

Cellular and molecular mechanism of the benzodiazepines addiction

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Update by topics

SUMMARY

Benzodiazepines (BZD) are a group of psychoactive drugs widely prescribed since their introduction into clinical practice in the early 1960's. These drugs have a high therapeutic efficiency in anxiety treatment. Its mechanism of action over the Central Nervous System at a molecular level is well known, nevertheless, concern between different health systems about the effects of pharmacological addiction they provoke has always existed. The objective of this article is to address the results recently obtained in basic research and to describe the mechanism by which benzodiazepines produce their addictive effects at a molecular level, as well as informing about the epidemiologic impact of their use.

Key words: Benzodiazepines, addiction, drugs of abuse, mechanism of action, epidemiology.

RESUMEN

Las benzodiazepinas (BZD) son un grupo de psicofármacos ampliamente prescritos desde su introducción en la práctica clínica a principios de los años 1960. Estos fármacos cuentan con una alta eficacia terapéutica en el tratamiento de la ansiedad. Su mecanismo de acción sobre el Sistema Nervioso Central a nivel molecular es bien conocido, sin embargo siempre ha existido preocupación entre distintos sistemas de salud por los efectos de adicción farmacológica que provocan. El objetivo de este trabajo es abordar los resultados obtenidos recientemente en investigación básica y describir el mecanismo por el cual las benzodiazepinas producen sus efectos adictivos a nivel molecular, así como informar sobre el impacto epidemiológico que tiene su uso.

Palabras clave: Benzodiazepinas, adicción, drogas de abuso, mecanismo de acción, epidemiología.

INTRODUCTION

It was in 1930 when Leo Sternback discovered benzodiazepines (BZD), but until 1957 the use of chlordiazepoxide (anxiolytic benzodiazepines) was introduced into clinical practice.¹ Since 1963, with the introduction of diazepam (Valium) BZDs were massively used thanks to the increase of the margin of safety and therapeutic efficacy with respect to the substances used until then as anxiolytics, which rapidly led to the replacement of barbiturates.²

Thus, BZDs currently make up the most widely used group of drugs for the treatment of anxiety. More than 15 kinds of BZDs are used for the treatment of a wide range of psychological and physical discomforts, because they cause fewer side effects than barbiturates, are relatively safe in case of overdose and have fewer risk of causing dependen-

cy.² Beside their anxiolytic action, and because they reduce neuronal excitability, BZDs have been given other applications in clinical practice such as: anticonvulsants, muscle relaxants and sleep inducers.¹

For some years certain public associations have expressed their concern over the possibly excessive prescription of these drug group and psychiatrists have also warned about the risk they have at producing serious side effects as well as producing pharmacological dependence (especially BZDs with high potency and medium short life span).

After this, even though the amount of short term prescriptions for anxiety has decreased, its wide usage in the long term treatment of insomnia continues, and it still is one of the most widely prescribed pharmacological groups, as it will be described hereafter.³

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BENZODIAZEPINE DEPENDENCE EPIDEMIOLOGY

The WHO establishes that the adequate use of medications implies that patients receive drugs that are appropriate for their clinical needs, with doses adjusted to their particular situation, during an adequate period of time and at the minimal cost possible for them and the community. The clinically inappropriate or economically inefficient use of the medications implies a very serious problem worldwide: it is estimated that more than half of all the medications are prescribed, dispensed or sold inappropriately. This, combined with the addictive potential of some of them, makes the problem more complicated and with risk of aggravation.⁴

The usage and prescription of BZDs is related to a high risk of abuse and dependency due to its inadequate handling since, even while regularly administered at therapeutic levels, they have a greater dependency potential than other anxiolytic action drugs. Additionally, drug tolerance has been reported when the prescription lasts longer than four weeks, as well as the appearance of drug withdrawal syndrome in 30% of the patients after an eight week treatment.⁵⁻⁷

Even though data about the prevalence of its consumption show variability between countries, it generally reflects a high consumption rate. For example, in the last year, in western countries, between 10 and 20% of the population recognizes the consumption of BZDs and between 1 and 3% has consumed them on a daily basis for over a year.⁸ In Spain, during 2006, data from the National Health System reflect prescriptions for a total of 69.9 DDD (defined daily doses per 1000 inhabitants per day) was reported, double than in 1993. In 2010 that meant that approximately 6 to 7% of Spain population was being treated with BZDs.⁹ In Mexico, data reported in the last National Poll on Addictions mark BZDs as the prescription drugs most widely used in men and women from 12 to 65 years of age.⁶ Despite the reports in different countries regarding the prevalence of their usage, the numbers keep increasing and difficulty lowering them can be observed.⁹

Regarding the variables and factors of risk for the dependency development, three factors have a crucial influence: 1. the drug's nature, 2. the individual himself and 3. The environment.¹⁰

Among the factors related to the drug's nature, BZDs' kinetics has proved to be an element that has an influence over their abuse. BZDs with more lipophilicity, and hence, more absorption, have a greater chance of producing this effect, compared to the ones that absorb more slowly. For example, in European countries flunitrazepam, a drug with high lipophilicity, is the BZD reported with greater abuse, while in the United States it is diazepam.¹⁰

The individual's genetic is an element that also has influence over their abuse.^{10,11} Midazolam, for example, is a BZD that is metabolized exclusively through the family of

CYP3A enzymes, which is a part of the family of the cytochrome p-450, and in spite of being a non-polymorphic enzyme its activity varies considerably between people.¹² In the case of diazepam and flunitrazepam, most of their metabolism is made through the enzyme CYP2C19 (40% or more). Between 17 and 20% of Asian people are slow metabolizers of CYP2C19, which means that approximately 250 million people do not metabolize these drugs properly.¹⁰

Age also influences the use of addictive substances. When it comes to the consumption of BZDs, studies made in various countries and different social strata reveal people of older age consume them more than young people, mostly due to greater insomnia prevalence in mature age.¹⁰

A study performed in the community of Valencia, Spain, showed that between 14 and 25% of elderly people on treatment receive an inadequate prescription, in which the misuse of medium life span BZDs such as dipyridamole stands out, as well as with other drugs such as amitriptyline and chlorpropamide, causing a strengthening of adverse side effects and a greater risk of drug interaction.¹³

In general, men exceed women in the consumption of legal and illegal drugs, except for BZDs. Women between 35 and 64 years of age turn to them the most. Additionally, being exposed to different levels of progesterone from puberty to menopause, women are more sensitive to the negative consequences of the usage of BZDs. It has been proved that levels of progesterone strengthen the effect of BZDs and boost their use and abuse.^{14,15} Alcohol, an external factor, has influence over the abuse of BZDs as well because it enhances their pharmacological effect due to them acting over the same receptors. It has been reported that between 15 and 20% of the alcoholic patients on treatment display an abuse of these drugs; many of them have taken them as self-medication for treating abstinence symptoms or anxiety, as euphorants or (as it was stated before), to boost the effects of alcohol itself.¹⁶

Thus, nowadays the good use and rational prescriptions of these drugs is essential.¹⁷ In the last decades very important progress in the knowledge of the neurobiological bases of addictions has been made, and this has allowed for a change in the conceptualization of this disorder, which evolved from being regarded as a vice to focus it as a psychorganic disorder that requires an specialized treatment.¹⁸

MECHANISM OF ACTION OF BZDS

γ -aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the Central Nervous System in mammals. GABA can activate two kinds of receptors, one that is metabotropic (GABA_B), coupled to one G protein with a presynaptic and a postsynaptic function, and one that is ionotropic (GABA_A) with a synaptic function located at the postsynaptic membrane.^{19,20}

GABA_A receptor is the inhibitory receptor coupled to a ionic channel which is most abundant in the Central Nervous System, its conduction pore being selective for Cl⁻^{19,20} ions and it is allosterically modulated by different drugs such as BZDs, barbiturates and also by ethanol. This receptor is a heteropentameric glycoprotein made up by the combination of multiples polypeptide subunits.¹⁹

There are seven different classes de pore-forming subunits ($\alpha, \beta, \gamma, \delta, \epsilon, \theta$ y ρ) as well as several isoforms for each class. At present, 18 subunits have been identified. However, the most frequent stoichiometry of receptors is where they are formed by two α subunits, two β and one γ .¹⁹

The specific binding site for BZD at the GABA_A receptor is called benzodiazepine binding site, and it is constituted mainly by an amino acid, histidine, in the position 101 of $\alpha 1$ subunit and in homologous sites in the other $\alpha 1$ subunits.¹⁹ Substitution of this amino acid by a different one in this position prevents pharmacologic effect of BZDs. The ligands of these sites are not limited to drugs of benzodiazepine structure. Other drugs such as zolpidem and zopiclone are also bound to this site benzodiazepine. The mechanism of activation of GABA_A receptor, seems to be the same both for benzodiazepines as for non benzodiazepines.^{19,20}

Coupling of BZD to their binding site at the GABA_A receptor generates an increment at the opening frequency of the ionic channel with respect to the moment when the GABA_A receptor is alone in presence of the GABA neurotransmitter. In other words, BZDs potentiate the effect of GABA neurotransmitter on its ionotropic receptors, which allows for a greater amount of chlorine ion going into the neurons favoring hyperpolarization of the membrane potential; the neuron becomes less susceptible to activating stimuli (less excitable) and a state of neuronal inhibition supervenes.¹⁹

The effect of BZDs on the GABA_A receptor is known as allosteric modulation since it modifies the tridimensional disposition of the receptor, thus potentiating the effect of opening the Cl⁻ channel thanks to the action of GABA.²¹

Pharmacological action of BZDs depends on the kind of α subunit contained by the GABA_A receptor. The site receptor of benzodiazepines of $\alpha 1$ subunit is the most abundant one in the Central Nervous System, and it regulates the anticonvulsive, hypnotic and sedative actions of BZDs; this subunit is mainly expressed in the cortex of the brain and cerebellum. The site receptor to BZDs of $\alpha 2$ subunit regulates anxiolytic actions and their expression predominates in the amygdala of the temporal lobe (especially in the central nucleus), the hippocampus and corpus striatum. The benzodiazepine site of $\alpha 3$ subunit is also known as a peripheral receptor, pharmacological action of BZDs on this subunit is related to the relaxing effect on muscles. The localization of subunits $\alpha 1, \alpha 2$ y $\alpha 3$ are mainly synaptic, whereas $\alpha 5$ subunit (also related to the relaxing muscular effect) has a predominantly extra-synaptic localization).^{19,22}

GABA_A receptors which contain subunits $\alpha 1, \alpha 2, \alpha 3$ y $\alpha 5$, combined with subunits β y γ , bind with classic BZDs, e.g. diazepam, while GABA_A receptors containing subunits $\alpha 4$ y $\alpha 6$ do not bind with classic BZD. Essentially all BZDs indicated for clinical use bind with GABA_A receptor containing subunits $\alpha 1, \alpha 2, \alpha 3$ y $\alpha 5$. Zolpidem, mentioned above, is the only clinically indicated drug which has specific selectivity: it has a high affinity with GABA_A receptor with $\alpha 1$ subunit, medium affinity with GABA_A receptor with subunits $\alpha 2$ or $\alpha 3$ and does not have affinity with GABA_A receptor with $\alpha 5$ subunit.^{20,23}

GENERAL MECHANISM OF ADDICTION TO DRUGS OF ABUSE

Dependency to certain substances is due to a neurobiological dysfunction of brain, mesencephalic, limbic and cortical structures, and of brain circuits implied in motivation and in behavior reinforcement processes.¹⁶

Substances causing addiction (such as cocaine, opioids or nicotine), indirectly increase the release or concentration of dopamine (DA) at the nucleus *accumbens* (NAC). El nucleus which liberates DA to NAC is the ventral tegmental area (VTA), located in the mesencephalon, and which innervates not only NAC but also a large number of regions of the encephalon.²⁴

VTA as well as NAC are regions of the brain which are related with reward and learning.²⁵ That is, these addictive substances act similarly to natural reward normally causing gratification or pleasure such as those necessary conducts for the preservation of the species, e.g. eating, sexual behavior and social relations.¹⁶ The stimulus creates a reinforcement in the consumption of such drugs and repeated exposure to this stimulus is transformed into physical dependence.²⁶

At the level of neuron networks, drugs of abuse increase DA concentration in the NAC through three different mechanisms. The first is the one produced by drugs such as amphetamines and cocaine, where the DA released into the NAC is maintained longer in the synaptic space due to the inhibition of the recapture of this neurotransmitter.²⁷

There is another, where the drugs directly activate the dopaminergic neuron and it liberates a greater amount of DA into the NAC. This is the mechanism employed by nicotine, which activates acetylcholine receptors expressed in dopaminergic neurons of the VTA.^{27,28}

In a third mechanism called disinhibition, the DA liberation by the VTA to the NAC is uncontrolledly increased since inhibitory control of dopaminergic neurons of the VTA is lost by the GABAergic interneurons of the same nucleus. As an example of drugs which produce disinhibition we can mention opioids and cannabinoids whose pharmacologic targets, μ -opioid receptor and CB1 receptor, are predominantly expressed by GABAergic interneurons, which,

once activated, repress their electric activity, releasing the inhibitory control (disinhibition) which they exert on dopaminergic neurons, which results in a greater DA release in the NAC²⁷ (figure 1).

NEUROBIOLOGICAL MECHANISM OF ADDICTION TO BZDS

Recently, Kelly Tan et al., from the University of Geneva, in Switzerland, proved the similarity of the mechanism of addiction to BZDs with regards to other addictive drugs.²⁶

For a better understanding of this mechanism it is necessary to understand that VTA is a nucleus whose neuronal population is composed as follows: 70% dopaminergic neurons, 15% de GABAergic interneurons and 15% glutamatergic neurons.^{2,27} The function of interneurons is to reduce electrical activity of dopaminergic neurons, thus, the amount of DA released to the NAC is under the release control of GABA neurotransmitter by interneurons.²⁶

An important discovery was that even when all neurons express GABA_A receptor, it is different in its molecular composition between dopaminergic neurons and GABAergic interneurons. Subunit α of the GABA_A receptor of dopaminergic neurons of VTA is mainly $\alpha 3$, while at interneurons it is $\alpha 1$.²⁶

Based on this, it was possible to determine that the effect of BZDs on the VTA-NAC circuit is mainly determined

by its effect on $\alpha 1$ subunit of the GABA_A receptors expressed by inhibitory interneurons.²⁴

By means of the study of the synaptic activity of both types of neurons (interneurons and dopaminergic neurons) it was observed that the activation $\alpha 1$ receptor of the interneurons produces miniature inhibitory postsynaptic potentials (mIPSP) of a greater amplitude than those generated by the activation of $\alpha 3$ receptors of dopaminergic neurons in the presence of BZDs. From these results it can be concluded that while both neuron types are susceptible to the effect of BZDs, interneurons are susceptible at a greater degree, while they stop releasing GABA to dopaminergic neurons. As a result, there is a loss inhibition of these neurons which promotes the excitatory input of VTA dopaminergic neurons to have no synaptic counterweight, which in the end produces the uncontrolled release of DA into the NAC.²⁴

To corroborate that these electrophysiological findings have a behavioral correlate, the use of transgenic mice proved to be very important; the expression of mutant forms $\alpha 1$ subunit of GABA_A channel was induced in them, whereby histidine of the binding site to BZDs was substituted by an arginine, leaving the receptor insensitive to BZDs. In these experiments mice were conditioned to drink water with sugar added, to which midazolam was later added. Under these conditions, wild-type animals ($\alpha 1$ subunit non- mutants) the consumption of water added with BZD significantly increased with regards to the basal average (previous to the drug), while $\alpha 1$ subunit mutant animals (H101R) maintained an average consumption with BZD, similarly when water did not contained the drug. From these results it can be concluded that $\alpha 1$ subunit of GABA_A receptor effectively mediates the adverse effect of physical dependency to BZD, and that there is a functional-behavioral correlate between the disinhibition of the VTA-NAC circuit and the compulsive behavior consumption of the drug.²⁴

No doubt these discoveries will allow for the design of new BZDs which pose a lower risk of provoking dependency in patients, granting thus safer ways of treatment which improve their life quality. For instance, from the knowledge obtained by means of the study of model animals it can be deduced that an almost ideal anxiolytic would be a BZD specific agonist to $\alpha 2$ subunit, which would lack the secondary effect of sedation and amnesia and still produce the anxiolytic effect. No doubt it would be less addictive for such reason. The search for drugs which produce the less possible secondary effects on people is one of the most ambitious challenges of contemporary medicine.

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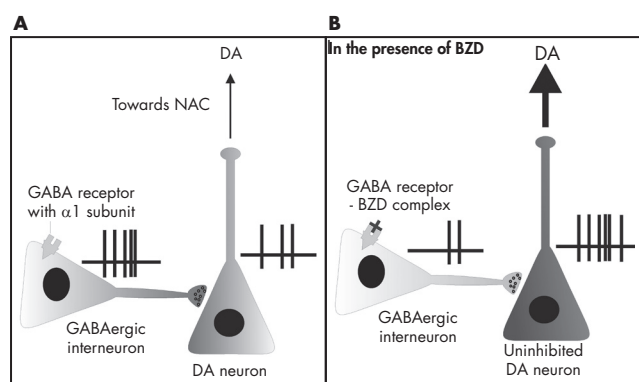


Figure 1. Scheme of the mechanism proposed for the generation of physical dependency and addiction to benzodiazepines (BZD). In (A) are shown two VTA neurons: inhibitory interneuron (left) makes synaptic contact with the dopaminergic neuron (shown in the right). A putative shot frequency for both neurons is exemplified in the inserts. Dopaminergic neuron projects nucleus *accumbens* and releases its neurotransmitter in this region: dopamine (DA). The amount of DA released at nucleus *accumbens* depends on the activity of the dopaminergic neuron, which depends on the activity of the inhibitory interneuron. In (B) is shown what is supposed to happen when BZD is administered. This is bound to the GABA_A receptor in the inhibitory interneuron containing α -1 protein. As a result there is a decrease in the inhibition towards the dopaminergic neuron, which increases its shot frequency. The final result is a greater DA release at the nucleus *accumbens* due to the decrease in inhibition, which reinforces the consumption of BZDs and generates addiction as well as physical dependency (modified from Mercado et al.²⁶).

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