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Investigation of allyphenyline efficacy in the treatment of alcohol withdrawal symptoms

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ABSTRACT

We have recently demonstrated that allyphenyline, behaving as α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor agonist and α_{2A}-adrenoceptor antagonist, in mice enhanced morphine analgesia, attenuated morphine withdrawal symptoms, showed significant antidepressant-like activity and was devoid of sedative side effects. Opioid and alcohol withdrawal shares several common neurobiological and molecular mechanisms. Therefore, in this study we expanded our analysis of the pharmacological properties of allyphenyline by investigating its ability to prevent the expression of somatic withdrawal signs, anxiety-like behavior and hyperlocomotion associated with chronic ethanol intoxication. Rats were subjected to induction of ethanol dependence via repeated daily intragastric ethanol (20%) administration for 4 consecutive days. Twelve hours after the last alcohol administration, somatic alcohol withdrawal signs were scored. Results revealed a significant expression of physical withdrawal signs that were not affected by intraperitoneal (i.p.) administration of allyphenyline at the doses of 0.05, 0.275 and 0.5 mg/kg. In contrast, allyphenyline (0.05 and 0.275 mg/kg i.p.) significantly reduced hyperanxiety-like behavior observed 6 days after alcohol intoxication as measured using the defensive burying test. Allyphenyline also reduced open field hyperlocomotor activity associated with alcohol withdrawal. Notably, the anxiolytic effect of the compound, as well as the already reported antidepressant action, was observed at very low doses, suggesting the involvement of its α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor agonism. Therefore, the present investigation suggests that allyphenyline might represent an interesting pharmacological tool to investigate the potential of compounds exhibiting α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor agonism and α_{2A}-adrenoceptor antagonism in the treatment of hyperanxiety and hyperlocomotion occurring during alcohol withdrawal in dependent subjects.

1. Introduction

Alcoholism is a chronic relapsing disease characterized by withdrawal symptoms emerging after cessation of drinking (Koob, 2008). Withdrawal syndrome consists of physical as well as emotional and affective symptoms, such as anxiety and depressed mood (Bokström et al., 1989, 1991; Roelofs, 1985; Roelofs and Dikkenberg, 1987).

A large body of evidence indicated that negative emotional symptoms are strongly correlated to relapse to compulsive drinking, which can occur after few days, but also after months or years of abstinence (Annis et al., 1998; Cloninger, 1987; De Soto et al., 1989; Hershon, 1977; Miller and Harris, 2000; Parsons et al., 1990; Willinger et al., 2002). Consistently, laboratory animal studies have revealed increased anxiety-like responses during protracted withdrawal (Valdez et al., 2003; Cippitelli et al., 2008; Braconi et al., 2010). The use of anxiolytics is an adjuvant therapy in the treatment of alcoholism and benzodiazepines are commonly utilized to treat withdrawal symptoms. However, benzodiazepines themselves are intoxicating, because they enhance the palatability of ethanol and increase alcohol consumption (Soderpalm and Hansen, 1998). Hence, their use in alcoholic patients is questionable.

An alternative approach in alcohol withdrawal treatment consists of the use of α_2-adrenoceptor agonists such as clonidine or guanfacine (Ungur et al., 2013; Muzyk et al., 2011). These compounds are beneficial not only in the treatment of alcohol withdrawal symptoms but also in preventing recidivism to drug use associated with stress or cue exposure in animal models and in humans (Fox et al., 2014; Smith and Aston-Jones, 2011; Erb et al., 2000; Lê et al., 2011). Nevertheless, the major problems associated with the use of non subtype selective α_2-adrenoceptor agonists are hypotension and sedation side effects.

We recently synthesized allyphenyline, an advantageous compound able to decrease the expression of morphine withdrawal

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symptoms in opioid dependent mice and endowed with antidepressant-like properties (Del Bello et al., 2010, 2012).

Opioid and alcohol dependence shares several common features. For instance, for both cases, abstinence symptoms not only consist of somatic signs but also negative affective states like hyperanxiety and depression.

Based on by these considerations, in the present study we examined the potential of allyphenyline to modulate the expression of somatic signs, anxiety-like behavior and hyperlocomotor activity associated with ethanol withdrawal in alcohol dependent rats.

2. Materials and methods

2.1. Drug

Allyphenyline (2-(1-(2-allylphenoxy)ethyl)-4,5-dihydro-1H-imidazole) (Gentili et al., 2008) was obtained from 2-(2-allylphenoxy)propanenitrile (Voronina et al., 1984) by treatment with ethylenediamine in the presence of sodium methoxide, as reported in Fig. 1.

2.2. Allyphenyline solution preparation

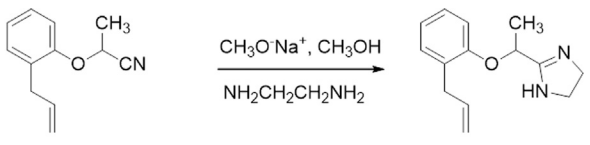
Allyphenyline was dissolved in saline and administered intraperitoneally (i.p.) at doses of 0.05, 0.275 and 0.5 mg/kg/ml. The same vehicle (1 ml/kg of saline) was administered to the control group (Veh).

2.3. Animals

Male Wistar rats (Charles River, Calco, Italy), weighting 250–300 g at the beginning of experiments, were housed two per cage on a reversed 12 h light/dark cycle (light off at 10:00 h) in a temperature- and humidity-controlled vivarium. Rats were handled daily for 5 min during the first week after arrival and had ad libitum access to standard rat chow and water throughout the course of the study. All alcohol intragastric intubation, defensive burying test and open field sessions were initiated during the dark cycle. All procedures were conducted in accordance with the National Institutes of Health Guide and the European Community Directive for the Care and Use of Laboratory Animals.

2.4. Ethanol solution preparation

Ethanol intubation solution (final concentration, 20% w/v) was prepared by diluting 95% ethanol in a solution consisting of powdered milk (baby formula), sucrose, and water. Specifically, 1 l of solution contained 166 g powdered milk, 60 g sucrose, 211 ml 95% ethanol, and 250 ml water. The solution was gently warmed and stirred until the powdered milk and sucrose were completely dissolved. Water was then added to a final volume of



2-(2-Allylphenoxy)propanenitrile Allyphenyline

Fig. 1. Synthesis of Allyphenyline (50% yield). The free base was transformed into the oxalate salt; which was recrystallized from 2-PrOH: mp 154–155 °C. 1 H NMR (DMSO): δ 1.53 (d, 3, CH_3), 3.42 (m, 2, CH_2CH), 3.88 (s, 4, NCH_2CH_2N), 5.06 (dd, 2, $CH=CH_2$), 5.40 (q, 1, OCH), 5.95 (m, 1, $CH=CH_2$), 6.92–7.26 (m, 4, ArH), 7.81 (br s, 1, NH, exchangeable with D_2O). Anal. Calcd for $C_{14}H_{18}N_2O\ H_2C_2O_4$: C, 56.80; H, 6.55; N, 8.28. Found: C, 56.69; H, 6.71; N, 8.13.

1 l (Braconi et al., 2010). The dietary liquid vehicle (powdered milk for newborn) was used to reduce the incidence of gastrointestinal irritations that could influence behavioral measure. Moreover, the dietary liquid provides the nutritional elements to the animals that present a compromised nutritional status caused by the alcohol treatment.

Preparation of the vehicle solution was identical, with the exception that ethanol was substituted with an equicaloric dose of sucrose. All solutions were freshly prepared daily and administered by intragastric intubation via a standard 10 ml syringe equipped with polyethylene 50 tubing (5–6 cm length) connected to the tip of a blunted 18-gauge needle

2.5. Intragastric intubation procedure

Rats were subjected to induction of ethanol dependence (ethanol group, $n=12$) via repeated intragastric ethanol intubation. The day before the beginning of intoxication an intragastric administration of water by gavage took place, in order to accustom the animals to the experimental procedure. For alcohol intoxication the 4-day binge treatment developed by Majchrowicz, 1975, was used. Ethanol was administered three times per day for four consecutive days. Rats were treated with three fractional doses of ethanol administered at 8 h intervals. Rats serving as controls (vehicle group, $n=8$) received intragastric administration of vehicle (milk) in a total volume identical to those in ethanol-treated rats. All rats were weighted daily.

2.6. Blood alcohol levels

Tail blood (approximately 200 μl) was collected on days 3 and 4, 1 h after the last daily dose of ethanol. Samples were collected on ice and then immediately centrifuged (10 min, 5000 rpm). Ethanol content was then assayed from 5 μl plasma aliquots using an oxygen-rate alcohol analyzer (Analox Instruments, Lunenburg, MA).

2.7. Somatic ethanol withdrawal signs

Rats were examined for physical signs of withdrawal 12 h after the last ethanol intubation (Majchrowicz, 1975) by an experimenter blind to treatment conditions. Using a withdrawal rating scale adapted from Macey et al. (1996), somatic ethanol withdrawal signs, including ventro-medial limb retraction, irritability to touch (vocalization), tail rigidity, and body tremors, were scored. To each sign was assigned a score of 0–2, based on the following severity scale: 0=no sign, 1=moderate, 2=severe. The sum of the four observation scores (0–8) was used as a quantitative measure of withdrawal severity. For these behavioral observations, animals were individually transferred from their home cages to a quiet observation room to avoid excessive stimulation.

2.8. Defensive burying

This test is used to measure anxiety-like behavior following a single shock from a novel object (De Boer and Koolhaas, 2003). The defensive burying apparatus is a modified home cage with 4 cm wood chip bedding material evenly distributed throughout the cage. One end of the cage contained a 0.75 cm hole through which a shock probe was inserted into the cage. A constant current generator was connected to the shock probe and delivered a shock of 1.5 mA upon contact with the probe. Each animal was placed individually into the testing apparatus facing away from the shock probe for a 15 min test. At the end of the session test, the apparatus was cleaned and the new bedding was placed into the test cage for the next rat. All behavioral testing sessions were monitored for later analysis of (i) latency to begin burying after

contact with the shock probe, and (ii) duration of burying. Burying was defined as any spraying or throwing of the bedding with the head or paws in the direction of the shock probe. Increased duration of burying and decreased latency to burying indicate an increase in rats anxiety.

2.9. Open field test

This test is used to measure locomotor activity of animals. The open field apparatus is an automated locomotor activity box (Med Associates, VT 05478) as previously reported (Nasuti et al., 2008). Locomotor activity was recorded for 5 min, starting 1 min after placing the animal in the test cage. Each rat was automatically recorded by interruptions of two orthogonal light beams (3.5 cm and 13.0 cm above the activity box floor), which were connected to automatic software (Activity Monitor, Med Associates). The behavioral parameters observed were ambulatory counts (number of horizontal episodes).

2.10. Experiment 1: effect of allyphenyline treatment on the expression of somatic alcohol withdrawal signs

To evaluate the effect of allyphenyline on alcohol withdrawal signs, animals ($n=46$) were divided into 4 groups at the end of the intoxication cycle 12 h after the last ethanol (or vehicle) dose and received allyphenyline at doses of 0.0, 0.05, 0.275 and 0.5 mg/kg (i.p.) 30 min before behavioral measures of ethanol withdrawal. A fifth group of rats ($n=8$) was prepared in parallel and served as a control. This group received alcohol vehicle (milk) during the intoxication phase and allyphenyline vehicle (saline) injections prior to testing.

2.11. Experiment 2: effect of allyphenyline treatment on anxiety-like behavior following chronic ethanol intoxication

Alcohol withdrawal induced anxiety was tested by many authors at different time points ranging from 2 h till 6 weeks into withdrawal with various regimen of alcohol exposure (Sharma et al., 2007, 2014; Kliethermes, 2005). The procedure of binge alcohol exposure used in this study elicits anxiety symptoms about 6 days into withdrawal as showed by defensive burying and elevated plus maze tests (Ruggeri et al., 2010; Economidou et al., 2011; Lal et al., 1993 Prather et al., 1991; Aujla et al., 2013).

In the present investigation, to evaluate the effect of allyphenyline on alcohol-induced anxiety, animals ($n=46$) were divided into 3 groups 6 days after the last alcohol administration and received allyphenyline at doses of 0.0, 0.05 and 0.275 mg/kg (i.p.) 30 min before anxiety measures on the defensive burying. A fourth group of rats ($n=8$) was prepared in parallel and served as a control. This group received alcohol vehicle (milk) during the intoxication phase and allyphenyline vehicle (saline) injections prior to testing.

An additional test was carried out to assess anxiety behavior in rats treated with allyphenyline without a prior exposition to alcohol. One group of rats ($n=6$) received allyphenyline (i.p.) at the dose of 0.275 mg/kg 30 min prior to test anxiety-like behavior while another group ($n=6$) received vehicle and served as control.

2.12. Experiment 3: effect of allyphenyline treatment on locomotor activity following chronic ethanol intoxication

To evaluate the effect of allyphenyline on locomotor activity, animals ($n=46$) were divided into 2 groups at the end of the intoxication cycle and received allyphenyline at doses of 0.0 and 0.275 mg/kg (i.p.) 30 min before behavioral measures of open field that were carried out 6 days after the last ethanol (or vehicle) dose. A third group of rats ($n=8$) was prepared in parallel and served as a control. This group received alcohol vehicle (milk) during the intoxication phase and allyphenyline vehicle (saline) injections prior to testing.

To test the effect of allyphenyline on locomotor activity in naïve rats, an other group of rats ($n=6$) injected with the drug (0.275 mg/kg) and a control group injected with vehicle 30 min before the open field were used.

2.13. Statistical analysis

Results are expressed as mean $\pm$ $\pm$ S.E.M. Alcohol withdrawal signs**,** defensive burying and open field data were analyzed by two-way between-subjects Analysis of Variance (ANOVA). Significant main effects or interactions were confirmed by Newman-Keuls post-hoc tests. Experiments involving two groups such as open field and defensive burying of naïve wistar rats were analyzed by means of Student's *t*-test. Statistical significance was set at $P<0.05$.

3. Results

3.1. Blood alcohol levels

On days 3 and 4, blood alcohol levels, measured 1 h after the third and the fourth daily doses, were 347.92 ± 7.87 and 301.83 ± 11.14 mg/dl, respectively (Table 1). This indicates that, during alcohol administration, blood alcohol levels were maintained at intoxication levels (Braconi et al., 2010).

3.2. Experiment 1: effect of allyphenyline treatment on the expression of somatic alcohol withdrawal signs

According to what previously demonstrated (Majchrowicz, 1975; Ruggeri et al., 2010; Economidou et al., 2011; Faingold, 2008), 12 h after the last alcohol administration, rats showed a significant withdrawal score. Analysis of variance demonstrated a significant overall difference between vehicle and alcohol-treated rats [$F(1,24)=17.47$]. Post-hoc comparisons revealed a significant difference for all scored physical withdrawal signs ($P<0.01$) between alcohol intoxicated vs. non intoxicated rats.

When post-hoc tests were used to compare the withdrawal scores of intoxicated rats treated with allyphenyline (0.05, 0.275 and 0.5 mg/kg) vs intoxicated controls no difference was observed indicating a lack of effect of allyphenyline (Fig. 2). Since the higher dose of allyphenyline (0.5 mg/kg) also induced some irritability in the rats, it has been discarded in the successive experiments.

3.3. Experiment 2: effect of allyphenyline treatment on anxiety-like behavior following chronic ethanol intoxication

A four-days ethanol intoxication elicited, after 6 days, a marked anxiogenic-like response in the defensive burying test (Fig. 3), resulting in a significant increased duration of burying and decreased latency to burying in ethanol exposed rats ([F(3.28)=

Table 1
Blood alcohol levels measured on days 3 and 4, 1 h after the last daily dose of ethanol or vehicle (Veh) in rats subjected to the ethanol chronic intoxication procedure.

	Treatment	Alcohol (g/kg/day)	Blood alcohol level (mg/dl)
Day-3	EtOH	11	347.92 ± 7.87
	Veh	–	0.0
Day-4	EtOH	11	301.83 ± 11.14
	Veh	–	0.0

5.0, $P<0.01$] and [F(3.24)=4.39, $P<0.05$], respectively). Post-hoc analysis demonstrated a significant increase in duration ($P<0.05$) and a trend to a decrement in latency to burying in ethanol-treated rats compared to vehicle-treated controls. These effects were completely reversed by allyphenyline and post-hoc analyses showed a significant lower anxiety in alcohol intoxicated rats treated with allyphenyline (0.05 or 0.275 mg/kg) as compared to rats treated with drug vehicle (Fig. 3 A and B). The comparable anxyolitic effect induced by 0.05 and 0.275 mg/kg doses of allyphenyline in the defensive burying test prompted us to consider only one of them (0.275 mg/kg) in the successive experiments.

In ethanol free rats, allyphenyline (0.275 mg/kg) sligthly decreased the duration of burying and increased the latency to burying (Fig. 4 A and B). However, the analysis showed no statistically significant differences.

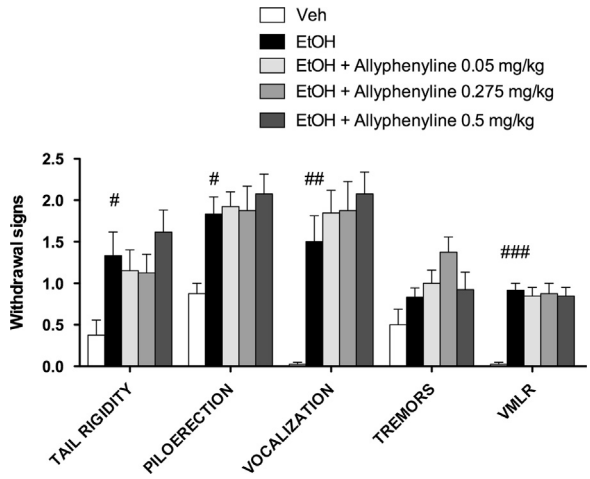


Fig. 2. Somatic withdrawal signs measured 12 h after the last ethanol administration. Rats were treated i.p. with allyphenyline 30 min before behavioral assessment of ethanol withdrawal ($n=6$ rats/group). Control non intoxicated rats (Veh) received allyphenyline and alcohol vehicles. VMLR, ventro-medial limb retraction. Data, analyzed by 2-way ANOVA followed by Newman–Keuls post-hoc test, represent mean (±S.E.M.) values. $^{\#}P<0.05$ $^{\#\#}P<0.01$ $^{\#\#\#}P<0.001$ significant difference from vehicle.

3.4. Experiment 3: effect of allyphenyline treatment on locomotor activity following chronic ethanol intoxication

For the ambulatory counts registered in the open field test, ANOVA revealed a significant overall difference ([F(2.21)=3.48, $P<0.05$]). Newman Keuls post-hoc tests showed that post dependent rats had significant higher locomotor activity on horizontal plane than non dependent controls ($P<0.05$). At the dose of 0.275 mg/kg allyphenyline significantly decreased ($P<0.05$) the ambulatory counts in post dependent rats (Fig. 5).

Nevertheless, at the same dose, it did not modify the locomotory activity of naïve wistar rats compared with vehicle-injected controls (Fig. 6).

4. Discussion

Allyphenyline, behaving as α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor agonist and as α_{2A}-adrenoceptor antagonist, is endowed with uncommon and interesting in vitro biological profile (Cardinaletti et al., 2009; Del Bello et al., 2012).

As known (Tan and Limbird, 2006), the α_{2C}-adrenoceptor is one of the three subtypes (namely α_{2A}, α_{2B}, and α_{2C}) of α_2 G-protein-coupled receptor superfamily. It is involved in many central nervous system processes, such as the startle reflex, stress responses, and control of locomotion as well as feedback inhibition of cathecolamine release. In addition, the α_{2C} subtype can contribute to spinal α_2-agonist mediated analgesia and adrenergic-opioid synergy. The α_{2A} adrenoceptor subtype mediates hypotension, sedation, and analgesia, as well as inhibition of monoamine release and metabolism in the brain. Moreover, α_{2A}-, and α_{2C}-adrenoceptors, as heteroreceptors, inhibit dopamine and serotonin release.

It has been reported that overactive signaling associated with α_{2C}-adrenoceptor overexpression leads to a depressive and anxious-like state (Tan and Limbird, 2006). Nevertheless, it has also been observed that α_{2A}- and α_{2C}-adrenoceptors can complement each other to integrate central nervous system functions and behavior (Fairbanks et al., 2009; Hein, 2006).

Among the serotonin 5-HT receptors, serotonin 5-HT_{1A} subtype has been the first to be pharmacologically characterized. Although its structure is not known, mutagenesis studies allowed the identification of amino acid residues responsible for ligand binding and G-protein coupling (Kalipatnapu and Chattopadhyay,

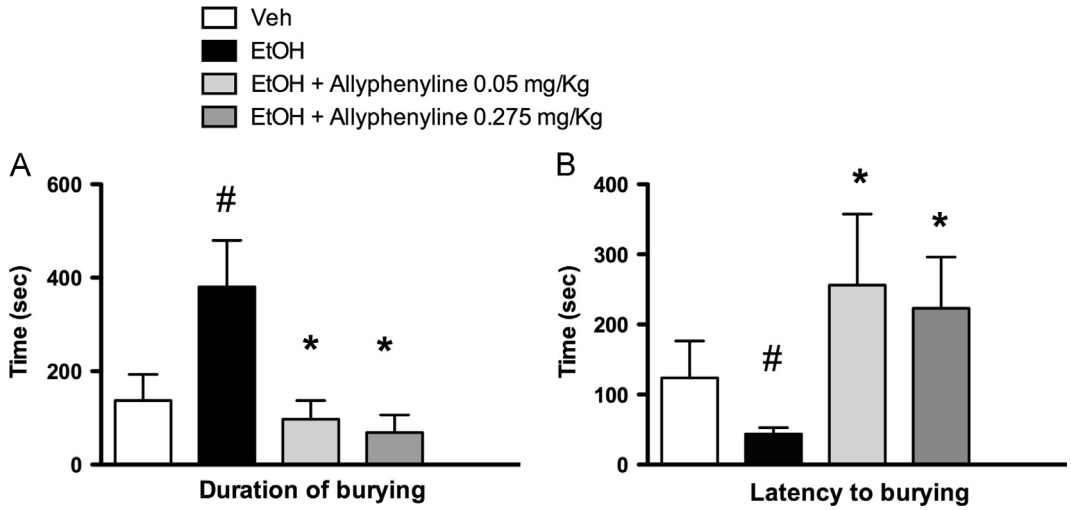


Fig. 3. Effect of allyphenyline (0.05 and 0.275 mg/kg, i.p.) or its vehicle (0.0) in post dependent rats as compared to non dependent controls (Veh) on (A) the duration of burying and (B) the latency to contact the shock probe 6 days following withdrawal from ethanol intoxication ($n=6$ rats/group). Control group (non intoxicated rats) received allyphenyline and alcohol vehicles. Data, analyzed by 2-way ANOVA followed by Newman-Keuls post-hoc test, represent mean (±S.E.M.) values. $^{\#}P<0.05$ significant difference from vehicle. $^{*}P<0.05$ significant difference from EtOH.

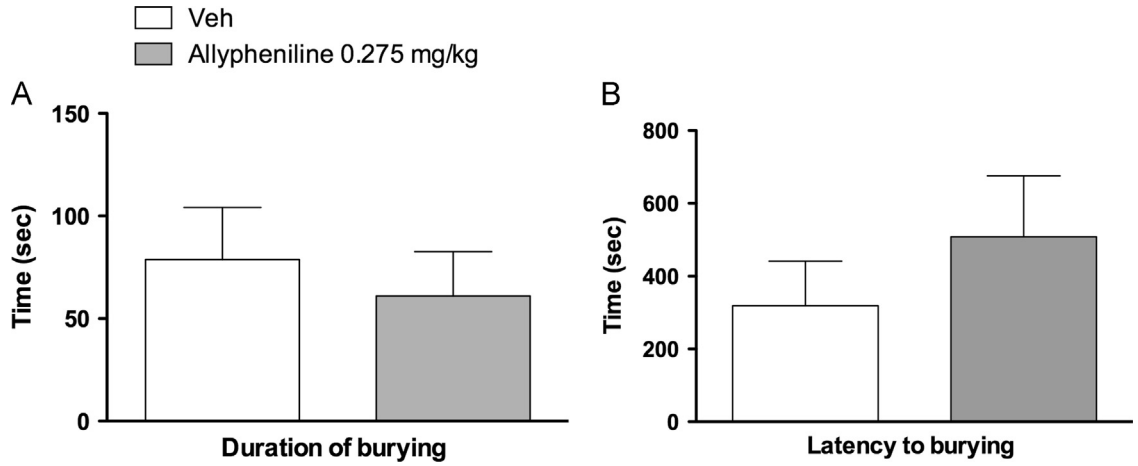


Fig. 4. Effect of allyphenyline (0.275 mg/kg, i.p.) or its vehicle (0.0) in wistar rats as compared to controls (Veh) tested on (A) the duration of burying and (B) the latency to contact the shock probe ($n=6$ rats/group). Data, analyzed by Student's t-test, represent mean (± S.E.M.) values.

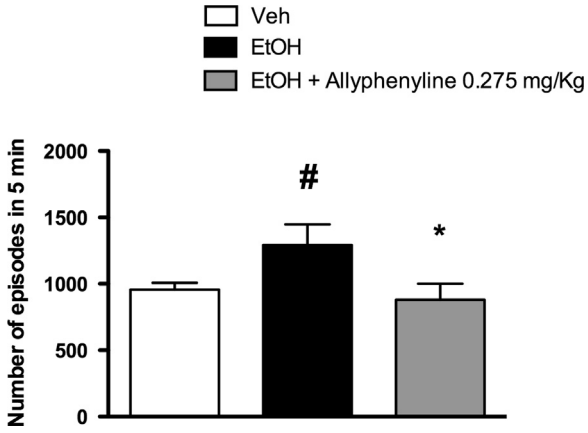


Fig. 5. Effect of allyphenyline (0.275 mg/kg, i.p.) or its vehicle (0.0) on post dependent rats and non dependent controls tested in the open field 6 days into alcohol withdrawal ($n=6$ rats/group). Control non intoxicated rats (Veh) received allyphenyline and alcohol vehicles. Data, analyzed by 2-way ANOVA followed by Newman-Keuls post-hoc test, represent mean (± S.E.M.) values. $^{\#}P<0.05$ significant difference from Veh; $^{*}P<0.05$ significant difference from EtOH.

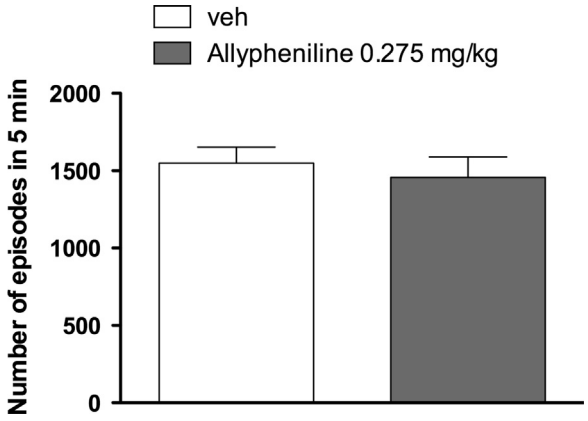


Fig. 6. Effect of allyphenyline (0.275 mg/kg, i.p.) or its vehicle (0.0) in wistar rats as compared to controls (Veh) tested in the open field ($n=6$ rats/group). Data, analyzed by Student's t-test, represent mean (± S.E.M.) values.

2007). Serotonin 5-HT_{1A} receptors are distributed throughout the central nervous system and are implicated in the pathogenesis of depressive disorders and anxiety. Mice with inactivated serotonin 5-HT_{1A} receptor gene develop an anxiety-like phenotype, whereas mice with overexpressed serotonin 5-HT_{1A} receptor display diminished anxiety when compared to wild-type animals (Bordukalo-Niksic et al., 2010).

Serotonin and norepineprine, both involved in psychiatric morbidities, have been proposed to play an important role in drug addiction (Weinshenker and Schroeder, 2007). Our recent studies demonstrated that allyphenyline enhanced morphine analgesia (due to its α_{2C}-adrenoceptor agonism), was devoid of sedative effect (due to its α_{2A}-adrenoceptor antagonism) (Cardinaletti et al., 2009), contrasted and prevented morphine tolerance and dependence at very low dose (0.05 mg/kg) (Del Bello et al., 2010). In addition, importantly, allyphenyline induced significant reduction of immobility time in the mouse forced swimming test that is consistent with an antidepressant-like effect (Del Bello et al., 2012). This effect was maximal at the dose of 0.05 mg/kg and comparable to that obtained with 20 mg/kg of the clinically used serotonin reuptake inhibitor fluoxetine (Page et al., 1999). The experiments carried out in the presence of the serotonin 5-HT_{1A} receptor antagonist WAY 100135 and the α_2-adrenoceptor antagonist yohimbine confirmed that dual α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor activation took part in the observed anti-depressant effect (Del Bello et al., 2012).

This background prompted us to examine the ability of allyphenyline to modulate the expression of somatic signs, anxiety-like behavior and hyperlocomotion associated to ethanol withdrawal in alcohol dependent rats. Though allyphenyline proved to be unable to prevent the expression of somatic alcohol withdrawal symptoms, the results of the present study supported the validity of our choice. Indeed, allyphenyline, devoid of anxiolytic effect on naïve wistar rats, exerted an interesting anxiolytic effect when the anxiety was triggered by an external stimulus such as prolonged ethanol abstinence. It is known that, during protracted withdrawal, the brain undergoes plastic changes that lead to an increased function of anxiolytic systems (Bison and Crews, 2003; Mitrirattanakul et al., 2007; Olling et al., 2009; Roberto and Siggins, 2006; Ruggeri et al., 2010).

The efficacious low doses (0.05 and 0.275 mg/kg) observed in the present study suggested that the anxiolytic effect of allyphenyline might be associated to its α_{2C}-adrenoceptor/serotonin 5-HT_{1A} receptor activation, according to what demonstrated for its antidepressant activity (Del Bello et al., 2012). Such consideration strengthened the existence of a peculiar relationship between α_2-adrenoceptor activation and serotonin 5-HT receptor function, also underlined by other authors (Mongeau et al., 1993; Redrobe and Bourin, 1998).

Locomotor hyperactivity is often observed during ethanol withdrawal (Uzbay and Kayaalp, 1995) and a positive correlation

between ethanol intake and motor activity in a novel environment has also been documented (Nadal et al., 2002). For example C57BL/6 mice are more active than DBA/2 in a novel environment (Rogers et al., 1999) and show higher inclination to consume drug of abuse including ethanol (Melinska et al., 1995).

Indeed, also in the present study post-dependent rats showed higher locomotor activity compared to control animals.

It is noteworthy that the significant reduction of the locomotor hyperactivity produced by allyphenyline cannot be attributed to a sedative effect, as demonstrated by its inability to affect locomotor activity on naïve wistar rats at the dose of 0.275 mg/kg. This result supported the previous in vivo study on mice showing that, at the dose of 0.05 mg/kg, allyphenyline did not increase the sleeping time induced by pentobarbital (Cardinaletti et al., 2009).

As aforementioned, α_{2C}-adrenoceptor is involved in locomotory activity. Indeed, mice with genetically inactivated α_{2C}-adrenoceptor displayed an increase in locomotor activity following injection of amphetamine; in contrast, mice overexpressing α_{2C}-adrenoceptor demonstrated to be significantly less active than wild-type counterparts after amphetamine injection (Tan and Limbird, 2006). Therefore, it is very likely that the ability of allyphenyline to reduce alcohol-related hyperlocomotion was induced by its α_{2C}-adrenoceptor agonism.

5. Conclusion

In conclusion, this study revealed the potential of allyphenyline to prevent the expression of affective signs such as anxiety associated with protracted alcohol withdrawal. Considering that psychiatric pathologies, induced by drug withdrawal, represent the primary cause of relapse in dependent patients, one could speculate that allyphenyline or other drugs endowed with α_{2C}-adrenoceptor/serotonin5-HT_{1A} receptor agonism and α_{2A}-adrenoceptor antagonism may be efficacious in relapse prevention. On the other hand, though benzodiazepines are the gold standard treatment for alcohol withdrawal, they are associated with the expression of several side effects including excessive sedation, memory deficits, and respiratory depression. Therefore, there is a wide need of novel effective and safer agents. In addition, allyphenyline offers the advantage of reducing the locomotor hyperactivity in ethanol withdrawal. The pharmacological properties of allyphenyline, emerged by the present and previous studies, may open the possibility to develop innovative, more efficacious and safer remedies to treat drug dependence.

Conflict of interest statement

The authors declare no conflict of interest.

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