Pharmacology, Biochemistry and Behavior 148 (2016) 119–127

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Opinion paper

Disulfiram inhibits chocolate self-administration and reinstatement to chocolate seeking in rats

Alessandro Zaru [a,b], Paola Maccioni [a], Giancarlo Colombo [a,*], Gian Luigi Gessa [a,b]

[a] Neuroscience Institute, National Research Council of Italy, Section of Cagliari, S.S. 554, km. 4500, I-09042 Monserrato, CA, Italy
[b] Department of Biomedical Sciences, University of Cagliari, S.S. 554, km. 4500, I-09042 Monserrato, CA, Italy

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 11 April 2016
Accepted 28 April 2016
Available online 8 May 2016

Keywords:
Disulfiram
Inhibition of dopamine β-hydroxylase (DBH)
Inhibition of aldehyde dehydrogenase (ALDH)
Chocolate
Operant self-administration
Rats

ABSTRACT

We recently found that the dopamine β-hydroxylase (DBH) inhibitor, nepicastat, reduced chocolate self-administration and reinstatement of chocolate-seeking behavior in free-fed rats and self-administration of regular food pellets in food-restricted rats. Here we show that disulfiram reproduced all these effects but exhibited a considerably different time-course. Wistar rats were trained to lever-respond for a chocolate solution (free-fed rats) or regular food pellets (food-restricted rats) under the Fixed Ratio (FR) 10 (FR10) schedule of reinforcement. Once lever-responding stabilized, rats were exposed to sessions under FR10 and Progressive Ratio (PR) schedule and reinstatement sessions. Acutely administered disulfiram (0, 25, 50, and 100 mg/kg, i.p.) inhibited, with similar potency and effectiveness, lever-responding for chocolate solution and regular food pellets under the FR10 schedule. Disulfiram-induced inhibition of lever-responding for chocolate solution and regular food pellets showed a biphasic time-course: an early inhibition at 2 h, which subsided within 24 h, and a second long-lasting inhibition from 48 to 96 h. Administered 48 h beforehand, disulfiram reduced lever-responding for chocolate solution under the PR schedule and prevented cue-induced reinstatement of chocolate-seeking behavior. Spontaneous locomotor activity was reduced at 2 but not 48 h after disulfiram administration. These results indicate that disulfiram reduced food seeking and consumption elicited by high palatability or appetite. It is suggested that the delayed inhibitory effect of disulfiram is likely mediated by a novel mechanism distinct from blockade of DBH or aldehyde dehydrogenase.

1. Introduction

Disulfiram is the oldest and most widely used medication for alcoholism, its effect being due to the inhibition – via its primary metabolite, S-methyl-N,N-diethylthiolcarbamate sulfoxide (DETC-MeSO) – of aldehyde dehydrogenase-1 and -2 (ALDH-1 and -2) (Hart and Faiman, 1994), resulting in acetaldehyde accumulation upon alcohol ingestion (see Suh et al., 2006; Skinner et al., 2014). Acetaldehyde is responsible for the flushing reaction and unpleasant symptoms that deter from alcohol consumption. More recently, disulfiram has emerged as one of the few promising treatments for cocaine dependence (see Shorter and Kosten, 2011).

The efficacy of disulfiram in cocaine addiction has been explained by its property to inhibit – through diethyldithiocarbamate – dopamine β-hydroxylase (DBH) (Musacchio et al., 1966), the enzyme that converts dopamine (DA) into noradrenaline (NA) in noradrenergic neurons. NA depletion may result in loss of the stimulatory noradrenergic tone on mesolimbic dopaminergic neurons, required for the stimulant effect of cocaine and for its capacity to trigger relapse to cocaine seeking (Leri et al., 2002; Lee et al., 2004; Zhang and Kosten, 2005; Platt et al., 2007). Consistent with this hypothesis, Schroeder and coworkers found, in a rat model of human relapse to cocaine seeking and consumption, that disulfiram attenuated cocaine-primed reinstatement of cocaine-seeking behavior (Schroeder et al., 2010) and that this effect was reproduced by the selective DBH inhibitor, nepicastat (Schroeder et al., 2013).

Alternatively, the inhibitory effect of disulfiram on reinstatement of cocaine-seeking behavior may be explained by its ability to inhibit ALDH-2. Accordingly, Yao et al. (2010) found that the selective ALDH-2 inhibitor, GS 455534, prevented both cocaine- and cue-induced reinstatement of cocaine-seeking behavior in rats, but – unlike nepicastat – also suppressed cocaine self-administration. These authors found that the combination of cocaine and GS 455534 resulted in the formation of tetrahydropapaveroline (THP), that inhibits tyrosine hydroxylase (the rate limiting enzyme for DA synthesis) and DA signaling in the nucleus accumbens, a condition required in cocaine seeking (Yao et al., 2010). Thus, disulfiram efficacy in the treatment of cocaine addiction might be explained by its ability to inhibit either ALDH-2, DBH, or both.

Abbreviations: ALDH, aldehyde dehydrogenase; ALDH-1, aldehyde dehydrogenase-1; ALDH-2, aldehyde dehydrogenase-2; DA, dopamine; DBH, dopamine β-hydroxylase; DETC-MeSO, S-methyl-N,N-diethylthiolcarbamate sulfoxide; FR, Fixed Ratio; NA, noradrenaline; PR, Progressive Ratio; RR, response requirement; THP, tetrahydropapaveroline.

* Corresponding author.
E-mail address: colomb@unica.it (G. Colombo).

http://dx.doi.org/10.1016/j.pbb.2016.04.008
0091-3057/

Considerable evidence suggests that common neural and molecular mechanisms underlie the reinforcing and motivational properties of drugs of abuse and natural rewards, including food (see Volkow and Wise, 2005; Volkow et al., 2008). Consistent with this hypothesis, we found that nepicastat suppressed in rats operant self-administration of (a) alcohol in selectively bred alcohol-preferring rats (Colombo et al., 2014), (b) chocolate solution in free-fed rats (Zaru et al., 2013), and (c) regular food pellets in food-restricted rats (Zaru et al., 2013). Nepicastat was also found to effectively prevent reinstatement of chocolate-seeking behavior (Zaru et al., 2013). In accordance with these observations, Bocarsly et al. (2014) found that GS 455534 suppressed binge eating of palatable food (sugar or fat) and attenuated DA release, elicited by sugar-bingeing, in the rat nucleus accumbens.

The aim of the present study was to clarify whether disulfiram shares with nepicastat and/or GS 455534 the ability to suppress operant self-administration of a chocolate solution in free-fed rats and of regular food pellets when driven by appetite (i.e., in food-restricted rats), as well as to prevent reinstatement of chocolate-seeking behavior.

Beside sharing with nepicastat and GS 455534 the ability to inhibit DBH and ALDH, respectively, disulfiram – via DETC-MeSO – is a potent carbamoylating agent for sulfhydryl groups (Jin et al., 1994), including those present in glutamate receptors (Nagendra et al., 1997). We suggest that comparisons between differences and similarities in the effects of disulfiram, nepicastat, and GS 455534 might provide important information on the molecular substrates involved in craving for drugs of abuse and natural rewards.

2. Material and methods

The experimental procedures employed in the present study were in accordance with the Italian Law on the "Protection of animals used for scientific reasons".

2.1. Animals

The present study employed adult, male Wistar rats (Harlan Laboratories, San Pietro al Natisone, Italy). At the start of the lever-responding training phase and pharmacological tests with disulfiram, rat age and body weight were approximately 200 g and 6 weeks and 300 g and 10 weeks, respectively. The only exception was represented by rats of Experiment 4, whose body weight was kept at approximately 85% of free-feeding values. Although an ideal design would have required identical housing for all rats, the large number of rats employed ($n = 174$) forced us to house rats differently, depending on their feeding regimen. Specifically, rats used in Experiments 1–3 and 5 were housed 4 per cage (cage floor area: 1820 cm^2); rats used in Experiment 4 were housed individually (cage floor area: 800 cm^2). Previous studies from this lab indicated however that self-administration of the chocolate solution did not differ between grouped and singly housed rats (this lab, unpublished data). All cages had wood chip bedding. The animal facility was under an inverted 12:12 h light-dark cycle (lights on at 9:30 p.m.), constant temperature of 22 ± 2 °C, and relative humidity of approximately 60%. Standard rat chow [diet code: 4RF21 (Mucedola, Settimo Milanese, Italy) in Experiments 1–3 and 5; diet code: 5001 (International Product Supplies Ltd., London, UK) in Experiment 4; these two diets were virtually identical in composition] and tap water were always available in the homecage, except as noted below. Rats were extensively habituated to handling and intraperitoneal injection; specifically, over the 2 weeks preceding the start of the pharmacological experiments, rats received a daily injection of 2 ml/kg saline. Each experiment used independent sets of rats.

2.2. Self-administration and reinstatement of seeking for chocolate solution

A detailed description of the procedure of chocolate self-administration used in the present study has recently been given elsewhere (see Maccioni and Colombo, 2016).

2.2.1. Chocolate solution

The chocolate solution was prepared diluting powdered Nesquik® (Nestlè Italiana, Milan, Italy) in tap water. Concentration of Nesquik® chocolate powder was 5% (w/v) throughout the study. This concentration was selected based on the results of previous experiments in which it had been largely preferred over a range of concentrations (Maccioni et al., 2008). The chocolate solution was prepared daily and sipper bottles (see below) were shaken immediately before the start of each session to prevent development of any deposit. The chocolate solution provided 0.8 kJ/g.

Previous experiments demonstrated that this chocolate solution possesses highly rewarding, reinforcing, and motivational properties in rats (e.g.: Gessa et al., 2006; Maccioni et al., 2008; Zaru et al., 2013).

2.2.2. Apparatus

Operant sessions were conducted in modular chambers (Med Associates, St. Albans, VT, USA), located in sound-attenuated cubicles, with fans for ventilation and background white noise. The front panel of each chamber was equipped with (a) one retractable response lever, (b) one green stimulus light mounted above the lever, and (c) the retractable spout of a liquid sipper bottle (250-ml capacity) located outside the chamber. A white house light was centered at the top of the back wall of each chamber. Achievement of the response requirement (RR; see below) resulted in exposure of the sipper bottle spout (lasting for 5 s in each phase of the experiment) and illumination of the green light for the period of exposure of the sipper bottle spout.

2.2.3. Experimental procedure

2.2.3.1. Training and maintenance phase. To facilitate the acquisition of lever-pressing behavior, rats were water-deprived in their homecage in the 12 h preceding the first 2 operant sessions. Self-administration sessions were conducted daily, 7 days per week, during the first 4 h of the dark phase of the light/dark cycle. Self-administration sessions lasted 20 min. During the first 2 sessions, rats were trained to lever-respond on a Fixed Ratio (FR) 1 (FR1) schedule of reinforcement for the chocolate solution. FR was progressively increased from FR1 to FR10 over 10 sessions. Subsequently, 20 additional sessions with FR10 were conducted (maintenance phase), so that number of lever-responses for and intake of the chocolate solution stabilized in all rats before the start of the test sessions (see below).

2.2.3.2. Testing under the FR schedule (Experiment 1). This experiment used $n = 32$ rats, divided into 4 groups of $n = 8$ matched for the number of lever-responses over the last 5 sessions of the maintenance phase. The test session, that lasted 20 min, was conducted at the end of the maintenance phase. In the test session, RR was maintained at the value of FR10. Disulfiram (Sigma-Aldrich, Steinheim, Germany) was suspended in saline with 1% (w/w) Tween 80 and administered acutely and intraperitoneally (injection volume: 2 ml/kg), at the doses of 25, 50, and 100 mg/kg, 2 h before the start of the first test session. Control (vehicle) condition (0 mg/kg disulfiram) was represented by administration of an equal volume of saline with 1% (w/w) Tween 80. Disulfiram dose-range, route of administration, and treatment time were chosen on the basis literature data suggesting its efficacy in suppressing reinstatement of cocaine-seeking behavior in rats (Schroeder et al., 2010).

Measured variables were (a) number of lever-responses and (b) amount of self-administered chocolate solution [expressed in ml/kg and determined by weighing the sipper bottle (0.01-g accuracy) before