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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

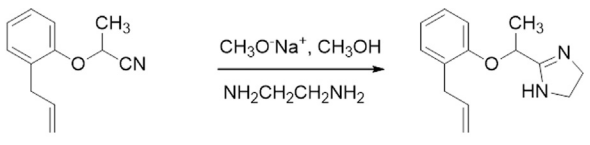
Allyphenyline was dissolved in saline and administered intraperitoneally (i.p.) at doses of 0.05, 0.275 and 0.5 mg/kg/ml. The same vehicle (1 ml/kg of saline) was administered to the control group (Veh).

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