



Role of GABA_B Receptor in Alcohol Dependence: Reducing Effect of Baclofen on Alcohol Intake and Alcohol Motivational Properties in Rats and Amelioration of Alcohol Withdrawal Syndrome and Alcohol Craving in Human Alcoholics

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The present paper describes the results of recent preclinical and clinical studies conducted in this laboratory in order to characterize the anti-alcohol properties of the GABA_B receptor agonist, baclofen. At a preclinical level, the repeated administration of non-sedative doses of baclofen dose-dependently suppressed the acquisition and maintenance of alcohol drinking behavior in selectively bred Sardinian alcohol-preferring (sP) rats tested under the homecage, 2-bottle "alcohol vs water" choice regimen. Acute injection of baclofen completely blocked the temporary increase in voluntary alcohol intake occurring after a period of alcohol abstinence (the so-called alcohol deprivation effect, which models alcohol relapses in human alcoholics). Acute treatment with baclofen also dose-dependently suppressed extinction responding for alcohol (an index of motivation to consume alcohol) in sP rats trained to lever-press for oral alcohol self-administration. Taken together, these results suggest the involvement of the GABA_B receptor in the neural substrate mediating alcohol intake and alcohol motivational properties in an animal model of excessive alcohol consumption. Further, acutely administered baclofen dose-dependently reduced the severity of alcohol withdrawal signs in Wistar rats made physically dependent upon alcohol.

Preliminary clinical surveys suggest that the anti-alcohol properties of baclofen observed in rats may generalize to human alcoholics. Indeed, a double-blind survey demonstrated that repeated daily treatment with baclofen was associated, when com-

pared to placebo, with a higher percentage of subjects totally abstinent from alcohol and a higher number of days of total abstinence. Treatment with baclofen also suppressed the number of daily drinks and decreased the obsessive and compulsive components of alcohol craving. Finally, a single non-sedative dose of baclofen resulted in the rapid disappearance of alcohol withdrawal symptomatology, including delirium tremens, in alcohol-dependent patients. In both clinical studies, baclofen was well tolerated with minimal side effects. These results suggest that baclofen may represent a potentially effective medication in the treatment of alcohol-dependent patients.

Keywords: Baclofen; GABA_B receptor; Alcohol; Alcoholics; Alcoholism; Sardinian alcohol-preferring (sP) rats

INTRODUCTION

In the treatment of alcoholism, the use of pharmacological agents is conceived to provide a substantial contribution to achievement of abstinence, facilitating the psychological support and social rehabilitation of alcoholic patients. Unfortunately, drugs currently available to physicians apparently possess limited therapeutic efficacy or may themselves provide problems of tolerance, dependence or even abuse liability. New medications are needed in order for their combination with psychological interventions to result in an enhancement of abstinence and prevention of relapses in alcoholics.

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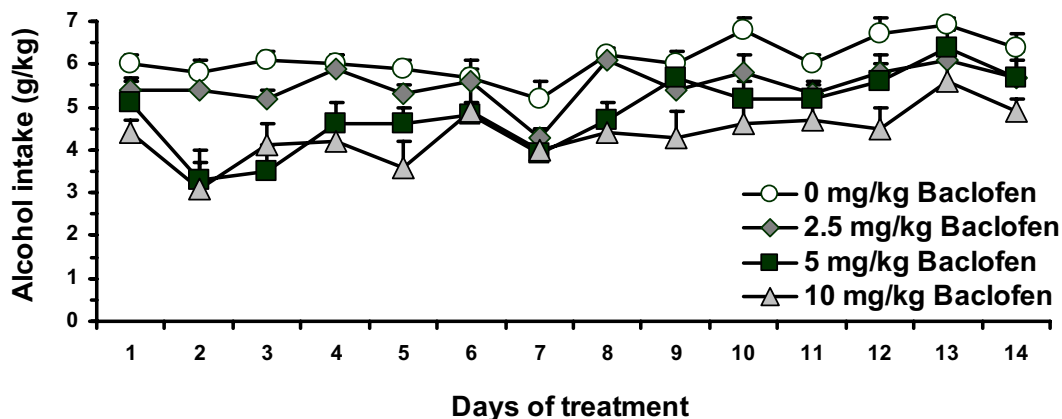


FIGURE 1 Reducing effect of baclofen on alcohol intake in alcohol-experienced sP rats tested under the 2-bottle choice regimen. Each point is the mean \pm SEM of $n=7$. Reprinted with permission from Lippincott Williams & Wilkins, in Colombo *et al.*, *Alcohol. Clin. Exp. Res.* 24: 58-66, 2000.

Elucidation of the mechanisms of alcohol action in brain and identification of the neural substrates targeted by alcohol are thought to be necessary conditions for designing novel and effective pharmacotherapies for alcoholism. Indeed, only the knowledge of how alcohol acts in brain may permit the rational design and development of selective drugs capable of correcting the neurochemical alterations underlying alcohol dependence.

Recent experimental results and preliminary clinical data suggest that the GABA_B receptor may be considered a novel player among the neural substrates controlling alcohol drinking behavior, alcohol reinforcement (in rats) and craving (in humans), and alcohol withdrawal symptomatology. The present paper is intended to provide a description of the "lab bench to bedside" studies conducted by this laboratory which characterize the GABA_B receptor agonist, baclofen, as a promising agent in the pharmacotherapy of alcoholism.

PRECLINICAL STUDIES

Reducing Effect of GABA_B Receptor Agonists on Alcohol Intake in Rats

Most of the studies conducted by this laboratory on the anti-alcohol properties of GABA_B receptor agonists have used the Sardinian alcohol-preferring (sP) rats, one of the few rat lines selectively bred worldwide for high alcohol preference and consumption. Specifically, when given a choice between a 10% (v/v) alcohol solution and water, under the standard homecage 2-bottle regimen with unlimited access for 24 hours/day, sP rats

display a clear preference for the alcohol solution and consume daily approximately 6 g/kg pure alcohol (e.g., Colombo *et al.*, 1995). Voluntary alcohol intake in sP rats gives rise to blood alcohol levels in the 30-60 mg% range (Agabio *et al.*, 1996; Colombo *et al.*, 1998; 2002b), and produces specific psychopharmacological effects, including amelioration of anxiety-related behaviors (Colombo *et al.*, 1995), motor activation (Colombo *et al.*, 1998) and stimulation of dopamine release in the shell of the nucleus accumbens (see below). Recent work demonstrated that sP rats acquire and maintain oral self-administration of alcohol under an operant procedure (namely, lever-pressing), suggesting that alcohol functions as a reinforcer in these rats (Vacca *et al.*, 2002).

The initial investigation by this laboratory on the anti-alcohol properties of baclofen evaluated its effect on voluntary alcohol intake in alcohol-experienced sP rats, that is rats in which the consumption of pharmacologically relevant doses of alcohol was already established before baclofen injection. These rats are thought to represent a model of the "maintenance" or "active drinking" phase of human alcoholism. In this study (Colombo *et al.*, 2000), adult male sP rats were singly housed and exposed to alcohol for approximately 2 months before the start of the drug treatment. Alcohol (10%, v/v) was offered under the 2-bottle "alcohol vs water" regimen with unlimited access. Food was always available. Rats were divided into 4 groups ($n=7$), matched for alcohol intake over the 3 days preceding the start of the baclofen treatment. Baclofen was injected intraperitoneally (i.p.) at the doses of 0, 2.5, 5 and 10 mg/kg once a day for 14 consecutive days.

Treatment with 5 and 10 mg/kg baclofen induced an initial reduction, by 40-50% in comparison to saline-treated rats, in daily alcohol consumption (FIG. 1); this

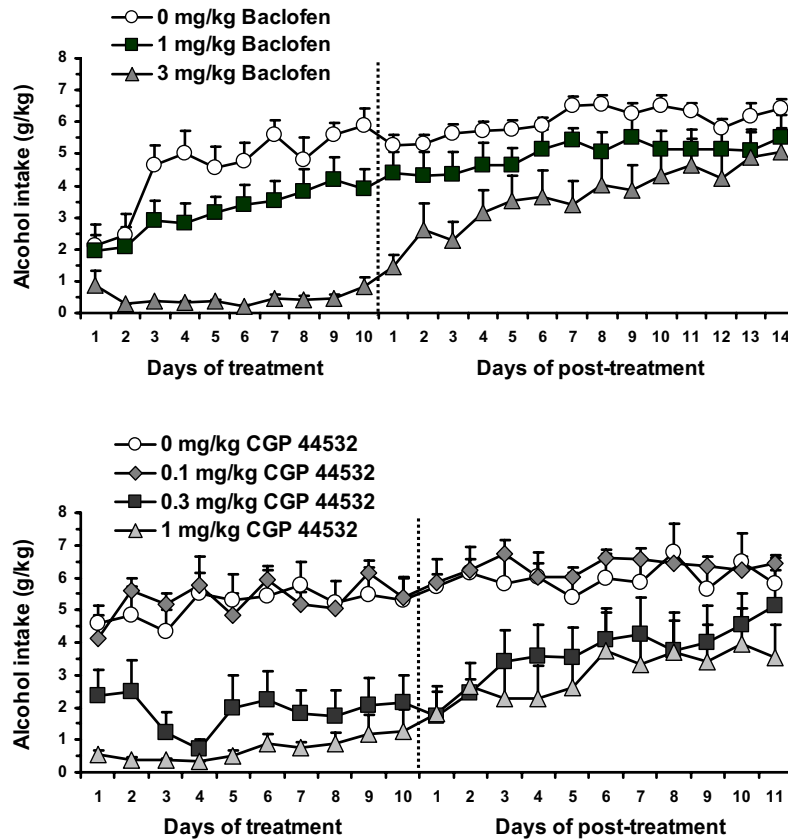


FIGURE 2 Suppressing effect of baclofen (top panel) and CGP 44532 (bottom panel) on the acquisition of alcohol drinking behavior in sP rats tested under the 2-bottle choice regimen. Each point is the mean \pm SEM of $n=7-9$. Reprinted with permission from Oxford University Press, in Gessa, *Alcohol Alcohol.* 37: 499-503, 2002a.

decrement tended to reduce on continuing treatment. An increase in daily water intake compensated the reduction in alcohol intake and left total fluid intake virtually unchanged. Food intake (a variable usually recorded in animal studies where alcohol intake is pharmacologically manipulated as a measure of animal malaise and non-selectivity of the drug action) was significantly altered only by treatment with 10 mg/kg baclofen; this effect was however limited to the first half of the treatment period.

We subsequently investigated the effect of baclofen and the newly synthesized GABA_B receptor agonist, CGP 44532 [(2*S*)-3-amino-2-hydroxypropylmethyl phosphinic acid], on the acquisition of alcohol drinking behavior in alcohol-naive sP rats, that is, rats which had never consumed alcohol before the start of the experiment; these rats may represent a model of the predisposition toward excessive alcohol consumption in individuals at genetic risk of alcoholism. In this study (Colombo *et al.*, 2002a), singly-housed adult male sP rats were divided into 3 groups ($n=9$) in the baclofen experiment and into 4 groups ($n=7$) in the CGP 44532 experiment. Baclofen (0, 1 and 3 mg/kg) and CGP

44532 (0, 0.1, 0.3 and 1 mg/kg) were administered i.p. once a day for 10 consecutive days. Alcohol (10%, v/v) and water were offered under the 2-bottle choice regimen immediately after the first injection of either baclofen or CGP 44532. Food was available *ad libitum*.

As expected, mean daily alcohol intake in saline-treated rats rose to 5-6 g/kg/day (i.e., the amount of alcohol usually consumed daily by sP rats) within 4-7 days in both experiments (FIG. 2). Both baclofen and CGP 44532 produced a dose-dependent suppression of daily alcohol intake throughout the treatment period (FIG. 2). Specifically, in the rat groups treated with 3 mg/kg baclofen and 1 mg/kg CGP 44532 daily alcohol intake was virtually suppressed throughout the 10-day treatment period. In both experiments, reduction in alcohol intake was associated with a compensatory increase in daily water intake, so that total daily fluid intake remained unchanged. Daily food intake tended to be higher in the rat groups treated with baclofen or CGP 44532 than in the saline-dosed groups, likely because in the control group part of the total caloric intake was provided by alcohol. After treatment completion, daily alcohol intake in the 3 mg/kg baclofen-treated group and 1 mg/kg CGP 44532-treated group

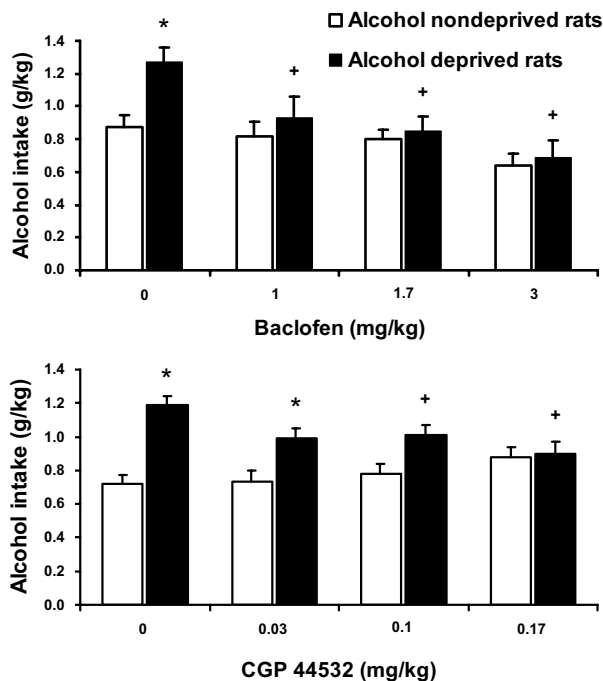


FIGURE 3 Suppressing effect of baclofen (top) and CGP 44532 (bottom) on alcohol deprivation effect (ADE) in sP rats given alcohol under the 2-bottle choice regimen. Each bar is the mean \pm SEM of $n=7-8$. * $P < 0.05$ with respect to saline-treated alcohol-nondeprived rats; + $P < 0.05$ with respect to saline-treated alcohol-deprived rats (Newman-Keuls test). Reprinted with permission from Elsevier Publ., in part from Colombo *et al.*, *Drug Alcohol Depend.* **70**, 105-108, 2003a.

increased progressively, reaching control values after approximately 10-14 days (FIG. 2); water intake progressively diminished.

The results of these experiments ("acquisition" and "maintenance" of alcohol drinking behavior) suggest that stimulation of the GABA_B receptor resulted in a virtually complete blockade of the disclosure and experience of those effects of alcohol that sustain alcohol drinking behavior, which is otherwise a phenomenon with a rapid onset and stable maintenance in sP rats, as indicated in undrugged rats by the constant daily intake of pharmacologically relevant amounts of alcohol from the very beginning of alcohol exposure.

A subsequent series of experiments investigated the effect of baclofen and CGP 44532 on the so-called alcohol deprivation effect (ADE), that is, the transient increase in alcohol intake which occurs in several animal species after a period of abstinence from alcohol (see Boening *et al.*, 2001; McBride *et al.*, 2002).

Interestingly, this phenomenon has been proposed to model the loss of control over alcohol and the episodes of alcohol relapse of human alcoholics. Rats of the sP line appear to constitute a proper model for this investigation, since they have been found to display a pronounced ADE during the first hour of re-access to alcohol after a period of deprivation from alcohol (Agabio *et al.*, 2000).

In the experiments testing the effect of baclofen (Colombo *et al.*, 2003a) and CGP 44532 on ADE, individually-housed adult male sP rats were initially offered alcohol (10%, v/v) and water under the standard 2-bottle choice with unlimited access for 8 consecutive weeks. Subsequently, rats were divided into 2 groups (matched for alcohol intake over the last 7 days): one group was deprived of alcohol for 14 consecutive days, during which water was the sole fluid available (alcohol-deprived rats); the second group continued to have unlimited access to alcohol and water (alcohol-nondeprived rats). At the end of the deprivation phase, rats of both groups (alcohol-deprived and -nondeprived) were further divided into 4 subgroups ($n=7-8$ in both experiments) and acutely injected with 0, 1, 1.7 and 3 mg/kg baclofen, or 0, 0.03, 0.1 and 0.17 mg/kg CGP 44532. Alcohol was re-presented at lights off and its consumption was recorded one hour later. Standard rat chow was available throughout the studies.

In both experiments, alcohol intake was higher, by 50-80%, in saline-treated alcohol-deprived rats than in saline-treated alcohol-nondeprived rats (FIG. 3), indicative of the development of a marked ADE. All doses of baclofen (FIG. 3, top panel) and the two highest doses of CGP 44532 (FIG. 3, bottom panel) resulted in a virtually complete suppression of the extra-intake of alcohol produced by alcohol deprivation. Importantly, no dose of baclofen and CGP 44532 affected water and food intake, tending to exclude that the action of baclofen and CGP 44532 on ADE had been secondary to their muscle-relaxant and sedative effects. Accordingly, complementary experiments of motor activity found that the doses of baclofen and CGP 44532 that suppressed ADE affected neither the time spent moving, distance traveled, nor number of rearings (measures of horizontal and vertical motor activities in rodents) in alcohol-consuming sP rats tested in an open-field arena (Colombo *et al.*, 2003a). These results suggest that a) the GABA_B receptor may be part of the neural substrate mediating ADE in sP rats, and b) GABA_B receptor agonists may possess some efficacy in preventing alcohol relapses in human alcoholics.

Despite the apparent consistency of the results demonstrating the reducing effect of baclofen on alcohol intake in sP rats tested under different experimental procedures ("maintenance", "acquisition" and "alcohol deprivation effect"), it should be noted however that the above results do not find unanimous consensus in the scientific literature. Indeed, other works reported that baclofen reduced, produced no change on, or even stimulated alcohol intake in rats given a choice between an alcohol solution and water. Specifically, four separate studies investigated the effect of baclofen on alcohol intake in alcohol-experienced rats ("maintenance" model) (Daoust *et al.*, 1987; Tomkins and Fletcher, 1996; Smith *et al.*, 1999; Perfumi *et al.*, 2002). An initial work by Daoust and coworkers (1987) found that baclofen, administered daily at the single dose of 3 mg/kg for 14 consecutive days, reduced alcohol intake in Long Evans rats, with no compensatory increase in water intake. The acute infusion of behaviorally active doses of baclofen (62.5 and 125 ng) directly into the dorsal raphe failed to alter alcohol intake in Wistar rats (Tomkins and Fletcher, 1996). Smith and colleagues (1999) reported that the repeated administration of a relatively high dose of baclofen (10 mg/kg) to Long Evans rats given alcohol on alternate days produced a nonspecific increase in alcohol intake, which was apparently secondary to an increase in the frequency of drinking bout. More recently, Perfumi and coworkers (2002) reported that the acute administration of 5, but not 2.5, mg/kg baclofen completely suppressed alcohol intake in a line of selectively bred alcohol-preferring rats; in contrast, 10 mg/kg baclofen produced a nonspecific reduction of all consummatory behaviors. One study (Smith *et al.*, 1992) investigated the effect of baclofen on the acquisition of alcohol drinking behavior in Long Evans rats. Alcohol was presented at increasing concentrations, from 2 to 10%, on alternate days; baclofen was administered at the single dose of 10 mg/kg on alcohol presentation days. Baclofen treatment resulted in an increase in alcohol intake, which was however accompanied by a proportional increase in total fluid intake. Differences in the rat strain (unselected or selectively bred alcohol-preferring rats), baclofen dose-range, route of baclofen administration, and procedure of alcohol exposure may explain, at least in part, these large discrepancies among the literature data as well as between the literature data and those collected in this laboratory.

The effect of baclofen on alcohol intake has also been examined using operant procedures, which require the rat to perform some "work" - such as pressing a lever - to gain access to alcohol (opposite to the relatively

"easy" access to alcohol represented by a simple lick of the bottle spout in the 2-bottle choice paradigm). Importantly enough, operant procedures allow investigations on the effect of a given drug on the reinforcing properties of alcohol, i.e., the capability of alcohol to direct and maintain a behavior such as lever-pressing. Again, mixed results have been collected to date: depending upon the drug dose or the experimental procedure, baclofen has been found to stimulate or decrease operant responding for alcohol in rats; when observed, this decrease in lever pressing for alcohol was not always highly selective, being often accompanied by a proportional decrease in lever pressing for an alternative reinforcer, such as sucrose. Specifically, Petry (1997) found that a low dose of baclofen (1.25 mg/kg) stimulated responding for a sweetened alcohol solution; however, a higher dose (5 mg/kg) nonspecifically reduced lever pressing for both the sweetened alcohol solution and a sucrose solution. More recently, Anstrom and colleagues (2003) reported that 3.2 and 5.6 mg/kg baclofen markedly reduced responding for alcohol in Long Evans rats; comparable reductions in lever pressing for a sucrose solution were however observed in a separate group of rats trained to lever press for sucrose. Janak and Gill (2003) found that 1 and 3 mg/kg baclofen reduced responding on the "active" lever (which delivered an alcohol solution), without altering responding on the "inactive" lever (pressing on which, had no consequences). When alcohol was concurrently available with a sucrose solution, the selectivity of the reducing effect of baclofen on alcohol responding had a narrow window, being limited to 1 but not 3 mg/kg. Finally, when tested under the "sipper tube" model of alcohol access, which permits some separation between the appetitive and consummatory aspects of alcohol self-administration, baclofen (0.3-3 mg/kg) decreased alcohol-seeking behavior (measured as the time spent in achieving the response requirement to gain access to the alcohol solution) and increased alcohol-consummatory behavior (the amount of alcohol actually consumed) in rats (Czachowski *et al.*, 2002).

Recent experimental work has investigated whether treatment with GABA_B receptor agonists alters the self-administration of other drugs of abuse. Specifically, baclofen and CGP 44532 have been reported to block the intravenous self-administration of cocaine, heroin, nicotine, methamphetamine, and γ -hydroxybutyric acid in rodents tested under multiple procedures (see Cousins *et al.*, 2002). Accordingly, baclofen has been reported to suppress the locomotor stimulation [a possible animal model of the euphori-

genic properties of a psychoactive drug (see Wise and Bozarth, 1987)] induced by cocaine, amphetamine, morphine, and the opioid μ -receptor agonist DAMGO [Tyr-d-Ala-Gly-N(me)Phe-Gly-ol-enkephalin] (see Cousins *et al.*, 2002).

It has been proposed that the suppressing effect of GABA_B receptor agonists on reinforcement produced by drugs of abuse may occur *via* a common mechanism, that is, the inhibition of the stimulatory effect of cocaine, heroin and nicotine on the mesolimbic dopamine system, the key neurochemical substrate mediating the reinforcing properties of drugs of abuse (see Di Chiara, 1995; Spanagel and Weiss, 1999). Accordingly, doses of baclofen in the range of those that suppressed the self-administration of abusive drugs have been reported to block the stimulation of dopamine release induced by heroin, morphine, cocaine and nicotine in the rat nucleus accumbens (Xi and Stein, 1999; Fadda *et al.*, 2003).

Reducing Effect of Baclofen on the Motivation to Consume Alcohol in Rats

The previous experiments, performed with the 2-bottle choice paradigm, focused on the effect of baclofen and CGP 44532 on some *consummatory* aspects of alcohol ingestive behavior in sP rats. Recently, this laboratory investigated whether baclofen could affect, in this rat line, also the *appetitive* or *motivational*, properties of alcohol. Specifically, we evaluated the effect of baclofen on the extinction responding for alcohol, defined as the maximal amount of "work" that a rat trained to lever-press for alcohol is willing to perform to obtain alcohol (Colombo *et al.*, 2003b). Extinction responding has been proposed to represent an index of the appetitive strength of alcohol (Samson *et al.*, 2001; 2003). Recent work has shown that sP rats trained to lever-press for alcohol displayed high values of extinction responding for alcohol (Vacca *et al.*, 2002), confirming that alcohol has strong motivational capacities in sP rats, and indicating the suitability of this rat line for the planned study.

Adult male sP rats ($n=8$) were initially trained to lever-press for oral alcohol (15%, v/v) in standard operant chambers under a fixed ratio 4 (FR4) schedule. Each 4 consecutive presses on the lever resulted in the presentation of a 0.1 ml-drop of alcohol solution. After approximately 20 daily sessions, all rats displayed a robust lever-pressing behavior; this responding resulted in a mean alcohol intake of 0.6 g/kg during the 30-min session and blood alcohol levels in the range of 40–50 mg%. A second group of sP rats ($n=8$) was trained

to lever-press for 3% (w/v) sucrose on an FR4 schedule. Extinction responding for alcohol or sucrose was defined as the maximal number of lever responses reached by each rat in the absence of alcohol or sucrose reinforcement, respectively; specifically, during extinction sessions, rats were exposed to the operant chamber for 30 min but lever-pressing did not result in any alcohol or sucrose presentation. On test sessions, baclofen was injected i.p. at the doses of 0, 1, 2 and 3 mg/kg. All four doses of baclofen were tested in each rat of both groups under a latin-square design.

Average values of extinction responding for alcohol and sucrose were virtually identical in saline-treated rats (FIG. 4), suggesting that 10% alcohol and 3% sucrose had comparable motivational properties. Pretreatment with baclofen resulted in a dose-dependent suppression of extinction responding for alcohol: responses on the lever in the rat groups treated with 1, 2 and 3 mg/kg baclofen was 64, 88 and 98% lower, respectively, than that recorded in saline-treated rats (FIG. 4, left panel). Notably, 7/8 rats in the 3 mg/kg baclofen-group did not press the lever at all. Conversely, only the highest dose of baclofen tested significantly affected extinction responding for sucrose (FIG. 4, right panel), suggesting that baclofen was more potent in reducing the motivation for alcohol than for sucrose. A separate experiment found that the above doses of baclofen did not affect spontaneous motor activity in sP rats tested in an open-field arena (Colombo *et al.*, 2003b), suggesting that the suppressing effect of baclofen on extinction responding was indeed secondary to a reduction in the appetitive strength of alcohol (and, to a lesser extent, that of sucrose) and not to the muscle-relaxant and sedative effects of baclofen.

These results a) implicate the GABA_B receptor in the neural system mediating alcohol reinforcement in sP rats, and b) suggest that the reducing effect of baclofen on alcohol intake in sP rats (see above) may be secondary to its ability to suppress the motivational properties of alcohol.

Hypothesized Mechanism of the Reducing Action of GABA_B Receptor Agonists on Alcohol Intake

The cellular mechanism by which GABA_B receptor agonists exert their reducing effect on alcohol intake and motivation to consume alcohol has yet to be defined. Different lines of experimental evidence suggest a role for the mesolimbic dopamine neurons, originating in the ventral tegmental area and projecting into the nucleus accumbens, in the regulation of alcohol

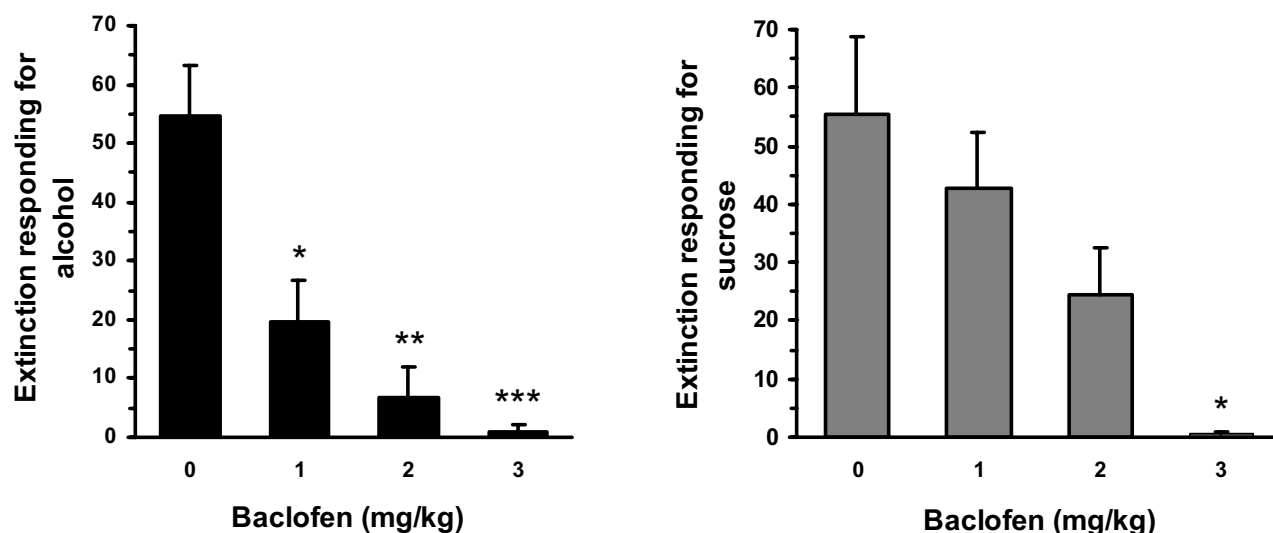


FIGURE 4 Differential effect of baclofen on the extinction responding for alcohol (left panel) and sucrose (right panel) in sP rats trained to self-administered alcohol under a standard operant procedure. Each bar is the mean \pm SEM of $n=8$. $P < 0.01$, $P < 0.001$, and $P < 0.0001$ with respect to saline-treated rats (Scheffé test). Reprinted with permission from Springer-Verlag, in Colombo *et al.*, *Psychopharmacology (Berl.)* 159: 181-187, 2003a.

preference and of the reinforcing properties of alcohol (see Weiss and Porrino, 2002). Accordingly, voluntarily consumed alcohol has been repeatedly reported to stimulate dopamine release in the nucleus accumbens of alcohol-preferring rats (Weiss *et al.*, 1993; Mendez *et al.*, 2002), including sP rats (this laboratory, unpublished results). GABA_B receptors located in the ventral tegmental area (Bowery *et al.*, 1987), both on the cell body of dopamine neurons and on the terminals of glutamatergic afferent neurons, have been suggested to contribute to the control of mesolimbic dopamine neurons, exerting - when stimulated - an inhibitory action on the latter neurons (Kalivas, 1993; Yoshida *et al.*, 1994; Westerink *et al.*, 1996). We hypothesize that these receptors might constitute the site of action of the anti-alcohol effects of baclofen; accordingly, we have recently conducted a preliminary microdialysis study to verify whether the decreasing effect of baclofen on alcohol intake is indeed associated with a reduction in alcohol-stimulated dopamine release in the nucleus accumbens shell of sP rats.

In this experiment, individually-housed adult male sP rats were initially habituated to consume alcohol (10%, v/v) under the 2-bottle choice regimen in daily drinking sessions of 30 min. Water was also available over the remaining 23 hours and 30 min. After a few days of habituation, alcohol intake stabilized at 0.6-0.7 g/kg, giving rise to blood alcohol levels in the 30-70 mg% range. Subsequently, rats were implanted with a permanent vertical probe in the shell of the nucleus accumbens by means of a stereotaxic apparatus. After

approximately one week, i.e., when rats had recovered their pre-surgery levels of alcohol intake, a microdialysis fiber was inserted through the guide probe. The test session was conducted on the following day. Saline and baclofen (3 mg/kg) were injected i.p. 30 min before the start of the drinking session to groups of $n=3-4$ rats. Dialysate samples were collected every 10 min and dopamine concentrations were determined using a HPLC with electrochemical detector.

Accumbal extracellular dopamine levels did not differ between saline- and baclofen-treated rats before the start of the drinking session. Saline- and baclofen-treated rats consumed 0.78 and 0.43 g/kg alcohol, respectively. In saline-treated rats, alcohol intake was associated with an increase in the levels of accumbal extracellular dopamine; this increase peaked at the first 10- and 20-min intervals of dopamine monitoring, averaging approximately 50% with respect to the values recorded prior to the drinking session. In contrast, extracellular DA levels in baclofen-treated rats did not differ among the pre-session, session and post-session periods of the experiment.

The preliminary nature of these results does not presently allow any definitive conclusion; however, should these results be confirmed by future experiments, it might be suggested that baclofen exerts its reducing effects on alcohol intake and appetitive properties by inhibiting alcohol-stimulated dopamine release in the mesolimbic system. This interpretation would be in close agreement with the lines of evidence suggesting that the suppressing effect of baclofen and

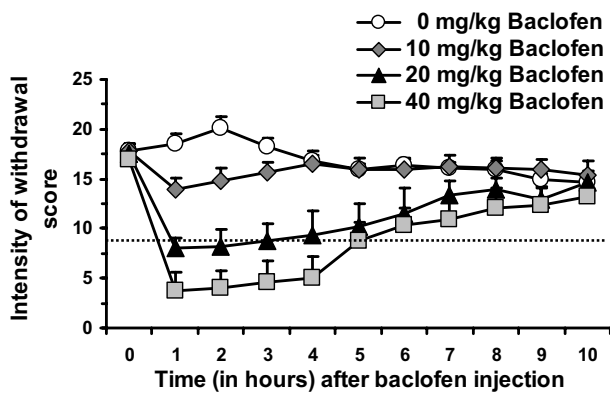


FIGURE 5 Suppressing effect of baclofen on the severity score of alcohol withdrawal syndrome in Wistar rats made physically dependent upon alcohol by the repeated administration of intoxicating doses of alcohol. The dashed line indicates the neutrality state, i.e., the score theoretically corresponding to healthy and undrugged rats. Each point is the mean \pm SEM of $n=8$. Reprinted with permission from Lippincott Williams & Wilkins, in Colombo *et al.*, *Alcohol. Clin. Exp. Res.* **24**: 58-66, 2000.

CGP 44532 on the self-administration of cocaine, heroin and nicotine in rats is likely secondary to their ability to block the release of dopamine evoked by these drugs of abuse in the shell of the rat nucleus accumbens (Xi and Stein, 1999; Fadda *et al.*, 2003).

Suppressing Effect of Baclofen on Alcohol Withdrawal Syndrome in Rats

A final experiment by this laboratory examined the effect of baclofen on the severity of alcohol withdrawal syndrome in rats made physically dependent upon alcohol by the repeated administration of intoxicating amounts of alcohol (Colombo *et al.*, 2000).

Specifically, adult male Wistar rats underwent a regimen of 4 daily intragastric administrations of alcohol (20%, w/v) for 6 consecutive days, conceived to maintain blood alcohol concentrations constant. After an initial dose of 4 g/kg alcohol, all subsequent doses were determined individually for each rat at each administration time on the basis of the observed degree of intoxication, according to the procedure by Majchrowicz (1975). Six successive stages of intoxication were defined: neutrality, sedation, ataxia 1, 2 and 3, loss of righting reflex. Alcohol doses, ranging from 0 to 5 g/kg, were inversely related to the degree of intoxication. The daily alcohol dose averaged approximately 10 g/kg. Food pellets were always available; body weight loss induced by the severe state of intoxication was partially compensated by the daily oral administration of a liquid diet.

Fifteen hours after the last alcohol administration, alcohol withdrawal syndrome reached maximal severity. By means of the scale proposed by Lal *et al.* (1988), we assessed the intensity of the following items: general activity, shakes, jerks, head tremors, bracing posture, general tremors, tail tremors, rigidity of muscle tone and tail rigidity, vocalizations and spontaneous convulsions. Rats were divided into 4 groups ($n=8$), matched for severity score at the 15-h observation. Baclofen was injected i.p. at the doses of 0, 10, 20 and 40 mg/kg immediately after the 15-h observation. Thereafter, withdrawal scoring was performed every hour for 11 consecutive hours.

Baclofen administration resulted in a dose-dependent reduction of the withdrawal score intensity (FIG. 5). This effect had a rapid onset, being maximally evident already at the first hourly observation after baclofen administration. The highest dose tested (40 mg/kg) induced a marked degree of muscle relaxation and sedation, as shown by a withdrawal score lower than that corresponding to healthy and undrugged rats; in contrast, at the dose of 20 mg/kg baclofen, no muscle flaccidity or loss of vigilance was observed, and the withdrawal score approached the neutrality-state set for a prolonged time (4-5 hours). When signs were evaluated singly, baclofen resulted to be maximally effective in suppressing tremors, muscle and tail rigidity and vocalizations. A separate experiment demonstrated that baclofen (20 mg/kg) was also effective in protecting alcohol-dependent rats from audiogenic seizures (Colombo *et al.*, 2000).

These results implicate GABA_B receptors in the occurrence of alcohol withdrawal signs. However, the existing literature on this issue is controversial. Data from our study are in agreement with those by File *et al.* (1991), demonstrating that administration of low doses of baclofen (1.25 and 2.5 mg/kg) reduced tremors and anxiogenic responses associated to alcohol withdrawal in rats. In contrast, Humeniuk *et al.* (1994) reported that baclofen (5-20 mg/kg) failed to protect against tremors and tail arch in alcohol-withdrawn mice. The reason for these discrepancies at present escapes our understanding; it should be noted however that occurrence, intensity and frequency of alcohol withdrawal signs are secondary to a number of experimental variables, including animal species and strain, and procedures employed for inducing physical dependence upon alcohol.

With regard to the mechanism of action, a baclofen-induced inhibition of the glutamate hyperfunctionality associated to alcohol withdrawal syndrome can be proposed. The GABA_B receptor system is indeed believed

to exert an inhibitory action on the release of excitatory aminoacids, including glutamate (see Misgeld *et al.*, 1995). Further, alcohol withdrawal hyperexcitability has been repeatedly associated with an increased function of the *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamate neurotransmission (see Krystal *et al.*, 2003). Thus, baclofen-induced activation of GABA_B receptors in alcohol-withdrawn rats might counterbalance the enhanced function of glutamate excitatory neurotransmission, resulting in the observed attenuation of alcohol withdrawal syndrome.

Interestingly, a further piece of evidence on the involvement of the GABA_B receptor in the neural substrate underlying alcohol withdrawal syndrome may come from the results of a recent study demonstrating that the acute administration of non-convulsive doses of the GABA_B receptor antagonist, SCH 50911 [(+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid], resulted in a dramatic facilitation of spontaneous seizure occurrence in alcohol-dependent Wistar rats undergoing alcohol withdrawal syndrome at the time of SCH 50911 administration (Carai *et al.*, 2002).

CLINICAL STUDIES

Reducing Effect of Baclofen on Alcohol Consumption and Craving for Alcohol in Alcoholics

Baclofen (Lioresal®, Novartis) has been widely used for over 30 years as an antispastic agent. This long-term, extensive use in the clinical practice, together with its relative safety, have made it possible to verify whether the above preclinical data would generalize to human alcoholics.

A recently completed double-blind survey (Addolorato *et al.*, 2002a) evaluated the effect of baclofen on the consumption of alcoholic beverages and on craving for alcohol (i.e., the obsessive and compulsive desire to consume alcohol). Thirty-nine alcoholic patients, diagnosed according to DSM IV criteria (American Psychiatric Association, 1994), were recruited and randomized as follows: 20 patients received baclofen [mean age: 46±11 years; mean daily drinks: 18±7; mean years of alcohol addiction: 13±5 (mean±SEM)] and 19 patients received placebo (tablets of identical size, color, shape and taste to baclofen tablets) [mean age: 49±10 years; mean daily drinks: 11±7; mean years of alcohol addiction: 11±3 (mean±SEM)]. Major psychiatric and medical disorders represented exclusion criteria. Baclofen was given

orally at doses of 15 mg/day for the first 3 days and 30 mg/day thereafter, fractioned in 3 daily administrations, for 30 consecutive days. Routine psychological support counseling was also provided. Patients underwent weekly outpatient visits. At each visit, the following parameters were evaluated: a) alcohol intake (expressed as the number of daily drinks, where each drink was defined as an alcoholic beverage containing 12 g pure alcohol); b) duration of alcohol abstinence [measured by means of the Cumulative Abstinence Duration (CAD), i.e., the total number of days of abstinence]; c) alcohol craving score [evaluated by administration of the Obsessive Compulsive Drinking Scale (OCDS) (Anton *et al.*, 1995)].

The study results showed that a greater proportion of patients in the baclofen group, when compared to those of the placebo group, completed the study [17/20 (85%) vs 11/19 (58%)], and achieved and maintained a complete abstinence from alcohol throughout the 4-week period [14/20 (70%) vs 4/19 (21%)]. Accordingly, CAD was approximately three-fold higher in baclofen- than placebo-treated patients [20±3 vs 6±2 (mean±SEM)].

In the baclofen group, the mean number of daily drinks was virtually suppressed within the first week of treatment, being reduced by approximately 18 (value recorded at the last visit preceding the start of the drug administration) to less than 0.5 (value recorded at each weekly visit); in the placebo group, the daily drinks were reduced by an initial mean value of approximately 10 to 3.5-4.5 during treatment. OCDS score in the baclofen group was constantly lower than that monitored in the placebo group throughout the treatment period. Interestingly, a pronounced reduction was observed in both the Compulsive and Obsessive subscales of OCDS.

Tolerability was fair in all patients; the most common side-effects were sleepiness (2 patients), tiredness (one patient), vertigo (one patient) in the baclofen group and abdominal pain (one patient) in the placebo group, which resolved within 1-2 weeks of drug treatment and did not recur. On drug discontinuation, neither drug withdrawal syndrome nor side-effect due to drug suspension was observed.

In spite of the limitation due to the low number of patients evaluated and the shortness of the treatment period, the results of this preliminary double-blind study suggest that the administration of relatively low doses of baclofen to alcohol-dependent patients is more effective than placebo in a) inducing and maintaining abstinence from alcohol (both in terms of number of patients reaching complete abstinence and cumulative

abstinence duration), b) suppressing alcohol consumption, and c) reducing alcohol craving in both its "obsessive" and "compulsive" features. Importantly, under a therapeutic point of view, baclofen-induced abstinence and reduction in alcohol consumption and craving score were achieved within the first week of treatment.

Suppressing Effect of Baclofen on Alcohol Withdrawal Symptomatology in Alcoholics

A separate, open-label study (Addolorato *et al.*, 2002b) assessed the effect of baclofen on the severity of alcohol withdrawal syndrome. Five alcoholic patients, diagnosed according to DSM IV criteria (American Psychiatric Association, 1994) and showing a score of alcohol withdrawal syndrome - according to the Clinical Institute Withdrawal Assessment for Alcohol-revised (CIWA-r) scale (Sullivan *et al.*, 1989) - higher than 20 (requiring therefore pharmacological treatment), were recruited. Major psychiatric and medical disorders as well as previous occurrence of delirium represented exclusion criteria. Initially, baclofen was given orally at the single dose of 10 mg. Subsequently, patients were treated with 10 mg baclofen every 8 hours for 4 weeks. At the time of the first baclofen administration, blood alcohol levels were lower than 0.1 g/l in all patients. The severity of alcohol withdrawal syndrome was evaluated every hour for the first 4-8 consecutive hours, every day for the first week, and once a week for the following 3 weeks.

According to the results of the study in alcohol-dependent rats (FIG. 5), baclofen induced a rapid and complete disappearance of alcohol withdrawal symptomatology in all patients. Specifically, the initial administration of a relatively low dose of baclofen resulted, within 1 to 3 hours, in the suppression of the CIWA-r score. Importantly, all patients reported a rapid elevation of general sensation of well-being and improvement of psychological distress. Throughout the 30-day outpatient follow-up, all patients were asymptomatic and remained abstinent from alcohol. They also manifested a high degree of satisfaction with the treatment.

More recently, Addolorato *et al.* (2003) reported that baclofen was also effective in rapidly suppressing delirium tremens associated to alcohol withdrawal.

CONCLUSIONS

Pharmacological stimulation of the GABA_B receptor by baclofen and CGP 44532 resulted in a marked and selective decrease in voluntary alcohol intake, under

experimental procedures proposed to model different aspects of human alcoholism, in alcohol-preferring rats of the sP line, as well as amelioration of the signs of alcohol withdrawal syndrome in Wistar rats made physically dependent upon alcohol. The reduction in alcohol intake, which occurred at doses of baclofen and CGP 44532 devoid of any sedative effect, was likely secondary to a decrease in the appetitive or motivational attributes of alcohol. Indeed, baclofen was found to completely suppress extinction responding for alcohol, a reliable index of the motivation to consume alcohol, in sP rats. These results extend to alcohol the anti-motivational properties of GABA_B receptor agonists, since it has been repeatedly shown that baclofen and CGP 44532 are capable of suppressing the self-administration of different drugs of abuse, including cocaine, heroin and nicotine, in rodents.

These results apparently generalize to human alcoholics. The two clinical studies conducted to date do not allow definitive conclusions to be drawn, because of the relatively low number of patients recruited and the short duration of drug treatment and follow-up. However, these appear to be convincing enough to promote replication of these studies on a larger scale and to consider baclofen a promising, novel pharmacotherapy for the treatment of alcohol dependence.

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References

- Addolorato G, F Caputo, E Capristo, M Domenicali, M Bernardi, L Janiri, R Agabio, G Colombo, GL Gessa and G Gasbarrini (2002a) Baclofen efficacy in reducing alcohol craving and intake - a preliminary double-blind randomised controlled study. *Alcohol Alcohol.* **37**, 504-508.
- Addolorato G, F Caputo, E Capristo, L Janiri, M Bernardi, R Agabio, G Colombo, GL Gessa and G Gasbarrini (2002b) Rapid suppression of alcohol withdrawal syndrome by baclofen. *Am. J. Med.* **112**, 226-229.
- Addolorato G, L Leggio, L Abenavoli, G DeLorenzi, A Parente, F Caputo, L Janiri, E Capristo, GL Rapaccini and G Gasbarrini (2003) Suppression of alcohol delirium tremens by baclofen administration: a case report. *Clin. Neuropharmacol.* **26**, 258-262.
- Agabio R, G Cortis, F Fadda, GL Gessa, C Lobina, R Reali and G Colombo (1996) Circadian drinking pattern of Sardinian alcohol-preferring rats. *Alcohol Alcohol.* **31**, 385-388.
- Agabio R, MAM Carai C Lobina, M Pani, R Reali, G Vacca, GL Gessa and G Colombo (2000) Development of short-lasting alcohol deprivation effect (ADE) in Sardinian alcohol-preferring rats. *Alcohol* **21**, 59-62.

- American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders, 4th Ed.* (American Psychiatric Association, Washington, DC, USA).
- Anstrom KK, HC Cromwell, T Markowski and DJ Woodward (2003) Effect of baclofen on alcohol and sucrose self-administration in rats. *Alcohol. Clin. Exp. Res.* **27**, 900-908.
- Anton RF, DH Moak and P Latham (1995) The Obsessive Compulsive Drinking Scale: a self-rated instrument for the quantification of thoughts about alcohol and drinking behavior. *Alcohol. Clin. Exp. Res.* **19**, 92-99.
- Boening JA-L, OM Lesch, R Spanagel, J Wolffgramm, M Narita, D Sinclair, BJ Mason and GA Wiesbeck (2001) Pharmacological relapse prevention in alcohol dependence: from animal models to clinical trials. *Alcohol. Clin. Exp. Res.* **25**, 127S-131S.
- Bowery NG, AL Hudson and GW Price (1987) GABA_A and GABA_B receptor site distribution in the rat central nervous system. *Neuroscience* **20**, 365-383.
- Carai MAM, G Brunetti, C Lobina, S Serra, G Vacca, G Minardi, G Colombo and GL Gessa (2002) Proconvulsive effect of the GABA_B receptor antagonist, SCH 50911, in rats undergoing ethanol withdrawal syndrome. *Eur. J. Pharmacol.* **445**, 195-199.
- Colombo G, R Agabio, C Lobina, R Reali, A Zocchi, F Fadda and GL Gessa (1995) Sardinian alcohol-preferring rats: a genetic animal model of anxiety. *Physiol. Behav.* **57**, 1181-1185.
- Colombo G, R Agabio, C Lobina, R Reali, G Vacca and GL Gessa (1998) Stimulation of locomotor activity by voluntarily consumed ethanol in Sardinian alcohol-preferring rats. *Eur. J. Pharmacol.* **357**, 109-113.
- Colombo G, R Agabio, MAM Carai, C Lobina, M Pani, R Reali, G Addolorato and GL Gessa (2000) Ability of baclofen in reducing alcohol intake and withdrawal severity: I - preclinical evidence. *Alcohol. Clin. Exp. Res.* **24**, 58-66.
- Colombo G, S Serra, G Brunetti, G Atzori, M Pani, G Vacca, G Addolorato, W Froestl, MAM Carai and GL Gessa (2002a) The GABA_B receptor agonists baclofen and CGP 44532 prevent acquisition of alcohol drinking behaviour in alcohol-preferring rats. *Alcohol Alcohol.* **37**, 499-503.
- Colombo G, S Serra, G Brunetti, R Gomez, S Melis, G Vacca, MAM Carai and GL Gessa (2002b) Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. *Psychopharmacology (Berl.)* **159**, 181-187.
- Colombo G, S Serra, G Brunetti, G Vacca, MAM Carai and GL Gessa (2003a) Suppression by baclofen of alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. *Drug Alcohol Depend.* **70**, 105-108.
- Colombo G, G Vacca, S Serra, G Brunetti, MAM Carai and GL Gessa (2003b) Baclofen suppresses motivation to consume alcohol in rats. *Psychopharmacology (Berl.)* **167**, 221-224.
- Cousins MS, DCS Roberts and H de Wit (2002) GABA_B receptor agonists for the treatment of drug addiction: a review of recent findings. *Drug Alcohol Depend.* **65**, 209-220.
- Czachowski CL, BH Legg and KH Stansfield (2002) Effects of the GABA(B) agonist, baclofen, on ethanol- and sucrose-seeking and self-administration. *Alcohol. Clin. Exp. Res.* **26**, 115A.
- Daoust M, C Saligaut, JP Lhuintre, N Moore, JL Flipo and F Boismare (1987) GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. *Alcohol* **4**, 469-472.
- Di Chiara G (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. *Drug Alcohol Depend.* **38**, 95-137.
- Fadda P, M Scherma, A Fresu, M Collu and W Fratta (2003) Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. *Synapse* **50**, 1-6.
- File SE, A Zarkovsky and K Gulati (1991) Effects of baclofen and nitrendipine on ethanol withdrawal responses in the rat. *Neuropharmacology* **30**, 183-190.
- Humeniuk RE, JM White and J Ong (1994) The effects of GABA_B ligands on alcohol withdrawal in mice. *Pharmacol. Biochem. Behav.* **49**, 561-566.
- Janak PH and TM Gill (2003) Comparison of the effects of allopregnanolone with direct GABAergic agonists on ethanol self-administration with and without concurrently available sucrose. *Alcohol* **30**, 1-7.
- Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. *Brain Res. Rev.* **18**, 75-113.
- Krystal JH, IL Petrakis, G Mason, L Trevisan and DC D'Souza (2003) N-methyl-D-aspartate glutamate receptors and alcoholism: reward, dependence, treatment, and vulnerability. *Pharmacol. Ther.* **99**, 79-94.
- Lal H, CM Harris, D Benjamin, AC Springfield, S Bhadra and MW Emmett-Oglesby (1988) Characterization of a pentylentetrazol-like interoceptive stimulus produced by ethanol withdrawal. *J. Pharm. Exp. Ther.* **247**, 508-518.
- Majchrowicz E (1975) Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia (Berl.)* **43**, 245-254.
- McBride WJ, A-D Le and A Noronha (2002) Central nervous system mechanisms in alcohol relapse. *Alcohol. Clin. Exp. Res.* **26**, 280-286.
- Melendez RI, ZA Rodd-Henricks, EA Engleman, T-K Li, WJ McBride and JM Murphy (2002) Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. *Alcohol. Clin. Exp. Res.* **26**, 318-325.
- Misgeld U, M Bijak and W Jarolimek (1995) A physiological role for GABA_B receptor and the effects of baclofen in the mammalian central nervous system. *Prog. Neurobiol.* **46**, 423-462.
- Perfumi M, M Santoni, R Ciccocioppo and M Massi (2002) Blockade of γ -aminobutyric acid receptors does not modify the inhibition of ethanol intake induced by *Hypericum perforatum* in rats. *Alcohol Alcohol.* **37**, 540-546.
- Petry NM (1997) Benzodiazepine-GABA modulation of concurrent ethanol and sucrose reinforcement in the rat. *Exp. Clin. Psychopharmacol.* **5**, 183-194.
- Samson HH, A Chappell, C Czachowski and A Sharpe (2001) Measuring ethanol-seeking behavior: the effect of using repeated extinction trials. *Alcohol* **24**, 205-209.
- Samson HH, CL Czachowski, A Chappell and B Legg (2003) Measuring the appetite strength of ethanol: use of an extinction trial procedure. *Alcohol* **31**, 77-86.
- Smith BR, J Robidoux and Z Amit (1992) GABAergic involvement in the acquisition of voluntary ethanol intake in laboratory rats. *Alcohol Alcohol.* **27**, 227-231.
- Smith BR, AEL Boyle and Z Amit (1999) The effects of GABA_B agonist baclofen on the temporal and structural characteristics of ethanol intake. *Alcohol* **17**, 231-240.
- Spanagel R and F Weiss (1999) The dopamine hypothesis of reward: past and current status. *Trends Neuroscience* **22**, 521-527.
- Sullivan JT, K Sykora, J Schneiderman, CA Naranjo and EM Sellers (1989) Assessment of alcohol withdrawal: the revised Clinical Institute Withdrawal Assessment for Alcohol scale (CIWA-Ar). *Br. J. Addiction* **84**, 1353-1357.
- Tomkins DM and PJ Fletcher (1996) Evidence that GABA_A but not

- GABA_B receptor activation in the dorsal raphe nucleus modulates ethanol intake in Wistar rats. *Behav. Pharmacol.* **7**, 85-93.
- Vacca G, S Serra, G Brunetti, MAM Carai, HH Samson, GL Gessa and G Colombo (2002) Operant self-administration of ethanol in Sardinian alcohol-preferring rats. *Alcohol. Clin. Exp. Res.* **26**, 1678-1685.
- Weiss F and LJ Porrino (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. *J. Neurosci.* **22**, 3332-3337.
- Weiss F, MT Lorang, FE Bloom and GF Koob (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *J. Pharm. Exp. Ther.* **267**, 250-258.
- Westerink BHC, H-F Kwint and JB deVries (1996) The pharmacology of mesolimbic dopamine neurons: a dual-probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. *J. Neurosci.* **16**, 2605-2611.
- Wise RA and MA Bozarth (1987) A psychomotor stimulant theory of addiction. *Psychol. Rev.* **94**, 469-492.
- Yoshida M, H Yokoo, T Tanaka, H Emoto and M Tanaka (1994) Opposite changes in the mesolimbic metabolism in the nerve terminal and cell body sites induced by locally infused in the rat. *Brain Res.* **636**, 111-114.
- Xi Z-X and EA Stein (1999) Baclofen inhibits heroin self-administration behavior and mesolimbic dopamine release. *J. Pharm. Exp. Ther.* **290**, 1369-1374.