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REVIEW ARTICLES

The Neurochemical Mechanisms of the Pharmacological Activities of Inverse Agonists of the Benzodiazepine Binding Site

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Abstract—Data on the basal (constitutive or spontaneous) activities of receptors, as well as their neurochemical and electrophysiological correlates, are presented. Inverse receptor agonists are a group of pharmacological drugs that can suppress constitutive activities. We describe the neurochemical mechanisms of the pharmacological activities of benzodiazepine binding site inverse agonists. These compounds inhibit chloride currents caused by gamma-aminobutyric acid at low non-physiological concentrations. This activity is the basis of the sobering action during the action of ethanol and the ability to suppress the addictive potential of psychoactive substances. In addition, the benzodiazepine binding site inverse agonists are able to activate memory formation processes and improve learning. The possible uses of drugs from this group in modern medicine are discussed. They may be used to treat a number of diseases in the fields of narcology, therapy, neurology, and psychiatry.

Keywords: inverse agonists for the benzodiazepine binding site, reduction of the intoxicating effects of ethanol, decrease in addictive potential of psychoactive substances, accelerated learning and memory, antidepressant actions

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INTRODUCTION

Inverse agonists are ligands that suppress the basal activities of receptors [1–3]. In turn, basal (constitutive or spontaneous) receptor activity is understood as the state of receptor excitation in the absence of agonists [3–5]. Constitutive activity is a phenomenon that is inherent to a greater degree in metabotropic receptors: opioid [6, 7], histamine receptors of the third subtype [8–10], serotonin $5HT_{2C}$ and cannabinoid CB_1 [11], adrenergic β_2 [12, 13], and others.

Inverse agonists of ionotropic receptors are also known. The inverse agonists of benzodiazepine (BD) binding sites have been most intensely studied [14–16]. The BD binding sites (formerly referred to as benzodiazepine receptors, BD receptors) are known to be part of a more sophisticated complex, the ionotropic $GABA_A$ receptor [17–20].

The physiological significance of constitutive neurotransmission is not entirely clear. It may provide a certain level of activity of a particular neurotransmitter system in the absence of an agonist [21]. The change in constitutive activity under certain conditions may be accompanied by pathological reactions at the level of the whole organism. Thus, after long-term exposure to opiate/opioid, the basal activity of opioid receptors increases. This phenomenon is considered as an element of the tolerance syndrome [21, 22] or the withdrawal syndrome [22–24]. Excessive constitutive activity of β_2-adrenergic receptors is associated with the formation of cardiac pathology in transgenic mice of the TG-35 line [12].

Admittedly, the clinical use of inverse agonists is very limited. This is determined by the lack of understanding by the scientific community of the role of constitutive receptor activities, which are modified by these drugs. In addition, there are difficulties at the methodical and practical levels. As an example, if a certain in vitro receptor system has this property, how can it be evaluated in vivo? Are there advantages of inverse agonists over neutral antagonists and vice versa in terms of their clinical application? Finally, it is unclear how the basal activity of receptors is regulated, and how acute (chronic) effects of chemical compounds alter the constitutive transduction and effectiveness of ligands [3–5, 23, 25]?

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This review attempts to answer some of the above questions for the inverse agonists of BD binding sites. Their neurochemical properties that allow prediction of their pharmacological activities are discussed. We consider the neurochemical, electrophysiological, and behavioral aspects of this group of agents to evaluate their prospects for clinical medicine.

NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL EFFECTS OF INVERSE AGONISTS

The phenomenon of inverse agonists is based on the assumption that a certain set of receptors may be subdivided into two populations: the active form R* and the inactive form R, between which there is an equilibrium (the binary model) [3, 5, 12, 26].

The second assumption is the presence of a third, special, population of active receptors that can trigger a transduction signal even in the absence of an agonist (the ternary receptor model). This population provides the basal signaling (constitutive) activity of receptors [3–5, 25].

The agonists interact with the R* form of the receptors, shift the equilibrium in the R ↔ R * system to the right, and trigger subsequent transducer events. Antagonists prevent the effects of agonists. Some antagonists inhibit the constitutive activity of the receptors by shifting the equilibrium R ↔ R* to the left and stabilizing the receptor in an inactive conformation [3, 21]. In this regard, antagonists that inhibit basal transmission and eliminate its neurochemical correlates are designated as inverse agonists or antagonists with intrinsic negative activity, while neutral antagonists (which do not have internal activity) do not affect constitutive transmission. Neutral antagonists eliminate the effects of both agonists and inverse agonists [27, 28].

Inverse agonists modify the parameters of spontaneous receptor activity, which is considered as the neurochemical and electrophysiological equivalents of their effects. As an example, in metabotropic receptors, inverse agonists alter various indicators of basal activity:

—the magnitude of the specific binding of radioactive nonhydrolyzable analogs of GTP (one of the most frequent is [^{35}S] GTPγS, {guanosine-5 '-(γ-thio)-triphosphate}). This allows us to evaluate the activation of the G protein necessary for the subsequent involvement of secondary messenger systems (adenylate cyclase, guanylate cyclase, phosphoinositide, calcium, etc.) [5, 10, 21, 23];

—the activities of adenylate cyclase, guanylate cyclase, mitogen-activated protein kinases, and phospholipases C, D [4, 8, 9, 21];

—the permeability of ion channels, for example, calcium channels [4, 5, 21, 23, 26].

An electrophysiological correlate of the basal activity of ionotropic $GABA_A$ receptors is chloride currents initiated by the corresponding agonists at low (nonphysiological) concentrations. Inverse agonists of binding sites for benzodiazepines[2] inhibit chloride currents, while agonists potentiate them [17, 20, 34, 35].

Neurochemical and electrophysiological equivalents of the action of inverse agonists are mainly evaluated in cells of various types that express certain receptors (in vitro experiments). In some cases, neurons of rodents with a knockout of some receptor (or receptor subunit) are used for experiments [5, 7, 14, 15, 21]. Neurons of wild-type animals are used as the control in these studies. Obviously, the properties of the inverse agonist in a particular preparation may depend both on the characteristics of the cell system and on the properties of the receptor construct. The characteristics of the incubation medium, the preliminary action of agonists, and other factors also play their roles. It may be concluded that the intrinsic negative activity of an inverse agonist is not a constant characteristic of a particular agent; it varies depending on the experimental model that is used, the type of cells, the state of the receptors, etc. Therefore, inverse agonists are described by the usual terms in neurochemistry: a full or partial inverse agonist (if the intrinsic negative activities of several drugs are compared), and a mixed antagonist/inverse agonist [3, 5, 21].

The cases where the pharmacological profile of the same drug is completely or partially transformed under various experimental conditions between the roles "complete agonist–partial agonist–neutral antagonist–partial inverse agonist–complete inverse agonist," are referred to as the phenomenon of "protean agonism," according to the name of the mythological creature Proteus, who was able to take the shapes of different characters [24, 36–38].

INVERSE AGONISTS OF BENZODIAZEPINE BINDING SITES

Inverse agonists of binding sites for benzodiazepines, or inverse BD agonists, constitute a rather large group of drugs from different chemical classes: β-carbolines, imidazobenzodiazepines, triazolopyridazines, pyrazolotriazines, imidazothienodiazepinones, etc. [1, 14, 16, 27, 39]. To determine the intrinsic negative activity of a putative inverse BD agonist, its effect on the GABA-induced chloride currents at a low concentration is examined. Typically, an amino acid is used at a concentration many times below the

[2] These compounds are often called inverse benzodiazepine agonists (inverse BD agonists) [29, 30]. Quite often the term "inverse agonists of benzodiazepine receptors" is also used [31, 32]. In accordance with the proposal of the International Union of Pharmacologists (IUPHAR), instead of the term "BD receptors" it is recommended to use the designation "BD site," or "binding sites for benzodiazepines" [33]. Here, the term "inverse BD agonists" is used more often.

EC_{50}, for example, EC_3 [15], EC_{10} [35], and EC_{20} [34, 40]. At these concentrations, it is possible to avoid desensitization of $GABA_A$ receptors [40]. Usually, different variants of electrophysiological methods are used to evaluate the kinetics of one ion channel (patch clamps). Substances with intrinsic positive activities, for example 1,4-benzodiazepines and other BD agonists, enhance these currents by increasing the frequency of $GABA_A$ receptor ionophore opening [17–20]. Inverse agonists, in contrast, suppress GABA-induced chloride currents and weaken the GABA-induced inhibition of neurons [1, 14, 15, 18]. If benzodiazepine agonists increase the GABA affinity to the corresponding receptors, the inverse agonists have the opposite effect [41, 42]. Flumazenil, which is an antagonist of BD binding sites, prevents the effects of inverse BD agonists on GABA-induced chloride currents [43–45]; however, alone it does not affect them [18, 43, 44]. Another approach is the study of long-term potentiation (frequently in hippocampal slices), which is enhanced by inverse BD agonists [1, 14, 15, 46, 47].

Radioligand analysis is widely used to evaluate the affinity of inverse BD agonists for $GABA_A$ receptors of different subunit compositions (using the dissociation constant, K_d, or the inhibition constant, K_i), as well as the density of binding sites (Bmax). The labeled ligands [^{3}H] flumazenil and [^{3}H] Ro 15-4513 are used to determine the affinity of agents to BD binding sites using K_i as an index [1, 27, 31, 34, 46].

The behavioral equivalents of the effects of inverse BD agonists are improved memory, accelerated learning, sobering action, anxiogenic and convulsive effects, and increased aggression [27, 39, 48, 49].

Given the heterogeneity of the binding sites of GABA agonists and BD ligands it is possible to designate BD agonists as positive allosteric modulators of $GABA_A$ receptors, and inverse BD agonists as negative allosteric modulators [1, 14, 39, 50].

The question arises of which $GABA_A$ receptors are the target of inverse BD agonists. That is, which receptor structures are inhibited by the analyzed agents and where do these receptors have to be located?

NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL MECHANISMS OF MODULATION OF $GABA_A$ RECEPTORS BY INVERSE BENZODIAZEPINE AGONISTS

$GABA_A$ receptors are divided into postsynaptic, extrasynaptic, and presynaptic types. The first mediate rapid phasic inhibition and the second mediate long tonic inhibition. Presynaptic $GABA_A$ receptors are involved in the regulation of exocytosis of both GABA itself and other neurotransmitters [18–20, 51].

The receptor complex is often a pentamer that consists of subunits of different classes: α, β, γ, δ, π, θ, ρ, and χ. In turn, some subunits are divided into subtypes: α_{1-6}, β_{1-3}, γ_{1-3}, and ρ_{1-3}. The γ_2 subunit is also represented by two forms that differ in the length of the polypeptide chain: γ_{2L} (long) and γ_{2S} (short) [17, 19, 20, 50]. The central $GABA_A$ receptors are frequently composed of subunits of three types: α, β, and γ. Some receptors contain a δ subunit instead of γ. These receptors have pre- or extrasynaptic localization and have the subunit composition of $\alpha_{4/6}\beta_3\delta$ [18, 19, 51, 52]. The level of these receptors in the mammalian brain is no more than 10% [53]. It appears that α_{1-3}-containing $GABA_A$ receptors are located inside the synapses and they mediate phasic inhibition. α_{4-6}-containing receptors are often located outside the synapses. These receptors initiate tonic inhibition [18–20, 51].

Postsynaptic $GABA_A$ receptors are usually represented by combinations of $2\alpha + 2\beta + 1\gamma$, $2\alpha + 1\beta + 2\gamma$, and $1\alpha + 2\beta + 2\gamma$. If the receptor includes two subunits of the same type, often they are identical polypeptides ($\alpha_1 + \alpha_1$; $\beta_2 + \beta_2$; $\gamma_{2S} + \gamma_{2S}$ etc.). It is possible that the brain of mammals also contains receptors that consist of four subunits. In addition, it is likely that structures that are composed of subunits of two or even one type are present [17, 18, 20]. Postsynaptic mammalian receptors often include the α_1-, β_2-, and γ_2-subunits, while the extrasynaptic receptors include the α_4-, β_2-, and δ-subunits. The most common combination is $2\alpha_1 + 2\beta_2 + 1\gamma_{2S}$. At least 75–80% of the $GABA_A$ receptors in the mammalian brain include the γ_2-subunit, and approximately half include the α_1- and β_2-subunits [17–20].

The distribution of the α_1, β_{1-3}, and γ_2 subunits in the structures of the rat brain is rather homogeneous. The α_{2-6}, γ_1, and δ subunits have been identified mainly in certain regions: α_2, in the forebrain and the cerebellum; α_5, in the dorsal hippocampus and prefrontal cortex (the subunit composition is usually $\alpha_5\beta_{2-3}\gamma_2$; often, they are referred to as α_5 $GABA_A$ receptors[3]); and α_6, in the cerebellar granule cells and in the cochlear nuclei. Joint expression was established for the α_4- and δ-subunits in the thalamus, striatum, the outer layers of the cerebral cortex, the hippocampal dentate gyrus, and the cerebellum [18–20].

The binding sites of GABA and GABA agonists are located on the α and β subunits. Benzodiazepines interact with the α- and γ-subunits. 1,4-Benzodiazepines (diazepam, flunitrazepam, etc.) have the greatest affinity for receptors with the following subunit

[3] There are α_1, α_2, and α_3 $GABA_A$ receptors [20, 54]. These all contain binding sites for benzodiazepines. Therefore, inverse BD agonists for α_5-containing receptors are often called inverse agonists of α_5 $GABA_A$ receptors [1, 14, 39, 54]. In some cases, this definition is used in this review, although it is not very correct, since it is an issue of negative allosteric modulators of $GABA_A$ receptors.

compositions: $\alpha_1\beta_{2-3}\gamma_2$, $\alpha_2\beta_{2-3}\gamma_2$, $\alpha_3\beta_{2-3}\gamma_2$, and $\alpha_5\beta_{2-3}\gamma_2$. The $\alpha_{4/6}\beta_{2-3}\gamma_2$ receptors are insensitive to 1,4-benzodiazepines, but are capable of binding the benzodiazepine antagonist flumazenil [18–20]. In addition, the structure of the GABA-ionophore complex has sites for specific binding of $GABA_A$-antagonists, sites for binding of chloride ionophore blockers, barbiturates, neurosteroids, butyrolactones, ions of zinc, terbium, and lanthanum, melatonin, ethanol, antidepressants, and polyunsaturated fatty acids [18–20, 42, 50].

It is seen that $GABA_A$ receptors have a considerable heterogeneity both in subunit composition and in localization. Despite this variety, it is quite unusual that there are not many selective targets for inverse BD agonists. It was found that the inverse agonists L-655708 (related to imidazobenzodiazepines) and MRK-016 (a representative of the triazolothriazine class) suppressed the chloride currents in the α_5-containing $GABA_A$ receptors. Hippocampal neurons from Swiss mice were used as a biological system. No electrophysiological effects of inverse BD agonists were observed in the neurons of "knockout" mice that lack the α_5-subunit [14]. MRK-016 also exhibited properties of an inverse agonist for $\alpha_5\beta_3\gamma_2$ receptors expressed in mouse fibroblasts. A similar effect was observed with triazolophthalazine α5IA. Suppression of chloride currents by both drugs in $\alpha_{1-4}\beta_3\gamma_2$ receptors was insignificant (neutral antagonism). MRK-016 suppressed prolonged tonic inhibition in hippocampal slices of C57 mice. This indicates the manifestation of the properties of the inverse agonist with respect to BD binding sites on extrasynaptic α_5-containing $GABA_A$ receptors. The α5IA agent was ineffective under these conditions [1]. Imidazobenzodiazepine PWZ-029, which specifically binds to the benzodiazepine site, inhibited the GABA-induced chloride current in $\alpha_5\beta_3\gamma_2$ receptors (expressed in the *Xenopus laevis* oocytes); i.e., it acted as an inverse agonist. However, in the $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$ receptors the drug had the properties of a BD agonist; i.e., it increased the chloride current and showed an intrinsic positive activity [15].

The study [34] showed that imidazo-triazolo-benzodiazepine Ro 4938581 has a high affinity for rat $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors expressed in HEK 293 cells (Human Embryonic Kidney 293). [^{3}H]flumazenyl was used as a radioligand. The affinity to the $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$ receptors was significantly lower. The properties of the inverse BD agonist Ro 4938581 with respect to $\alpha_5\beta_3\gamma_2$ receptors were expressed in its ability to suppress chloride currents induced by GABA at low concentrations (EC_{20}). These data were obtained using the patch-clamp technique in the above-mentioned HEK 293 cells. In cells that expressed $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$ receptors no inhibition of chloride currents occurred. Ro 4938581 enhanced the long-term potentiation in the hippocampal slices of DBA2/J mice, which also indicates that it has characteristics of an inverse BD agonist of the α_5 $GABA_A$ receptor, since the long-term potentiation in the hippocampus is under the negative control of α_5 $GABA_A$ receptors [1, 47, 54 , 55]. The suppression of chloride currents by Ro4938581 was also established in human $\alpha_5\beta_3\gamma_2$ receptors expressed in *Xenopus laevis* oocytes (chloride currents were initiated by the addition of 3 μM GABA to the incubation medium). Ro 4938581 was ineffective in $\alpha_1\beta_3\gamma_2$ receptors [56]. Another representative of imidazo-triazolo-benzodiazepines, Ro 4882224, had a similar neurochemical and electrophysiological profile [35].

Inverse BD agonists that predominantly block α_5-containing receptors are often called selective inverse agonists for α_5 $GABA_A$ receptors, or inverse α_5 $GABA_A$ agonists (see note 2) [1, 14, 34, 39, 57, 58].

Many inverse BD agonists do not differ in selectivity and are able to inhibit the basal activity of receptors containing α_{1-3} and α_5 subunits. This holds for the imidazobenzodiazepines Ro 15-4513 [59–61] and RY080 [61–62], β-carbolines FG-7142, β-CCM, DMCM, β-CCE, β-CCP [27].

The neurochemical mechanisms of the pharmacological activities of inverse BD agonists are mainly determined by their ability to suppress the constitutive activity of $GABA_A$ receptors [1, 14, 39]. The scenario of the response behavior is determined by the specific receptor structure that the agent affects.

As noted above, the presence of α- and γ-subunits is most important for the manifestation of benzodiazepine activity [18–20, 50]. The pharmacological effects of BD agonists determine the type of α-subunit. α_1-containing receptors are involved in sedative reactions, anterograde amnesia, and, partially, in the anticonvulsant action of benzodiazepines. $GABA_A$ receptors containing α_2- and α_3-subunits are responsible for the formation of anxiolytic effects and, in part, anticonvulsant action and myorelaxation. α_5-containing receptors are involved in the development of amnesia caused by benzodiazepines [17, 18, 20, 48, 50].

Inverse BD agonists suppress the function of $GABA_A$ receptors, and their pharmacological profile is also determined by the characteristics of the subunit composition. As an example, it is likely that the sobering properties of these agents are realized via α_5-containing $GABA_A$ receptors [59, 63]. Receptors containing the α_5-subunit are also implicated in the realization of the procognitive[4] effects of inverse BD agonists [1, 14, 15, 39], their antidepressant activity [55, 64–

[4] In the context of this article, the terms "procognitive action" and "procognitive effect" mean the ability of the drug to increase attention, memory, weaken amnesia, and accelerate learning. The use of the term "nootropic action" is considered inappropriate, since its interpretation is much broader.

66], and suppression of the addictive potential of ethanol [59].

In mammals, α_5 $GABA_A$ receptors are present mainly in the hippocampus and have both synaptic and extrasynaptic localization. In this structure, α_5 $GABA_A$ receptors are localized mainly on the dendrites of pyramidal neurons of the CA1 field. They mediate tonic inhibition through extrasynaptic $GABA_A$ receptors. Synaptic receptors of pyramidal neurons provide phasic inhibition [1, 46, 58]. It was proposed that the procognitive effect of α_5-selective inverse agonists in the hippocampus is associated with inhibition of $\alpha_5\beta_3\gamma_2$ $GABA_A$ receptors (predominantly extrasynaptic) [46]. On the other hand, the ability of inverse BD agonists to inhibit neurotransmission mediated by α_1-, α_2-, and α_3-containing $GABA_A$ receptors explains the undesirable pharmacological activities of these drugs (an anxiogenic effect, lowering the convulsive threshold, increasing aggressiveness) [16, 39, 62]. As an example, imidazobenzodiazepine RY080, which possesses sobering and procognitive properties [59, 63], inhibits α_5 $GABA_A$ receptors, which is a neurochemical basis of its pharmacological activity [54, 67]. Inhibition of α_{1-3}-containing receptors, explains the convulsive properties of RY080 [61, 62].

The selective inverse BD agonist of the α_5 $GABA_A$ receptor Ro 4938581 did not exhibit anxiogenic and convulsive activity in experiments on rats [34]. In contrast, the non-selective inverse BD agonists Ro 19-4603 and FG-7142 had opposite properties (the studies were also performed in rats) [68–70].

As follows from the above data, the neurochemical and electrophysiological profiles of inverse BD agonists are often transformed depending on the experimental conditions (agonist–neutral antagonist–inverse agonist). This should be taken into account when evaluating the prospective areas of possible clinical use of the considered drugs and is important for understanding the neurochemical mechanisms of pharmacological activity [1, 15, 27, 32, 71, 72].

THE BEHAVIORAL EFFECTS OF INVERSE BENZODIAZEPINE AGONISTS

Initially, these agents were supposed to be used to reduce the toxic effects of ethanol [73–75]. In particular, hopes were associated with imidazobenzodiazepine Ro 15-4513. In the late 1980's and early 1990's it was shown that Ro 15-4513 weakened motor disorders, sedative, amnesic, and anxiolytic effects of ethanol in rodents [73, 75–79]. The alcohol doses used, as a rule, did not exceed 2 g/kg. With an increase in the dose of ethanol, the efficiency of Ro 15-4513 decreased [79, 80]. The drug did not affect the hypothermic effect of ethanol [79, 80] and its lethality [71]. Later, it was found that the sobering effects of Ro 15-4513 are most likely due to its ability to compete for ethanol binding sites on extrasynaptic $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ receptors [40, 42, 67, 81]. The neurochemical profile of the drug corresponded to the properties of a partial inverse BD agonist for $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$ receptors (rat receptors expressed in HEK 293 cells). This was seen as inhibition of chloride currents initiated by GABA. This effect was absent in experiments with $\alpha_5\beta_2\gamma_2$ receptors (neutral antagonism) [82]. It is not clear whether the suppression of the basal activity of the $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, and $\alpha_3\beta_3\gamma_2$ $GABA_A$ receptors is related to the sobering activity of Ro 15-4513 or it constitutes the neurochemical basis of its anxiogenic and convulsive properties [40, 59, 67]. The clinical prospects of the drug are highly questionable [67, 72].

Sobering properties were found in other non-selective partial inverse BD agonists. Thus, R.G. Lister and M.J. Durcan [83] showed in Swiss mice the ability of the imidazothienodiazepinone derivative Ro 19-4603 to attenuate the sedative effect of ethanol. Ro 19-4603 was used at doses of 0.1–0.3 mg/kg (5 minutes after ethanol at a dose of 2.4 g/kg, both intraperitoneally). The same researchers established a sobering effect of another partial inverse BD agonist Ro 15-3505 (imidazobenzodiazepine) [84]. As in the case of Ro 15-4513, the exact mechanism of the sobering action of the inverse BD agonists Ro 19-4603 and Ro 15-3505 is not fully understood.

In the following years, the family of sobering agents was extended by the imidazobenzodiazepines RY008, RY023, RY024, RY080, Ro16-0154 (iomazenil), and triazolophthalazine α5IA. These are considered as partial inverse agonists of the α_5-containing $GABA_A$ receptor [32, 59, 60, 63, 67, 85]. Sobering properties of these drugs have been established both in animal experiments [59, 60, 63] and in human studies [32, 86]. As an example, in clinical trials on healthy volunteers, the α5IA drug weakened the amnesic effect of ethanol (0.8 g/kg, orally). The effect on the sedative effects of alcohol was weaker. Subjective signs of intoxication in the presence of α5IA did not change [32].

An important characteristic of partial inverse BD agonists is their ability to lower the addictive potential of psychoactive substances (an anti-addictive effect). This effect has been studied in detail based on the example of formation of the syndrome of dependence on ethanol. Using the method of oral self-administration of an alcohol solution by rodents, a decrease in the ethanol consumption via non-selective inverse agonists by BD agonists Ro 15-4513 [87, 88] and Ro 19-4603 was shown [60, 89]. As an example, we can describe the data from [88]. In female C57BL/6J mice with a formed dependence on ethanol, Ro 15-4513 (2.5–5.0–10.0 mg/kg, intraperitoneally) dose-dependently inhibited alcohol consumption. The role of the ventral tegmental area (an important component of reinforcement systems) in the realization of the addictive potential of alcohol was evaluated. To this end, Ro 15-4513 was injected into the anterior or posterior regions of the tegmentum at a dose of 1 ng/mouse.

A multifold decrease in intake was observed after microinjection of the drug into the posterior region of the ventral tegmental area. No changes in consumption were found after drug administration in the anterior part of the structure. It was assumed that the modulation of the addictive potential of ethanol by Ro 15-4513 involves $GABA_A$ receptors in the posterior part of the ventral tegmentum that contain the α_4 and α_6 subunits [88]. Apparently, the issue is competition between Ro 15-4513 and ethanol for common binding sites of $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ receptors (see above).

On the other hand, suppression of the addictive potential of ethanol by the inverse BD agonist Ro 19-4603 may be due to its binding to α_4-containing $GABA_A$ receptors in the nucleus accumbens (one of the key structures of the reinforcement systems) [60].

Inverse BD agonists that bind predominantly to α_5 $GABA_A$ receptors also possess the ability to reduce the addictive properties of ethanol: RY023, RY024, RY028, L-655708, etc. [59, 63, 90, 91]. Thus, in alcohol-preferring (P) rats, a partial inverse agonist from the imidazobenzodiazepine group RY028 (intraperitoneally, at doses of 1–10 mg/kg) significantly inhibited alcohol consumption [59]. The partial inverse BD agonist RY024 also effectively suppressed ethanol consumption in experiments with male Long Evans rats. The drug was administered intraperitoneally at doses of 0.5–3.5 mg/kg [63].

It was assumed that the inhibition of the reinforcing properties of ethanol by the inverse BD agonists RY023, RY024, and RY028 is due to the suppression of the activity of the α_5 $GABA_A$ receptors in the hippocampus. In [90], female P rats were trained to perform oral self-administration of an ethanol solution. Further microinfusion of RY023 in the hippocampus (fields CA1 and CA3) was performed. Under these conditions, there was a significant decrease in the consumption of the ethanol solution, while the behavior of self-administration of sucrose did not change. The antagonist of BD binding sites ZK 93426 (a derivative of β-carbolines) during combined microinfusions eliminated the ability of the drug RY023 to inhibit ethanol self-administration. If the inverse BD agonist was injected into the ventral tegmental area or into the nucleus accumbens, then no reduction in ethanol consumption was observed. The authors explained the revealed discrepancy by the low density of α_5 $GABA_A$ receptors in the ventral tegmental area and nucleus accumbens.

L-655708, another selective inverse BD agonist of α_5-containing $GABA_A$ receptors inhibited the consumption of a 2% ethanol solution by male *Macaca mulatta* monkeys. Animals were previously trained to perform oral self-administration of an alcohol solution. During the self-administration session, the dose of ethanol reached 1.8 g/kg (baseline), and its blood concentration ranged from 0.9–1.6 g/L. For 5–7 days, primates received L-655708 10 min before the start of the next session (intramuscularly, the dose range was 0.1–1.8 mg/kg). In the presence of the drug, the amount of ethanol consumed was significantly lower. At a dose of L-655708 of 1.8 mg/kg the volume of alcohol consumed was reduced by 40% relative to the baseline level. The drug-induced suppression of the alcohol self-administration behavior remained during a week of the daily preliminary administration of the inverse agonist, which indicated the absence of formation of tolerance to L-655708. The selective antagonist of the α_5 $GABA_A$ receptor XLi-093 (0.3 mg/kg, intramuscularly, simultaneously with L-655708) eliminated the ability of the inverse agonist L-655708 (1.8 mg/kg) to suppress the ethanol consumption. It was considered that inverse BD agonists of α_5 $GABA_A$ receptors can be a promising group of drugs for the treatment of alcohol dependence [91].

There is evidence that Ro 15-4513 has the ability to suppress the addictive effects of toluene, methamphetamine, and diazepam in male C57BL/6J mice (electric self-stimulation of the brain was used). The inverse BD agonist was administered subcutaneously at doses of 0.3 or 1.0 mg/kg. In a separate series of experiments using the microdialysis method, the ability of methamphetamine to enhance the dopamine (DA) release from the nucleus accumbens (a phenomenon that is a neurochemical equivalent of the narcotic potential of psychoactive substances) and the influence of Ro 15-4513 on this process was evaluated. It was found that the drug increased the exocytosis of the neurotransmitter by a factor of 9 and the inverse BD agonist halved the methamphetamine-induced increase in dopamine release. This indicates that the inverse BD agonist modulates the functional state of the dopaminergic neurotransmitter systems, which play a key role in the pathogenesis of addictions [72].

Neurochemical mechanisms of the anti-addictive effects of inverse BD agonists are based on changes in the functional state of $GABA_A$-neurons involved in the regulation of reinforcement systems. In particular, in the ventral tegmental region, GABA-interneurons that give projections to dopaminergic cells suppress DA-neuron activity. This is accompanied by weakening of the dopamine release in the nucleus accumbens. It was assumed that the inverse BD agonist Ro 15-4513 blocks the activating effect of GABA on the α_4 and α_6 $GABA_A$ receptors of interneurons in the posterior region of the ventral tegmental area. Under these conditions, the activity of primary interneurons in the chain increases with subsequent enhancement of the inhibition of dopaminergic cells and a decrease in the release of dopamine in the nucleus accumbens [72, 88]. Currently, there is no direct evidence that GABA-interneurons that regulate dopaminergic cells in the ventral tegmental area contain the α_4 and α_6 subunits in the $GABA_A$ receptor. Nevertheless, this is very likely. As

an example, GABA interneurons in the periaqueductal gray of rats express $\alpha_4\beta_1\delta$ receptors [92, 93].

The presented hypothesis of anti-addictive activity of Ro 15-4513 appears to be incomplete, since it does not reflect the possibility of modulation by the given agent of GABA-neurons in other structures of the reinforcement system (locus coeruleus, amygdala, prefrontal cortex, etc.). In addition, the neurochemical mechanisms of the addictive effects of psychoactive substances and drugs that suppress the addictive potential include the modification of many neurotransmitter systems (glutamatergic, noradrenergic, opioid, serotonergic, endocannabinoid, etc.) [94]. The neurochemical and neurophysiological mechanisms of the anti-addictive activity of inverse BD agonists cannot be considered as fully studied.

There are data on the ability of inverse BD agonists to activate memory-formation processes and improve the training of experimental animals (both intact and subjected to any pharmacological effects) [1, 15, 16, 39, 47]. In some models, an anti-amnestic effect was detected after intoxication with the muscarinic receptor antagonist scopolamine, the blocker of channels of N-methyl-D-aspartate receptors (NMDA receptors) MK-801, and ethanol [16, 30, 39, 78].

It was found that some imidazo-triazolo-benzodiazepines (Ro 4882224, Ro 4938581, etc.) have a pronounced procognitive effect. They exhibit the properties of selective inverse BD agonists of α_5 $GABA_A$ receptors; the electrophysiological equivalent is the enhancement of prolonged potentiation in the hippocampus. This is the basis of the pharmacological activity of drugs from this group [34, 35, 95]. Drugs Ro 4882224 and Ro 4938581 attenuated memory impairments in rats caused by scopolamine and diazepam [34, 35]. The agent Ro 4938581 also accelerated learning of monkeys in the food search test [34]. These studies suggest the inverse BD agonist α_5 $GABA_A$ receptors as promising drugs for weakening intellectual-mnestic disorders (for example, in Alzheimer's disease) [30, 31, 34, 57, 96].

In [56] it was found that the inverse agonist Ro 4938581 attenuated memory impairment in adult and newborn Lister hooded male rats after multiple injections of the NMDA receptors blocker phencyclidine (one of the experimental models used to study cognitive disorders in schizophrenia). The authors believe that inverse DB agonists that selectively inhibit the basal activity of α_5 $GABA_A$ receptors may constitute a new class of drugs for the treatment of cognitive disorders in schizophrenia. On the other hand, the inverse agonist of the α_5 $GABA_A$ receptor MRK-016, which improved the training of male Sprague–Dawley rats in the Morris water maze, has a very short half-life. This worsens its clinical prospects [1, 58].

Weakening of impairments of memory and attention and accelerated learning by inverse agonists of α_5 $GABA_A$ receptors suggested that they are promising drugs for the treatment of Down's disease [48, 58]. The procognitive ability of the α5IA and Ro 4938581 compounds was shown in Ts65Dn mice with segmental trisomy on chromosome 16, which is used to model Down's disease [2, 58]. Currently, one more possible treatment for Down's syndrome, the drug Basmisanil (RG-1662, Ro 5186582), is undergoing clinical trials. Although the first results do not confirm the high effectiveness of the compound, the search for promising drugs with pro-cognitive properties among the inverse BD agonists will continue [48, 97].

It was noted above that behavioral equivalents of inverse BD agonists may be different depending on the neurochemical and electrophysiological profiles of the agent, the experimental conditions, the type of animals used, and other factors [15, 39]. This applies equally to the anti-amnestic action. As an example, amnestic drugs MK-801 and scopolamine caused spatial memory disturbances in male ddY mice (evaluated using a Y-shaped labyrinth and in an object-recognition test). The inverse BD agonist AC-3933 (a derivative of 1,6-naphthyridine) showed significant procognitive effect in both models of amnesia, while another inverse BD agonist FG-7142 was effective only in experiments with scopolamine. If AC-3933 was used together with the benzodiazepine antagonist flumazenil, the anti-amnestic effect of the inverse agonist disappeared only in the scopolamine amnesia model. Consequently, the pro-cognitive effect of AC-3933 in the presence of the NMDA receptor blocker MK-801 is not related to its ability to inhibit the basal activity of $GABA_A$ receptors [30].

As another example, the drug PWZ-029 improved the training of rats in the passive-avoidance test but was ineffective during active avoidance training [15]. PWZ-029 also exhibited anti-amnesic properties in rats treated with scopolamine (in the object-recognition test). The anti-amnestic effect was not observed under the conditions of the water maze [39].

Recently, the heterogeneity of the sites of specific binding of $GABA_A$ receptor ligands has been actively discussed. One these elements is the F-loop of the extracellular amino terminal domains of α-subunits [20]. It has been proposed that the F-loop is involved in the binding of competitive antagonists of α_5 $GABA_A$ receptors, for example, tricyclic oxazolo-2,4-benzodiazepines, which have procognitive activity. The best-known drug of this group is the compound S44819, which is undergoing preclinical trials [98–100]. It is possible that this fragment of the amino terminal of the α_5 subunit is involved in the procognitive effects and effects of negative allosteric modulators of α_5 $GABA_A$ receptors. Such data have been obtained for the inverse DB agonist triazolophthalazine α5IA [101]. Whether this is related to inverse DB agonists of a different chemical nature is not yet clear.

It has been reported that inverse BD agonists have antidepressant activity [55, 64–66]; MRK-016 and L-655708 have this characteristic. As an example, a single intraperitoneal injection of the inverse agonist L-655708 to male Sprague–Dawley rats at a dose of 3 mg/kg had a significant antidepressant effect in the forced-swim test. This effect lasted for 1 week. It was hypothesized that administration of the agent is accompanied by activation of the hippocampus, which serves as a basis of antidepressant action. L-655708 has no psychotomimetic and addictive characteristics and did not cause anxiety [55]. The compounds MRK-016 and L-655708 inhibit the basal activity of α_5-containing $GABA_A$ receptors [1, 14].

CONCLUSIONS

The sobering effect of partial inverse BD agonists during the action of ethanol, suppression of the addictive potential of alcohol and other psychoactive substances, anti-amnestic and antidepressant effects, and the ability to activate learning processes show that these agents are a very promising group of medications. Their introduction into clinical practice is limited by the presence of anxiogenic and convulsive activities, as well as accelerated metabolism [16, 39, 61, 62, 102]. These problems may be solved by the creation of selective partial benzodiazepine agonists that have acceptable pharmacokinetic parameters [34, 46, 50, 62, 102].

REFERENCES

1. Atack, J.R., Maubach, K.A., Wafford, K.A., O'Connor, D., Rodrigues, A.D., Evans, D.C., Tattersall, F.D., and Chambers, M.S., Macleod A.M., Eng, W.S., Ryan, C., Hostetler, E., Sanabria, S.M., Gibson, R.E., Krause, S., Burns, H.D., Hargreaves, R.J., Agrawal, N.G., McKernan, R.M., Murphy, M.G., Gingrich, K., Dawson, G.R., Musson, D.G., and Petty, K.J., *J. Pharmacol. Exp. Ther.,* 2009, vol. 331, no. 2, pp. 470–484.
2. Braudeau, J., Delatour, B., Duchon, A., Pereira, P.L., Dauphinot, L., de Chaumont, F., Olivo-Marin, J.C., Dodd, R.H., Hérault, Y., and Potier, M.C., *J. Psychopharmacol.*, 2011, vol. 25, no. 8, pp. 1030–1042.
3. Sato, J., Makita, N., and Iiri, T., *Endocr. J.,* 2016, vol. 63, no. 6, pp. 507–514.
4. Bhowmik, M., Khanam, R., and Vohora, D., *Br. J. Pharmacol.,* 2012, vol. 167, no. 7, pp. 1398–1414.
5. Monczor, F., Fernandez, N., Fitzsimons, C.P., Shayo, C., and Davio, C., *Eur. J. Pharmacol.,* 2013, vol. 715, nos. 1–3, pp. 26–32.
6. Corder, G., Doolen, S., Donahue, R.R., Winter, M.K., Jutras, B.L., He, Y., Hu, X., Wieskopf, J.S., Mogil, J.S., Storm, D.R., Wang, Z.J., McCarson, K.E., and Taylor, B.K., *Science*, 2013, vol. 341, no. 6152, pp. 1394–1399.
7. Polter, A.M., Barcomb, K., Chen, R.W., Dingess, P.M., Graziane, N.M., Brown, T.E., and Kauer, J.A., *Elife*, 2017, vol. 6, article e23785.
8. Arrang, J.M., Morisset, S., and Gbahou, F., *Trends Pharmacol. Sci.,* 2007, vol. 28, no. 7, pp. 350–357.
9. Morisset, S., Rouleau, A., Ligneau, X., Gbahou, F., Tardivel-Lacombe, J., Stark, H., Schunack, W., Ganellin, C.R., Schwartz, J.C., and Arrang, J.M., *Nature*, 2000, vol. 408, no. 6814, pp. 860–864.
10. Rouleau, A., Ligneau, X., Tardivel-Lacombe, J., Morisset, S., Gbahou, F., Schwartz, J.C., and Arrang, J.M., *Br. J. Pharmacol.,* 2002, vol. 135, no. 2, pp. 383–392.
11. Meye, F.J., Ramakers, G.M., and Adan, R.A., *Transl. Psychiatry,* 2014, vol. 4.
12. Bond, R.A., Leff, P., Johnson, T.D., Milano, C.A., Rockman, H.A., McMinn, T.R., Apparsundaram, S., Hyek, M.F., Kenakin, T.P., Allen, L.F., and Lefkowitz, R.J., *Nature*, 1995, vol. 374, no. 6519, pp. 272–276.
13. Walwyn, W.M., Chen, W., Kim, H., Minasyan, A., Ennes, H.S., McRoberts, J.A., and Marvizon, J.C., *J. Neurosci.,* 2016, vol. 36, no. 1, pp. 204–221.
14. Lecker, I., Yin, Y., Wang, D.S., and Orser, B.A., *Br. J. Anaest*, vol. 110, no. Suppl. 1, pp. i73–i81.
15. Savić, M.M., Clayton, T., Furtmüller, R., Gavrilović, I., Samardzić, J., Savić, S., Huck, S., Sieghart, W., and Cook, J.M., *Brain Res.,* 2008, vol. 1208, pp. 150–159.
16. Stamenic, T., Joksimovic, S., Biawat, P., Stankovic, T., Markovic, B., Cook, J.M., and Savic, M.M., *J. Psychopharmacol.*, 2015, vol. 29, no. 9, pp. 1013–1024.
17. Chua, H.C. and Chebib, M., *Adv. Pharmacol.,* 2017, vol. 79, pp. 1–34.
18. Olsen, R.W. and Sieghart, W., *Pharmacol. Rev.*, 2008, vol. 60, no. 3, pp. 243–260.
19. Olsen, R.W., *Adv. Pharmacol.,* 2015, vol. 73, pp. 167–202.
20. Sigel, E. and Steinmann, M.E., *J. Biol. Chem.*, 2012, vol. 287, no. 48, pp. 40224–40231.
21. Sadee, W., Wang, D., and Bilsky, E.J., *Life Sci.*, 2005, vol. 76, no. 13, pp. 1427–1437.
22. Sirohi, S., Dighe, S.V., Madia, P.A., and Yoburn, B.C., *J. Pharmacol. Exp. Ther.,* 2009, vol. 330, no. 2, pp. 513–519.
23. Raehal, K.M., Lowery, J.J., Bhamidipati, C.M., Paolino, R.M., Blair, J.R., Wang, D., Sadee, W., and Bilsky, E.J., *J. Pharmacol. Exp. Ther.*, 2005, vol. 313, no. 3, pp. 1150–1162.
24. Wang, D., Raehal, K.M., Lin, E.T., Lowery, J.J., Kieffer, B.L., Bilsky, E.J., and Sadée, W., *J. Pharmacol. Exp. Ther.*, 2004, vol. 308, no. 2, pp. 512–520.
25. Alyautdin, R.N. and Romanov, B.K., *Bezopasnost' i risk farmakoterapii* (Safety and Risk of Pharmacotherapy), 2014, no. 4, pp. 6–11.
26. Hodavance, S.Y., Gareri, C., Torok, R.D., and Rockman, H.A., *J. Cardiovasc. Pharmacol.,* 2016, vol. 67, no. 3, pp. 193–202.
27. Evans, A.K. and Lowry, C.A., *CNS Drug Rev,* 2007, vol. 13, no. 4, pp. 475–501.
28. Sally, E.J., Xu, H., Dersch, C.M., Hsin, L.W., Chang, L.T., Prisinzano, T.E., Simpson, D.S., Giuvelis, D., Rice, K.C., Jacobson, A.E., Cheng, K., Bilsky, E.J., and Rothman, R.B., *Synapse*, 2010, vol. 64, no. 4, pp. 280–288.

29. Barbalho, C.A., Nunes-de-Souza, R.L., and Canto-de-Souza, A., *Brain Res.,* 2009, vol. 1267, pp. 65–76.
30. Hashimoto, T. and Iwamura, Y., *Pharmacol. Biochem. Behav.,* 2016, vol. 144, pp. 45–52.
31. Hashimoto, T., Kiyoshi, T., Kohayakawa, H., Iwamura, Y., and Yoshida, N., *Neuroscience*, 2014, vol. 256, pp. 352–359.
32. Nutt, D.J., Besson, M., Wilson, S.J., Dawson, G.R., and Lingford-Hughes, A.R., *Neuropharmacology*, 2007, vol. 53, no. 7, pp. 810–820.
33. Barnard, E.A., Skolnick, P., Olsen, R.W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A.N., and Langer, S.Z., *Pharmacol. Rev.*, 1998, vol. 50. no. 2, pp. 291–313.
34. Ballard, T.M., Knoflach, F., Prinssen, E., Borroni, E., Vivian, J.A., Basile, J., Gasser, R., Moreau, J.L., Wettstein, J.G., Buettelmann, B., Knust, H., Thomas, A.W., Trube, G., and Hernandez, M.C., *Psychopharmacology* (Berlin), 2009, vol. 202, nos. 1-3, pp. 207–223.
35. Knust, H., Achermann, G., Ballard, T., Buettelmann, B., Gasser, R., Fischer, H., Hernandez, M.C., Knoflach, F., Koblet, A., Stadler, H., Thomas, A.W., Trube, G., and Waldmeier, P., *Bioorg. Med. Chem. Lett.,* 2009, vol. 19, no. 20, pp. 5940–5944.
36. De Min, A., Matera, C., Bock, A., Holze, J., Kloeckner, J., Muth, M., Traenkle, C., De Amici, M., Kenakin, T., Holzgrabe, U., Dallanoce, C., Kostenis, E., Mohr, K., and Schrage, R., *Mol. Pharmacol.,* 2017, vol. 91, no. 4, pp. 348–356.
37. Gbahou, F., Rouleau, A., Morisset, S., Parmentier, R., Crochet, S., Lin, J.S., Ligneau, X., Tardivel-Lacombe, J., Stark, H., Schunack, W., Ganellin, C.R., Schwartz, J.C., and Arrang, J.M., *Proc. Natl. Acad. Sci. U.S.A.,* 2003, vol. 100, no. 19, pp. 11086–11091.
38. Riddy, D.M., Cook, A.E., Diepenhorst, N.A., Bosnyak, S., Brady, R., Mannoury la Cour, C., Mocaer, E., Summers, R.J., Charman, W.N., Sexton, P.M., Christopoulos, A., and Langmead, C.J., *Mol. Pharmacol.,* 2017, vol. 91, no. 2, pp. 87–99.
39. Milić, M., Timić, T., Joksimović, S., Biawat, P., Rallapalli, S., Divljaković, J., Radulović, T., Cook, J.M., and Savić, M.M., *Behav. Brain Res.,* 2013, vol. 241, pp. 206–213.
40. Wallner, M., Hanchar, H.J., and Olsen, R.W., *Proc. Natl. Acad. Sci. U.S.A.,* 2006, vol. 103, no. 22, pp. 8540–8545.
41. Haefely, W.E., *Eur. Arch. Psychiatry Neurol. Sci.,* 1989, vol. 238, nos 5-6, pp. 294–301.
42. Olsen, R.W., *Neurochem. Res.,* 2014, vol. 39, no. 10, pp. 1924–1941.
43. Mehta, A.K. and Ticku, M.K., *J. Pharmacol. Exp. Ther.,* 1988, vol. 246, no. 2, pp. 558–564.
44. Mehta, A.K. and Ticku, M.K., *J. Pharmacol. Exp. Ther.,* 1989, vol. 249, no. 2, pp. 418–423.
45. Wang, F., Xu, Z., Yuen, C.T., Chow, C.Y., Lui, Y.L., Tsang, S.Y., and Xue, H., *Neuropharmacology,* 2007, vol. 53, no. 4, pp. 574–582.
46. Clayton, T., Poe, M.M., Rallapalli, S., Biawat, P., Savic, M.M., Rowlett, J.K., Gallos, G., Emala, C.W., Kaczorowski, C.C., Stafford, D.C., Arnold, L.A., and Cook, J.M., *Int. J. Med. Chem.,* 2015, vol. 2015, article ID 430248.
47. Hashimoto, T., Hatayama, Y., Nakamichi, K., and Yoshida, N., *Eur. J. Pharmacol.,* 2014, vol. 745, pp. 123–128.
48. Möhler, H., *Adv. Pharmacol.,* 2015, vol. 72, pp. 1–36.
49. Venault, P. and Chapouthier, G., *Scientific World Journal,* 2007, vol. 7, pp. 204–223.
50. Guerrini, G. and Ciciani, G., *Expert Opin. Ther. Pat.,* 2013, vol. 23, no. 7, pp. 843–866.
51. Farrant, M. and Nusser, Z., *Nat. Rev. Neurosci.,* 2005, vol. 6, no. 3, pp. 215–229.
52. Hoestgaard-Jensen, K., Dalby, N.O., Krall, J., Hammer, H., Krogsgaard-Larsen, P., Frolund, B., and Jensen, A.A., *J. Neurosci.,* 2014, vol. 34, no. 49, pp. 16256–16272.
53. Jia, F., Chandra, D., Homanics, G.E., and Harrison, N.L., *J. Pharmacol. Exp. Ther.,* 2008, vol. 326, no. 2, pp. 475–482.
54. Atack, J.R., *Curr. Top. Med. Chem.,* 2011, vol. 11, no. 9, pp. 1203–1214.
55. Carreno, F.R., Collins, G.T., Frazer, A., and Lodge, D.J., *Int. J. Neuropsychopharmacol.,* 2017, vol. 20, no. 6, pp. 504–509.
56. Redrobe, J.P., Elster, L., Frederiksen, K., Bundgaard, C., de Jong, I.E., Smith, G.P., Bruun, A.T., Larsen, P.H., and Didriksen, M., *Psychopharmacology* (Berlin), 2012, vol. 221, no. 3, pp. 451–468.
57. Atack, J.R., *Pharmacol. Ther.,* 2010, vol. 125, no. 1, pp. 11–26.
58. Martínez-Cué, C., Delatour, B., and Potier, M.C., *Neurosci. Biobehav. Rev.,* 2014, vol. 46., Pt. 2, pp. 218–227.
59. Cook, J.B., Foster, K.L., Eiler, W.J. 2nd., McKay P.F., Woods J., 2nd., Harvey S.C., Garcia M., Grey C., McCane S., Mason D., Cummings R., Li X., Cook J.M., and June H.L. *Alcohol. Clin. Exp. Res.,* 2005, vol. 29, no. 8, pp. 1390–1401.
60. June, H.L., Torres, L., Cason, C.R., Hwang, B.H., Braun, M.R., and Murphy, J.M., *Brain Res.,* 1998, vol. 784, nos. 1–2, pp. 256–275.
61. Liu, R., Hu, R.J., Zhang, P., Skolnick, P., and Cook, J.M., *J. Med. Chem.,* 1996, vol. 39, no. 9, pp. 1928–1934.
62. Atack, J.R., Bayley, P.J., Fletcher, S.R., McKernan, R.M., Wafford, K.A., and Dawson, G.R., *Eur. J. Pharmacol.,* 2006, vol. 548, nos. 1–3, pp. 77–82.
63. McKay, P.F., Foster, K.L., Mason, D., Cummings, R., Garcia, M., Williams, L.S., Grey, C., McCane, S., He, X., Cook, J.M., and June, H.L., *Psychopharmacology* (Berlin), 2004, vol. 172, no. 4, pp. 455–462.
64. Fischell, J., Van Dyke, A.M., Kvarta, M.D., LeGates, T.A., and Thompson, S.M., *Neuropsychopharmacology,* 2015, vol. 40, no. 11, pp. 2499–2509.
65. Samardzic, J., Puskas, L., Obradovic, M., Lazic-Puskas, D., and Obradovic, I.D., *Acta Veterinaria-Beograd,* 2014, vol. 64, no. 1, pp. 52–60.
66. Zanos, P., Nelson, M.E., Highland, J.N., Krimmel, S.R., Georgiou, P., Gould, T.D., and Thompson, S.M., *eNeuro,* 2017, vol. 4, no. 1, article e0285-16.2017.
67. Wallner, M. and Olsen, R.W., *Br. J. Pharmacol.,* 2008, vol. 154, no. 2, pp. 288–298.

68. Corda, M.G., Giorgi, O., Mele, S., and Biggio, G., *Brain Res. Bull.,* 1987, vol. 19, no. 3, pp. 379–385.

69. Kubova, H. and Mares, P., *Naunyn Schmiedeberg's Arch. Pharmacol.,* 1994, vol. 350, no. 4, pp. 393–397.

70. Nicolas, L.B. and Prinssen, E.P., *Psychopharmacology* (Berlin), 2006, vol. 184, no. 1, pp. 65–74.

71. Nutt, D.J., Lister, R.G., Rusche, D., Bonetti, E.P., Reese, R.E., and Rufener, R., *Eur. J. Pharmacol.,* 1988, vol. 151, no. 1, pp. 127–129.

72. Tracy, M.E., Banks, M.L., and Shelton, K.L., *Psychopharmacology* (Berlin), 2016, vol. 233, no. 4, pp. 715–725.

73. Bonetti, E.P., Burkard, W.P., Gabl, M., Hunkeler, W., Lorez, H.P., Martin, J.R., Moehler, H., Osterrieder, W., Pieri, L., Polc, P., Richards, J.G., Schaffner, R., Scherschlicht, R., Schoch, P., and Haefely, W.E., *Pharmacol. Biochem. Behav.,* 1988, vol. 31, no. 3, pp. 733–749.

74. Lister, R.G. and Nutt, D.J., *Adv. Alcohol Subst. Abuse,* 1988, vol. 7, nos. 3–4, pp. 119–123.

75. Suzdak, P.D., Paul, S.M., and Crawley, J.N., *J. Pharmacol. Exp. Ther.,* 1988, vol. 245, no. 3, pp. 880–886.

76. Dar, M.S., *Pharmacol. Biochem. Behav.,* 1992, vol. 42, no. 3, pp. 473–479.

77. Glowa, J.R., Crawley, J., Suzdak, P.D., and Paul, S.M., *Pharmacol. Biochem. Behav.,* 1988, vol. 31, no. 3, pp. 767–772.

78. Nabeshima, T., Tohyama, K., and Kameyama, T., *Eur. J. Pharmacol.,* 1988, vol. 155, no. 3, pp. 211–217.

79. Syapin, P.J., Gee, K.W., and Alkana, R.L., *Brain Res. Bull.,* 1987, vol. 19, no. 5, pp. 603–605.

80. Hoffman, P.L., Tabakoff, B., Szabó, G., Suzdak, P.D., and Paul, S.M., *Life Sci.,* 1987, vol. 41, no. 5, pp. 611–619.

81. Hanchar, H.J., Chutsrinopkun, P., Meera, P., Supavilai, P., Sieghart, W., Wallner, M., and Olsen, R.W., *Proc. Natl. Acad. Sci. U.S.A.,* 2006, vol. 103, no. 22, pp. 8546–8551.

82. Benson, J.A., Löw, K., Keist, R., Mohler, H., and Rudolph, U., *FEBS Lett.,* 1998, vol. 431, no. 3, pp. 400–404.

83. Lister, R.G. and Durcan, M.J., *Brain Res.,* 1989, vol. 482, no. 1, pp. 141–144.

84. Durcan, M.J. and Lister, R.G., *Pharmacol. Biochem. Behav.,* 1989, vol. 32, no. 3, pp. 667–670.

85. Sternfeld, F., Carling, R.W., Jelley, R.A., Ladduwahetty, T., Merchant, K.J., Moore, K.W., Reeve, A.J., Street, L.J., O'Connor, D., Sohal, B., Atack, J.R., Cook, S., Seabrook, G., Wafford, K., Tattersall, F.D., Collinson, N., Dawson, G.R., Castro, J.L., and Macleod, A.M., *J. Med. Chem.,* 2004, vol. 47, no. 9, pp. 2176–2179.

86. ClinicalTrials.gov, Ability of partial inverse agonist, iomazenil, to block ethanol effects in humans. NCT01590277. https://clinicaltrials.gov/ct2/show/NCT01590277.2017.

87. McBride, W.J., Murphy, J.M., Lumeng, L., and Li, T.K., *Pharmacol. Biochem. Behav.,* 1988, vol. 30, no. 4, pp. 1045–1050.

88. Melón, L.C. and Boehm, S.L. 2nd, *Behav. Brain Res.,* 2011, vol. 220, no. 1, pp. 230–237.

89. June, H.L., Greene, T.L., Murphy, J.M., Hite, M.L., Williams, J.A., Cason, C.R., Mellon-Burke, J., Cox, R., Duemler, S.E., Torres, L., Lumeng, L., and Li, T.K., *Brain Res.,* 1996, vol. 734, nos. 1–2, pp. 19–34.

90. June, H.L., Harvey, S.C., Foster, K.L., McKay, P.F., Cummings, R., Garcia, M., Mason, D., Grey, C., McCane, S., Williams, L.S., Johnson, T.B., He, X., Rock, S., and Cook, J.M., *J. Neurosci.,* 2001, vol. 21, no. 6, pp. 2166–2177.

91. Rüedi-Bettschen, D., Rowlett, J.K., Rallapalli, S., Clayton, T., Cook, J.M., and Platt, D.M., *Alcohol. Clin. Exp. Res.,* 2013, vol. 37, no. 4, pp. 624–634.

92. Griffiths, J.L. and Lovick, T.A., *Neuroscience*, 2005, vol. 136, no. 2, pp. 457–466.

93. Lovick, T.A., Griffiths, J.L., Dunn, S.M., and Martin, I.L., *Neuroscience*, 2005, vol. 131, no. 2, pp. 397–405.

94. Korpi, E.R., den Hollander, B., Farooq, U., Vashchinkina, E., Rajkumar, R., Nutt, D.J., Hyytiä, P., and Dawe, G.S., *Pharmacol. Rev.,* 2015, vol. 67, no. 4, pp. 872–1004.

95. Buettelmann, B., Ballard, T.M., Gasser, R., Fischer, H., Hernandez, M.C., Knoflach, F., Knust, H., Stadler, H., Thomas, A.W., and Trube, G., *Bioorg. Med. Chem. Lett.,* 2009, vol. 19, no. 20, pp. 5958–5961.

96. Hatayama, Y., Hashimoto, T., Kohayakawa, H., Kiyoshi, T., Nakamichi, K., Kinoshita, T., and Yoshida, N., *Neuroscience*, 2014, vol. 265, pp. 217–225.

97. Contestabile, A., Magara, S., and Cancedda, L., *Front. Cell. Neurosci.,* 2017, vol. 11, article 54.

98. Etherington, L.A., Mihalik, B., Pálvölgyi, A., Ling, I., Pallagi, K., Kertész, S., Varga, P., Gunn, B.G., Brown, A.R., Livesey, M.R., Monteiro, O., Belelli, D., Barkóczy, J., Spedding, M., Gacsályi, I., Antoni, F.A., and Lambert, J.J., *Neuropharmacology*, 2017, vol. 125, pp. 353–364.

99. Gacsályi, I., Móricz, K., Gigler, G., Wellmann, J., Nagy, K., Ling, I., Barkoczy, J., Haller, J., Lambert, J.J., Szénási, G., Spedding, M., and Antoni, F.A., *Neuropharmacology*, 2017, vol. 125, pp. 30–38.

100. Mihalik, B., Pálvölgyi, A., Bogár, F., Megyeri, K., Ling, I., Barkóczy, J., Bartha, F., Martinek, T.A., Gacsályi, I., and Antoni, F.A., *Eur. J. Pharmacol.,* 2017, vol. 798, pp. 129–136.

101. Pálvölgyi, A., Móricz, K., Pataki, Á., Mihalik, B., Gigler, G., Megyeri, K., Udvari, S., Gacsályi, I., and Antoni, F.A., *Neuropharmacology*, 2018, vol. 128, pp. 408–415.

102. Atack, J.R., *CNS Neurosci. Ther.,* 2008, vol. 14, no. 1, pp. 25–35.