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LEADING ARTICLE

Potential of $GABA_B$ Receptor Positive Allosteric Modulators in the Treatment of Alcohol Use Disorder

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Abstract
The orthosteric γ-aminobutyric $acid_B$ ($GABA_B$) receptor agonist baclofen is currently considered a therapeutic option for alcohol use disorder (AUD); however, the safety profile of baclofen is a concern, thus arousing interest in the positive allosteric modulators (PAMs) of the $GABA_B$ receptor ($GABA_B$ PAMs), a new class of ligands expected to possess a better safety profile. The present paper summarizes the several lines of experimental evidence indicating the ability of $GABA_B$ PAMs to inhibit multiple alcohol-motivated behaviors in rodents. All $GABA_B$ PAMs tested to date have invariably been reported to reduce, or even suppress, excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking, and alcohol-induced locomotor stimulation and conditioned place preference in rats and mice. The use of validated animal models of several aspects of AUD confers translational value to these findings. The reducing effects of $GABA_B$ PAMs on alcohol-motivated behaviors (1) occurred at doses largely lower than those inducing sedation, suggesting that $GABA_B$ PAMs may possess, if compared with baclofen, a higher therapeutic index and a more favorable safety profile, and (2) were often not associated with reductions on other non-drug consummatory behaviors. Additional findings with therapeutic potential were (1) the lack of tolerance, after repeated treatment, to the reducing effect of $GABA_B$ PAMs on alcohol drinking and self-administration; (2) the efficacy of $GABA_B$ PAMs after intragastric administration; and (3) the ability of $GABA_B$ PAMs to selectively potentiate the suppressing effect of baclofen on alcohol self-administration. The recent transition of the first $GABA_B$ PAMs to the initial steps of clinical testing makes investigation of the efficacy of $GABA_B$ PAMs in AUD patients a feasible option.

1 Introduction

In the early 2000s, several research projects converged in demonstrating that treatment with the prototypic γ-aminobutyric $acid_B$ ($GABA_B$) receptor agonist baclofen suppressed multiple alcohol-related behaviors in laboratory rodents [1–6], as well as alcohol consumption and craving for alcohol in individuals with alcohol use disorder (AUD) [7–9]. These initial data, together with several additional lines of experimental and clinical evidence published in the following years, have led to baclofen being considered a therapeutic option for AUD [10].

In the same years, chemists at Novartis, Basel, Switzerland, synthesized the first in vivo effective positive allosteric modulators (PAMs) of the $GABA_B$ receptor ($GABA_B$ PAMs), 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) [11] and *N*,*N*'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) [12], discovering a new mode to pharmacologically modulate the $GABA_B$ receptor-mediated neurotransmission. Pharmacologists were immediately intrigued by verifying whether $GABA_B$ PAMs reproduced and potentiated the pharmacological effects of baclofen. This research appeared to be highly interesting because of the mechanism of action of $GABA_B$ PAMs and their expected, more favorable toxicological profile. $GABA_B$ PAMs indeed interact with a binding site on the $GABA_B$ receptor that is topographically distinct from the orthosteric binding site of endogenous GABA or baclofen [13, 14]. $GABA_B$ PAMs have no, or limited, intrinsic activity per se (i.e. they do not activate the GABA binding site in the absence of GABA); they modify the conformation of the $GABA_B$ receptor, increasing agonist affinity and/or efficacy. Due to this use-dependent mechanism of action (i.e. activating the $GABA_B$ receptor only at those synapses where and when GABA has been released), $GABA_B$ PAMs are predicted to display a large separation between the 'desired' pharmacological actions and the

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Key Points

The γ-aminobutyric acid$_B$ ($GABA_B$) receptor agonist baclofen is currently considered a therapeutic option for alcohol use disorder in view of its ability to suppress alcohol craving and consumption; however, its safety profile is a concern, mainly due to a narrow separation between therapeutic doses and doses producing side effects.

Positive allosteric modulators (PAMs) of the $GABA_B$ receptor ($GABA_B$ PAMs) constitute a new class of $GABA_B$ receptor ligands. They potentiate endogenous GABA only where and when it has been released, i.e. a more physiological binding mechanism than that of $GABA_B$ receptor agonists.

Accumulating lines of experimental evidence unanimously indicate that $GABA_B$ PAMs reproduced the effect of baclofen on multiple alcohol-motivated behaviors, reducing excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking in rodents, and alcohol-induced locomotor stimulation and conditioned place preference. These effects occurred at doses largely lower than those producing sedation.

'unwanted' toxicological actions, overcoming a major drawback of the pharmacological profile of baclofen [14].

In the alcohol research field, preclinical pharmacological investigations confirmed both hypotheses: (1) $GABA_B$ PAMs reproduced and potentiated the reducing effects of baclofen on multiple alcohol-motivated behaviors in rats and mice; (2) the effects of $GABA_B$ PAM on alcohol-motivated behaviors occurred at doses largely lower than those inducing behavioral toxicity (motor incoordination and sedation). The present paper intends to provide a brief overview of these research data (summarized in Table 1), demonstrating the ability of all tested $GABA_B$ PAMs—from the class 'founders', CGP7930 and GS39783, to *N*-[(1*R*,2*R*,4*S*)-bicyclo[2.2.1]hept-2-yl]-2-methyl-5-[4-(trifluoromethyl)phenyl]-4-pyrimidinamine (BHF177), (R,S)-5,7-di-tert-butyl-3-hydroxy-3-trifluoromethyl-3H-benzofuran-2-one (*rac*-BHFF), 2-{1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinyl} ethanol (CMPPE), and the more recently synthesized N-(5-(4-(4-chloro-3-fluorobenzyl)-6-methoxy-3,5-dioxo-4,5-dihydro-1,2,4-triazin-2(3H)-yl)-2-fluorophenyl)acetamide (ADX71441), methyl2-(4-chlorophenylcarboxamido)-4-ethyl-5-methylthiophene-3-carboxylate (COR659), and (S)-1-(5-fluoro-2,3-dihydro-1H-inden-2-yl)-4-methyl-6,7,8,9-tetrahydro-[1,2,4]triazolo[4,3-a]quinazolin-5(4H)-one (ORM-27669) (Fig. 1) [15] —to inhibit seeking for and drinking of alcohol in validated rodent models of AUD.

2 Effects of $GABA_B$ Positive Allosteric Modulators (PAMs) on Alcohol Drinking

Alcohol drinking in rodents is usually modeled by simply giving rats and mice access to alcohol in their homecage environment [16]; this is also how alcohol drinking has been measured in the studies testing $GABA_B$ PAMs. To facilitate recordings of alcohol intake from each single animal, rats or mice are usually housed individually. The vast majority of techniques and studies employ the free choice paradigm, in which alcohol solution(s) and water are offered concomitantly, allowing measurement of the amounts of alcohol that animals consume voluntarily. Most studies employ single alcohol concentrations, although the concurrent exposure to multiple alcohol concentrations may result in, at least in some experimental instances, higher alcohol intakes [17, 18]. Exposure to alcohol may be temporally limited (i.e. in daily sessions of brief duration, resulting in relatively high intakes) or unlimited (i.e. lasting 24 h/day, with most alcohol drinking occurring during the dark phase of the daily light/dark cycle) [16, 19].

2.1 Excessive Alcohol Drinking

The excessive alcohol drinking that characterizes AUD patients is usually modeled by exposing genetically selected, alcohol-preferring rats to a free choice between alcohol solution(s) and water. Rats consume voluntarily psychopharmacologically relevant amounts of alcohol, with chronic alcohol drinking resulting in the development of tolerance and behavioral dependence [20].

Repeated (once daily for 5 consecutive days), intragastric treatment with CGP7930 (50 and 100 mg/kg) or GS39783 (50 and 100 mg/kg intragastrically) reduced, in a dose-related manner, daily alcohol intake in selectively bred, alcohol-experienced male Sardinian alcohol-preferring (sP) rats exposed to the homecage two-bottle 'alcohol (10% v/v) versus water' choice regimen [21]. The reducing effect of CGP7930 and GS39783 on daily alcohol intake was associated with a fully compensatory increase in daily water intake; daily food intake (recorded to assess the selectivity of the reducing effect of CGP7930 and GS39783 on alcohol intake) tended to be higher in CGP7930- and GS39783-treated rats than vehicle-treated rats [21]. These data were subsequently confirmed by a similar experiment testing the $GABA_B$ PAM, *rac*-BHFF: repeated (once daily for 7 consecutive days) treatment of male sP rats with *rac*-BHFF (50–200 mg/kg intragastrically) resulted in a marked and stable reduction in daily alcohol intake, fully compensated by a proportional increase in daily water intake [22]; daily food intake was decreased only by treatment with 200 mg/kg *rac*-BHFF, suggesting that the lowest doses tested (50 and

Table 1 Summary of the effect of the PAMs of the GABA$_B$ receptor (GABA$_B$ PAMs) on multiple alcohol-motivated behaviors in rodents

GABA$_B$ PAM	Alcohol-related behavior	Experimental procedure	Drug dose and route of administration	Treatment duration	Animal species, strain/line, and sex	Effect	Selectivity/specificity	Reference
ADX71441	Binge-like drinking	Drinking In the Dark	3, 10, and 30 mg/kg; IG	Acute	C57BL/6J mouse; male	Dose-dependent suppression	NI	[26]
	Excessive alcohol drinking	Intermittent (once every other day) access to alcohol	3, 10, and 17 mg/kg; IG	Acute	C57BL/6J mouse; male	Dose-related reduction	Yes (no effect on water intake)	[26]
	Operant alcohol self-administration	FR (FR2–FR3)	1, 3, 10, and 30 mg/kg; IP	Acute	Wistar rat; male	Dose-dependent suppression (increased potency in alcohol-dependent rats)	No (dose-dependent suppression of saccharin self-administration)	[44]
	Operant alcohol self-administration	PR	3 and 10 mg/kg; IP	Acute	Wistar rat; male	Dose-dependent suppression	NI	[44]
	Reinstatement of alcohol seeking	Cue- and stress (footshock)-induced	3 and 10 mg/kg; IP	Acute	Wistar rat; male	Dose-independent suppression	NI	[44]
BHF177	Operant alcohol self-administration	FR (FR4)	12.5, 25, and 50 mg/kg; IG	Acute	sP rat; male	Dose-related reduction	Yes (no effect on sucrose self-administration)	[40]
	Operant alcohol self-administration	PR	12.5, 25, and 50 mg/kg; IG	Acute	sP rat; male	Dose-related reduction	Yes (no effect on sucrose self-administration)	[40]
	Operant alcohol self-administration	Sipper	10 and 20 µg/hemisphere; intra-VTA	Acute	Long Evans rat; male	Suppression of seeking	Yes (no effect on sucrose seeking)	[64]
CGP7930	Alcohol drinking (acquisition of)	Two-bottle 'alcohol vs. water' choice; unlimited access	25, 50, and 100 mg/kg; IG	Repeated (once daily for 5 consecutive days)	sP rat; male	Dose-related suppression	Yes (compensatory increase in water intake)	[21]
	Alcohol drinking (maintenance of)	Two-bottle 'alcohol vs. water' choice; unlimited access	50 and 100 mg/kg; IG	Repeated (once daily for 5 consecutive days)	sP rat; male	Dose-related reduction	Yes (compensatory increase in water intake)	[21]
	Operant alcohol self-administration	FR (FR3)	10 and 20 mg/kg; IP	Acute	P rat; male	Dose-related reduction	No (reduction in locomotor activity)	[34]
	Operant alcohol self-administration	FR (FR3)	10 mg/kg; IP	Acute	P rat; male	Potentiation of baclofen (2 mg/kg IP) effect	Yes (no effect of 'CGP7930 + baclofen' combination on locomotor activity)	[34]
	Operant alcohol self-administration	FR (FR4)	5, 10, and 20 µg; intra-VTA	Acute	sP rat; male	Dose-related reduction	Yes (no effect on motor performance)	[62]
	Operant alcohol self-administration	FR (FR4)	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; male	Dose-related reduction	NI	See Fig. 2

Table 1 (continued)

$GABA_B$ PAM	Alcohol-related behavior	Experimental procedure	Drug dose and route of administration	Treatment duration	Animal species, strain/line, and sex	Effect	Selectivity/specificity	Reference
CMPPE	Relapse-like drinking	Alcohol deprivation effect	10 and 30 mg/kg; IP	Five injections across the phases of alcohol deprivation and alcohol reaccess	Wistar rat; male	Dose-related reduction	Yes (no effect on locomotor activity)	[28]
	Reinstatement of alcohol seeking	Cue-induced	10 and 30 mg/kg; IP	Acute	Wistar rat; male	Dose-related suppression	NI	[28] (see also Fig. 4)
	Operant alcohol self-administration	FR (FR5)	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; female	Dose-related reduction	Yes (no effect on chocolate self-administration)	[42]
	Operant alcohol self-administration	PR	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; female	Dose-related reduction	NI	[42]
	Reinstatement of alcohol seeking	Cue-induced	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; female	Dose-related suppression	NI	[42]
COR659	Operant alcohol self-administration	FR (FR4)	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; male	Dose-related suppression	Yes (no effect on locomotor activity)	[39]
	Operant alcohol self-administration	FR (FR4)	2.5, 5, and 10 mg/kg; IP	Repeated (once daily for 10 consecutive days)	sP rat; male	Dose-related reduction	NI	[43]
	Operant alcohol self-administration	PR	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; male	Dose-related suppression	Yes (no effect on locomotor activity)	[39]
	Reinstatement of alcohol seeking	Cue-induced	2.5, 5, and 10 mg/kg; IP	Acute	sP rat; male	Dose-related suppression	NI	[43]
	Binge-like drinking	Four-bottle 'alcohol vs. water' choice; limited and unpredictable access	2.5, 5, 10, 20, and 40 mg/kg; IG	Acute	sP rat; male	Dose-related reduction	Yes (no effect on food intake)	Our laboratory, unpublished results
GS39783	Alcohol drinking (acquisition of)	Two-bottle 'alcohol vs. water' choice; unlimited access	6.25, 12.5, and 25 mg/kg; IG	Repeated (once daily for 5 consecutive days)	sP rat; male	Dose-related suppression	Yes (compensatory increase in water intake)	[21]

Table 1 (continued)

GABA$_B$ PAM	Alcohol-related behavior	Experimental procedure	Drug dose and route of administration	Treatment duration	Animal species, strain/line, and sex	Effect	Selectivity/specificity	Reference
	Alcohol drinking (maintenance of)	Two-bottle 'alcohol vs. water' choice; unlimited access	50 and 100 mg/kg; IG	Repeated (once daily for 5 consecutive days)	sP rat; male	Dose-related reduction	Yes (compensatory increase in water intake)	[21]
	Operant alcohol self-administration	FR (FR4)	2.5, 5, 10, 25, 50, and 100 mg/kg; IG	Acute	sP rat; male	Dose-related reduction	Yes (no effect on sucrose or food self-administration)	[36, 37, 39]
	Operant alcohol self-administration	FR (FR4)	25, 50, and 100 mg/kg; IG	Acute	P rat; male	Dose-related suppression	Yes (no effect on food self-administration)	[37]
	Operant alcohol self-administration	FR (FR4)	25, 50, and 100 mg/kg; IG	Acute	AA rat; male	Dose-related reduction	Yes (no effect on food self-administration)	[37]
	Operant alcohol self-administration	FR (FR4)	50 mg/kg; IG	Repeated (once daily for 10 consecutive days)	sP rat; male	Reduction; lack of tolerance	Yes (no effect on sucrose self-administration)	[35] (see also Fig. 3)
	Operant alcohol self-administration	FR (FR4)	5 mg/kg; IG	Acute	sP rat; male	Potentiation of baclofen (1 mg/kg IP) effect	Yes (no effect of 'GS39783 + baclofen' combination on sucrose self-administration)	[35]
	Operant alcohol self-administration	PR	25, 50, and 100 mg/kg; IG (but not lower doses)	Acute	sP rat, male	Dose-related reduction	Yes (no effect on sucrose self-administration)	[37, 39, 45]
	Operant alcohol self-administration	PR	25, 50, and 100 mg/kg; IG	Acute	P rat; male	Dose-unrelated suppression	NI	[37]
	Operant alcohol self-administration	PR	25, 50, and 100 mg/kg; IG	Acute	AA rat; male	Non-significant tendency toward a reduction	NI	[37]
	Operant alcohol self-administration	Sipper	25, 50, and 100 mg/kg; IG	Acute	sP rat, male	Dose-unrelated reduction (both seeking and drinking)	NI	[47]
	Operant alcohol self-administration	Sipper	5, 10, and 20 µg/hemisphere; intra-VTA	Acute	Long Evans rat; male	Suppression of seeking	Yes (no effect on sucrose seeking)	[63, 64]
	Binge-like drinking	Drinking In the Dark	30 mg/kg; IP	Acute	C57BL/6J mouse; male	Suppression	Yes (no effect on locomotor activity)	[31]

Table 1 (continued)

$GABA_B$ PAM	Alcohol-related behavior	Experimental procedure	Drug dose and route of administration	Treatment duration	Animal species, strain/line, and sex	Effect	Selectivity/specificity	Reference
	Binge-like drinking	Four-bottle 'alcohol vs. water' choice; limited and unpredictable access	25, 50, and 100 mg/kg; IG	Acute	sP rat; male	Dose-related reduction	Yes (no effect on food intake)	[29]
	Locomotor stimulation	Hyperlocomotion induced by acute alcohol (2 g/kg IP)	1, 3, 10, and 30 mg/kg; IP	Acute	DBA/2J mouse; male	Attenuation	Yes (no effect when administered alone)	[51]
	Locomotor sensitization	Hyperlocomotion induced by repeated alcohol (2.5 g/kg IP)	30 mg/kg	Repeated (once daily for 11 consecutive days; concomitant to alcohol)	DBA/2J mouse; male	Potentiation	NI	[51]
	Locomotor sensitization	Hyperlocomotion induced by repeated alcohol (2.5 g/kg IP)	30 mg/kg	Acute (test session when sensitization had developed)	DBA/2J mouse; male	No effect	NI	[51]
ORM-27669	Binge-like drinking	Drinking In the Dark	100 mg/kg; IP	Acute	C57BL/6J mouse; male	Suppression	Yes (no effect on locomotor activity)	[32]
	Conditioned place preference	Eight conditioning sessions, four with saline and four with alcohol (0.5 g/kg IP)	100 mg/kg; IP	Repeated (prior to each 'alcohol' conditioning session)	C57BL/6J mouse; male	Reduction	Yes (no effect on locomotor activity)	[32]

Table 1 (continued)

GABA$_B$ PAM	Alcohol-related behavior	Experimental procedure	Drug dose and route of administration	Treatment duration	Animal species, strain/line, and sex	Effect	Selectivity/specificity	Reference
rac-BHFF	Operant alcohol self-administration	FR (FR4)	50, 100, and 200 mg/kg; IG	Acute	sP rat; male	Dose-related suppression	Yes (no effect on sucrose self-administration at 50 and 100 mg/kg)	[41]
	Operant alcohol self-administration	FR (FR4)	50 mg/kg; IG	Repeated (once daily for 5 consecutive days)	sP rat; male	Reduction; lack of tolerance	Yes (no effect on sucrose self-administration, at least on the first 3 days)	[35]
	Operant alcohol self-administration	FR (FR4)	5 mg/kg; IG	Acute	sP rat; male	Potentiation of baclofen (1 mg/kg IP) effect	Yes (no effect of '*rac*-BHFF + baclofen' combination on sucrose self-administration)	[35]
	Alcohol drinking (maintenance of)	Two-bottle 'alcohol vs. water' choice; unlimited access	50, 100, and 200 mg/kg; IG	Repeated (once daily for 7 consecutive days)	sP rat; male	Dose-related suppression	Yes (compensatory increase in water intake)	[22]
	Binge-like drinking	Drinking in the Dark	30 mg/kg; IP	Acute	C57BL/6J mouse; male	Reduction	Yes (no effect on locomotor activity)	[32]
	Conditioned place preference	Eight conditioning sessions, four with saline and four with alcohol (0.5 g/kg IP)	30 mg/kg; IP	Repeated (prior to each 'alcohol' conditioning session)	C57BL/6J mouse; male	Reduction	Yes (no effect on locomotor activity)	[32]

AA Alko Alcohol, *FR* fixed ratio, *GABA$_B$* γ-aminobutyric acid$_B$, *IG* intragastric, *IP* intraperitoneal, *NI* not investigated, *P* Indiana alcohol-preferring, *PAMs* positive allosteric modulators, *PR* progressive ratio, *sP* Sardinian alcohol-preferring, *VTA* ventral tegmental area

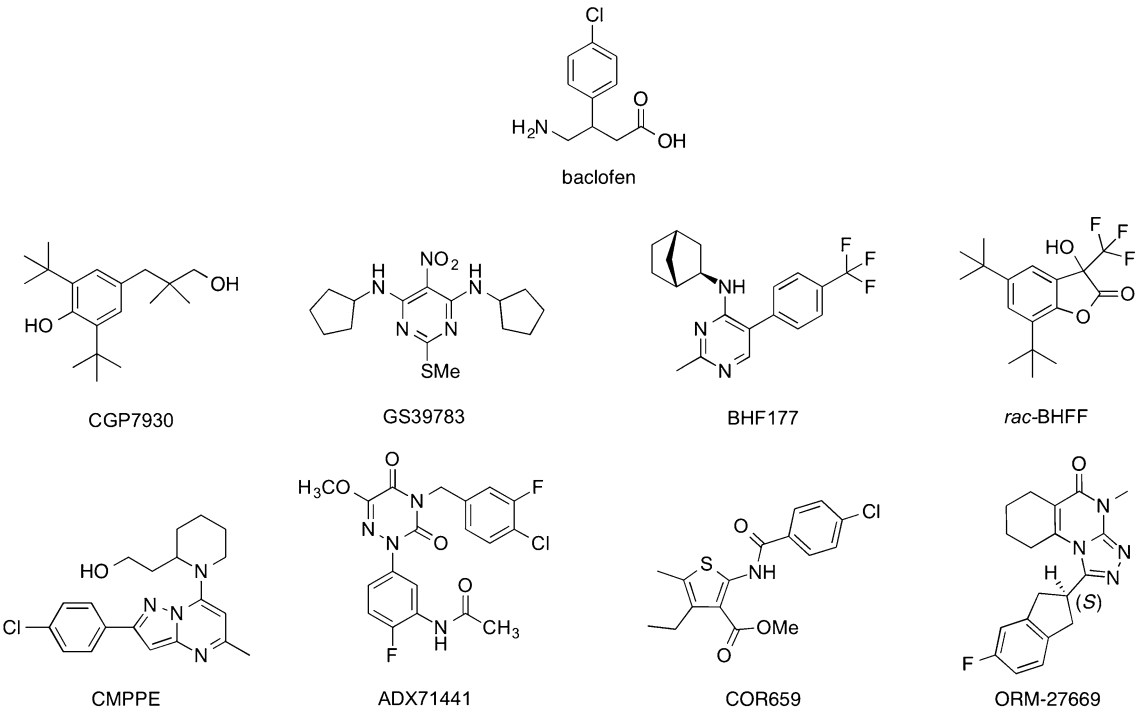

Fig. 1 Chemical structure of the orthosteric $GABA_B$ receptor agonist baclofen, and the positive allosteric modulators of the $GABA_B$ receptor tested to date on multiple alcohol-motivated behaviors in rodents. *$GABA_B$* γ-aminobutyric $acid_B$

100 mg/kg) selectively reduced daily alcohol intake [22]. The greater efficacy of *rac*-BHFF, compared with CGP7930 and GS39783, in reducing daily alcohol intake in sP rats was explained by its longer-lasting half-life [23], likely resulting in a more appropriate drug bioavailability over the 24-h drinking phase.

CGP7930 and GS39783 had also been tested in alcohol-naive male sP rats: repeated (once daily for 5 consecutive days) treatment with CGP7930 (25–100 mg/kg intragastrically) or GS39783 (6.25–25 mg/kg intragastrically) started before rat exposure to the two-bottle 'alcohol (10% v/v) versus water' choice regimen [21]. Treatment with both compounds resulted in a dose-related suppression of daily alcohol intake; rats started consuming alcohol only when treatment with CGP7930 or GS39783 had been terminated [21]. These data suggest that treatment with CGP7930 and GS39783 prevented the detection of the psychopharmacological effects of underlying alcohol drinking. The suppressing effect of CGP7930 and GS39783 on daily alcohol drinking was fully compensated by an increase in daily water intake [21]. In agreement with the notion that pharmacological activation of the $GABA_B$ receptor may stimulate eating [24], treatment with CGP7930 and GS39783 resulted in higher daily chow intakes [21].

ADX71441 has recently been tested in a rodent model of excessive alcohol drinking based on the intermittent access to alcohol; this procedure generates cycles of alcohol bingeing and withdrawal, promoting dramatic escalations in alcohol drinking in rats and mice [25]. ADX71441 (3–17 mg/kg intragastrically) was administered acutely to male C57BL/6 J mice (an inbred mouse strain with a high alcohol preference) repeatedly exposed (i.e. once every other day) to a free choice between alcohol (20% *w*/*v*) and water [26]. Treatment with ADX71441 reduced alcohol drinking; the effect of ADX71441 on alcohol intake was selective (as water intake was unaltered by ADX71441 treatment), long-lasting (as still evident at the end of the 24-h drinking phase), and of larger magnitude than the effects produced by intraperitoneal administration of the positive reference compound naltrexone (0.1–10 mg/kg) [26].

2.2 Relapse-Like Drinking

To date, CMPPE is the only $GABA_B$ PAM tested on alcohol deprivation effect (ADE), i.e. the transient increase in voluntary alcohol intake occurring after a relatively long period of forced abstinence, or deprivation, from alcohol; ADE is a validated rodent model of relapse drinking in AUD patients [27]. CMPPE was tested in alcohol-dependent male Wistar rats exposed to long periods of alcohol drinking interposed with periods of alcohol abstinence [28]. CMPPE (10 and 30 mg/kg intraperitoneally) was injected repeatedly across the last deprivation phase and the subsequent phase of

re-exposure to alcohol. Treatment with CMPPE reduced, in a dose-related manner, the extra intake of alcohol on the first 2 post-abstinence days, with no effect on daily water intake and spontaneous locomotor activity [28].

2.3 Binge-Like Drinking

GS39783, ADX71441, *rac*-BHFF, and ORM-27669 have been tested in rodent models of binge drinking. Binge drinking is modeled in rats and mice by experimental procedures in which brief sessions of alcohol drinking generate intoxicating blood alcohol levels [25].

Specifically, the study testing GS39783 employed male sP rats exposed to multiple alcohol concentrations (10, 20, and 30% v/v), under the four-bottle 'alcohol versus water' choice regimen, in daily 1-h drinking sessions occurring, with an unpredictable time schedule, over the dark phase of the light/dark cycle [29]. When the drinking session took place in the final hours of the dark phase, sP rats consumed intoxicating, binge-like amounts of alcohol [29]. Acute treatment with GS39783 (25–100 mg/kg intragastrically) completely abolished the extra intake of alcohol representing binge-like drinking [29]. Full selectivity was indicated by the lack of effect of GS39783 on water and food intake [29].

Three mouse studies tested the effect of GS39783, ADX71441, *rac*-BHFF, and ORM-27669. These studies employed an experimental procedure named Drinking In the Dark (DID), based on access to alcohol, often as a single option, in daily sessions of limited duration (2–4 h) starting a few hours after the dark phase has commenced [30]. When applied to C57BL/6J mice, this procedure generates intakes of intoxicating amounts of alcohol, mimicking human binge drinking [30]. Acute treatment of male C57BL/6J mice with GS39783 (30 mg/kg intraperitoneally) suppressed alcohol drinking over the first 15 min of the drinking session (i.e. the time period during which alcohol drinking was more intense), abolishing the 'front-loading' characteristic of the alcohol drinking pattern developed by mice after repeated exposure to DID sessions [31]. Water intake and spontaneous locomotor activity were completely unaltered by treatment with GS39783, indicative of the selectivity of the suppressing effect of GS39783 on alcohol drinking [31]. Acute treatment of male C57BL/6J mice with ADX71441 (3–30 mg/kg intragastrically) dose-dependently suppressed alcohol intake over the entire 4-h time period of the DID session [26]. The effect of ADX71441 was longer-lasting and of larger magnitude than that produced by naltrexone (0.1–10 mg/kg intraperitoneally) [26]. Acute treatment of male C57BL/6J mice with non-sedative doses of *rac*-BHFF (30 mg/kg intraperitoneally) and ORM-27669 (100 mg/kg intraperitoneally) reduced and suppressed, respectively, alcohol intake over the 4-h DID session [32].

3 Effects of $GABA_B$ PAMs on Operant Alcohol Self-Administration

The majority of currently available, in vivo effective $GABA_B$ PAMs have been tested in rats exposed to operant procedures of alcohol self-administration. At variance with the above models of alcohol drinking, operant procedures of alcohol self-administration require the animals to perform a given task (usually pressing a lever or nose-poking inside a hole for a given number of times) to access alcohol. In addition to alcohol consumption, operant procedures provide a measure of the animals' willingness to 'work' for alcohol. Accordingly, when the workload requirement is high, operant procedures successfully model the AUD diagnostic criterion of 'excessive amount of time spent in obtaining and using alcohol' [16, 20].

Studies with $GABA_B$ PAMs have basically used two different procedures of operant alcohol self-administration: (1) a fixed ratio (FR) schedule of reinforcement, in which the response requirement (RR), i.e. the number of operant responses needed to access alcohol, is kept fixed throughout the self-administration session (providing a measure of the reinforcing properties of alcohol); and (2) a progressive ratio (PR) schedule of reinforcement, in which RR is increased progressively over the self-administration session up to breakpoint, defined as the highest RR achieved or the lowest RR not achieved (providing a measure of the motivational properties of alcohol) [33].

3.1 Fixed Ratio Schedule of Reinforcement

The first $GABA_B$ PAM tested on operant alcohol self-administration was CGP7930 [34]. Acute treatment with 10 and 20 mg/kg CGP7930 intraperitoneally halved lever responding for alcohol (10% v/v) in selectively bred, male alcohol-preferring Indiana P rats exposed to the FR3 schedule of reinforcement. Treatment with CGP7930 did not affect lever responding for water. These data have subsequently been replicated in male sP rats: acute treatment with CGP7930 (2.5–10 mg/kg intraperitoneally) reduced, by up to approximately 45% at the highest dose tested, lever responding for alcohol (15%, v/v) [Fig. 2, top panel] and the amount of self-administered alcohol (Fig. 2, bottom panel) in sP rats exposed to the FR4 schedule of reinforcement.

The initial study on CGP7930 [34] also reported that combination of subthreshold doses of CGP7930 (10 mg/kg intraperitoneally) and baclofen (2 mg/kg intraperitoneally) resulted in a substantial reduction in lever responding for alcohol in P rats. These data have recently been extended to GS39783 and *rac*-BHFF [35]: combination of per se ineffective doses of GS39783 (5 mg/kg intragastrically) or

rac-BHFF (5 mg/kg intragastrically) and baclofen (1 mg/kg intraperitoneally) synergistically reduced lever responding for alcohol and the amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement. Both combinations (GS39783 + baclofen and *rac*-BHFF + baclofen) were totally ineffective on self-administration of a sucrose solution (1% w/v in water), indicating the selectivity of the reducing effect of the drug combination on alcohol self-administration and ruling out that the reducing effect of the drug combination on alcohol self-administration was due to a potentiation of the sedative effects of each combination ingredient [35]. The results of these combination studies [34, 35] confirm the in vivo facilitatory ability of $GABA_B$ PAMs to activate the $GABA_B$ receptor [15].

Acute treatment with GS39783 (25–100 mg/kg intragastrically) effectively reduced lever responding for alcohol (15% v/v) and the amount of self-administered alcohol in alcohol-preferring male sP [36, 37], P [37], and Alko Alcohol (AA) [37] rats exposed to the FR4 schedule of reinforcement. The magnitude of the reducing effect of GS39783 on alcohol self-administration varied considerably among the three lines of alcohol-preferring rats, with GS39783 being more potent and effective in the rat line (P) displaying the strongest reinforcing and motivational properties of alcohol [37]. Of interest, similar data have been collected with baclofen, indicating it to be particularly potent and effective in rats characterized by high levels of responding for alcohol and large amounts of self-administered alcohol (P rats [37] and Wistar rats made physically dependent on alcohol by exposure to alcohol vapors [38]). No dose of GS39783 altered, even minimally, operant self-administration of food pellets (regular chow) in food-deprived sP, P, and AA rats [37], suggesting that GS39783 selectively reduced alcohol self-administration and was devoid of any sedative or motor incoordinating effect. A more recent study found that the ability of GS39783 to reduce lever responding for alcohol and the amount of self-administered alcohol in male sP rats also extended to doses as low as 5 mg/kg intragastrically [39].

The reducing effect of GS39783 50 mg/kg intragastrically on lever responding for alcohol (15% v/v) [Fig. 3, top panel] and the amount of self-administered alcohol (Fig. 3, bottom panel) was also unaltered after repeated treatment (10 consecutive daily self-administration sessions) in male sP rats [35], demonstrating no development of tolerance on continuing treatment.

Highly consistent results have been collected in studies testing other $GABA_B$ PAMs in rats exposed to the FR schedule of reinforcement. Specifically, acute treatment with BHF177 (12.5–50 mg/kg intragastrically) [40], *rac*-BHFF (50–200 mg/kg intragastrically) [41], and COR659 (2.5–10 mg/kg intraperitoneally) [39] effectively reduced lever responding for alcohol (15% v/v) and the amount of

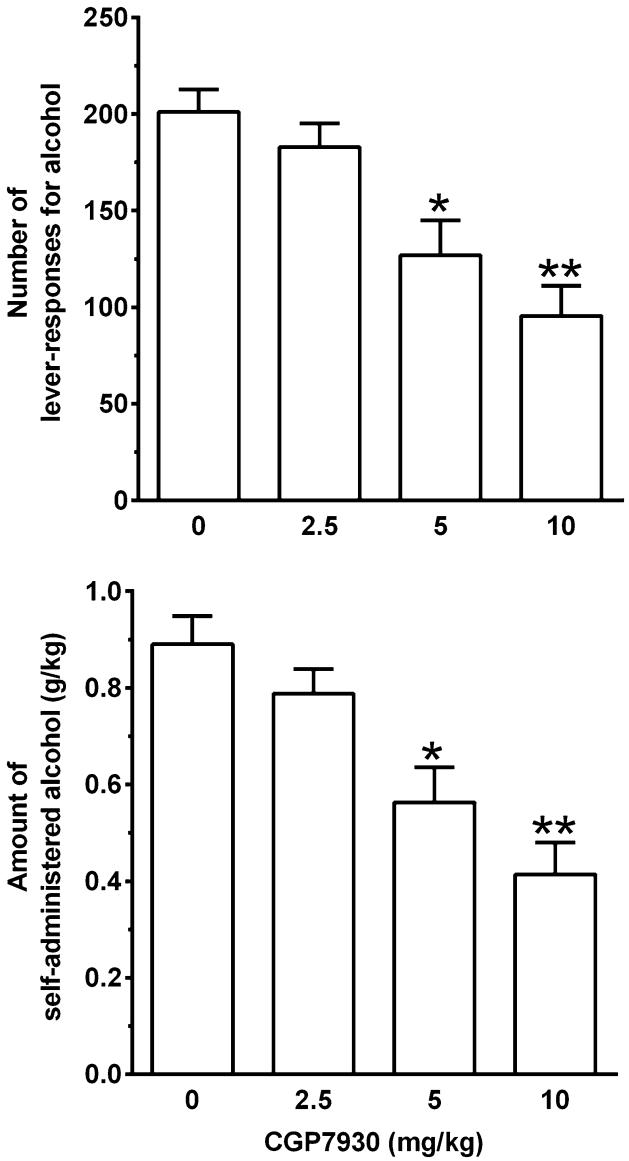

Fig. 2 Reducing effect of acutely administered 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) on operant oral alcohol self-administration in selectively bred sP rats. Male sP rats were initially trained to lever respond for 15% (v/v) alcohol (FR4) and water (FR1) in daily 30-min self-administration sessions in two-lever operant chambers. Once lever responding had stabilized, rats were tested with CGP7930 under the same FR schedule of reinforcement (alcohol: FR4; water: FR1). CGP7930 was suspended in saline with a few drops of Tween 80 and injected intraperitoneally (injection volume: 2 ml/kg) at doses of 0, 2.5, 5, and 10 mg/kg 30 min before the start of the self-administration session. All four doses of CGP7930 were tested in each rat under a Latin-square design. Measured variables were (1) number of lever responses on the alcohol lever; and (2) amount of self-administered alcohol (expressed in g/kg and estimated from the number of earned reinforcers). Each bar is the mean ± standard error of mean of $n = 12$ rats. ANOVA for number of lever responses for alcohol: $F(3,33) = 8.62$, $p < 0.0005$; ANOVA for the amount of self-administered alcohol: $F(3,33) = 9.27$, $p < 0.0005$. *$p < 0.05$ and **$p < 0.005$ in comparison with the vehicle-treated rat group (Tukey's test). *sP* Sardinian alcohol-preferring, *FR* fixed ratio, *ANOVA* analysis of variance

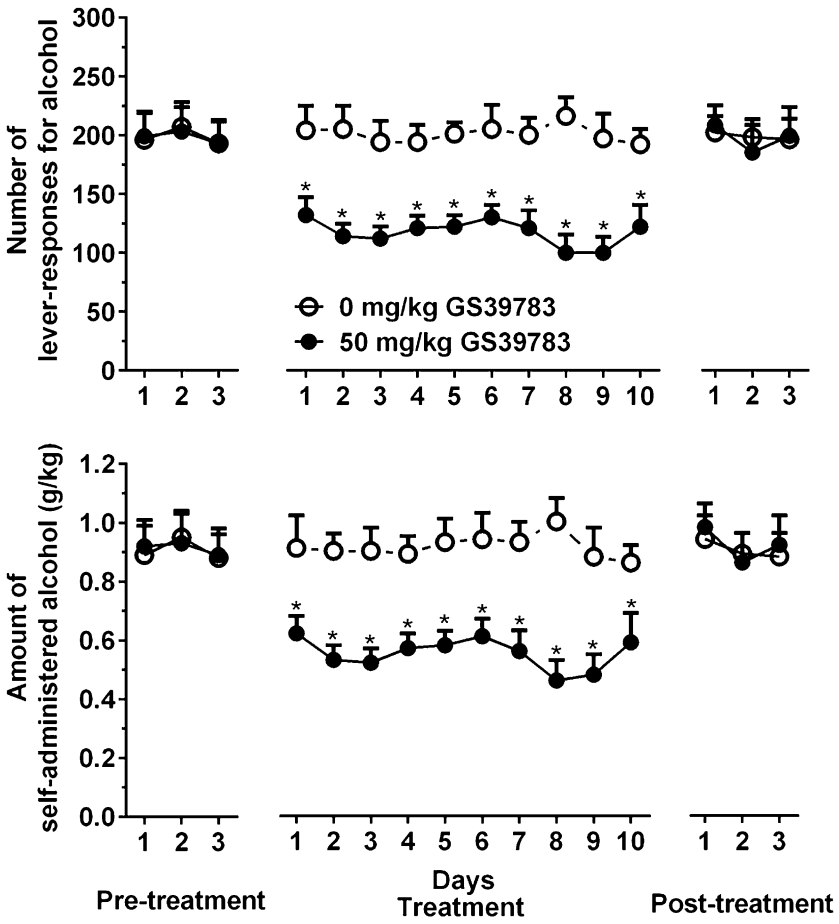

Fig. 3 Lack of tolerance to the reducing effect of repeated (10 consecutive days) treatment with *N*,*N*'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) on operant oral alcohol self-administration in selectively bred sP rats. Each point is the mean ± standard error of mean of $n = 12$ rats. *$p < 0.001$ in comparison to the vehicle-treated rat group (Newman–Keuls test). *sP* Sardinian alcohol-preferring. Adapted from Maccioni et al. [35], with permission from Springer

self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement. Similar data have been collected in the only study using female rats: acute treatment with CMPPE (2.5–10 mg/kg intraperitoneally) effectively reduced lever responding for alcohol (15% v/v) and the amount of self-administered alcohol in female sP rats exposed to the FR5 schedule of reinforcement [42]. The reducing effect of BHF177, *rac*-BHFF, and CMPPE was selective for alcohol as no compound affected self-administration of alternative, non-drug reinforcers (sucrose [0.7–1% w/v in water] or chocolate [5% w/v Nesquik® powder in water] solution). Conversely, selectivity for alcohol of the reducing effect of COR659 was low; because of its peculiar and composite mechanism of action, involving interactions with the cannabinoid CB_1 receptor, treatment with COR659 also suppressed sucrose self-administration in sP rats [39]. However, the reducing effects of COR659 on alcohol (and sucrose) self-administration occurred at doses far lower than those producing hypomotility, as indicated by a therapeutic index higher than 16 [39]. These data suggest that the reducing effect of COR659 on alcohol (and sucrose) self-administration was not influenced by possible sedative effects. When tested, as in the case of *rac*-BHFF [35] and COR659 [43], the reducing effect on alcohol (15% v/v) self-administration in sP rats was maintained after repeated drug treatment.

Acute treatment with ADX71441 (1–30 mg/kg intraperitoneally) dose-dependently suppressed lever responding for alcohol (20% v/v) in male Wistar rats exposed to the FR2 schedule of reinforcement [44]. ADX71441 was more potent in male Wistar rats made dependent on alcohol by long-term exposure to alcohol vapors (and exhibiting stronger reinforcing properties of alcohol) than alcohol-non dependent rats exposed to the FR3 schedule of reinforcement [44]. These results are in line with the previously observed higher sensitivity of P rats to the reducing effect of GS39783 on alcohol self-administration [37]. Treatment with ADX71441 also dose-dependently suppressed lever responding for a saccharin (0.2% w/v in water) solution, with potency and efficacy comparable to that of the suppressing effect on alcohol self-administration [44], suggesting that the pharmacological profile of ADX71441 may differ from that of several other GABA$_B$ PAMs.

3.2 Progressive Ratio Schedule of Reinforcement

Several highly consistent results have also been collected in studies testing $GABA_B$ PAMs on alcohol self-administration under the PR schedule of reinforcement. Specifically, acute treatment with GS39783 (25–100 mg/kg intragastrically, but not lower doses) [37, 39, 45], BHF177 (12.5–50 mg/kg intragastrically) [40], COR659 (2.5–10 mg/kg intraperitoneally) [39], and CMPPE (2.5–10 mg/kg intraperitoneally) [42] reduced lever responding and breakpoint for alcohol in male or female sP rats. Conversely, treatment with neither GS39783 nor BHF177 altered lever responding and breakpoint for alternative, non-drug reinforcers (sucrose [3% *w/v*] solution); treatment with COR659 reduced lever responding and breakpoint for sucrose (likely because of the dual mechanism of action described above) but it did not affect spontaneous locomotor activity at the doses reducing lever responding and breakpoint for alcohol [39]. As seen in the FR study, potency and efficacy of GS39783 in reducing breakpoint for alcohol among sP, P, and AA lines of alcohol-preferring rats paralleled the strength of the motivational properties of alcohol: GS39783 was indeed potent and effective in P rats (i.e. the rat line displaying the highest breakpoint value) and ineffective in AA rats (i.e. the rat line displaying the lowest breakpoint value) [37]. Finally, and in close agreement with the above data, acute treatment with ADX71441 (3 and 10 mg/kg intraperitoneally) suppressed breakpoint for alcohol in male Wistar rats [44].

3.3 Sipper Procedure

GS39783 has been the only $GABA_B$ PAM tested in the so-called sipper procedure of alcohol self-administration. At variance with FR procedures, based on the repetition (within a single session) of multiple, brief sequences of lever responding (or nose poking) and access to limited amounts of alcohol, the sipper procedure is based on completion of a single, elevated RR followed by access to alcohol for a relatively long period of time [46]. The major advantage of the sipper procedure is that of providing a clear separation between the appetitive (seeking) and consummatory phases of alcohol self-administration within a single session. In the study testing GS39783 [47], male sP rats were trained to lever respond under an RR55 for alcohol; achievement of RR55 resulted in a 20-min presentation of a sipper bottle containing 15% (v/v) alcohol. Acute treatment with GS39783 (25–100 mg/kg intragastrically) affected both the seeking and consummatory phases; each GS39783 dose virtually halved the number of rats achieving RR55, mean value of achieved RR, and amount of consumed alcohol (available once RR was achieved) [47].

4 Effects of $GABA_B$ PAMs on Reinstatement of Alcohol Seeking

Several $GABA_B$ PAMs have also been tested for their ability to suppress reinstatement of alcohol-seeking behavior, another validated animal model of loss of control over alcohol, and relapse into heavy drinking in AUD patients [27]. In this procedure, animals are initially trained to perform a given behavior (lever-pressing or nose-poking) to access alcohol. Once established, this behavior is extinguished, being non-reinforced for a given period of time. Alcohol-seeking (but not drinking, as operant responding is still non-reinforced) is then reinstated by (1) environmental stimuli previously associated with alcohol availability; (2) limited availability of alcohol; (3) exposure to stressors; or (4) administration of specific drugs (e.g. nicotine and cannabinoids).

Data collected to date have consistently reported that $GABA_B$ PAMs suppressed reinstatement of alcohol seeking in rats. Specifically, acute treatment with ADX71441 (3 and 10 mg/kg intraperitoneally) suppressed reinstatement of alcohol seeking induced by both alcohol-predictive environmental (olfactory and visual) cues and exposure to a stressful event such as intermittent footshock in male Wistar rats [44]. Notably, even the lowest ADX71441 dose tested (3 mg/kg) fully suppressed reinstatement-associated lever responding. Treatment with CMPPE (10 and 30 mg/kg intraperitoneally) abolished reinstatement of alcohol seeking induced in male Wistar rats by presentation of alcohol-associated cues (Fig. 4) [28]. Similar data were collected in a subsequent study testing acutely administered CMPPE (2.5–10 mg/kg intraperitoneally) on reinstatement of alcohol seeking induced in female sP rats by presentation of a complex of different alcohol-associated (olfactory, visual, and auditory) cues [42]. In the latter study [42], the suppressing effect of CMPPE was evident at doses as low as 5 mg/kg. Finally, acute treatment with COR659 (2.5–10 mg/kg intraperitoneally) resulted in a complete suppression of cue-induced reinstatement of alcohol seeking in male sP rats [43].

5 Effects of $GABA_B$ PAMs on Alcohol-Stimulated Locomotor Activity and Alcohol-Induced Locomotor Sensitization

Acute administration of low to moderate doses of alcohol stimulates locomotor activity in rats and mice [48, 49]. Alcohol-induced hyperlocomotion in rodents, and euphoria in humans, are homologous phenomena as they are mediated by activation of common neural systems [50].

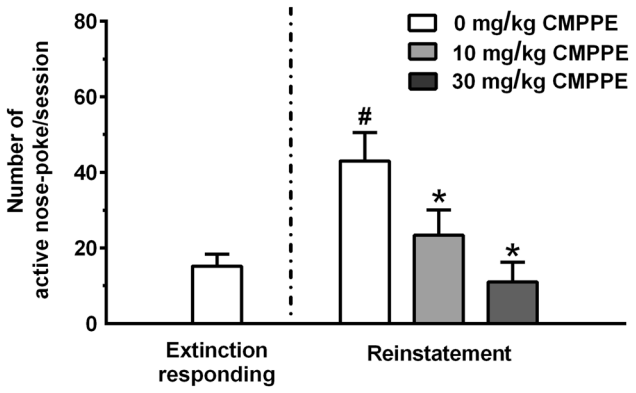

Fig. 4 Suppressing effect of acutely administered 2-{1-[2-(4-chlorophenyl)-5-methylpyrazolo[1,5-a]pyrimidin-7-yl]-2-piperidinyl}ethanol (CMPPE) on cue-induced reinstatement of alcohol seeking in Wistar rats. Each bar is the mean ± standard error of mean of $n = 10$ rats. # $p < 0.05$ in comparison with the last extinction session (Newman–Keuls test); * $p < 0.05$ in comparison with the vehicle-treated rat group in the reinstatement session (Newman–Keuls test). Adapted from Vengeliene et al. [28], with permission from Springer

Pharmacological blockade of alcohol-induced hyperlocomotion in rodents has therefore repeatedly been tested in the search for drugs with suppressing potential on the euphorigenic and rewarding properties of alcohol [48, 49]. When this line of research was applied to GABA$_B$ PAMs, it was found that acute treatment with per se ineffective doses of GS39783 (1–30 mg/kg intraperitoneally) attenuated hyperlocomotor induced by acute administration of 2 g/kg alcohol intraperitoneally in male DBA/2J mice [51]. These data extend to previous observations of GABA$_B$ PAMs on baclofen-induced suppression of alcohol stimulatory effects [52].

Repeated (under specific time schedules) treatment with alcohol results in robust, progressive enhancement of its locomotor-stimulating effect in rats and mice [53, 54]. This phenomenon, named locomotor sensitization, is considered a form of alcohol-induced behavioral plasticity facilitating the development and maintenance of alcohol-seeking and alcohol-taking behavior [55]. The treatment of male DBA/2J mice with GS39783 had differential effects on alcohol-induced locomotor sensitization, depending on whether GS39783 was administered concomitant to or at the end of alcohol treatment [51]. When administered repeatedly (once daily for 11 consecutive days) and concomitant to alcohol (2.5 g/kg intraperitoneally), GS39783 (30 mg/kg intraperitoneally) enhanced alcohol-associated locomotor sensitization [51]. Conversely, when administered acutely, once alcohol-associated locomotor sensitization had already been established, GS39783 (30 mg/kg intraperitoneally) was completely ineffective [51].

6 Effects of GABA$_B$ PAMs on Alcohol-Induced Conditioned Place Preference

Conditioned place preference (CPP) is a pharmacological behavioral technique validated to investigate the rewarding properties of psychoactive drugs [56, 57]. In this procedure, rodents are trained to associate the interoceptive cues produced by a psychoactive drug (e.g. alcohol) with the external (neutral) stimuli of a specific environment, and the absence of those effects (e.g. saline) with the stimuli of a different environment. After a number of conditioning sessions, animals are given a choice between the two environments: if the animal increases the time spent in the drug-paired context, it is inferred that the drug possesses rewarding properties [56, 57].

A recent study investigated the effect of *rac*-BHFF and ORM-27669 on alcohol-induced CPP [32]. Male C57BL/6J mice were initially exposed to eight conditioning sessions, four with saline injection and four with alcohol (0.5 g/kg intraperitoneally) injection; in each 'alcohol' conditioning session, alcohol administration was preceded by treatment with either *rac*-BHFF (30 mg/kg intraperitoneally) or ORM-27669 (100 mg/kg intraperitoneally). The injection-free test session was conducted 48 h after the last conditioning session. Pretreatment with both GABA$_B$ PAMs, administered at doses devoid of any sedative effect, partially attenuated the development of alcohol-induced CPP [32], also suggesting that the rewarding properties of alcohol may be manipulable by GABA$_B$ PAMs.

7 Mechanism of Action of GABA$_B$ PAM Effects on Alcohol-Motivated Behaviors

The results of the few experimental studies conducted to date with the intent of identifying the neural substrates mediating the *anti*-alcohol effects of GABA$_B$ PAMs support the hypothesis of the involvement of the ventral tegmental area (VTA). The VTA is the brain region in which the mesolimbic dopamine reward neurons originate [58]. GABA$_B$ receptors are densely expressed in VTA [59], where they are located both presynaptically (on GABA and glutamate afferent neurons) and postsynaptically (on dopamine efferent neurons), providing a substrate through which GABA$_B$ receptor ligands may control mesolimbic dopamine neurons and likely exert their *anti*-addictive properties [60, 61]. Accordingly, acute intra-VTA microinjection of CGP7930 (5–20 μg) halved lever responding for alcohol (15% v/v) and the self-administered amount of alcohol in male sP rats exposed to the FR4 schedule of reinforcement [62]; the effect of CGP7930 on alcohol self-administration was site-specific and was not associated with any motor

incoordinating effects. Additionally, intra-VTA microinjection of GS39783 (5–20 μg/hemisphere) and BHF177 (10 and 20 μg/hemisphere) decreased alcohol seeking in Long Evans rats exposed to the sipper procedure of alcohol (10% v/v) self-administration [63, 64]. As further evidence in favor of the 'dopamine' hypothesis on the *anti*-alcohol properties of $GABA_B$ PAMs, intra-VTA microinjection of GS39783 (10 and 20 μg/hemisphere) and BHF177 (10 and 20 μg/hemisphere) decreased dopamine release, stimulated by cues anticipating alcohol availability, in the core of nucleus accumbens (i.e. the brain area to which mesolimbic dopamine neurons project their axons) of Long Evans rats [64]. To summarize, $GABA_B$ PAM-induced activation of $GABA_B$ receptors located in the VTA likely hyperpolarizes the mesolimbic dopamine neurons, preventing alcohol-induced stimulation of the latter and dopamine release in the nucleus accumbens, with the ultimate result of decreasing the rewarding and reinforcing properties of alcohol.

The 'dopamine' hypothesis is also supported by data on the reducing effect of GS39783 on alcohol-induced hyperlocomotion [51], a phenomenon known to be mediated by activation of the mesolimbic dopamine system [50]. Data on the potentiating, rather than inhibiting, effect of GS39783 on the induction of locomotor sensitization to alcohol were interpreted as the consequence of a downregulation, produced by repeated treatment with GS39783, of VTA $GABA_B$ receptors, resulting in a reduced inhibition by GS39783 of mesolimbic dopamine transmission [51].

Involvement of the VTA as the main target structure for $GABA_B$ PAMs is further confirmed by the results of a recent electrophysiological, ex vivo study demonstrating that alterations of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptor currents in dopamine neurons of the dorsomedial posterior VTA, induced by acute treatment with 2 g/kg alcohol intraperitoneally in TH-EGFP mice, were suppressed by acute pretreatment with *rac*-BHFF (30 mg/kg intraperitoneally) and ORM-26779 (100 mg/kg intraperitoneally) [32]. These results are of interest as increases in the AMPA/NMDA receptor current ratio represent a process of alcohol-induced synaptic plasticity: treatment with $GABA_B$ PAMs apparently has the potential to prevent the development of some processes of alcohol-induced neuroplasticity [32].

A recent c-Fos immunohistochemical study found that the ability of 3 mg/kg ADX71441 intraperitoneally to suppress stress-induced reinstatement of alcohol seeking in male Wistar rats was associated with inhibition of neuronal activity in a network of brain structures, including dorsal raphe nucleus, nucleus accumbens shell, and medial prefrontal cortex [44].

An additional hypothesis (although not yet experimentally tested) may come from recent data indicating reduced levels of the GABA transporter GAT3 and, subsequently, high concentrations of extracellular GABA in the amygdala of alcohol-dependent rats [65]. It has been proposed that baclofen-induced activation of amygdalar presynaptic $GABA_B$ receptors would lower extracellular GABA levels, reducing the enhanced tonic inhibition of amygdala and, in turn, excessive alcohol drinking [66]. Extension of this hypothesis to $GABA_B$ PAMs merits experimental evaluation.

8 Conclusions

Several important conclusions, most with therapeutic potential, can be drawn from an analysis of the current preclinical literature on the *anti*-alcohol effects of $GABA_B$ PAMs. First, all $GABA_B$ PAMs tested to date have invariably been found to reduce excessive alcohol drinking, binge-like drinking, relapse-like drinking, and relapse-like alcohol seeking, as well as alcohol reinforcing, motivational, stimulating, and rewarding properties in rats. The predictive validity of the experimental models used in these studies confers remarkable translational value to the collected results.

At a preclinical level, $GABA_B$ PAMs retain the ability of baclofen to affect several alcohol-motivated behaviors [52]. These data confirm that the $GABA_B$ receptor is a major part of the neural substrate controlling alcohol drinking and mediating the reinforcing, motivational, stimulating, and rewarding properties of alcohol. These data also suggest that positive modulation of the allosteric binding site(s) is an effective mechanism, in addition to activation of the orthosteric binding site, to potentiate $GABA_B$ receptor-mediated neurotransmission and inhibit alcohol-motivated behaviors.

Comparison of data on baclofen and $GABA_B$ PAMs provides interesting food for thought. First, in the majority of studies, the magnitude of the reducing effect of $GABA_B$ PAMs on alcohol-motivated behaviors did not exceed 40–50%, being lower than that observed in several baclofen studies (in which treatment with baclofen resulted in a virtually complete suppression of the recorded behavior [52]). A possible explanation may reside in the use-dependent mechanism of action of $GABA_B$ PAMs: $GABA_B$ PAMs potentiate endogenously released GABA, being ineffective, or limitedly effective, in activating $GABA_B$ receptors per se (as orthosteric agonists do); therefore, their action depends on synaptic concentrations of GABA, and the halving of a given effect, rather than its suppression, is the likely consequence of the maximal behavioral outcome in terms of potentiation of extracellular GABA. Second, the reducing effects of $GABA_B$ PAMs are often selective as they are not associated with any effect on other non-drug consummatory behaviors, and are always specific as they occur at doses largely lower than those producing motor incoordination and sedation. The high therapeutic index, i.e. the

large separation between the 'desired' *anti*-addictive effects and the 'unwanted' sedative effects, appears to be a major advantage of $GABA_B$ PAMs in comparison to baclofen; it is predictive of a more favorable safety profile in clinics. In this perspective, the two baclofen + $GABA_B$ PAM combination studies [34, 35] have yielded interesting results: $GABA_B$ PAM pretreatment potentiated baclofen effect on alcohol self-administration but not on motor incoordination; if transposed to humans, these results would predict suppression of alcohol craving and consumption with doses of baclofen lower than those producing the well-known side effects.

From a translational point of view, three additional aspects seem to be important. First, the results of the studies testing repeated treatment with $GABA_B$ PAMs, indicating retained efficacy in reducing alcohol drinking [21, 22] and self-administration [35, 43], are of interest as they predict unaltered efficacy after prolonged treatment. Second, the *anti*-alcohol effects of the majority of $GABA_B$ PAMs tested to date have been observed after intragastric administration, suggesting that they may also be effective after oral administration (the preferred and most convenient route of drug administration in clinics). Finally, the experimental results indicating that $GABA_B$ PAMs are more potent and effective in rats displaying the strongest reinforcing properties of alcohol [37, 38, 44] suggest that $GABA_B$ PAMs might display differential efficacy among specific subpopulations, or typologies, of AUD patients; similar to baclofen [67], $GABA_B$ PAMs might be more effective in patients with severe AUD.

We are aware of at least two $GABA_B$ PAMs (ADX71441 [68] and ODM-106 [69]) entering the initial phases of clinical testing, moving closer to the possibility of testing whether the series of promising data collected in rats may be translated to individuals with AUD. In the meantime, preclinical research should address additional urgent questions. These questions include, among others, whether $GABA_B$ PAMs (1) substitute for the discriminative stimulus effects of alcohol (i.e. the animal correlate of the human subjective feelings perceived after alcohol ingestion), providing hints on their possible abuse potential; (2) potentiate the intoxicating effects of alcohol, predicting, as in the case of baclofen [10], possible limitations or need for particular caution in the treatment of AUD patients; (3) alter alcohol metabolism or taste; (4) are also effective in female rats (as only one study conducted to date has used only female rats [42]); and (5) also exert anxiolytic effects [70] in rodent models of anxiety associated with alcohol withdrawal syndrome, suggesting another potential beneficial effect in AUD therapy.

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Compliance with Ethical Standards

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Conflict of interest Paola Maccioni and Giancarlo Colombo declare that they have no conflicts of interest relevant to the contents of this article.

Research involving animals Data depicted in Fig. 1 were collected in an experiment that fully complied with European Directive no. 2010/63/EU and subsequent Italian Legislative Decree no. 26, 4 March 2014, on the 'Protection of Animals Used for Scientific Purposes'.

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