years represented a synonym for BDZ group of drugs, and only later a huge number of BDZ ligands were synthesized. Diazepam has a wide spectrum of indications and shows anxiolytic, hypnotic, myorelaxant, anticonvulsant and other effects. Moreover, the examination of diazepam, as a reference BDZ, on genetically modified animals provided linkage of certain effects with specific receptor subtype and enabled further development of selective drugs with new, narrow profiles of action [35, 95, 96]. The past decade of research has led to an increased understanding of the specific GABA-A receptor subtypes responsible for various pharmacological effects of BDZs (**Table 1**) [97, 98].

Effect	Receptor subtypes			
	α_1	α_2	α_3	α_5
Sedation	+	−	−	−
Anxiolytic activity	−	+	−	−
Anterograde amnesia	+	−	−	+
Myorelaxation	−	+	+	+
Anticonvulsive activity	+	−	−	−
Addiction	+	−	−	−

Table 1. Pharmacological effects of BDZs and the corresponding subtypes of GABA-A receptors.

So far, 19 subunits of GABA-A receptor complex have been cloned, and subunits are classified into several structurally connected subfamilies comprising highly homologous isoforms (α1–6, β1–3, γ1–3, δ, ε, θ, π, ϱ1–3). The most commonly represented receptor population is a complex consisted of two α and two β and one γ subunit [10, 99]. Concerning the fact that two different α and/or β subunit can be present in the same receptor, in the brain, it is highly probable that there are more than 500 different GABA-A receptor subtypes. However, molecular studies show that the number of main subtypes of GABA-A receptors is less than 10, with the α1β2γ2 present in about 43%, α2β2γ3 in 18%, and α3$\beta\gamma$2/3 in 17% of receptors [100, 101]. On the other hand, extensive immunochemical studies which investigated the distribution of 13 most common subunits (α1–6, β1–3, γ1–3, δ) in the rat brain, have shown that the great majority of receptors contain γ2 subunit, while δ and γ1 subunits are confined to a very small number of brain regions. Among α subunits, α1 showed the most pronounced immunoreactivity in practically all areas of the brain. Other α subunits are more confined to particular parts of the brain [38].

The development of ligands selective for α1 subunit (e.g. zolpidem and zaleplon) has been accomplished. Zolpidem represents selective agonist for GABA-A receptors containing α1 subunit, and it is widely used in the treatment of insomnia [102]. Zolpidem is characterized by 5–14 times greater affinity for α1 in relation to α2 and α3 receptor subtypes, as well as by very low affinity for α5GABA-A receptors [103]. Zaleplon is another α1GABA-A selective agonist from the so-called Z-hypnotics group, which in relation to zolpidem has two times

lower affinity for α1GABA-A receptors, and unlike zolpidem shows certain affinity for α5GABA-A receptors [104]. Only sporadic reports can be found on behavioural effects of ligands characterized by selective affinity/efficiency at α2- and α3-subtype of GABA-A receptor complex, which in preclinical conditions have demonstrated anxiolytic action. For current pharmacological trials, particularly α5 subunit of GABA-A receptor represents the most interesting drug target, primarily from the aspect of new pro-cognitive drugs development. The results of the study with genetically modified mice have shown that decreased expression of α5 subunit acts facilitatory on performing certain memory tests, so over the next couple of years, various inverse agonists with selective affinity or efficiency at α5GABA-A receptors were synthesized [43, 105, 106]. L-665, 708, partial inverse agonist at all four subtypes of GABA-A receptors, with greater functional selectivity at α5GABA-A receptors, improves learning and motivation in rats during forced swimming [107]. In addition, α5IA is a substance with similar profile, which has also shown promnestic activity, as well as selective influence on the acquisition and retrieval of memory [108]. Finally, RO4938581, a selective inverse agonist at α5GABA-A receptors has successfully antagonized impairing effects of scopolamine and diazepam on rats learning in Morris water maze [109]. Clinical studies have shown that in healthy volunteers, pre-treatment with α5 selective inverse agonist has greatly decreased amnesic effect caused by alcohol intake, confirming the concept validity of α5GABA-A receptors significance in hippocampal-dependent memory processes [110]. However, more clinical studies remain to be conducted.

5.3. Natural compounds as BDZ receptor ligands

Extracts of many plants, as well as their natural and synthetic derivatives, are increasingly proposed as an integral part of the clinical treatment of psychiatric diseases, particularly mood disorders, due to higher compliance and fewer side effects in patients [111, 112]. One of the first studies on natural compounds as CNS ligands was reported by Roche researchers, in which they isolated a few '*diazepam-like*' isoflavan derivatives with low affinity for BDZ receptor [113]. Chrysin, the first flavonoid described as a specific partial agonist of BDZ receptor, is almost equipotent to diazepam as an anxiolytic, but does not exert myorelaxant and sedative effects [114]. In subsequent preclinical studies, the flavonoid cirsiliol showed a very low affinity for BDZ receptors and was devoid of anxiolytic actions, while kaempferol, quercetin and myricetin were active after oral application, exerting anxiolytic effects [115]. Apigenin, a common flavonoid found in many plants including chamomile, was initially described as a BDZ antagonist with anxiolytic effects [116]. However, later studies have showed that apigenin fits more into pharmacological profile of an inverse BDZ agonist, exerting sedative, but not anxiolytic effect [117]. Since this initial search for natural/alternative BDZ ligands, a significant number of successful derivatives have been isolated [112]. *In vitro* and *in vivo* studies have sufficiently clarified the pharmacological actions of flavonoid and flavonol derivatives on BDZ receptors; however, their precise mechanism seems to be much more complex.

Besides flavonoids, terpenoids have been also reported to affect GABA-A receptors in various ways, including modulation of BDZ binding site [118]. The most widely studied terpenoid is

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picrotoxin, a convulsant and non-competitive antagonist at GABA-A receptors. A structurally similar to picrotoxin is bilobalide, a terpenoid from the *Ginkgo biloba* plant, which acts as a negative allosteric modulator of GABA-A receptors, partially explaining cognition-enhancing effects of *Ginkgo biloba* extracts [119]. Studies have shown that bilobalide particularly modulate the peripheral BDZ receptors [120]. Furthermore, the extracts of *Valeriana* contain a large number of substances including terpenoids and flavonoids, many of which are considered to be active at GABA-A receptor complex [121]. More studies are still required in order to fully understand the pharmacological profile of natural CNS ligands and their potential in the treatment of neuropsychiatric disorders.

6. Conclusion

Thanks to a very wide range of pharmacological actions, including anxiolytic, sedative-hypnotic, anticonvulsant and myorelaxant effects, BDZs became the most frequently used group of psychoactive drugs in clinical medicine in the last 50 years. They have been used to treat a wide range of disorders, from anxiety, affective disorders and insomnia to epilepsy, alcohol withdrawal and muscle spasms. However, in spite of rapid onset of efficacy, relative safety and mild side-effects of these drugs, the knowledge about the development of tolerance and dependence after long-term BDZ treatment, as well as their abuse potential, has somewhat limited their previous widespread use. Although some other classes of drugs are currently considered as a better choice for long-term treatment, BDZs to date still remain indispensable drugs in clinical medicine. They are widely prescribed as a first line treatment in anxiety disorders, with high levels of evidence existing for the short-term BDZ use in PD and GAD treatment, intermediate for SAD therapy and poor in PTSD and OCD. Further research for new alternative drugs has been encouraged by the increased understanding of the mechanisms of BDZ action. Information and knowledge about the structure and function of GABA-A receptor complex provided the molecular basis for further elucidation of the nature of interactions between BDZs and their receptors. As a result, the selective substances, such as zopiclone, zolpidem and zaleplon, which also interact with the BDZ receptors, have been developed. Future studies will probably result in the development of new selective drugs that act via BDZ receptors with novel, narrow profile of action—therapeutic as well as adverse. Furthermore, the research on alternative therapeutic approaches of neuropsychiatric disorders has shifted the focus onto therapeutic potential of natural BDZ ligands.

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