Chapter 12
Recent Advances on $GABA_B$ Receptor Positive Allosteric Modulators as Potential Pharmacotherapies for Alcohol Use Disorder

Paola Maccioni and Giancarlo Colombo

Abstract To date, ten different positive allosteric modulators (PAMs) of the $GABA_B$ receptor have been tested in laboratory rodents for their suppressing effects on a series of alcohol-related behaviors. This list includes CGP7930, GS39783, BHF177, *rac*-BHFF, ADX71441, CMPPE, COR659, ASP8062, KK-92A, and ORM-27669. The large body of collected data indicates a remarkable similarity in $GABA_B$-PAM effects: all compounds reduced, or even suppressed, excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and conditioned place preference in mice and rats exposed to validated experimental procedures that measure the reinforcing, motivational, rewarding, and stimulatory effects of alcohol. Aspects with translational value include large separation between the pharmacological and toxicological effects, limited development of tolerance, comparable efficacy in both sexes, and efficacy retained after per os administration. The current clinical testing of ASP8062 will prove to what extent these data may translate to patients with alcohol use disorder.

Keywords $GABA_B$ receptor · Positive allosteric modulators · Alcohol-related behaviors · Alcohol drinking · Alcohol self-administration · Fendiline · Mice · Rats

12.1 Introduction

Research on the ability of positive allosteric modulators (PAMs) of the γ-aminobutyric acid (GABA) type-B ($GABA_B$) receptor to suppress multiple alcohol-related behaviors in laboratory rodents stemmed from the several lines of experimental and

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clinical evidence demonstrating that the prototypic $GABA_B$ receptor agonist, baclofen, suppressed alcohol seeking and drinking in mice, rats, and baboons (see Colombo and Gessa 2018; Holtyn and Weerts 2022), as well as alcohol consumption, craving for alcohol, and signs of alcohol withdrawal syndrome in patients affected by alcohol use disorder (AUD) (see Agabio et al. 2023; see Chap. 6 of this volume for an extensive review on baclofen as a pharmacotherapy for AUD).

The discovery of modulatory binding site(s) in the structure of the $GABA_B$ receptor, together with the synthesis of the first $GABA_B$ PAMs, led several labs to investigate whether the suppressing effects of baclofen on a variety of alcohol-motivated behaviors (each modeling specific aspects of human AUD) in mice and rats extended to $GABA_B$ PAMs. Data generated to date unanimously indicate that acute or repeated treatment with all $GABA_B$ PAMs tested reduced, and in most instances even suppressed, excessive alcohol drinking, relapse- and binge-like drinking, operant oral alcohol self-administration, reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and conditioned place preference (CPP) in mice and rats.

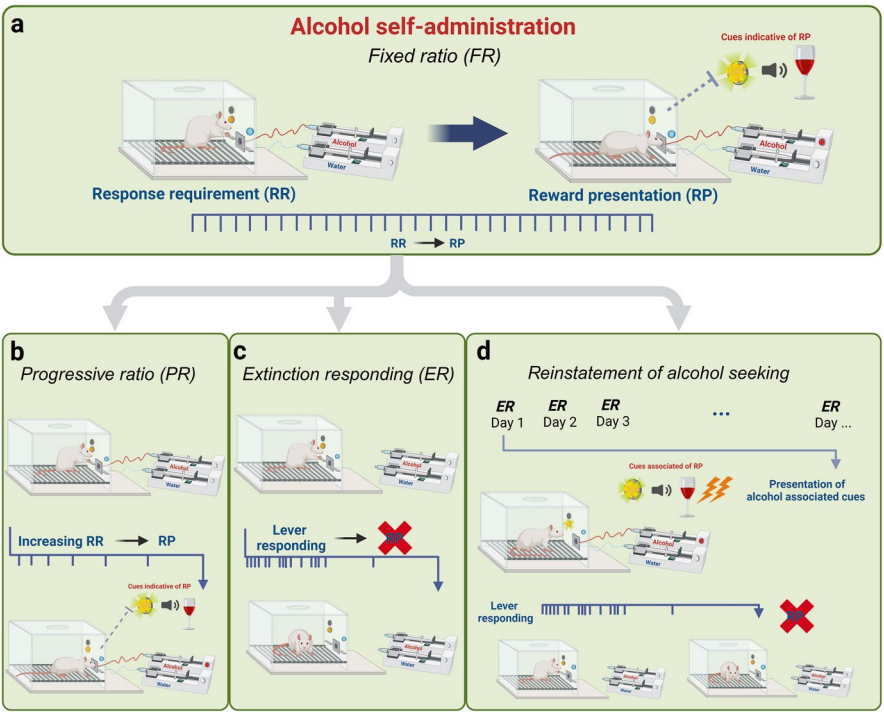
Further to their aim of reproducing baclofen effects on alcohol-related behaviors, the majority of these studies attentively investigated the breadth of separation between the pharmacological effects of $GABA_B$ PAMs and their toxicological effects. Based on their use-dependent mechanism of action (i.e., potentiation of GABA- or baclofen-induced receptor activation with no intrinsic activity), $GABA_B$ PAMs are indeed expected to display a larger separation—compared to baclofen—between the "desired," or expected, pharmacological effects and the "unwanted," off-target, toxicological effects (see Urwyler 2016; see also Chap. 2 of this volume), thus ideally overcoming a relevant shortcoming of baclofen use.

This paper devotes a separate paragraph to each distinct $GABA_B$ PAM, providing a brief description of its effects on alcohol-related behaviors in validated rodent models of AUD. This list of $GABA_B$ PAMs includes CGP7930, GS39783, BHF177, *rac*-BHFF, ADX71441, CMPPE, COR659, ASP8062, KK-92A, and ORM-27669 (see Chap. 8 of this volume for details on their synthesis and chemical structure).

12.2 Overview of the Suppressing Effects of $GABA_B$ PAMs on Alcohol-Related Behaviors

12.2.1 CGP7930

CGP7930 is the first $GABA_B$ PAM made available for in vivo studies (Urwyler et al. 2001; Carai et al. 2004). A few years after CGP7930 synthesis, the research team headed by Andrew J. Lawrence at the University of Melbourne, Australia, reported the results of a study investigating the effect of treatment with CGP7930 on operant oral alcohol self-administration (Box 12.1a) in selectively bred Indiana alcohol-preferring (P) rats (Liang et al. 2006). This was the first paper to document the reducing effect of a $GABA_B$ PAM on an alcohol-related behavior. Specifically, acute



Box 12.1 This picture depicts an operant chamber (also called Skinner box) for rodents. It features a conventional setup comprised of two retractable response levers (one dispensing the alcohol solution and one plain water), one dual liquid receptacle, two syringe pumps located outside the chamber, two stimulus lights (associated to each lever), and one tone generator. This chamber is used for a variety of operant procedures involving alcohol-seeking and alcohol-taking behaviors. Panel (**a**) oral alcohol self-administration under the fixed ratio (FR) schedule of reinforcement, in which each single drop of the alcohol solution (reinforcer) is made available once the animal has pressed the lever for an established, or indeed fixed, number of times [response requirement (RR): one to five in most studies]; animals can freely repeat this sequence of events (lever-responding, consuming alcohol, lever-responding, consuming alcohol, and so on) throughout the duration of the self-administration session; FR schedules of reinforcement provide a measure of the reinforcing properties of alcohol (see Markou et al. 1993). Panel (**b**) oral alcohol self-administration under the progressive ratio (PR) schedule of reinforcement, in which the number of lever-responses required to access each single alcohol reinforcer increases progressively over the self-administration session and up to breakpoint, i.e., the last completed ratio before lever-responding for alcohol is abandoned; breakpoint for alcohol provides a measure of the motivational properties of alcohol (see Markou et al. 1993). Panel (**c**) extinction responding (ER), in which animals are initially trained to lever-respond for alcohol under standard FR schedules of reinforcement and then exposed to an ER session during which lever-responding for alcohol is never reinforced, irrespective of the number of lever-responses; ER (i.e., the number of lever-responses performed during the ER session) provides an additional measure of the motivational properties of alcohol (Samson et al. 2003). Panel (**d**) reinstatement of alcohol-seeking behavior, in which lever-responding for alcohol is initially established under standard FR schedules of reinforcement, then extinguished over a series of consecutive ER sessions, and finally resumed—or indeed reinstated—by (i) non-contingent presentation of visual, olfactory, auditory, and/or gustatory cues previously associated to alcohol availability; (ii) exposure to stressful events (in most instances, a mild footshock delivered by the metal grid floor); or (iii) injections of given, triggering drugs; lever-responding during the reinstatement session models human loss of control over alcohol (see Martin-Fardon and Weiss 2013). (Created with BioRender.com)

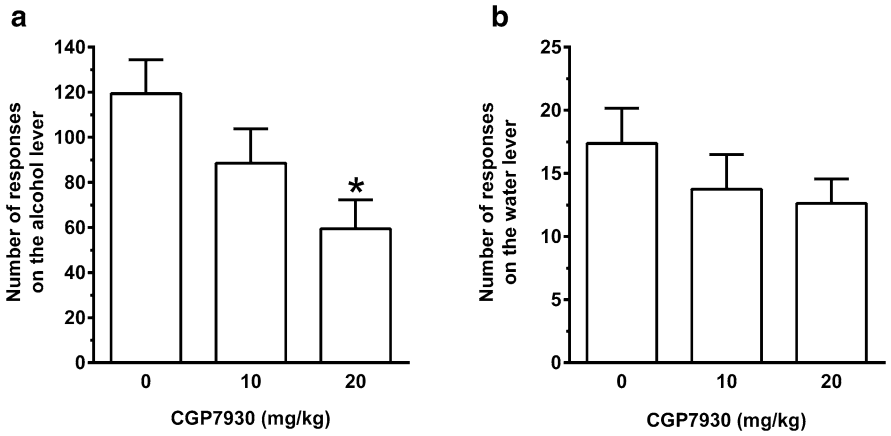


Fig. 12.1 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the $GABA_B$ receptor, CGP7930, on number of lever-responses for alcohol (panel **a**) and water (panel **b**) in male Indiana alcohol-preferring P rats. Rats were initially trained to lever-respond for oral alcohol (10% v/v) and water under the fixed ratio (FR) 3 schedule of reinforcement in daily 40-min self-administration sessions. Once lever-responding had stabilized, rats were tested with CGP7930 under the same FR schedule of reinforcement. CGP7930 was administered 120 min before of the self-administration session. Each bar is the mean ± SEM of $n = 8$ rats. *: $P < 0.05$ in comparison to the rat group treated with 0 mg/kg CGP7930 (Student-Newman-Keuls test). (Adapted from Liang et al. (2006) with permission from Elsevier)

and intraperitoneal (i.p.) treatment with CGP7930 (10 and 20 mg/kg) virtually halved the number of lever-responses of alcohol in male P rats trained to lever-respond for alcohol (10% v/v) under the fixed ratio (FR) 3 (FR3) schedule of reinforcement (Fig. 12.1a) (Liang et al. 2006). CGP7930 effect was selective for alcohol, as responding for a concurrently available, water-dispensing lever was unaffected by CGP7930 treatment (Fig. 12.1b), but not fully specific, as acute treatment with 20 mg/kg CGP7930 also reduced spontaneous locomotor activity (a reliable index of behavioral toxicity) (Liang et al. 2006). In terms of magnitude, the reducing effect of CGP7930 on alcohol self-administration was comparable to that produced by the acute treatment of 3 mg/kg baclofen (i.p.) (Liang et al. 2006).

Similar data were collected in a subsequent study using Sardinian alcohol-preferring (sP) rats, selectively bred—in the same way as P rats—for high alcohol preference and consumption. Acute treatment with 2.5–10 mg/kg CGP7930 (i.p.) reduced, in a dose-related fashion, number of lever-responses for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule reinforcement for alcohol (15% v/v) (Maccioni and Colombo 2019).

CGP7930 and its reducing effect on alcohol self-administration were also used in a mechanistic study aimed at investigating the neural substrate underlying the ability of $GABA_B$ receptor ligands to affect alcohol-related behaviors (Maccioni et al. 2018). This study focused on the ventral tegmental area (VTA), i.e., the area of the brain "reward" mesolimbic system in which dopamine neurons originate.

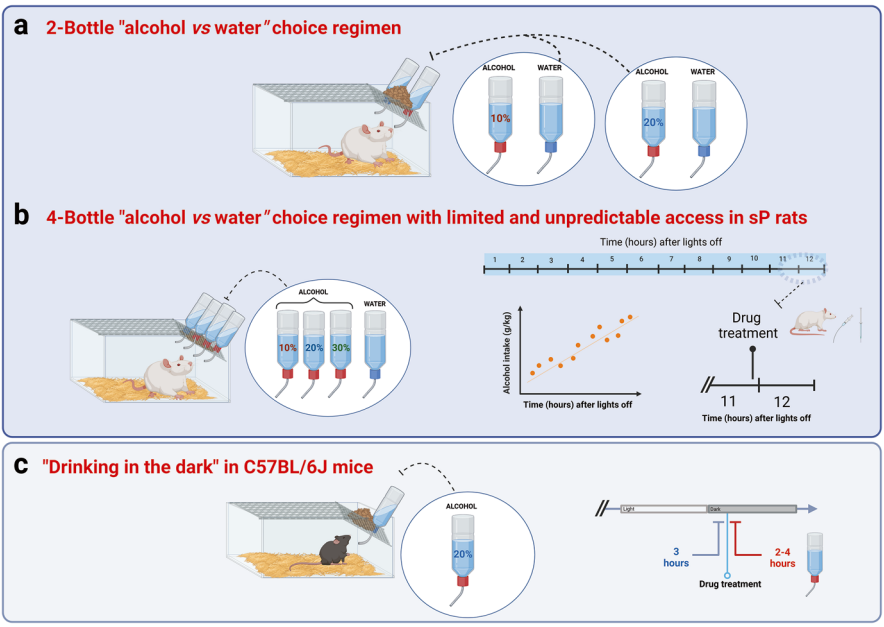
$GABA_B$ receptors are densely located in the VTA, both presynaptically on GABA and glutamate afferent neurons and postsynaptically on dopamine efferent neurons (see Castelli and Gessa 2016). It has been hypothesized that their pharmacological activation inhibits alcohol-induced stimulation of mesolimbic dopamine neurons and therefore the reinforcing, stimulating, and rewarding properties of alcohol (see Frankowska et al. 2016; Lalive and Lüscher 2016).

Specifically, male sP rats originally trained to lever-respond for alcohol (15% v/v) under the FR4 schedule of reinforcement were first implanted with a cannula aimed at the left hemisphere of the VTA and then exposed to a series of self-administration sessions occurring immediately after the intra-VTA microinjection of 5–20 μg CGP7930 (Maccioni et al. 2018). Treatment with CGP7930 was highly effective, as it halved both number of lever-responses for alcohol and amount of self-administered alcohol, with no effect on motor performance (measured by exposing the rats to the inverted screen test immediately before the self-administration session) (Maccioni et al. 2018). CGP7930 effect was also site-specific, as a 20-μg dose of CGP7930 was completely ineffective when infused directly into the deep mesencephalic nucleus (Maccioni et al. 2018).

An early study reported the ability of CGP7930 to decrease alcohol drinking in male sP rats exposed to the conventional, homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Orrù et al. 2005). Specifically, repeated (once daily for 5 consecutive days) and intragastric (i.g.) treatment with 25–100 mg/kg CGP7930 (i) prevented acquisition of alcohol drinking behavior in rats that had never been previously exposed to alcohol before the start of CGP7930 treatment and (ii) reduced daily alcohol intake in rats displaying a consolidated alcohol drinking behavior at the start of CGP7930 treatment (Orrù et al. 2005). Compensatory increases in daily water intake proved the selectivity for alcohol intake of CGP7930 effect (Orrù et al. 2005).

More recent studies suggested that CGP7930 may target binding sites other than the positive allosteric modulatory binding site of the $GABA_B$ receptor. CGP7930 has indeed been reported to activate the $GABA_B$ receptor also in the absence of GABA, thus displaying an ago-allosteric profile at the $GABA_B$ receptor (see Mugnaini and Corelli 2016). Additionally, CGP7930 exerted allosteric modulation (positive and negative, depending upon the concentration) of the GABA type-A ($GABA_A$) receptor (Hannan et al. 2023); these effects occurred at CGP7930 concentrations overlapping those found to modulate the $GABA_B$ receptor, leading to hypothesize a virtually equivalent contribution of ionotropic $GABA_B$ and metabotropic $GABA_B$ receptors to the neuropharmacological actions of CGP7930 (Hannan et al. 2023).

This reduced selectivity of CGP7930 apparently limits its usefulness in studies aimed at assessing the contribution of the positive allosteric modulatory binding site of the $GABA_B$ receptor to a given behavior, including—relative to the scopes of the present chapter—alcohol drinking and self-administration. It may however explain some unexpected results, including the development of partial tolerance to the reducing effect of CGP7930 on alcohol drinking (Orrù et al. 2005); the agonistic activity of CGP7930 at the $GABA_B$ receptor might indeed result in receptor



Box 12.2 This picture depicts different procedures of alcohol drinking in rodents. Specifically, panel (**a**) depicts the conventional two-bottle "alcohol vs. water" choice regimen in which animals have free access to an alcohol solution (10% or 20% v/v in most studies) and tap water. Access may be unlimited (24 h/day), limited (drinking sessions of limited periods of time), or intermittent (usually on alternate days); panel (**b**) depicts a procedure of binge-like drinking recently developed in Sardinian alcohol-preferring (sP) rats and based on daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) (Colombo et al. 2014); panel (**c**) depicts the setup used for the so-called drinking in the dark (DID), the binge-like procedure in which C57BL/6 J mice are exposed to daily drinking sessions of 2–4 h, occurring during the dark phase of the daily light/dark cycle, and access to a single alcohol (20% v/v) bottle (see Thiele and Navarro 2014). (Created with BioRender.com)

desensitization and development of tolerance, a sequence of events not theoretically attributable to a "pure" PAM (see Urwyler 2016).

12.2.2 GS39783

GS39783 is likely the most used $GABA_B$ PAM, at least in the alcohol research field. Notably, the results of all studies focusing on GS39783 were consonant in indicating its ability to effectively reduce different alcohol-related behaviors in mice and rats. Specifically, acute and i.g. treatment with doses of GS39783 ranging from 5 to 100 mg/kg reduced, in a dose-related fashion, lever-responding for alcohol and amount of self-administered alcohol in male, alcohol-preferring P, sP, and Alko Alcohol (AA) rats exposed to the FR4 schedule reinforcement for alcohol (15% v/v)

(Maccioni et al. 2007, 2012, 2017; Lorrai et al. 2019) (Box 12.1a). When compared under identical experimental conditions, treatment with GS39783 came out to be more potent and effective in rats (P line) displaying the most robust lever-responding behavior and self-administering the largest amount of alcohol (Maccioni et al. 2012).

The reducing effect of GS39783 on alcohol self-administration was (i) reproduced, with minimal sex differences, in female sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) and treated with 25–100 mg/kg GS39783 (i.g.) (Lorrai et al. 2019) and (ii) maintained unaltered throughout 10 days of repeated treatment (50 mg/kg, i.g.) in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015).

In the above studies, no dose of GS39783 affected self-administration of alternative, nondrug reinforcers, including regular food pellets and sucrose solutions (Maccioni et al. 2007, 2012, 2015, 2017), indicative of a complete selectivity of the reducing effect of GS39783 for alcohol reinforcement.

GS39783 was also tested in a slightly different alcohol self-administration procedure, the daily sessions of which comprised an initial phase of lever-responding [up to achievement of a relatively high response requirement (RR); seeking behavior] and a subsequent, relatively long period of free access to alcohol (consummatory behavior). More specifically, male sP rats were initially trained to lever-respond under an RR55 for alcohol; achievement of RR55 gave access to alcohol (15% v/v) for 20 consecutive min. Acute treatment with GS39783 (25–100 mg/kg, i.g.) affected both "seeking" and "consummatory" components, as it effectively reduced number of rats achieving RR55 and amount of alcohol consumed by the rats that achieved RR55 (Maccioni et al. 2010a).

GS39783 was also profitably used, together with baclofen, in a "combination" experiment. Combination of a per se ineffective dose of GS39783 (5 mg/kg, i.g.) with a per se ineffective dose of baclofen (1 mg/kg, i.p.) resulted in a marked reduction in lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015). These results represent a clear example of how the facilitatory ability of $GABA_B$ PAMs on $GABA_B$ receptor functionality can be reproduced in vivo. The lack of any effect of the "GS39783 + baclofen" combination on sucrose self-administration indicated that the potentiating effect of the drug association was selective for alcohol reinforcement and did not extend to sedative or motor-incoordinating, adverse-like effects (Maccioni et al. 2015).

Reduction of the motivational properties of alcohol is another consolidated aspect of the pharmacological profile of GS39783. Studies employing the progressive ratio (PR) schedule of reinforcement, in which the number of lever-responses needed to access alcohol increases progressively up to breakpoint (Box 12.1b), demonstrated indeed that acute treatment with GS39783 (25–100 mg/kg, i.g.) reduced the number of lever-responses and breakpoint for alcohol in male P, sP, and AA rats firstly trained under the FR4 schedule of reinforcement for alcohol (15% v/v) and then exposed to a conventional PR schedule of reinforcement (Maccioni et al. 2008, 2012). Similar to the results of the FR experiment, the reducing effect of GS39783 on the motivational properties of alcohol ranged from virtually no effect

in the rat line (AA rats) displaying a relatively low breakpoint value to a dose-unrelated suppression in the rat line (P rats) displaying a high breakpoint value (Maccioni et al. 2012). Selectivity of GS39783 effect for alcohol breakpoint was demonstrated by the lack of any drug effect of breakpoint for a sucrose solution (Maccioni et al. 2008).

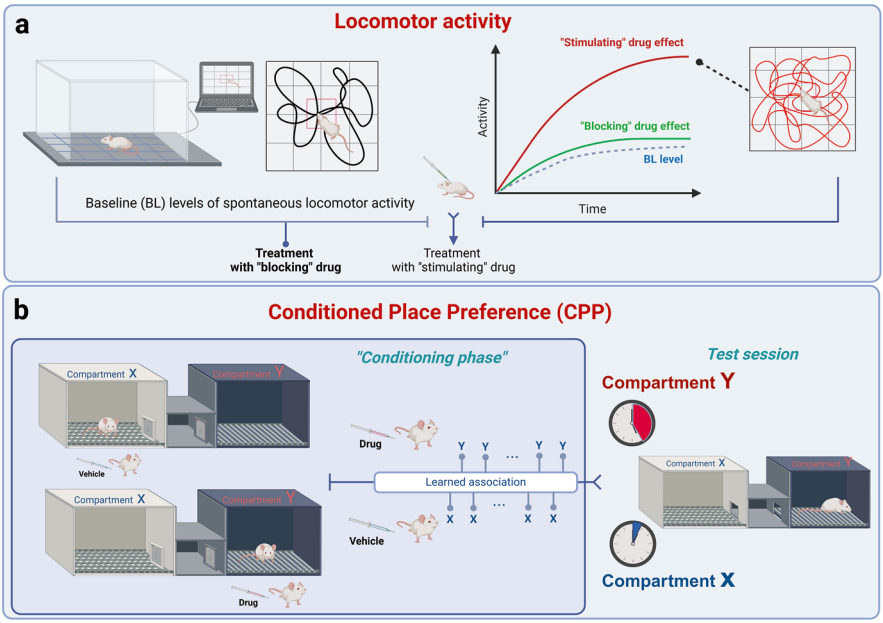
Three additional studies investigated the effect of treatment with GS39783 on different aspects of excessive alcohol drinking in rodents. The first study demonstrated that repeated (once a day for 5 consecutive days) treatment with relatively low doses of GS39783 (6.25–25 mg/kg, i.g.) prevented acquisition of alcohol drinking behavior in male sP rats exposed to the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Orrù et al. 2005); higher doses of GS39783 (50 and 100 mg/kg, i.g.) were however needed to reduce daily alcohol drinking once it was already consolidated (Orrù et al. 2005). In both experiments, reduction in daily alcohol intake was associated to a compensatory increase in daily water intake, indicative of the selectivity of GS39783 effect on alcohol drinking (Orrù et al. 2005).

The other two studies employed validated models of binge drinking. In detail, acute treatment with 30 mg/kg GS39783 (i.p.) suppressed alcohol intake in C57BL/6 J mice exposed to the "drinking in the dark" (DID) procedure (Linsenbardt and Boehm 2014), an experimental condition made of daily 2–4-h drinking sessions occurring during the dark phase of the daily light/dark cycle and under which C57BL/6 J mice consume intoxicating amounts of alcohol (Box 12.2c) (see Thiele and Navarro 2014). GS39783 treatment did not alter spontaneous, homecage locomotor activity (Linsenbardt and Boehm 2014). In the second study, acute treatment with 25–100 mg/kg GS39783 (i.g.) suppressed alcohol intake in sP rats exposed to daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) (Colombo et al. 2015); when exposed to this peculiar drinking regimen, and when the drinking session occurs at the last hours of the dark phase, sP rats consume alcohol up to intoxication (Box 12.2b) (Colombo et al. 2014). Water intake was not affected by treatment with GS39783 (Colombo et al. 2015).

Finally, acute administration of per se ineffective doses of GS39783 (1–30 mg/kg, i.p.) attenuated hyperlocomotion induced by acute alcohol treatment (2 g/kg, i.p.) in male DBA/2 J mice (Box 12.3a) (Kruse et al. 2012). These data are of relevance as they constitute the first line of experimental evidence on the ability of a $GABA_B$ PAM to block the stimulating, euphorigenic-like properties of alcohol.

12.2.3 BHF177

BHF177 is a GS39783 derivative with reduced genotoxicity (see Chap. 8 of this volume). Its pharmacological effects—related to alcohol-motivated behaviors—were highly consonant with those of the parent compound. Indeed, acute treatment with BHF177 (12.5–50 mg/kg, i.g.) reduced, in a dose-related fashion,



Box 12.3 Panel (**a**) of this picture depicts an open field arena used to assess locomotor activity in rodents; distance covered (index of horizontal activity) and the number of rearings on the hindlegs (index of vertical activity) are measured by means of infrared beams positioned around the box and recorded by computerized tracking systems. Panel (**b**) depicts a standard equipment for conditioned place preference (CPP), comprised of two contextually different compartments; rodents are initially trained to associate alcohol-induced interoceptive cues with the distinguishable, visual, and tactile stimuli of one compartment and absence of those effects (e.g., after saline injection) with the stimuli of the other comportment; after a proper number of conditioning sessions, animals are given a choice between the two compartment: preference for the alcohol-paired compartment infers that alcohol has exerted rewarding properties (see Davis 2017). (Created with BioRender.com)

lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2009). Acute treatment with 50 mg/kg BHF177 (i.g.), but not lower doses, reduced breakpoint for alcohol in male sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR4 schedule of reinforcement and then tested with BHF177 under a PR schedule of reinforcement (Box 12.1a) (Maccioni et al. 2009). Acute treatment with BHF177 was totally devoid of any effect on lever-responding for a sucrose solution, amount of self-administered sucrose solution, and breakpoint for the sucrose solution in male sP rats, indicative of the selectivity of BHF177 effect for alcohol reinforcing and motivational properties (Maccioni et al. 2009).

12.2.4 rac-BHFF

The first study testing *rac*-BHFF in the alcohol research field found that acute treatment with 50–200 mg/kg *rac*-BHFF (i.g.) dose-dependently suppressed lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2010b). The effect of *rac*-BHFF on alcohol self-administration was selective and specific, as only treatment with 200 mg/kg *rac*-BHFF reduced lever-responding for a sucrose solution and no dose of *rac*-BHFF altered spontaneous locomotor activity in male sP rats (Maccioni et al. 2010b). Notably, and as a likely consequence of the long-lasting half-life of *rac*-BHFF (Malherbe et al. 2008), the reducing effect of *rac*-BHFF on alcohol self-administration was still detectable 24 h after treatment (Maccioni et al. 2010b).

When *rac*-BHFF (50 mg/kg, i.g.) was given repeatedly (once daily for 5 consecutive days) to male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v), magnitude of its reducing effect on lever-responding for alcohol and amount of self-administered alcohol increased progressively over time, as the likely result of tissue accumulation and long-lasting bioavailability (Maccioni et al. 2015). Accordingly, alcohol self-administration was still reduced over the first 2 days of the posttreatment phase (Maccioni et al. 2015).

Similar to GS39783 (see above), acute treatment with a per se ineffective dose of *rac*-BHFF (5 mg/kg. i.g.) interacted synergistically with a per se ineffective dose of baclofen (1 mg/kg, i.p.), unraveling a marked reduction in lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Maccioni et al. 2015). Conversely, the drug combination was totally ineffective—similarly to each drug when given alone—on sucrose self-administration (Maccioni et al. 2015).

Treatment with *rac*-BHFF yielded interesting results also when tested on excessive alcohol drinking.

Repeated (once a day for 7 consecutive days) treatment with 50–200 mg/kg *rac*-BHFF suppressed, in a dose-related fashion, daily alcohol intake in male sP rats offered alcohol under the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Loi et al. 2013). Reduction in daily alcohol intake was fully compensated by an increase in daily water intake, so that daily total fluid intake was virtually unaltered by treatment with *rac*-BHFF (Loi et al. 2013). Additionally, acute treatment with 30 mg/kg *rac*-BHFF (i.p.) virtually halved binge-like, alcohol intake in C57BL/6 J mice exposed to the DID procedure (Box 12.2c) (de Miguel et al. 2019). Specificity was proven by the lack of any effect of 30 mg/kg *rac*-BHFF (i.p.) on spontaneous locomotor activity in a companion set of mice (de Miguel et al. 2019).

rac-BHFF was also found to diminish the rewarding properties of alcohol, measured in male C57BL/6 J mice exposed to a CPP test (Box 12.3b) (de Miguel et al. 2019). Specifically, injection of 30 mg/kg *rac*-BHFF prior to alcohol injection (0.5 g/kg, i.p.) in each of the four "alcohol" conditioning sessions prevented the development of alcohol-induced CPP in the final, drug-free test session (de Miguel et al. 2019).

12.2.5 ADX71441

Acute administration of ADX71441 (1–30 mg/kg, i.p.) resulted in a dose-dependent, virtually complete suppression of lever-responding for alcohol and number of earned alcohol reinforcers in male Wistar rats trained to lever-respond for alcohol (20% v/v) under the FR2 schedule of reinforcement (Box 12.1a) (Augier et al. 2017). Only the highest dose tested (30 mg/kg) affected spontaneous locomotor activity, thus limiting its specificity (Augier et al. 2017).

This initial set of data was corroborated by a series of additional, relevant results. Acute treatment with ADX71441 (1 and 3 mg/kg, i.p.) was more potent and effective in reducing alcohol self-administration under an FR3 schedule of reinforcement for alcohol (20% v/v) in male Wistar rats made alcohol-dependent by prolonged exposure to alcohol vapors than control, air-exposed rats (Augier et al. 2017); specifically, treatment with 1 mg/kg ADX71441 halved lever-responding for alcohol in alcohol-dependent rats while being ineffective in alcohol-nondependent rats (Augier et al. 2017). Additionally, acute treatment with relatively low doses of ADX71441 (3 and 10 mg/kg, i.p.) suppressed breakpoint for alcohol in male Wistar rats exposed to a PR schedule of reinforcement (Box 12.1b) (Augier et al. 2017).

At variance with data collected with most of the other $GABA_B$ PAMs, treatment with ADX71441 (1–10 mg/kg, i.p.) also suppressed self-administration of a saccharin solution in male Wistar rats exposed to the FR2 schedule of reinforcement (Augier et al. 2017), suggesting the relatively unique ability of ADX71441 to affect a broad range of reinforcers.

ADX71441 was the first $GABA_B$ PAM tested on reinstatement of alcohol-seeking behavior, a validated and widely used experimental model of human loss of control over alcohol and relapse into heavy drinking (Box 12.1d) (see Martin-Fardon and Weiss 2013). In this study, male Wistar rats were initially trained to lever-respond for alcohol (20% v/v) under the FR2 schedule of reinforcement; once established, lever-responding was first extinguished (lever-responses were not reinforced) and then reinstated by intermittent (i) exposure to a conventional stressor such as mild footshock or (ii) presentation of environmental cues previously associated with alcohol availability (Augier et al. 2017). Under both experimental circumstances, acute treatment with 3 and 10 mg/kg ADX71441 (i.p.) completely suppressed alcohol-seeking, lever-responding behavior in the final reinstatement session (Augier et al. 2017).

ADX71441 was also effective in reducing alcohol intake in two mouse models of excessive alcohol drinking. In the first study (Hwa et al. 2014), acute treatment with ADX71441 (3–30 mg/kg, i.g.) produced a dose-dependent suppression of alcohol intake in male C57BL/6 J mice exposed to the binge-like DID procedure (Box 12.2c). In the second study (Hwa et al. 2014), acute treatment with ADX71441 (3–17 mg/kg, i.g.) reduced alcohol intake in C57BL/6 J mice exposed to the intermittent (once every other day) access to two bottles containing alcohol (20% v/v) and water, respectively (Box 12.2a), a procedure known to generate remarkable escalation of alcohol drinking in rodents (see Carnicella et al. 2014).

12.2.6 CMPPE

In line with data from all other $GABA_B$ PAMs, acute treatment with CMPPE (2.5–10 mg/kg, i.p.) produced a dose-related reduction in lever-responding for alcohol and amount of self-administered alcohol in female sP rats trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement (Box 12.1a) (Maccioni et al. 2019b). Selectivity of this effect was demonstrated by the inability of the same dose range of CMPPE to alter lever-responding for a chocolate solution in a companion set of female sP rats (Maccioni et al. 2019b). Acute treatment with CMPPE (2.5–10 mg/kg, i.p.) also reduced, still in a dose-related fashion, breakpoint for alcohol in female sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed to a test session under a PR schedule of reinforcement (Box 12.1b) (Maccioni et al. 2019b).

CMPPE was also profitably tested under the reinstatement procedure (Box 12.1d). Specifically, in a first study, acute treatment with CMPPE (10 and 30 mg/kg, i.p.) suppressed cue-induced reinstatement of alcohol seeking in male Wistar rats initially trained to nose-poke for alcohol (10% v/v) under the FR3 schedule of reinforcement (Vengeliene et al. 2018). In a subsequent study, acute treatment with CMPPE (10 and 30 mg/kg, i.p.) suppressed cue-induced reinstatement of alcohol seeking in female sP rats initially trained to lever-respond for alcohol (15% v/v) under an FR5 schedule of reinforcement (Fig. 12.2) (Maccioni et al. 2019b). In both cases, reinstatement of alcohol-seeking behavior was virtually completely abolished by the highest doses of CMPPE.

In addition to reinstatement of alcohol seeking, human episodes of relapse drinking can be effectively modeled by the so-called alcohol deprivation effect (ADE), i.e., the temporary, although substantial, increase in voluntary alcohol drinking occurring in rodents after a period of alcohol deprivation (mimicking human abstinence from alcohol) (Box 12.2a). Repeated (five injections occurring across the phases of alcohol deprivation and alcohol reaccess) treatment with CMPPE (10 and 30 mg/kg, i.p.) resulted in a dose-related reduction of ADE in male Wistar rats exposed to a four-bottle "alcohol (5%, 10%, and 20% v/v) vs. water" choice regimen (Vengeliene et al. 2018).

12.2.7 COR659

Acute treatment with doses of COR659 ranging between 2.5 and 10 mg/kg (i.p.) suppressed, in a dose-related fashion, lever-responding for alcohol and amount of self-administered alcohol in male sP rats exposed to the FR4 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2017, 2019a; Ferlenghi et al. 2020). This effect was maintained—with relatively limited development of tolerance—after repeated treatment (2.5–10 mg/kg, i.p.; injections occurring before ten consecutive daily self-administration sessions) (Maccioni et al. 2019a). Notably,

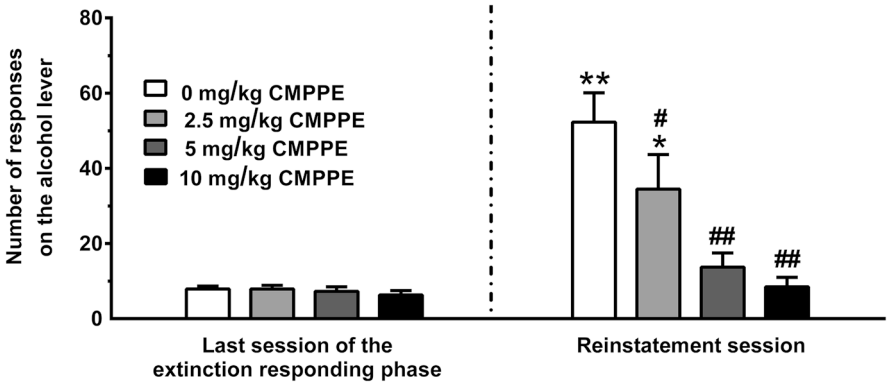


Fig. 12.2 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the GABA$_B$ receptor, CMPPE, on cue-induced reinstatement of alcohol-seeking behavior in female Sardinian alcohol-preferring (sP) rats. Rats were initially trained to lever-respond for oral alcohol (15% v/v) and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were exposed to an extinction responding (ER) phase during which lever-responding was unreinforced. The reinstatement session occurred once each single rat had achieved the extinction criterion (≤12 responses on the alcohol lever per session for 2 consecutive sessions). In the reinstatement session, unreinforced lever-responding was resumed by five repeated presentations of a stimulus complex—comprised of auditory (tone), visual (turning on of the stimulus lights), and gustatory (0.1 ml alcohol solution in the liquid receptacle)—previously associated with alcohol availability. Reinstatement sessions lasted 60 min. CMPPE was administered 30 min before start of the reinstatement session. Each bar is the mean ± SEM of n = 10–11 rats. * is $P < 0.001$ and ** is $P < 0.0001$ in comparison with the same rat group in the last session of the extinction responding phase (Tukey's test); # is $P < 0.05$ and ## is $P < 0.001$ in comparison with the vehicle-treated rat group in the reinstatement session (Tukey's test). (Adapted from Maccioni et al. (2019b), with permission from Elsevier)

COR659 doses suppressing alcohol self-administration were far lower than those affecting spontaneous locomotor activity (Maccioni et al. 2017).

Acute treatment with COR659 (2.5–10 mg/kg, i.p.) (i) markedly reduced breakpoint for alcohol in male sP rats initially trained to lever-respond for alcohol (15% v/v) under an FR4 schedule of reinforcement and then exposed to a test session under a PR schedule of reinforcement (Box 12.1b) (Maccioni et al. 2017) and (ii) completely abolished cue-induced reinstatement of alcohol seeking in male sP rats previously trained to lever-respond for alcohol (15% v/v) under an FR4 schedule of reinforcement (Box 12.1d) (Maccioni et al. 2019a).

COR659 was also effective in experimental procedures of excessive alcohol drinking. Specifically, acute treatment with COR659 (2.5–10 mg/kg, i.p.) reduced alcohol intake in male sP rats exposed to the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Ferlenghi et al. 2020). However, when given repeatedly (5–20 mg/kg, i.p.; injections occurring before seven consecutive daily drinking sessions), tolerance to the suppressing effect of COR659 on voluntary intake of alcohol developed relatively rapidly (Lorrai et al. 2022b). Finally,

acute treatment with COR659 suppressed binge-like drinking: (i) injection of 2.5–40 mg/kg COR659 (i.p.) markedly decreased alcohol intake in male sP rats exposed to the experimental procedure comprised of daily drinking sessions with limited (1 h) and unpredictable access to water and three different alcohol concentrations (10%, 20%, and 30% v/v) and known to produce, at least in this rat line, escalations in alcohol drinking up to intoxication (Box 12.2b); (ii) injection of 10–40 mg/kg COR659 (i.p.) suppressed alcohol intake in male C57BL/6 J mice exposed to the DID procedure (Box 12.2c) (Lorrai et al. 2022a). A subsequent experiment found that tolerance to the suppressing effect of COR659 on alcohol drinking under the DID model developed rapidly when COR659 (10–40 mg/kg, i.p.) was administered repeatedly (i.e., before seven consecutive daily drinking sessions) (Lorrai et al. 2022b).

The results of a series of additional experiments revealed that COR659 displays a unique pharmacological profile, made of at least two distinct mechanisms of action: positive allosteric modulation of the $GABA_B$ receptor and antagonism/inverse agonism at cannabinoid type-1 (CB_1) receptor (Ferlenghi et al. 2020). At a behavioral level, this dual mechanism of action was confirmed by (i) the ability of pretreatment with the $GABA_B$ receptor antagonist, SCH50911, to partially block the suppressing effect of COR659 on alcohol self-administration in sP rats and (ii) the ability of pretreatment with the neutral cannabinoid CB_1 receptor antagonist, AM4113, to fully block the reducing effect of COR659 on chocolate self-administration in Wistar rats (Maccioni et al. 2017). This off-target mechanism of action may help to explain why tolerance developed to the reducing effect of COR659 on alcohol drinking, a phenomenon not expected when testing a PAM (see above). Conversely, and taking into account the well-known ability of the prototypic cannabinoid CB_1 receptor antagonist/inverse agonist, rimonabant, to suppress alcohol intake in mice and rats, it is likely that the cannabinoid CB_1 receptor component of the complex mechanism of action of COR659 contributed to the reducing effect of COR659 on alcohol drinking and was the molecular substrate involved in the development of tolerance to the reducing effects of COR659 on alcohol drinking (Lorrai et al. 2022b).

12.2.8 ASP8062

A recent experimental study tested ASP8062 on alcohol self-administration in rats (Haile et al. 2021). Specifically, ASP8062 was administered i.g., at doses ranging between 1 and 10 mg/kg, before four consecutive self-administration sessions to both female and male Sprague-Dawley rats trained to lever-respond for alcohol (10% v/v) under the FR2 schedule of reinforcement (Box 12.1a). Treatment with ASP8062 suppressed lever-responding for alcohol and number of earned alcohol reinforcers, with male rats resulting to be more sensitive than female rats to the suppressing effect of ASP8062 on alcohol reinforcement (Fig. 12.3). No dose of ASP8062 affected spontaneous locomotor activity in both female and male rats.

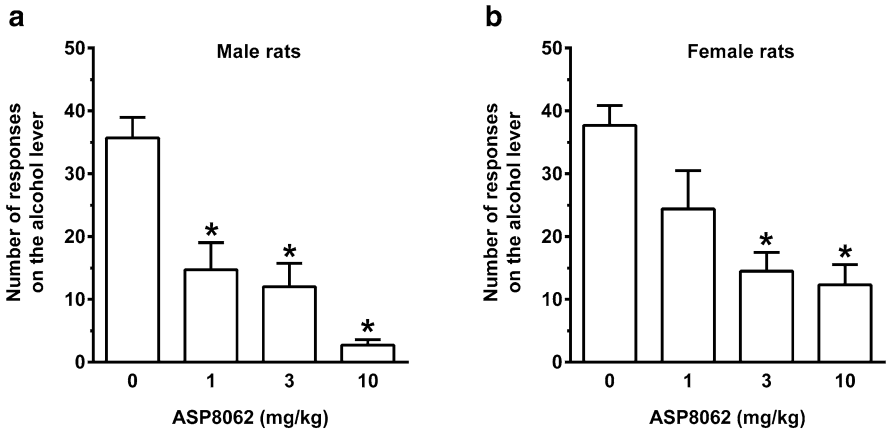


Fig. 12.3 Effect of repeated, intragastric (i.g.) treatment with the positive allosteric modulator of the $GABA_B$ receptor, ASP8062, on number of lever-responses for alcohol in male (panel a) and female (panel b) Sprague-Dawley rats. Rats were initially trained to lever-respond for oral alcohol (10% v/v) and water under the fixed ratio (FR) 2 schedule of reinforcement in daily 60-min self-administration sessions. Once lever-responding had stabilized, rats were tested with ASP8062 under the same FR schedule of reinforcement. ASP8062 was administered 30 min before start of consecutive self-administration sessions. Data depicted here refer only to day 4 of treatment. Each bar is the mean ± SEM of $n = 10$ rats. *: $P < 0.001$ in comparison with the rat group treated with 0 mg/kg ASP8062 (Dunnett's test). (Adapted from Haile et al. (2021) with permission from Springer Nature)

Based on these promising results, ASP8062 has recently reached clinical testing. More specifically, an initial phase I study reported safety and tolerability of oral ASP8062, even when administered in combination with alcohol (Ito et al. 2022). A subsequent trial has investigated the effect of oral ASP8062 on alcohol craving and consumption in subjects with moderate-to-severe AUD (ClinicalTrial.gov 2024); when available, data from this study will constitute the first line of clinical evidence on the therapeutic potential of $GABA_B$ PAMs in the AUD research field (see Burnette et al. 2022).

12.2.9 KK-92A

KK-92A is one of the $GABA_B$ PAMs most extensively evaluated, at least based on the number of different experimental procedures, for its suppressing effects on alcohol-related behaviors. Acute treatment with KK-92A (5–20 mg/kg, i.p.) dose-dependently suppressed lever-responding for alcohol and amount of self-administered alcohol in female sP rats exposed to the FR5 schedule of reinforcement for alcohol (15% v/v) (Box 12.1a) (Maccioni et al. 2021). When given repeatedly (once daily for 10 consecutive days), partial tolerance developed to the suppressing effect of KK-92A on alcohol reinforcement in female sP rats (Maccioni et al. 2022);

the agonistic component of the ago-allosteric profile of KK-92A (Li et al. 2017) likely produced some degree of receptor desensitization, thus being responsible for the observed development of partial tolerance (Maccioni et al. 2022).

The suppressing effect of KK-92A was not fully selective for alcohol, as acute treatment with KK-92A (5–20 mg/kg, i.p.) also affected—although less efficaciously—lever-responding for a sucrose solution in female sP rats exposed to the FR5 schedule of reinforcement (Maccioni et al. 2021). Again, the agonistic activity of KK-92A might be the key element in producing an effect repeatedly observed with baclofen (see Colombo and Gessa 2018).

The FR schedule of reinforcement was used to generate two additional sets of data. First, combination of a per se ineffective dose of KK-92A (2.5 mg/kg, i.p.) with a per se ineffective dose of baclofen (1 mg/kg, i.p.) synergistically reduced number of lever-responses for alcohol and amount of self-administered alcohol in female sP rats lever-responding for alcohol (15% v/v) (Maccioni et al. 2021), replicating the potentiating effect on baclofen action previously observed with GS39783 and COR659 (see above). Second, acute and i.g. administration of KK-92A (20 and 40 mg/kg) reduced lever-responding for alcohol and amount of self-administered alcohol in female sP rats tested under the FR5 schedule for alcohol (15% v/v) reinforcement (Maccioni et al. 2023), indicating that the ability of KK-92A to reduce alcohol self-administration is maintained also after per os treatment.

Acute treatment with KK-92A (5–20 mg/kg, i.p.) suppressed, in a dose-related fashion, lever-responding and breakpoint for alcohol in female sP rats initially trained to self-administer alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed, in the test session, to a conventional PR schedule of reinforcement (Fig. 12.4a; Box 12.1) (Maccioni et al. 2021). The suppressing effect of KK-92A on the motivational properties of alcohol was then extended to an alternative experimental procedure named extinction responding (ER) and characterized by the absence of any alcohol reinforcement whatever the number of lever-responses (Box 12.1c). Specifically, acute treatment with KK-92A (5–20 mg/kg, i.p.) reduced ER for alcohol in female sP rats initially trained to self-administer alcohol (15% v/v) under the FR5 schedule of reinforcement and then exposed, in the test session, to alcohol-seeking non-reinforced lever-pressing (Fig. 12.4b) (Maccioni et al. 2023).

In line with data previously collected with ADX71441, CMPPE, and COR659 (see above), treatment with KK-92A was also extremely effective in suppressing reinstatement of alcohol-seeking behavior (Box 12.1d). Indeed, acute treatment with 5–20 mg/kg KK-92A (i.p.) completely abolished cue-induced reinstatement of alcohol seeking in female sP rats initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement, ultimately leading to a virtually complete extinguishing of alcohol-seeking, lever-pressing behavior, and finally exposed animals to a session of cue-induced reinstatement (Maccioni et al. 2021).

Repeated (once daily for 7 consecutive days) treatment with KK-92A (5–20 mg/kg, i.p.) markedly reduced, with limited development of tolerance, excessive alcohol drinking in male sP rats exposed to daily 1-h drinking sessions under the homecage two-bottle "alcohol (10% v/v) vs. water" choice regimen (Box 12.2a) (Maccioni et al. 2023).

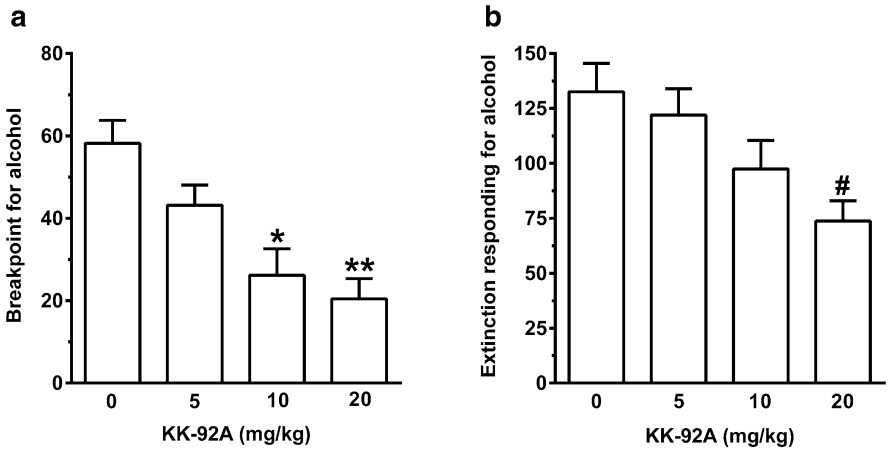


Fig. 12.4 Effect of acute, intraperitoneal (i.p.) treatment with the positive allosteric modulator of the GABA$_B$ receptor, KK-92A, on breakpoint for alcohol (panel **a**) and extinction responding (ER; panel **b**) in female Sardinian alcohol-preferring (sP) rats. In both studies, rats were initially trained to lever-respond for oral alcohol (15% v/v) and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats of the "breakpoint" study were exposed to a single, 60-min self-administration session under a conventional progressive ratio (PR) schedule of reinforcement; breakpoint was defined as the lowest response requirement not achieved. Rats of the "ER" study were exposed to a single, 60-min ER session in which lever-responding was not reinforced. In both studies, KK-92A was administered 30 min before start of the test session. In panel a, each bar is the mean ± SEM of $n = 12$ rats; *: $P < 0.001$ and **: $P < 0.0001$ in comparison to the rat group treated with 0 mg/kg KK-92A (Tukey's test). In panel b, each bar is the mean ± SEM of $n = 16$ rats; #: $P < 0.005$ in comparison with the rat group treated with 0 mg/kg KK-92A (Dunn's test). (Panel **a**: adapted from Maccioni et al. (2021). Panel **b**: adapted from Maccioni et al. (2023), with permission from Elsevier)

Two final, and somewhat ancillary, experiments demonstrated that none of the i.p. administered doses of KK-92A found to suppress alcohol-related behaviors altered (i) alcohol palatability in male sP rats (Maccioni et al. 2023) and (ii) spontaneous locomotor activity (Maccioni et al. 2021) in female sP rats, thus providing evidence that the reducing effects of KK-92A were not due to an increase in taste aversiveness of alcohol solutions or sedative and motor-incapacitating effects, respectively.

12.2.10 ORM-27669

To date, the latest synthesized GABA$_B$ PAM, ORM-27669, has been tested solely in DID and CPP procedures. Specifically, acute treatment with 100 mg/kg ORM-27669 (i.p.) suppressed alcohol intake in male C57BL/6 J mice exposed to the binge-like DID procedure (Box 12.2c) (de Miguel et al. 2019). The same ORM-27669 dose,

given prior to alcohol injection (0.5 g/kg, i.p.) in each "alcohol" conditioning session, prevented the development of alcohol-induced CPP in the final, drug-free test session in male C57BL/6 J mice (Box 12.3b) (de Miguel et al. 2019). Specificity of these reducing effects of ORM-27669 on alcohol drinking and alcohol rewarding properties was demonstrated by the lack of any effect of acute treatment with 100 mg/kg ORM-27669 (i.p.) on spontaneous locomotor activity in male C57BL/6 J mice (de Miguel et al. 2019).

12.3 The Putative $GABA_B$ PAM, Fendiline, and Alcohol Self-Administration

Fendiline is a human-approved, L-type Ca^{2+} channel blocker. Approximately 20 years ago, an intriguing debate arose on the ability of fendiline to act as a $GABA_B$ PAM in addition to blocking the L-type Ca^{2+} channels. Fendiline application to rat neocortical slices enhanced baclofen-induced hyperpolarization, an effect blocked by SCH50911 (Kerr et al. 2002). Similarly, fendiline application induced a leftward shift of baclofen concentration-response curve in rat midbrain slices (Chen et al. 2005). A subsequent study led by Stephan Urwyler (the chemist of Novartis, Basel, Switzerland, who headed the synthesis of CGP7930 and GS39783) challenged these data reporting the complete inability of fendiline to enhance (i) $GABA_B$ receptor-mediated guanosine 5-O-(3-[^{35}S]thio)triphosphate (GTPγ^{35}S) binding (an assay that evaluates functionality of the receptor by measuring its activation via the G-protein) in membrane preparations from CHO cells and rat brain cortex as well as (ii) affinity of GABA for $GABA_B$ receptors in a competition, radioligand binding assay using rat brain cortex (Urwyler et al. 2004).

Two more recent behavioral studies reported that fendiline exerted anti-addictive, $GABA_B$ PAM-like effects, revamping some interest on this compound. Specifically, (i) repeated treatment with fendiline prevented and abolished (once established) methamphetamine-induced CPP in rats (Voigt et al. 2014) and (ii) acute treatment with fendiline attenuated cue- and cocaine-induced reinstatement of cocaine seeking in rats (Cunningham et al. 2015). Unfortunately, no pharmacological blockade experiment investigated which component of the dual mechanism of action of fendiline (i.e., blockade of the L-type Ca^{2+} channels and positive allosteric modulation of the $GABA_B$ receptor) was actually responsible for these effects.

Nevertheless, intrigued by these anti-addictive effects of fendiline clearly evocative of those of $GABA_B$ PAMs (see Chap. 11 of this volume), we recently investigated fendiline effect on operant oral alcohol self-administration (Box 12.1a). Specifically, female sP rats were initially trained to lever-respond for alcohol (15% v/v) under the FR5 schedule of reinforcement in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were exposed to a test session preceded by acute treatment with 0, 1.7, 3, and 5.6 mg/kg fendiline (i.p.). Fendiline dose range was identical to that tested in the "methamphetamine" (Voigt et al. 2014) and "cocaine" (Cunningham et al. 2015) studies. Selectivity was investigated

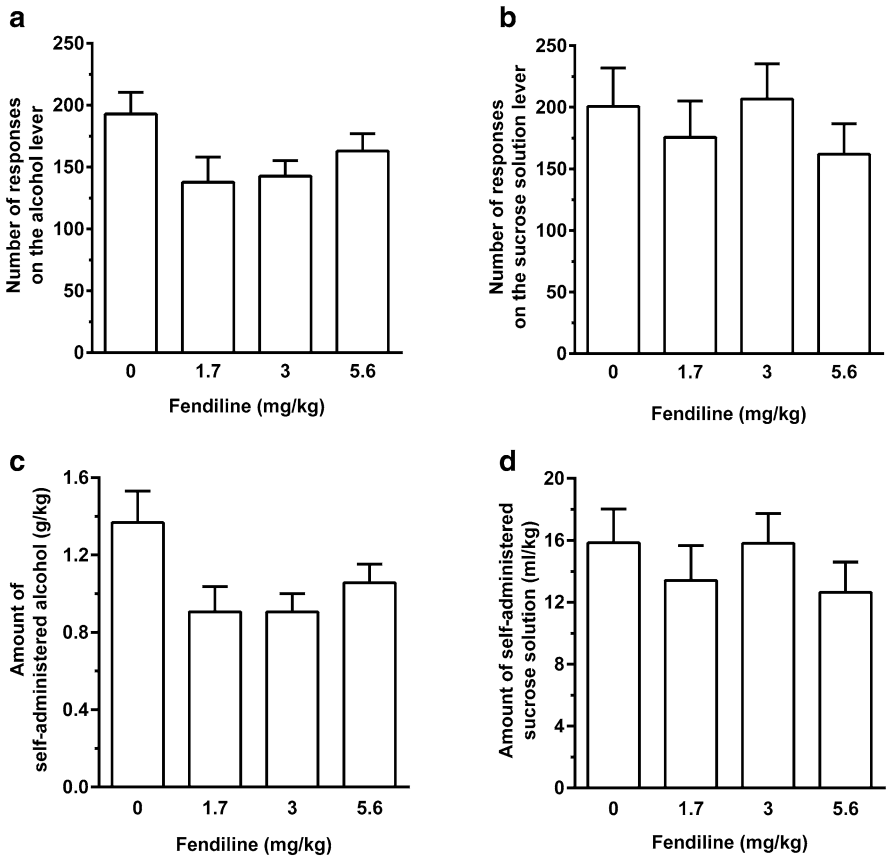


Fig. 12.5 Effect of acute, intraperitoneal (i.p.) treatment with the L-type Ca^{2+} channel blocker and putative positive allosteric modulator of the $GABA_B$ receptor, fendiline, on number of lever-responses for alcohol (panel **a**), amount of self-administered alcohol (panel **c**), number of lever-responses for a sucrose solution (panel **b**), and amount of self-administered sucrose solution (panel **d**) in female Sardinian alcohol-preferring (sP) rats. Rats were initially trained to lever-respond for oral alcohol (15% v/v) [or sucrose solution (0.3% w/v)] and water under the fixed ratio (FR) 5 and FR1 schedules of reinforcement, respectively, in daily 30-min self-administration sessions. Once lever-responding had stabilized, rats were tested with fendiline under the same FR schedule of reinforcement. Fendiline was administered 20 min before start of the self-administration session. Each bar is the mean ± SEM of n = 11–12 rats. Analysis of variance (ANOVA) for number of lever-responses for alcohol: $F(3,47) = 7.40$, $P > 0.05$; ANOVA for amount of self-administered alcohol: $F(3,47) = 7.70$, $P > 0.05$; ANOVA for number of lever-responses of the sucrose solution: $F(3,40) = 0.55$, $P > 0.05$; ANOVA for amount of self-administered sucrose solution: $F(3,40) = 0.63$, $P > 0.05$

testing the same fendiline doses on self-administration of a sucrose solution (0.3% w/v) in an independent set of female sP rats exposed to the FR5 schedule of reinforcement. Basal levels of lever-responding for the sucrose solution equated those sustained by alcohol.

As depicted in Fig. 12.5, no dose of fendiline altered either number of lever-responses for alcohol and amount of self-administered alcohol or number of

lever-responses for sucrose solution and amount of self-administered sucrose solution. Testing higher doses of fendiline was not advisable, as they were found to produce motor incoordination in rats (Voigt et al. 2014). The lack of any fendiline effect on alcohol self-administration prevented subsequent, pharmacologically blockade experiments from being conducted with $GABA_B$ receptor antagonists.

12.4 Conclusions

To summarize, all $GABA_B$ PAMs tested to date have invariably been reported to reduce, or even suppress, multiple alcohol-motivated behaviors, including excessive alcohol drinking, binge-like alcohol drinking, relapse-like alcohol drinking, operant oral alcohol self-administration, alcohol seeking, cue- and stress-induced reinstatement of alcohol seeking, and alcohol-induced hyperlocomotion and CPP, in mice and rats.

These data possess remarkable translational value, synthesized in the following bullet points:

- Data were generated using validated, experimental procedures that effectively model single aspects of human AUD (see Bell et al. 2017).
- The reducing, or suppressing, effects of $GABA_B$ PAMs on alcohol-related behaviors occurred at doses largely lower than those producing sedation and motor incoordination. When calculated (Maccioni et al. 2017, 2021), the resulting Therapeutic Index was remarkably high, suggestive of a large separation between the pharmacological and toxicological effects of $GABA_B$ PAMs.
- Development of tolerance to the reducing effects of repeatedly administered $GABA_B$ PAMs on alcohol drinking and alcohol self-administration was relatively limited, in line with the expected, minimal propensity of PAMs to produce receptor desensitization.
- $GABA_B$ PAMs came out to be more potent and effective in those experimental conditions under which rats displayed strong reinforcing and motivational properties of alcohol and self-administered large amounts of alcohol.
- No major sex differences were observed in the (still relatively few) studies comparing the effects of $GABA_B$ PAMs on alcohol-related behaviors in female and male rats.
- Virtually all $GABA_B$ PAMs maintain their ability to affect alcohol-related behaviors when given per os.

The recent transition of ASP8062 to clinical testing will soon reveal whether these promising preclinical data replicate in humans and whether $GABA_B$ PAMs may represent a feasible option among pharmacotherapies for AUD.

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