Orexin receptor blockers: A tool for lowering alcohol intake and alcohol addictive behavior in the light of preclinical studies

Kamila Czora-Poczwadowska^{*1}, Radosław Kujawski¹, Julia Stryko-Krzyżostaniak, Przemysław Ł. Mikołajczak^{1,2}, Michał Szulc¹

¹Department of Pharmacology, Poznań University of Medical Sciences, Poznań, Poland

²Department of Pharmacology and Phytochemistry, Institute of Natural Fibers and Medicinal Plants, Pleszew, Poland

Abstract

Alcohol use disorder (AUD) is a severe and globally widespread neurological and psychiatric problem. The treatment with currently used drugs often does not bring the expected effect. New optimization methods or directions in pharmacotherapy are still being sought. The group of bioactive ligands, targeted at neuropeptides called orexins (OXs) and their receptors (OXRs), affects a number of functions including ingestion, sleep-wake regulation, as well as the brain reward system which is the basis of addiction.

The purpose of this paper is to systematize the knowledge in the field of preclinical behavioral studies on rodents (rats and mice) in several models of alcohol consumption using the OXRs antagonists.

The results of the experiments indicated a potential efficacy of particular OXRs antagonists in the AUD treatment, especially those selectively blocking the OX1R. Among them, SB-334867 in the lowest effective dose of 3 mg/kg i.p. was most studied, as shown in the model of two-bottle choice using C57BL/6 mice. Moreover, this compound did not affect the reduction of cognitive functions. GSK1059865 was also involved in the selective reduction of ethanol intake, and simultaneously did not alter the consumption of sugar solution. The other group of selective OX2R antagonists, such as TCS-OX2-29 and LSN2424100, was less efficient.

In summary, the OX1R antagonists proved to have the potential in AUD therapy, not only through the reduction of ethanol consumption but also in the treatment of coexisting behavioral and physiological disorders, such as insomnia and anxiety.

Keywords

orexins • orexin receptors • AUD • reward system • two-bottle choice model • SB-334867

Received: 16.08.2020, Accepted: 16.04.2021

Introduction

At the moment, in the pharmacological treatment of alcoholism there are only a few clinically effective synthetic drugs, i.e., disulfiram, acamprosate, naltrexone, and recently, nalmefene. These drugs are known to reduce the craving and a relapse into alcohol of dependent patients [1]. Since alcoholism is one of the most prevalent neuropsychiatric diseases, it has enormous health and socioeconomic impact [2]. Therefore, the studies on the introduction of new drugs with anti-alcoholic properties seem to be a high priority.

Alcohol addiction, according to the current state of knowledge, is closely related to the brain reward and punishment system—a complicated network of neurons that convey stimulation to specific brain structures, constituting

the anatomical basis of the neural mechanisms underlying reward-seeking and motivation [3, 4, 5]. It is known that the mesocorticolimbic system, containing, among other things, such substructures as the ventral tegmental area (VTA) and nucleus accumbens (NAc), is essential for dopamine neurotransmission [6, 7]. The evidence confirming the role of dopamine in ethanol reward shows that it increases the dopamine release in NAc [5, 8] and in the prefrontal cortex [9]. Acting on dopaminergic systems, these drugs, both excitatory and inhibitory, influence alcohol consumption [10, 11, 12, 13].

The dopaminergic neurons propagating neurotransmission, through the above-mentioned and other substructures of the mesolimbic system and their dopaminergic receptors, are not the only crucial components contributing to the complexity of cognitive and behavioral reactions leading to ethanol reward-seeking. The participation of other neurotransmission systems, affecting the mesolimbic neurons and modulating the action of

*Corresponding author e-mail: kczora@ump.edu.pl

dopamine, is also associated with the AUD-related processes. For example, a release of endogenous opioids from NAc is linked to an increase in alcohol consumption [14]. As it is currently proposed, rewarding effects of alcohol are the result of a number of coexisting brain mechanisms, which has been already described in more detail in other publications [3, 13, 15, 16, 17]. Thus, a significant amount of data indicate the functional connection of the mesolimbic system with glutaminergic, GABA, acetylcholine, and serotonin neuronal networks, which act in VTA or NAc modulating compulsive alcohol or drug intake [13, 16].

Over the years, a growing number of studies on the role of orexins (OXs), also known as hypocretins, have been conducted, particularly in a brain reward system [18, 19, 20]. One of the potential targets of alcohol addiction therapy may be the effect on the receptor level of these neuropeptides [18, 20]. Although it is known that OXs are able to trigger responses in the central nervous system (CNS) as well as in many other tissues, the responses are often different, surprising, and unexpected [21]. A number of experimental studies and functional analyses of their receptors (OXRs) have been documented, including the use of more or less selective ligands [18]. However, the exact role of OXs in the etiopathogenesis of alcohol dependence still remains not fully understood.

Aim

The aim of this mini-review is to systematize the current state of knowledge in the field of usage of OXR antagonists in preclinical behavioral studies, along with the description of major experimental *in vivo* models and the xenobiotics focused on a potential AUD pharmacotherapy.

Basics of the OXs and OXR neurobiology

OXs are neuropeptides synthesized entirely by neurons of the lateral hypothalamic area (LH)—the lateral, perifornical, and dorsomedial hypothalamus, as well as in regions of the subthalamus including the zona incerta and subthalamic nuclei [22, 23].

There are two OX proteins, Orexin A (OX-A) and B (OX-B), originally formed as a result of cleavage of prepro-orexin during their maturation [22, 23]. The amino acid sequence is similar to the gut hormone secretin, and it is strongly conservative among a number of animal species (rat, mouse, dog, cow, sheep, pig) and humans [18]. Interestingly, this group of proteins has not been found in invertebrates [20, 24]. OX-A consists of 33 amino acids (3562 Da), it contains a N-terminal pyroglutamyl residue, two intramolecular disulfide

bridges between Cys6–Cys12 and Cys7–Cys14, and also a C-terminal amidation [22, 23]. OX-B (2937 Da) consists of 28 amino acids that build a linear peptide with a C-terminal amidation and probably forms two alpha helices. The human OX-B differs from the pig and dog orexine by one amino acid and by two amino acids in rats and mice. This protein is 46% amino acid-compatible with OX-A. The C-terminus determines mostly the similarity in amino acid sequence, whereas the N-terminal end is more variable in that matter [24]. Although OXs are formed only in the hypothalamus [23], OXs system fibers form connections with the above mentioned VTA and NAc regions of the brain mesolimbic system [18, 20, 24]. However, no significant affinity to neuropeptide Y, secretin, or other peptides was observed, despite their convergence in amino acid composition [19, 23].

These proteins act via their two appropriate G-protein-coupled receptors: orexin 1 and 2 (OX1R and OX2R, respectively) [23], distributed throughout the central nervous system [20, 22, 23]. OX-A has an equal affinity for OX1R and OX2R, while OX-B preferentially binds to OX2R [23, 25]. In other words, OX1R is considered to be more selective for OX-A, requiring half of the maximally effective concentration (EC_{50}) 30 nM of OX-A but EC_{50} 2500 nM of OX-B, whereas OX2R binds both peptides equally [25], requiring a concentration of EC_{50} 34 nM of OX-A and EC_{50} 60 nM of OX-B for a half-maximum response [23].

The receptor peptides are detected quite widely throughout the brain. Their projections are most highly concentrated in different limbic regions involved in arousal and emotion [18]. OX1R is more densely located in the brain regions involved in motivation: the medial prefrontal cortex (mPFC) and amygdala, whereas OX2R is located in areas associated with regulation of sleep and arousal, such as the hypothalamic and brainstem nuclei [25]. The expression of OX1R is also observed in several other regions, for example, the infralimbic cortex, hippocampus, paraventricular thalamic nucleus, ventromedial hypothalamic nucleus, dorsal raphe nucleus, and locus coeruleus. In turn, OX2R transcripts are detected in the cerebral cortex, hippocampus, septal nuclei, raphe nuclei, dorsomedial nucleus, paraventricular nucleus, medial thalamic groups, and ventral premammillary nucleus [26].

The two receptors interact with the Gq, Gs, and Gi proteins [27] and are identified as located pre- and postsynaptically [18, 28]. For instance, there are a couple of regions where neurons show direct postsynaptic responses to applied OXs; however, with their transcript levels no higher than background levels (e.g., the *substantia nigra pars reticulata*, medial dorsal nucleus of the thalamus), suggesting that functional receptors may be present in neurons having low mRNA levels or in regions with low densities of expressing neurons. This implies that functionally important groups of orexin-responding neurons have not yet been discovered [21]. The main activity

Table 1. Summary of behavioral preclinical experiments using antagonists of OX_R along with a brief description of some experimental details

Antagonist	<i>In vitro</i> study (Cellular measurements)	<i>In vivo</i> (Proven action)	Procedure	Dose/Administration	Effect specificity	Author's
SB-334867	N/I	+	P female rat 2-bottle choice	3, 10, or 30 mg/kg, i.p.	N/I	[4]
	N/I	-	P female rat Operant progressive ratio		N/I	
	N/I	+	Male C57BL/6 mice Binge drinking		-	
			All procedures 15%EtOH			
SB-334867	+	+	Male C57BL/6 mice Binge drinking 20%EtOH	5 mg/kg, i.p.	N/I	[48]
SB-334867	N/I	+	Male P rat Operant progressive ratio 10%EtOH	3 µg VTA	+	[61]
SB-334867	N/I	+	Female P rat Operant progressive ratio for EtOH->PSR ->Relapse ->SB administration>PSR ->Relapse 15%EtOH	10 or 20 mg/kg, i.p.	N/I	[59]
SB-334867	+	+	Male P rat Operant progressive ratio 10%EtOH	5 or 10 mg/kg i.p.	+	[57]
SB-334867	+	+	Male C57BL/6 mice 2-bottle choice 15%EtOH	0.3, 1, 3 or 10 mg/kg i.p.	+	[49]
SB-334867	+	mNash + mPFC +	Male C57BL/6 mice Binge drinking 15%EtOH	3 µg mNash and mPFC	+	[50]
		+	Male Long Evans rat Operant progressive ratio 20%EtOH		N/I	
SB-334867	+	+	Male P rat Operant responding ratio 10%EtOH	20 mg/kg, i.p.	+	[58]
GSK1059865	+	+	Male C57BL/6 mice intermittent ethanol or air expo- sure in inhalation chambers 15%EtOH	10, 25, 50 mg/kg i.p	+	[60]
SB-334867	N/I	+	Male Wistar rat 2-bottle choice 10%EtOH	3 ng, 6 ng NAcc	-	[44]

Antagonist	In vitro study (Cellular measurements)	In vivo (Proven action)	Procedure	Dose/Administration	Effect specificity	Author's
SB-334867	N/I	+	Male Sprague Dawley rat 2-bottle choice 10%EtOH	30 mg/kg, i.p.	+	[46]
SB-334867	N/I	+	Male Sprague-Dawley rat Operant progressive ratio cue-induced reinstatement	10, or 20 mg/kg i.p.	+	[47]
SB-334867	+	+	Male C57BL/6 mice Binge drinking 20%EtOH	5 or 10 mg/kg, i.p.	-	[51]
SB-334867	+	+	Male C57BL/6 mice Binge drinking 20%EtOH	6.0 µg VTA and CeA	+	[55]
SB-334867	-	+	Male Long-Evans rat Operant progressive ratio 10%EtOH	5, 10, 15, 20 mg/kg, i.p.	+	[43]
SB-408124	+	-	Male DBA/2 mice CPP	3, 10, 30 mg/kg, s.c.	N/I	[45]
		-	Male Wistar rat Operant progressive ratio All procedures 8%EtOH			
SB-334867	+	-	Male DBA/2J mice CPP	10, 15, 20, 30, 40 mg/kg, i.p.	N/I	[52]
LSN2424100	N/I	-	P female rat 2-bottle choice	10 or 30 mg/kg, i.p.	N/I	[4]
	N/I	+	P female rat Operant progressive ratio	3, 10, 30 mg/kg i.p.	N/I	
	N/I	+	Male C57BL/6 mice Binge drinking	5, 30, 60 mg/kg i.p.	-	
TCS-OX2-29	+	aPVT + pPVT -	All procedures 15%EtOH Male Long-Evans rat 2-bottle-choice 20%EtOH	10 nmol aPVT, pPVT	aPVT + pPVT -	[42]

Antagonist	<i>In vitro</i> study (Cellular measurements)	<i>In vivo</i> (Proven action)	Procedure	Dose/Administration	Effect specificity	Author's
TCS-OX2-29	+	NAcc + NAcsh –	Male P rats cue-induced reinstatement of ethanol-seeking Operant responding ratio 10%EtOH	100 µg or 300 µg NAcsh or NAcc	+	[56]
TCS-OX2-29	+	-	Male C57BL/6 mice 2-bottle choice 15%EtOH	3 or 10 mg/kg i.p.	N/I	[49]
TCS-OX2-29	+	-	Male C57BL/6 mice Binge drinking 15%EtOH Operant progressive ratio 20%EtOH	3 µg mNash	N/I	[50]
TCS-OX2-29	+	-	Male C57BL/6 mice Binge drinking 20%EtOH	5.0, or 7.5 µg VTA, CeA	-	[55]
JNJ10397049	+	-	Male DBA/2 mice CPP Male Wistar rat Operant progressive ratio All procedures 8%EtOH	1, 3, and 10 mg/kg, s.c.	+	[45]
Almorexant	N/I	+	P female rat 2-bottle choice P female rat Operant progressive ratio Male C57BL/6 mice Binge drinking All procedures 15%EtOH	60 or 100 mg/kg, i.p. 10, 30, or 60 mg/kg, i.p. 5, 50, or 100 mg/kg, i.p.	N/I N/I -	[4]
Almorexant	+	VTA + SN -	Male Long-Evans rat Operant progressive ratio 20%EtOH	3, 10 and 15 mg/kg, i.p. VTA and SN	-	[62]

(+) - confirmed effect, (-) - no effect, N/I – not investigated.

Antagonists of OX2 - TCS-OX2-29, JNJ10397049, LSN2424100; Dual Antagonist – Almorexant.
 aPVT – anterior Paraventricular nucleus of the Thalamus, CeA - Central nucleus of the Amygdala, CPP - conditioned place preference, EtOH – Ethanol, mNash - medial Nucleus Accumbens shell, mPFC - medial Prefrontal Cortex, NAcc - core of the Nucleus Accumbens, NAcsh - Nucleus Accumbens shell, pPVT - posterior Paraventricular nucleus of the Thalamus, PSR - Pavlovian Spontaneous Recovery, SN - Substantia Nigra, VTA - Ventral Tegmental Area.

of OXs in CNS cells is described as capable of producing a slow and long-lasting depolarization that can be large enough to initiate firing, or—if the neuron is already firing—to increase its firing rate. OXs are therefore considered as excitatory neuropeptides because their receptors coupled to effectors produce depolarization. In addition to participation in mediating of neurons membrane depolarization, it is believed that native OXRs alter the integrative properties of their target neurons by modulating postsynaptic ion channels and thus strongly influence how synaptic inputs from other sources get converted into firing [21]. Functional experimental studies have shown the presence of OXRs at or near presynaptic terminals of some glutamate- and GABA-releasing neurons. This ability to release glutamate and/or GABA by increasing miniature postsynaptic current frequency has been observed in CNS [29], although the underlying signaling mechanisms have not been investigated. In some structures, OXs preferentially release GABA or glutamate [29], typically in conjunction with postsynaptic actions. Presumably, this allows OXs to amplify or attenuate information transmission arising from particular afferents [21]. These relationships may be the reason for finding a reduction of OX-A levels in patients addicted to ketamine, a drug whose pharmacological profile involves blocking NMDA receptors in the glutamatergic system [30].

Looking at the basic mechanisms of intracellular signal transduction and the differences between the above discussed, it should be mentioned that OX1R is thought to couple to Gq, while OX2R is considered to signal through Gq or Gi/Go. However, the coupling mechanisms seem to be cell type specific and have not been fully described in neurons [24, 31].

Apart from their main occurrence in brain structures involved in food and/or drug-seeking motivation—especially in the limbic part of the reward system—there is a growing amount of evidence that they are also involved in the regulation of sleep and emotional states [18, 20, 24]. At the beginning of studies on the OX system, most of the research back then focused on determining its impact on the regulation of food-related behaviors. OX neurons activities occur during long-term fast, restricted nutrition and resulting hypoglycemia. The activation of these neurons is reduced after food intake. OXR expression is elevated after 48 to 72 hours without food [23, 32, 33]. With a limited access to nutrition, OX neurons are more active just prior to planned food availability, and the OX gene knock-out mice show reduced awakening and motor activity during the food waiting period [34].

It has become clear that these neuropeptides are involved in many other behaviors, many of which are used to promote survival. Apart from the above-mentioned feeding-related activities, the stimulation of the OX system also affects other aspects of behavior like stress [35] and arousal [36].

Behavioral preclinical models in the studies on OXRs antagonists

In the behavioral experiments to explain the role of OXs and their receptors in the course of alcohol addiction, there are three dominant models of alcohol consumption by rodents:

- **Drinking in the dark (DiD) paradigm** – corresponds to the human model of binge-like alcohol drinking. It takes advantage of the most active circadian period in rodents (the third hour of the dark cycle). Their water bottle is replaced with an unsweetened ethanol solution for a short period of two to four hours each day. The procedure promotes high levels of ethanol drinking and relevant blood ethanol concentrations. Numerous variations on DiD have been developed [37].
- **Two-bottle choice paradigm** – the animals have access to ethanol solution and water. This design allows the researcher to determine the preference for ethanol solution over water, as well as the total fluid intake, under normal conditions and in separate cages [38].
- **Operant self-administration paradigm** – allows to observe how much effort an animal will put into obtaining ethanol and accordingly, the extent to which ethanol acts as a reinforcer. The animal has to press the lever to receive ethanol. One of the main benefits of this strategy is that it can independently evaluate the motivational as opposed to the consummatory components of self-administration behavior. A negative aspect is the amount of time required for training [38].

In the light of the current state of knowledge, the proportion of P-strain rats (who prefer drinking alcohol) prevails in the studies involving OXRs antagonists. They are considered more relevant to the studies on ethanol drinking behavior due to the fact that these animals have been selectively bred in accordance with the procedures aimed at strengthening the ethanol effects [39]. Thus, they are less sensitive to the calming and aversive effects of ethanol and more sensitive to its stimulating effects, they have a genetic predisposition to alcohol abuse and alcohol dependence, and they can be used in many models of alcohol preference [40]. For that purpose, not only male P-strain rats but females also have been studied, as in the study by Anderson [4], in which a home cage with a two-bottle choice and the progressive operant responding (lever presses) were applied. Alcohol consumption and preference seemed to be higher in females than in males, in particular in the first stages of this process, which, over time, might be equalized at a similar level in both sexes [41].

Some papers highlight the differences in the amount of alcohol consumed or the effects of alcohol using different

strains of rats, for example: Long-Evans [42, 43], Wistar [44, 45] and Sprague-Dawley [46, 47].

In addition to the above-mentioned rats, especially P-strain, male C57BL mice are also used for the OXR antagonist investigations in the studies on alcoholism [4, 48, 49, 50, 51]. Another mouse strain—DBA—is used with much less frequency due to the fact that their daily ethanol consumption capacity is small [45, 52]. For example, the C57BL mice can consume 1.68 g/kg of ethanol, while DBA ones—only 0.25 g/kg [53].

Due to the fact that animals principally do not consume large amounts of alcohol willingly, the administration of alcohol is very often forced [38, 54]. Therefore, more frequent use of animals with a genetically higher ethanol consumption feature has been postulated, so there will be a greater chance of success in the study.

The usage of OX1R antagonists

SB-334867—a selective OX1R antagonist—was the most effective in three standard models of alcohol drinking in mice [4, 48, 49, 50, 51, 53, 55] and in rats [4, 43, 44, 46, 47, 53, 56, 57, 58]. The lowest effective dose after the i.p. administration was 3 mg/kg in C57BL/6 mice [49] and 5 mg/kg in rats [48, 51, 57]. The alcohol-conditioned place preference was disturbed after SB-334867 treatment [52]. Moreover, the compound induced the reduction of alcohol drinking relapse [59]. Another selective OX1R antagonist—GSK1059865—also significantly reduced the alcohol consumption in C57BL/6J mice [60]. Besides, OXR antagonists were directly administered to the VTA and NAc substructures. For example, the cannulation of SB-334867 into VTA [55, 61], CeA (central nucleus of the amygdala) [55], NAc core [44], mNash (medial Nucleus Accumbens Shell), and mPFC [50] reduced the alcohol consumption in all behavioral paradigms. Almorexant, a nonselective blocker of both types of OXRs, reduced the consumption of ethanol but also the sugar intake in all three models, both in mice and rats, in the dose range of 15–100 mg/kg, i.p., [4, 62]. Its infusion into VTA significantly reduced the animals response to the operant progressive responding ratio [62].

Apart from the above-mentioned studies, there is some evidence indicating the modulation of the reward system by OXs in the increase of extracellular dopamine [63] or the reduction of cocaine-induced dopamine levels in NAc [64] due to the intra-VTA OX1R antagonist administration.

The usage of OX2R antagonists

Compared with studies on OX1R antagonists, the number of studies in the context of assessing the use of OX2R antagonists in the possibility of their influence on ethanol drinking is relatively poor. For example, a selective OX2R antagonist—TCS-OX2-29—nonspecifically reduced alcohol consumption after the cannulation into a PVT [42] and NAc core [56]. In another study, TCS-OX2-29 was not effective [50]. Another selective OX2R antagonist—LSN2424100—administered i.p. decreased the self-administration of ethanol, but selectively—only in the binge-like drinking paradigm [4]. JNJ10397049 attenuated the ethanol self-administration, reinstatement, and place preference in mice, but at a lower degree than the positive control, naltrexone [45]. Apart from Shoblock *et al.* [45], no one has done any appropriate research to determine the effect of OX2R blockers on dopamine circulation or the expression of dopaminergic receptors in the limbic system. According to these authors, the assessment of the JNJ10397049 activity showed that the ethanol-induced increase in dopamine level in the NAc was not changed after the OX2R blockade, which was a surprising result [45].

The table summarizes the state of behavioral preclinical experiments using antagonists of OXRs along with a brief description of experimental details [Table 1].

Conclusions

There are many factors to be considered when targeting the OX system as a treatment against alcohol motivation. It is postulated that changes in the OXRs' activity in specific brain structures, associated directly with pathologically enhanced motivation, drive compulsive alcohol consumption. Modulation of activity of the reward system, which is closely related to addiction, may play a major role in inhibiting alcohol consumption with the administration of OXR antagonists, but the accurate role of OXs in AUD still remains not fully understood [18, 20]. A number of experiments have been undertaken to assess the effectiveness of OXR antagonists and to evaluate the behavioral and physiological consequences of their usage [18, 20, 24]. There are also other studies confirming the possible use of OXR blockers in the treatment of nicotine, cocaine, or morphine addictions [20, 65, 66].

In most experiments related to the effects of alcohol, the activity of SB-334867 is specific, but almost all OX2R and OX1/2R antagonists exhibit a similar trend in the decrease of alcohol (and sugar) intake, and sometimes they may indicate a problem with excessive sedation. The dual antagonists almorexant and suvorexant especially show such a tendency. Therefore, these two compounds represent possible groups of new hypnotics. Moreover, suvorexant has a rather low addictive

potential in comparison with zolpidem [67]. Suvorexant shows overall sleep-promoting effects in the ethanol-treated group after 24 hours following alcohol withdrawal in rats [68]. So it appears that the sleep-promoting action may be an additional benefit because sleep disorders are often associated with addiction and treating them improves the abstinence rates [69]. For example, the occurrence of insomnia in people with AUD is an important factor causing the recurrence of ethanol drinking [70]. The majority of alcohol addicts suffer from insomnia and they often self-medicate this condition with alcohol [70]. The additional hypnotic effect of substances that reduce ethanol consumption can be considered as an advantage in some cases.

Furthermore, SB-334867 seems to have an impact on cognitive function and anxiety, which could lead to doubts as to whether this substance significantly reduces alcohol consumption in various models of this addiction [20]. However, blocking OX1R with SB-334867 has a minimal effect on the inhibition of measured response in a rat model of the so-called stop-signal reaction time task (a different experimental model designed to verify whether a given xenobiotic affects cognitive function or attention) [71], which clearly indicates its impact on drug-seeking behavior without disturbance of cognitive functions.

Of all the studies described above, the use of naltrexone as a positive control was found only in Shoblock et al. [45] and Anderson et al. [4]. The researchers not only checked the effectiveness of the substances studied but also compared the effects they produce with the drug mostly used in the treatment of alcoholism.

ORXs signaling via OX1Rs has a strong effect on lowering the alcohol consumption. SB-334867 (its activity and selectivity on OX1Rs) was the most frequently studied compound, showing the desired behavioral effects. The use of selective OX2Rs antagonists, i.e., TCS-OX2-29 or LSN2424100, did not produce such effective results; they either nonspecifically reduced alcohol consumption [42, 56] or were active only in the binge-like drinking paradigm [4].

The verification of behavioral and physiological consequences of using OX1R antagonists (mostly targeting OX1Rs) provides crucial knowledge regarding their pharmacological profile in the field of experimental alcohol addiction *in vivo* models. Such knowledge may be valuable, so increasing the number of experimental studies should be postulated. Last but not least, it would be beneficial to expand the scope of currently published clinical trials involving OX1R antagonists and to successfully treat those aspects of AUD that coexist with other behavioral or physiological disorders, such as insomnia or anxiety.

Authors' Contributions

K.C.-P.: Research concept and design, acquisition of data; analysis and interpretation of data, literature review, writing the manuscript, final proofreading and approval of the version for publication; R.K.: Acquisition of data, analysis and interpretation of data, writing the manuscript, drafting the article or revising it critically for important intellectual content; J.S.-K.: Literature review, writing the manuscript; P.Ł.M.: Drafting the article or revising it critically for important intellectual content, supervising the project; M. S.: Literature review, writing the manuscript.

Conflict of Interest

The authors have no potential conflicts of interest to declare.

References

- [1] Aubin H.J., Reimer J., Nutt D.J., Bladström A., Torup L., François C., Chick J.: Clinical relevance of as needed treatment with nalmefene in alcohol-dependent patients. *Eur. Addict. Res.*, 2015; 21: 160–168
- [2] Xuan Z., Naimi T.S., Kaplan M.S., Bagge C.L., Few L.R., Maisto S., Saitz R., Freeman R.: Alcohol policies and suicide: A review of the literature. *Alcohol Clin. Exp. Res.*, 2016; 40: 2,043–2,055
- [3] Alasmari F., Goodwani S., McCullumsmith R.E., Sari Y.: Role of glutamatergic system and mesocorticolimbic circuits in alcohol dependence. *Prog. Neurobiol.*, 2018; 171: 32–49
- [4] Anderson R.I., Becker H.C., Adams B.L., Jesudason C.D., Rorick-Kehn L.M.: Orexin-1 and orexin-2 receptor antagonists reduce ethanol self-administration in high-drinking rodent models. *Front. Neurosci.*, 2014; 8: 33
- [5] Imperato A., Di Chiara G.: Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J. Pharmacol. Exp. Ther.*, 1986; 239: 219–228
- [6] Korotkova T.M., Sergeeva O.A., Eriksson K.S., Haas H.L., Brown R.E.: Excitation of ventral tegmental area dopaminergic and non-dopaminergic neurons by orexins/hypocretins. *J. Neurosci.*, 2003; 23: 7–11
- [7] Patyal R., Woo E.Y., Borgland S.L.: Local hypocretin-1 modulates terminal dopamine concentration in the nucleus accumbens shell. *Front. Behav. Neurosci.*, 2012; 6: 82
- [8] Vena A.A., Gonzales R.A.: Temporal profiles dissociate regional extracellular ethanol versus dopamine concentrations. *ACS Chem. Neurosci.*, 2015; 6: 37–47
- [9] Schier C.J., Dilly G.A., Gonzales R.A.: Intravenous ethanol increases extracellular dopamine in the medial prefrontal cortex of the Long-Evans rat. *Alcohol Clin. Exp. Res.*, 2013; 37: 740–747

- [10] Adinoff B.: Neurobiologic processes in drug reward and addiction. *Harv. Rev. Psychiatry*, 2004; 12: 305–320
- [11] Samson H.H., Pfeffer A.O., Tolliver G.A.: Oral ethanol self-administration in rats: Models of alcohol-seeking behavior. *Alcohol. Clin. Exp. Res.*, 1988; 12: 591–598
- [12] Samson H.H., Tolliver G.A., Pfeffer A.O., Sadeghi K, Haraguchi M.: Relation of ethanol self-administration to feeding and drinking in a nonrestricted access situation in rats initiated to self-administer ethanol using the sucrose-fading technique, *Alcohol*, 1988; 5: 375–385
- [13] Volkow N.D., Michaelides M., Baler R.: The neuroscience of drug reward and addiction. *Physiol. Rev.*, 2019; 99: 2,115–2,140
- [14] Mitchell J.M., O'Neil J.P., Janabi M., Marks S.M., Jagust W.J., Fields H.L.: Alcohol consumption induces endogenous opioid release in the human orbitofrontal cortex and nucleus accumbens. *Sci. Transl. Med.*, 2012; 4: 116ra6
- [15] Addolorato G., Leggio L., Abenavoli L., Gasbarrini G., Alcoholism Treatment Study Group: Neurobiochemical and clinical aspects of craving in alcohol addiction: A review. *Addict. Behav.*, 2005; 30: 1,209–1,224
- [16] Koob G.F., Volkow N.D.: Neurobiology of addiction: A neurocircuitry analysis. *Lancet Psychiatry*, 2016; 3: 760–773
- [17] Weiss F., Porrino L.J.: Behavioral neurobiology of alcohol addiction: Recent advances and challenges. *J. Neurosci.* 2002; 22: 3,332–3,337
- [18] Barson J.R., Leibowitz S.F.: Orexin/hypocretin system: Role in food and drug overconsumption. *Int. Rev. Neurobiol.*, 2017; 136: 199–237
- [19] Holmqvist T., Akerman K.E., Kukkonen J.P.: High specificity of human orexin receptors for orexins over neuropeptide Y and other neuropeptides. *Neurosci Lett.*, 2001; 305: 177–180
- [20] James M.H., Mahler S.V., Moorman D.E., Aston-Jones G.: A decade of orexin/hypocretin and addiction: Where are we now? *Curr. Top. Behav. Neurosci.*, 2017; 33: 247–281
- [21] Leonard C.S., Kukkonen J.P.: Orexin/hypocretin receptor signalling: A functional perspective. *Br. J. Pharmacol.*, 2014; 171: 294–313
- [22] de Lecea L., Kilduff T.S., Peyron C., Gao X., Foye P.E., Danielson P.E., Fukuhara C., Battenberg E.L., Gautvik V.T., Bartlett F.S. 2nd, et al.: The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. USA*, 1998; 95: 322–327
- [23] Sakurai T., Amemiya A., Ishii M., Matsuzaki I., Chemelli R.M., Tanaka H., Williams S.C., Richardson J.A., Kozlowski G.P., Wilson S., et al.: Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, 1998; 92: 573–585
- [24] Scammell T.E., Winrow C.J.: Orexin receptors: Pharmacology and therapeutic opportunities. *Annu. Rev. Pharmacol. Toxicol.*, 2011; 51: 243–266
- [25] Sakurai T.: The role of orexin in motivated behaviours. *Nature Rev. Neurosci.*, 2014; 15: 719–731
- [26] Marcus J.N., Aschkenasi C.J., Lee C.E., Chemelli R.M., Saper C.B., Yanagisawa M., Elmquist J.K.: Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.*, 2001; 435: 6–25
- [27] Magga J., Bart G., Oker-Blom C., Kukkonen J.P., Akerman K.E., Näslman J.: Agonist potency differentiates G protein activation and Ca²⁺ signalling by the orexin receptor type 1. *Biochem. Pharmacol.*, 2006; 71: 827–836
- [28] van den Pol A.N., Gao X.B., Obrietan K., Kilduff T.S., Belousov A.B.: Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J. Neurosci.*, 1998; 18: 7,962–7,971
- [29] Acuna-Goycolea C., van den Pol A.N.: Neuroendocrine proopiomelanocortin neurons are excited by hypocretin/orexin. *J. Neurosci.*, 2009; 29: 1,503–1,513
- [30] Huang M.C., Chen C.H., Chen L.Y., Chang H.M., Chen C.K., Lin S.K., Xu K.: Chronic ketamine abuse is associated with orexin-A reduction and ACTH elevation. *Psychopharmacology*, 2020; 237: 45–53
- [31] Randeve H.S., Karteris E., Grammatopoulos D., Hillhouse E.W.: Expression of orexin-A and functional orexin type 2 receptors in the human adult adrenals: Implications for adrenal function and energy homeostasis. *J. Clin. Endocrinol. Metab.*, 2001; 86: 4,808–4,813
- [32] Fujiki N., Yoshida Y., Ripley B., Honda K., Mignot E., Nishino S.: Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 hours and in response to food deprivation. *Neuroreport*, 2001; 12: 993–997
- [33] Moriguchi T., Sakurai T., Nambu T., Yanagisawa M., Goto K.: Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia. *Neurosci. Lett.*, 1999; 264: 101–104
- [34] Mieda M., Williams S.C., Sinton C.M., Richardson J.A., Sakurai T., Yanagisawa M.: Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. *J. Neurosci.*, 2004; 24: 10,493–10,501
- [35] Winsky-Sommerer R., Yamanaka A., Diano S., Borok E., Roberts A.J., Sakurai T., Kilduff T.S., Horvath T.L., de Lecea L.: Interaction between the corticotropin-releasing factor system and hypocretins (orexins): A novel circuit mediating stress response. *J. Neurosci.*, 2004; 24: 11,439–11,448
- [36] Chemelli R.M., Willie J.T., Sinton C.M., Elmquist J.K., Scammell T., Lee C., Richardson J.A., Williams S.C., Xiong Y., Kisanuki Y., et al.: Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation. *Cell*, 1999; 98: 437–451
- [37] Thiele T.E., Navarro M.: "Drinking in the dark" (DID) procedures: A model of binge-like ethanol drinking in non-dependent mice. *Alcohol*, 2014; 48: 235–241
- [38] Leeman R.F., Heilig M., Cunningham C.L., Stephens D.N., Duka T., O'Malley S.S.: Ethanol consumption: How should we measure it? Achieving consilience between human and animal phenotypes. *Addict. Biol.*, 2010; 15: 109–124

- [39] Gauvin D.V., Moore K.R., Holloway F.A.: Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or the preparedness to learn? *Alcohol*, 1993; 10: 37–43
- [40] Bell R.L., Rodd Z.A., Lumeng L., Murphy J.M., McBride W.J.: The alcohol-preferring P rat and animal models of excessive alcohol drinking. *Addict. Biol.*, 2006; 11: 270–288
- [41] Moore C.F., Lynch W.J.: Alcohol preferring (P) rats as a model for examining sex differences in alcohol use disorder and its treatment. *Pharmacol. Biochem. Behav.*, 2015; 132: 1–9
- [42] Barson J.R., Ho H.T., Leibowitz S.F.: Anterior thalamic paraventricular nucleus is involved in intermittent access ethanol drinking: Role of orexin receptor 2. *Addict. Biol.*, 2015; 20: 469–481
- [43] Richards J.K., Simms J.A., Steensland P., Taha S.A., Borgland S.L., Bonci A., Barlett S.E.: Inhibition of orexin-1/hypocretin-1 receptors inhibits yohimbine-induced reinstatement of ethanol and sucrose seeking in Long-Evans rats. *Psychopharmacology*, 2008; 199: 109–117
- [44] Mayannavar S., Rashmi K.S., Rao Y.D., Yadav S., Ganaraja B.: Effect of orexin A antagonist (SB-334867) infusion into the nucleus accumbens on consummatory behavior and alcohol preference in Wistar rats. *Indian J. Pharmacol.*, 2016; 48: 53–58
- [45] Shoblock J.R., Welty N., Aluisio L., Fraser I., Motley S.T., Morton K., Palmer J., Bonaventure P., Carruthers N.I., Lovenberg T.W., et al.: Selective blockade of the orexin-2 receptor attenuates ethanol self-administration, place preference, and reinstatement. *Psychopharmacology*, 2011; 215: 191–203
- [46] Moorman D.E., Aston-Jones G.: Orexin-1 receptor antagonism decreases ethanol consumption and preference selectively in high-ethanol-preferring Sprague-Dawley rats. *Alcohol.*, 2009; 43: 379–386
- [47] Moorman D.E., James M.H., Kilroy E.A., Aston-Jones G.: Orexin/hypocretin-1 receptor antagonism reduces ethanol self-administration and reinstatement selectively in highly-motivated rats. *Brain Res.*, 2017; 1654: 34–42
- [48] Alcaraz-Iborra M., Navarrete F., Rodríguez-Ortega E., de la Fuente L., Manzanares J., Cubero I.: Different molecular/behavioral endophenotypes in C57BL/6J mice predict the impact of OX₁ receptor blockade on binge-like ethanol intake. *Front. Behav. Neurosci.*, 2017; 11: 186
- [49] Lei K., Wegner S.A., Yu J.H., Hopf F.W.: Orexin-1 receptor blockade suppresses compulsive-like alcohol drinking in mice. *Neuropharmacology*, 2016; 110: 431–437
- [50] Lei K., Wegner S.A., Yu J.H., Mototake A., Hu B., Hopf F.W.: Nucleus accumbens shell and mPFC but not insula orexin-1 receptors promote excessive alcohol drinking. *Front. Neurosci.*, 2016; 10: 400
- [51] Olney J.J., Navarro M., Thiele T.E.: Binge-like consumption of ethanol and other salient reinforcers is blocked by orexin-1 receptor inhibition and leads to a reduction of hypothalamic orexin immunoreactivity. *Alcohol. Clin. Exp. Res.*, 2015; 39: 21–29
- [52] Voorhees C.M., Cunningham C.L.: Involvement of the orexin/hypocretin system in ethanol conditioned place preference. *Psychopharmacology*, 2011; 214: 805–818
- [53] Lê A.D., Ko J., Chow S., Quan B.: Alcohol consumption by C57BL/6, BALB/c, and DBA/2 mice in a limited access paradigm. *Pharmacol. Biochem. Behav.*, 1994; 47: 375–378
- [54] Mendoza-Ruiz L.G., Vázquez-León P., Martínez-Mota L., Juan E.R., Miranda-Páez A.: Forced ethanol ingestion by Wistar rats from a juvenile age increased voluntary alcohol consumption in adulthood, with the involvement of orexin-A. *Alcohol*, 2018; 70: 73–80
- [55] Olney J.J., Navarro M., Thiele T.E.: The role of orexin signaling in the ventral tegmental area and central amygdala in modulating binge-like ethanol drinking behavior. *Alcohol. Clin. Exp. Res.*, 2017; 41: 551–561
- [56] Brown R.M., Khoo S.Y., Lawrence A.J.: Central orexin (hypocretin) 2 receptor antagonism reduces ethanol self-administration, but not cue-conditioned ethanol-seeking, in ethanol-preferring rats. *Int. J. Neuropsychopharm.*, 2013; 16: 2,067–2,079
- [57] Jupp B., Krivdic B., Krstew E., Lawrence A.J.: The orexin₁ receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats. *Brain Res.*, 2011; 1,391: 54–59
- [58] Lawrence A.J., Cowen M.S., Yang H.J., Chen F., Oldfield B.: The orexin system regulates alcohol-seeking in rats. *Br. J. Pharmacol.*, 2006; 148: 752–759
- [59] Dhafer R., Hauser S.R., Getachew B., Bell R.L., McBride W.J., McKinzie D.L., Rodd Z.A.: The orexin-1 receptor antagonist SB-334867 reduces alcohol relapse drinking, but not alcohol-seeking, in alcohol-preferring (P) rats. *J. Addict. Med.*, 2010; 4: 153–159
- [60] Lopez M.F., Moorman D.E., Aston-Jones G., Becker H.C.: The highly selective orexin/hypocretin 1 receptor antagonist GSK1059865 potently reduces ethanol drinking in ethanol dependent mice. *Brain. Res.*, 2016; 1,636: 74–80
- [61] Brown R.M., Kim A.K., Khoo S.Y., Kim J.H., Jupp B., Lawrence A.J.: Orexin-1 receptor signalling in the prelimbic cortex and ventral tegmental area regulates cue-induced reinstatement of ethanol-seeking in iP rats. *Addict. Biol.*, 2016; 21: 603–612
- [62] Srinivasan S., Simms J.A., Nielsen C.K., Lieske S.P., Bito-Onon J.J., Yi H., Hopf F.W., Bonci A., Bartlett S.E.: The dual orexin/hypocretin receptor antagonist, almorexant, in the ventral tegmental area attenuates ethanol self-administration. *PLoS One*, 2012; 7: e44726
- [63] Narita M., Nagumo Y., Hashimoto S., Narita M., Khotib J., Miyatake M., Sakurai T., Yanagisawa M., Nakamachi T., Shioda S., Suzuki T.: Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J. Neurosci.*, 2006; 26: 398–405
- [64] Wang B., You Z.B., Wise R.A.: Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: Independence from the local corticotropin-releasing factor network. *Biol. Psychiatry*, 2009; 65: 857–862

- [65] Łupina M., Tarnowski M., Baranowska-Bosiacka I., Talarek S., Listos P., Kotlińska J., Gutowska I., Listos J.: SB-334867 (an orexin-1 receptor antagonist) effects on morphine-induced sensitization in mice - a view on receptor mechanisms. *Mol. Neurobiol.*, 2018; 55: 8473–8485
- [66] Mahler S.V., Smith R.J., Moorman D.E., Sartor G.C., Aston-Jones G.: Multiple roles for orexin/hypocretin in addiction. *Prog. Brain Res.*, 2012; 198: 79–121
- [67] Schoedel K.A., Sun H., Sellers E.M., Faulknor J., Levy-Cooperman N., Li X., Kennedy W., Cha J.H., Lewis N.M., Liu W., et al.: Assessment of the abuse potential of the orexin receptor antagonist, suvorexant, compared with zolpidem in a randomized crossover study. *J. Clin. Psychopharmacol.* 2016; 36: 314–323
- [68] Sanchez-Alavez M., Benedict J., Wills D.N., Ehlers C.L.: Effect of suvorexant on event-related oscillations and EEG sleep in rats exposed to chronic intermittent ethanol vapor and protracted withdrawal. *Sleep*, 2019; 42: zsz020
- [69] Terán A., Majadas S., Galan J.: Quetiapine in the treatment of sleep disturbances associated with addictive conditions: A retrospective study. *Subst. Use Misuse.*, 2008; 43: 2,169–2,171
- [70] Brower K.J., Aldrich M.S., Robinson E.A., Zucker R.A., Greden J.F.: Insomnia, self-medication, and relapse to alcoholism. *Am. J. Psychiatry*, 2001; 158: 399–404
- [71] Wiskerke J., James M.H., Aston-Jones G.: The orexin-1 receptor antagonist SB-334867 reduces motivation, but not inhibitory control, in a rat stop signal task. *Brain Res.*, 2020; 1,731: 146222