Psychopharmacology (2006) 186: 82–92
DOI 10.1007/s00213-006-0346-y

ORIGINAL INVESTIGATION

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Effects of dexfenfluramine and 5-HT3 receptor antagonists on stress-induced reinstatement of alcohol seeking in rats

Received: 29 October 2005 / Accepted: 3 February 2006 / Published online: 7 March 2006

Abstract *Rationale and objectives:* We previously found that systemic injections of the 5-HT uptake blocker fluoxetine attenuate intermittent footshock stress-induced reinstatement of alcohol seeking in rats, while inhibition of 5-HT neurons in the median raphe induces reinstatement of alcohol seeking. In this study, we further explored the role of 5-HT in footshock stress-induced reinstatement of alcohol seeking by determining the effects of the 5-HT releaser and reuptake blocker dexfenfluramine, and the 5-HT receptor antagonists ondansetron and tropisetron, which decrease alcohol self-administration and anxiety-like responses in rats, on this reinstatement. *Methods:* Different groups of male Wistar rats were trained to self-administer alcohol (12% v/v) for 28–31 days (1 h/day, 0.19 ml per alcohol delivery) and then their lever responding for alcohol was extinguished over 9–10 days. Subsequently, the effect of systemic injections of vehicle or dexfenfluramine (0.25 or 0.5 mg/kg, i.p), ondansetron (0.001, 0.01, or 0.1 mg/kg, i.p), or tropisetron (0.001, 0.01, and 0.1 mg/kg, i.p) on reinstatement induced by 10 min of intermittent footshock (0.8 mA) was determined. *Results:* Systemic injections of dexfenfluramine, ondansetron or tropisetron attenuated footshock-induced reinstatement of alcohol seeking. Injections of dexfenfluramine, ondansetron, or tropisetron had no effect on extinguished lever responding in the absence of footshock. *Conclusions:* The present results provide additional support for the hypothesis that brain 5-HT systems are involved in stress-induced reinstatement of alcohol seeking. The neuronal mechanisms that potentially mediate the unexpected observation that both stimulation of 5-HT release and blockade of 5-HT3 receptors attenuate footshock-induced reinstatement are discussed.

Keywords 5-Hydroxytryptamine · Reinstatement · Footshock · 5-HT3 receptors · Relapse · Stress

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Introduction

Drugs that increase synaptic levels of 5-HT such as the 5-HT releasing and reuptake blocker dexfenfluramine and the reuptake blocker fluoxetine decrease alcohol intake in laboratory rats (Gardell et al. 1997; Le et al. 1999; Lu et al. 1994). Conversely, inhibition of 5-HT cell firing and 5-HT release by systemic injections of low doses or intraraphe injections of the 5-HT1a autoreceptor agonist 8-OH-DPAT increase alcohol intake in rats and squirrel monkeys (McKenzie-Quirk and Miczek 2003; Tomkins et al. 1994a). These results suggest that activation of central 5-HT neurons decreases alcohol intake. Relatively, little is known about the identity of the receptors mediating these inhibitory effects, although there is evidence for the participation of 5-HT1a, 5-HT1b, and 5-HT2a receptors (Overstreet et al. 1997; Tomkins et al. 1994b, 2002). Other classes of 5-HT receptors also play a role in alcohol consumption. Of these receptors, the 5-HT3 receptor has received the most attention. In laboratory rats and mice, systemic injections of 5-HT3 receptor antagonists decrease alcohol intake (Fadda et al. 1991; Hodge et al. 1993, 2004;

Knapp and Pohorecky 1992; McKenzie-Quirk et al. 2005; Tomkins et al. 1995). In humans, administration of the 5-HT3 receptor antagonist ondansetron was reported to reduce alcohol craving and to increase abstinence in alcohol-dependent subjects (Johnson 2004; Johnson et al. 2000; Sellers et al. 1994). These findings indicate that 5-HT3 receptors play an important role in alcohol-taking behavior. 5-HT3 antagonists have also been shown to decrease anxiogenic-like responses in rats, mice, and humans (Artaiz et al. 1998; Bilkei-Gorzo et al. 1998; Nowakowska et al. 1998; Olivier et al. 2000; Yoshioka et al. 1995), an effect that may be relevant to the understanding of the mechanisms underlying stress-induced alcohol seeking.

Studies in humans suggest that stress is associated with alcohol use and relapse (Brown et al. 1995; Cooper et al. 1992; Sinha 2001), and that high comorbidity exists between stress-related psychiatric disorders such as anxiety and depression and drug and alcohol use (Brady 1997; Goldman and Barr 2002; Kandel et al. 1997; King et al. 1996). The neuronal mechanisms underlying the relationship between stress and alcohol abuse and relapse are unknown. An animal model used to study factors involved in relapse to drug seeking is the reinstatement procedure (Bossert et al. 2005; de Wit and Stewart 1981; Shaham et al. 2003; Shalev et al. 2002; Stewart 2000). In this procedure, the effect of noncontingent exposure to drugs or nondrug stimuli on reinstatement of drug seeking is examined after training for drug self-administration and subsequent extinction of drug-reinforced behavior (Stewart and de Wit 1987). Studies using the reinstatement model have demonstrated that brief exposure to intermittent footshock stress reinstates cocaine (Ahmed and Koob 1997; Erb et al. 1996; Mantsch and Goeders 1999), and heroin (Ahmed et al. 2000; Shaham and Stewart 1995) seeking (for reviews, see Lu et al. 2003; Shaham et al. 2000). Based on these findings, we adapted the reinstatement procedure to studies with alcohol-trained rats and tested the effect of intermittent footshock on reinstatement of alcohol seeking (Le and Shaham 2002; Le et al. 1998). We found that this stressor reinstates alcohol seeking in alcohol-experienced rats, an effect that was independently replicated (Economidou et al. 2006; Liu and Weiss 2002; Martin-Fardon et al. 2000). More recently, we and others have reported that the effect of footshock on reinstatement of alcohol seeking generalizes to other stress manipulations, including exposure to cues paired with footshock (Liu and Weiss 2003) or social stress (Funk et al. 2005), and systemic injections of the anxiogenic agent yohimbine (Le et al. 2005).

Results from pharmacological studies suggest that central 5-HT neurons are involved in footshock stress-induced reinstatement of alcohol seeking. Low doses of the selective serotonin reuptake inhibitor (SSRI) fluoxetine attenuate footshock-induced reinstatement (Le et al. 1999), while intra-median raphe injections of the 5-HT1a agonist 8-OH-DPAT mimic the effect of the stressor on reinstatement (Le et al. 2002). However, the effects of fluoxetine on alcohol consumption (Amit et al. 1991) and feeding behavior (Grignaschi and Samanin 1992) are not consistently altered by 5-HT receptor antagonists or by neurotoxic lesions of 5-HT neurons. Therefore, our data with fluoxetine in reference to the role of 5-HT in footshock-induced reinstatement should be interpreted with caution. Based on the above considerations, one aim of the present experiments was to determine whether the effect of fluoxetine generalizes to the 5-HT reuptake inhibitor and releaser dexfenfluramine (Rowland and Charlton 1985). These compounds have been shown to increase extracellular levels of 5-HT to a comparable degree, and to have a comparable profile of selectivity (Goodnick and Goldstein 1998; Rothman et al. 2003; Tao et al. 2002). Thus, if the inhibitory effect of fluoxetine on footshock stress-induced reinstatement is mediated by increased 5-HT neurotransmission, dexfenfluramine should also attenuate this reinstatement.

Another aim of the present study is to help determine the role of the 5-HT3 receptor in footshock stress-induced reinstatement of alcohol seeking. In the context of the role of stress in alcohol-taking behavior, the inhibitory effects of 5-HT3 receptor antagonists on alcohol intake (see above) in rodents and their antirelapse effects in humans are of interest because, as mentioned above, injections of these antagonists decrease anxiety-like responses in rats (Costall and Naylor 1992; Vasar et al. 1993). In the present report, therefore, we also examined the effect of two 5-HT3 receptor antagonists, ondansetron, and tropisetron (Hoyer et al. 1994, 2002), on footshock-induced reinstatement of alcohol seeking.

Materials and methods

Subjects and apparatus

One-hundred and sixty male Wistar rats (Charles River, Montreal, Quebec) weighing 150–200 g at the start of the experiment were used. The rats were individually housed under a 12:12 h light–dark cycle (light on from 7:00 a.m. to 7:00 p.m.). Food and water were freely available in the home cage during all phases of the experiments and the temperature was maintained at 21±1°C. The experimental procedures followed the "Principles of laboratory animal care" (NIH publication no. 85–23, 1996) and were approved by the local animal care and use committee. The self-administration chambers were constructed locally and were equipped with two levers, symmetrically centered on a side panel. Responding on one lever (an active lever) activated the infusion pump (Razel Sci. Stamford, CT), while responding on the other lever (an inactive lever) was recorded, but did not activate the pump. Activation of the infusion pump resulted in the delivery of 0.19 ml of a 12% alcohol w/v solution into a liquid drop receptacle located between the two levers. The grid floors of the self-administration chambers were connected to electric shock generators (Med Associates, Georgia, VT, USA). All behavioral procedures were performed between 9:00 a.m. and 2:00 p.m.

Drugs

Dexfenfluramine (Sigma, St. Louis, MO, USA) was dissolved in saline and was injected i.p. 1 h before the start of the test sessions. The doses of dexfenfluramine used are based on previous reports on its effect on alcohol consumption (Higgins et al. 1992). Ondansetron (a gift from GlaxoSmithKline, Research Triangle Park, NC, USA) and tropisetron (Sigma) were dissolved in distilled water and were injected i.p. 15 min before the start of the test sessions. The doses of the 5-HT3 antagonists are based on previous reports on their effect on alcohol consumption (Jankowska et al. 1994; Tomkins et al. 1995).

Procedures

Alcohol self-administration training

Rats were trained to self-administer alcohol using the methods described in our previous studies (Le et al. 1998, 2000). Briefly, the rats were initially provided with access to alcohol solutions and water in Richter tubes for 30 min/day affixed to drinking cages (30×18×18 cm) that were distinct from their home cages. Alcohol solutions were provided in escalating concentrations: 3% for the first 5 days, 6% for the next 8 days, and 12% (w/v) for the last 10–12 days. Subsequently, self-administration of alcohol was initiated on a fixed ratio-1 (FR-1) 5-s timeout reinforcement schedule for 10–14 days (1-h/day). During the 5-s timeout period, a stimulus light above the active lever was turned on. The beginning of sessions were signaled by illumination of a houselight located at the top of the self-administration chamber, on the side opposite the levers; at the end of the 1-h sessions, the houselight was turned off. The requirement for alcohol delivery was then increased to an FR-2 schedule for five sessions. Subsequently, the schedule requirement was increased to an FR-3 schedule for 8–12 days until the rats demonstrated 3 days of stable alcohol-taking behavior (variability of less than 20% of the mean). At the end of the self-administration sessions, the receptacles were checked for unconsumed alcohol; the volume remaining in the receptacles was taken into account in calculating the number of alcohol reinforcements earned in each session. Both the active and inactive levers were present during all sessions in the self-administration chambers. As the reinstatement of alcohol-taking behavior was the dependent measure in our study, we excluded rats that did not demonstrate reliable self-administration of pharmacologically relevant doses of alcohol during training. A total of 35 rats were excluded from the study on this basis: operationally defined as estimated alcohol intake of less than 0.3 g/kg per 30 min during the last 5 days of the two-bottle choice phase of training (21 rats), or less than 0.4 g/kg per 1 h during the last five alcohol self-administration sessions (14 rats). No rats were excluded on the basis of their responding during the extinction or reinstatement testing phases of the experiments.

Extinction of the alcohol-reinforced behavior

The experimental procedures during the extinction sessions were the same as those of the alcohol self-administration training sessions with the exception that responding on the active lever did not lead to alcohol delivery. Tests for reinstatement started after 9–10 daily 1-h extinction sessions, when the rats reached the extinction criterion of fewer than 12 presses on the active lever. During the extinction phase, rats were injected three times with the appropriate vehicle (1 ml/kg saline or distilled water) to habituate them to the injection procedures.

Tests for reinstatement

Tests were conducted under extinction conditions. Intermittent footshock (0.8 mA, 0.5 s ON, a mean OFF period of 40 s, range of 10–70 s) was administered for 10 min immediately before the 1-h test sessions. These parameters of footshock are based on our previous work (Le et al. 1998, 2000; Shaham et al. 1997; Shaham and Stewart 1995). Footshock was delivered through a scrambler to the stainless steel grid floor of the self-administration chambers. Drug or vehicle was injected 60 min (Experiment 1) or 15 min (Experiment 2) before exposure to the shock or no shock (extinction) conditions (see below). At the time of the reinstatement tests, the mean (±SEM) body weight of the rats was 415±3.2 g and 435±3.1 g for Experiment 1 and 2, respectively.

Experiment 1. Dexfenfluramine

Three groups of rats (n=14–15 per group) were used. After reaching the extinction criterion, the effect of injections of saline (vehicle) or dexfenfluramine on footshock-induced reinstatement of lever responding was determined. A mixed design was used with dexfenfluramine dose (0, 0.25, and 0.50 mg/kg) as the between-subjects factor and stress condition (no shock, shock) as the within-subjects factor. During testing, each rat was injected 60 min before the two test sessions (that occurred on consecutive days) with vehicle or one of the doses of dexfenfluramine and exposed to 10 min of intermittent footshock, or no shock (regular extinction) in counterbalanced order; the test sessions were separated by 24 h. The footshock was administered just before the start of the 1-h test sessions.

Experiment 2. Ondansetron and tropisetron

Seven groups of rats (n=10–11 per group) were used; one of these groups served as a common vehicle control for the groups treated with ondansetron and tropisetron. After reaching the extinction criterion, the effects of vehicle (distilled water), ondansetron, or tropisetron on footshock-induced reinstatement of lever responding were determined. A mixed design was used with ondansetron

dose (0, 0.001, 0.01, and 0.1 mg/kg) or tropisetron dose (0, 0.001, 0.01, and 0.1 mg/kg) as the between-subjects factor and stress condition (no shock, shock) as the within-subjects factor. During testing, each rat was injected 15 min before the two test sessions (that occurred on consecutive days) with vehicle or one of the doses of ondansetron or tropisetron and exposed to 10 min of intermittent footshock, or no shock (regular extinction) in counterbalanced order; the test sessions were separated by 24 h.

Statistical analyses

Active and inactive lever responding data were analyzed with ANOVAs using the appropriate between- and within-subjects factors (see "Results"). Because the responding of rats on the active lever was not normally distributed in Experiment 1 ($p<0.05$, Kolmogorov–Smirnov test) the data were square-root transformed before analysis. In Experiment 2, the raw data were analyzed. Significant effects (p values<0.05) were followed by post hoc (Newman–Keuls) tests.

Results

As in our previous studies, the rats acquired alcohol self-administration and the lever responding was extinguished over days when alcohol was no longer available. Figures 1 and 2 show the mean (±SEM) number of alcohol reinforcements (a) and the mean responses on the active levers (b) during alcohol self-administration training in Experiments 1 and 2. The mean (±SEM) estimated alcohol intake (number of reinforcements×0.19 ml×weight of alcohol/rat body weight/1-h session) during the last 3 days of self-administration training of the rats was 0.97±0.06 and 1.05±0.06 g kg^{-1} h^{-1} for Experiments 1 and 2, respectively. Figure 3 shows the mean number of responses made on the previously active and inactive levers during the extinction phase in Experiment 1 (subpanel a) and Experiment 2 (subpanel b).

Experiment 1. Dexfenfluramine

The effect of dexfenfluramine injections on footshock-induced reinstatement of lever responding is shown in Fig. 4. Dexfenfluramine dose-dependently attenuated footshock-induced reinstatement of responding on the previously active lever (a), but had no effect on lever responding in the absence of shock. The statistical analysis revealed a significant interaction of dexfenfluramine dose by stress condition [$F(2,40)=3.6$, $p<0.05$]. Exposure to footshock or injections of dexfenfluramine had no effect on inactive lever responding (b). Post hoc group differences are shown in Fig. 4.

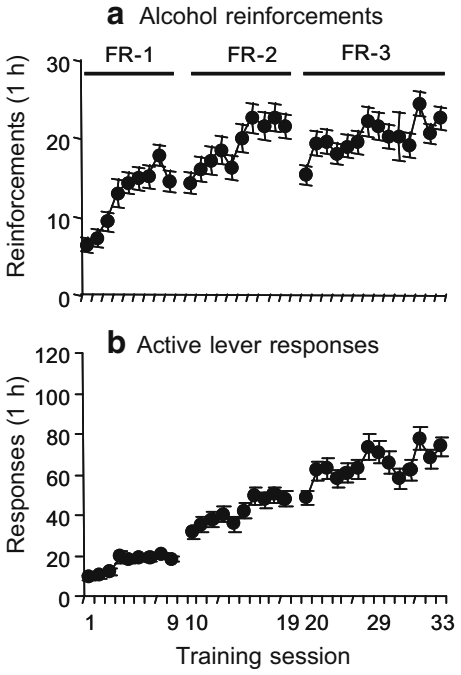

Fig. 1 Experiment 1: alcohol self-administration. Mean (±SEM) number of alcohol reinforcements (**a**) and total active lever responding under a fixed-ratio-3 schedule (**b**) during the training phase of "Experiment 1" (n=44)

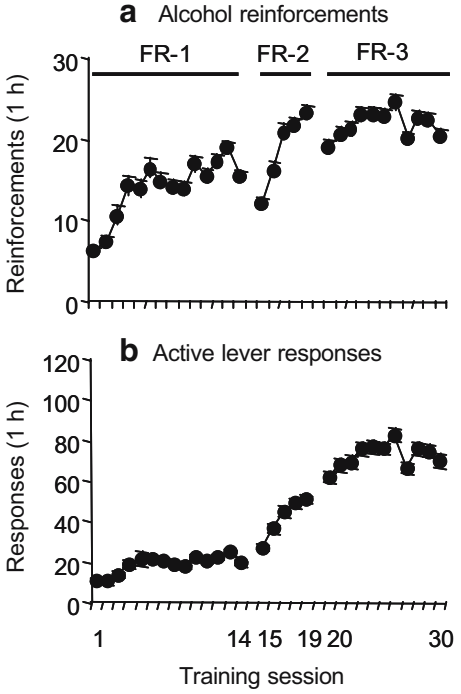

Fig. 2 Experiment 2: alcohol self-administration. Mean (±SEM) number of alcohol reinforcements (**a**) and total active lever responding under a fixed-ratio-3 schedule (**b**) during the training phase of "Experiment 2" (n=74)

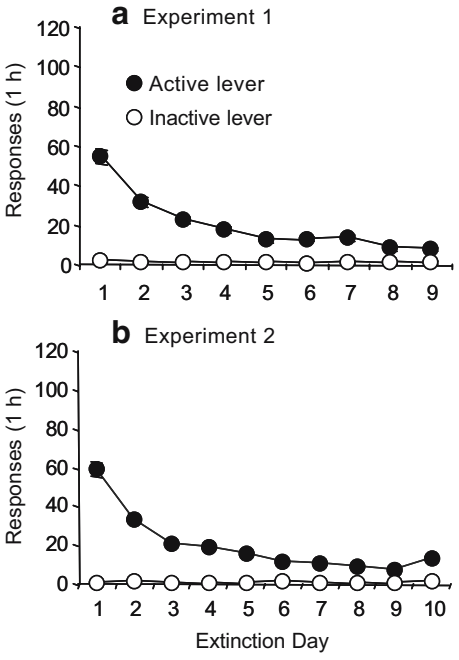

Fig. 3 Extinction of the alcohol-reinforced behavior. Mean (±SEM) number of lever presses on the previously active lever and on the inactive lever during the extinction phase during which lever responding are not reinforced with alcohol in Experiment 1 (**a**, n=44) and Experiment 2 (**b**, n=74)

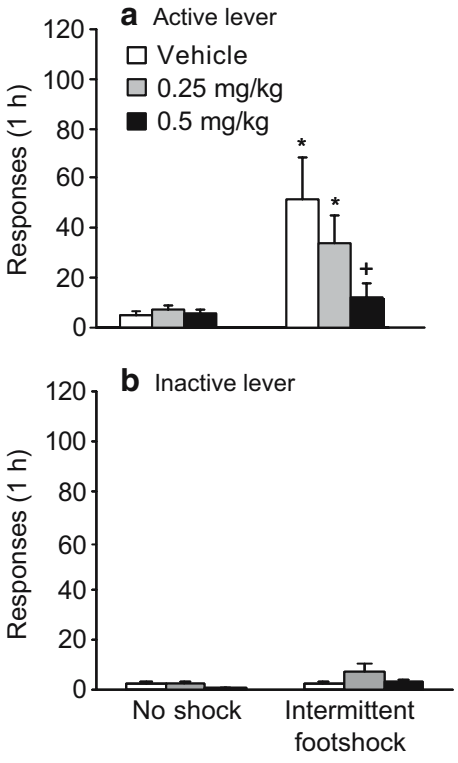

Fig. 4 Effect of the 5-HT releaser and reuptake blocker dexfenfluramine on footshock-induced reinstatement of alcohol seeking. The rats were injected with saline (vehicle) or dexfenfluramine (0.25 or 0.5 mg/kg i.p.) 60 min before the test sessions and were exposed to 10 min of intermittent footshock immediately before one of the test sessions. Data are expressed as the number of responses (±SEM) on the previously active (**a**) and inactive (**b**) levers. * Significant differences from the no shock condition (p<0.05). + Significant differences from the vehicle-footshock group (p<0.05); n=14–15 rats/dose

Experiment 2. Ondansetron and tropisetron

The effects of ondansetron (a) and tropisetron (b) injections on footshock-induced reinstatement of lever responding are shown in Fig. 5. Both drugs attenuated footshock-induced reinstatement of responding on the previously active lever (left panel), but had no effect on responding in the absence of shock. The statistical analysis revealed significant interactions of ondansetron dose by stress condition [F(3,40)=7.98, p<0.01] and tropisetron dose by stress condition [F(1,39)=7.1, p<0.01]. The attenuating effect of the two 5-HT3 antagonists on footshock-induced reinstatement was not dose-dependent; all doses of these drugs reduced footshock-induced reinstatement of lever responding to a similar degree. Exposure to footshock, or injections of ondansetron or tropisetron had no effect on inactive lever responding (right panel). Post hoc group differences are shown in Fig. 5.

Discussion

There are two main findings in this report. The first finding is that injections of the 5-HT releaser and reuptake blocker dexfenfluramine dose-dependently attenuated footshock-induced reinstatement of alcohol seeking. These findings are in agreement with our previous observation on the attenuation of footshock-induced reinstatement by the 5-HT reuptake blocker fluoxetine (Le et al. 1999). The second, and more novel finding, is that injections of the 5-HT3 receptor antagonists, ondansetron, and tropisetron, also attenuate footshock-induced reinstatement of alcohol seeking. Unlike dexfenfluramine, the effects of the 5-HT3 antagonists were not dose-dependent: all doses tested blocked footshock-induced reinstatement. Together, these findings provide further support to the idea that 5-HT transmission plays an important modulatory role in footshock-induced reinstatement of alcohol seeking (Le and Shaham 2002). In the section below, we address methodological considerations related to our data and also discuss neuronal mechanisms that can potentially account for the similar behavioral effects of dexfenfluramine, which increases 5-HT release, and ondansetron and tropisetron, which block 5-HT3 receptors, on footshock-induced reinstatement of alcohol seeking.

Methodological considerations

Dexfenfluramine, ondansetron, and tropisetron had no effects on active lever responding during the tests for reinstatement when they were injected in the absence of shock and also had no effect on inactive lever responding in the presence or absence of footshock. These data suggest that the effects of these compounds on footshock-induced

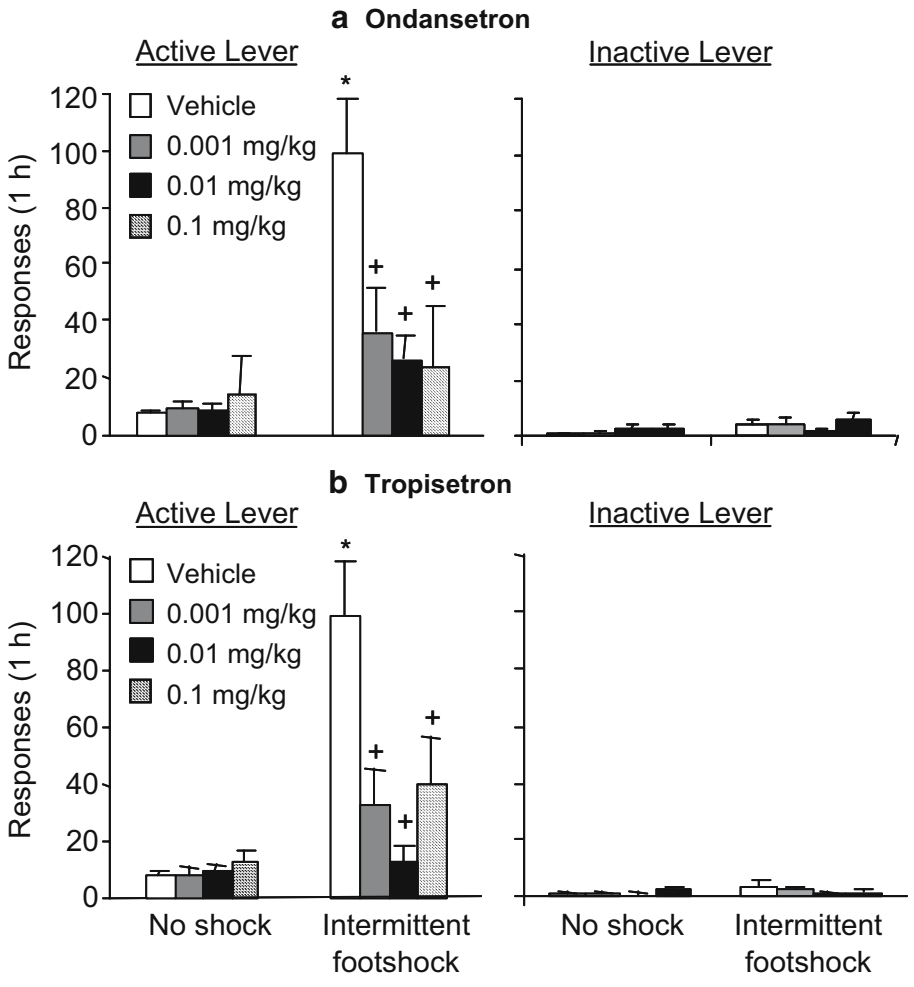

Fig. 5 Effects of the 5-HT3 receptor antagonists ondansetron and tropisetron on footshock-induced reinstatement of alcohol seeking. The rats were injected with saline (vehicle), ondansetron (0.001, 0.01, or 0.1 mg/kg, i.p.) (**a**), or tropisetron (0.001, 0.01, or 0.1 mg/kg, i.p.) (**b**) 15 min before the test sessions and were exposed to 10 min of intermittent footshock immediately before one of the test sessions. Data are expressed as the number of responses (±SEM) on the previously active (*left panel*) and inactive levers (*right panel*). * Significant differences from the no shock condition ($p<0.05$).+ Significant differences from the vehicle–footshock group ($p<0.05$); $n=10–11$ rats/dose

reinstatement are not due to nonspecific effects. However, inactive lever responding was very low in all phases of the experiments (Figs. 3, 4, and 5) and active lever responding was also low after extinction (no shock condition, Figs. 4 and 5). Thus, these measures are not optimal for testing the effect of pharmacological manipulations on nonspecific behavioral depression during reinstatement tests (Shalev et al. 2002). However, even with these considerations, it appears unlikely that behavioral depression can account for the effect of dexfenfluramine, ondansetron, and tropisetron on footshock-induced reinstatement of alcohol seeking. Previous studies reported that at the doses used in the present study, these compounds have no effect on locomotor activity (Arnold et al. 1995; Baumann et al. 2000; Corrigall and Coen 1994; Wilson et al. 1998) and on high rates of lever responding in drug discrimination procedures (De La Garza et al. 1996; Munzar et al. 1999; Paris and Cunningham 1991; Smith et al. 2002).

Another methodological issue to consider in the interpretation of the present data is that dexfenfluramine, ondansetron, and tropisetron may have decreased lever responding during the tests for footshock-induced reinstatement because of their potential analgesic effects. In other words, these drugs may block footshock-induced reinstatement by increasing pain threshold. However, this possibility is unlikely because administration of fenfluramine or ondansetron at doses higher than the ones employed in the present report had minimal effect on pain sensitivity (Sandrini et al. 2003; Wang et al. 1999). Moreover, tropisetron was reported to decrease shock-induced analgesia in mice, suggesting it may decrease pain threshold (Rodgers and Shepherd 1992).

The magnitude of the effect of footshock on reinstatement was greater in Experiment 2 than Experiment 1. This is consistent with our observations and those of others of large individual variations in the reinstatement response to footshock in rats with a history of alcohol (Economidou et al. 2006; Le et al. 1998), heroin (Shaham 1996; Shaham et al. 1997), cocaine (de Vries et al. 2001; Erb et al. 1996; Mantsch and Goeders 1999), and methamphetamine (Shepard et al. 2004) self-administration. The source of this variability is unknown at this time. Large individual differences in the rat's behavioral and physiological responses to stressors were reported in many studies (Funk et al. 2005; Hajos-Korcsok et al. 2003; Minor et al. 1994; Sgoifo et al. 1996). These individual differences in stress responses likely underlie the variable responding in studies on footshock-induced reinstatement of drug seeking. For example, individual differences in shock-induced freezing, a behavioral response that interferes with lever

pressing, likely contribute to the large variability in footshock-induced reinstatement of drug seeking.

A final methodological issue to consider in the interpretation of the present data is that we excluded rats that did not demonstrate reliable alcohol-taking behavior during training (see "Materials and methods" section). This percentage of alcohol "non-responding" rats in the present study (22%) is similar to what we and others have previously found using outbred strains (Le et al. 2001; Stromberg and Mackler 2005). In our studies, alcohol "non-responding" rats are excluded because our main dependent measure is reinstatement of alcohol-seeking behavior, a measure that cannot be readily assessed in rats that do not acquire alcohol-reinforced responding. In this regard, we reported that intermittent footshock selectively increased lever responding in rats previously trained to self-administer intravenous cocaine, but not saline (Wang et al. 2005).

Effect of dexfenfluramine on footshock stress-induced reinstatement

The attenuation of footshock stress-induced reinstatement of alcohol seeking by dexfenfluramine (present study) and fluoxetine (Le et al. 1999), drugs that increase synaptic levels of 5-HT, is surprising because several studies reported that exposure to certain shock parameters can increase brain 5-HT activity in the dorsal raphe and its terminal areas (Bliss et al. 1972; Hammack et al. 2002; Maier and Watkins 2005; Rueter et al. 1997). However, results from other studies demonstrate regional differences in the effect of stress on extracellular levels of 5-HT. Thus, exposure to swim stress increases serotonin levels in the striatum by about 90%, but decreases it in the amygdala and the septal area by about 40–50% (Kirby et al. 1995; Kirby and Lucki 1997). In addition, ventricular injections of the stress neurohormone corticotrophin-releasing factor (CRF) inhibit the firing of a subpopulation of dorsal raphe neurons (Kirby et al. 2000; Price et al. 1998).

The above findings are potentially relevant to the understanding of the present data on the effect of dexfenfluramine on footshock-induced reinstatement. At the same dose range used in the above studies, we found that ventricular CRF injections mimic to some degree the effect of footshock on reinstatement of alcohol or heroin seeking (Le et al. 2002; Shaham et al. 1997). Furthermore, blockade of CRF receptors attenuates footshock stress-induced alcohol (Le et al. 2000; Liu and Weiss 2002), as well as heroin and cocaine seeking (Erb et al. 1998; Shaham et al. 1997, 1998), and at least in the case of alcohol, blockade of reinstatement is also observed after injections of a CRF receptor antagonist into the median raphe (Le et al. 2002). Finally, local injections of CRF into the median raphe had effects similar to those produced by inhibition of these neurons with a 5-HT1a agonist (8-OH-DPAT) on reinstatement of alcohol seeking (Le et al. 2002). Taken together, based on the above findings, we argued previously that the effect of footshock stress on reinstatement of alcohol seeking involves decreases in 5-HT transmission and increases in CRF transmission (Le et al. 2002). The present findings with dexfenfluramine are consistent with this hypothesis. However, potentially inconsistent with the our hypothesis is the observation that dexfenfluramine increases the activity of CRF neurons in the paraventricular hypothalamus (Laflamme et al. 1996), the central component of the hypothalamic–pituitary–adrenal (HPA) stress axis (Dallman et al. 1995). However, in previous work, we found that the effect of intermittent footshock on reinstatement of alcohol seeking is independent of the stressor's effect on the HPA axis (Le et al. 2000), making it unlikely that dexfenfluramine's effects on the HPA axis are involved in its effect on stress-induced reinstatement.

Another potential mechanism that may be at play in the effects of dexfenfluramine on reinstatement of alcohol seeking is the drug's effect on feeding behavior. Administration of dexfenfluramine has been shown to reduce consumption of palatable foods (Roth and Rowland 1998). Although this mechanism cannot be completely ruled out as a potential explanation of the effect of dexfenfluramine on footshock-induced reinstatement, it seems relatively unlikely. First, during the reinstatement tests, alcohol was not available. Second, the rats were given free access to food in their home cage during the experiment and most likely were not hungry during these tests. Finally, we have found that footshock is ineffective in reinstating extinguished responding for a palatable sucrose solution, suggesting that modifications of caloric need and appetite do not mediate the effect of this stressor on reinstatement of alcohol seeking (Buczek et al. 1999).

Effect of 5-HT3 receptor antagonists on footshock stress-induced reinstatement

Ondansetron and tropisetron bind to the 5-HT3 receptor with similar affinity (Hoyer et al. 1994, 2002) and in the present study, these drugs were found to block footshock-induced reinstatement at all doses tested. The mechanisms underlying the effects of 5-HT3 antagonists that we observed are not clear. 5-HT3 receptors are found in brain regions associated with the behavioral effects of alcohol (frontal cortex, nucleus accumbens, ventral tegmental area, and the raphe nuclei) (Ge et al. 1997). The wide distribution of these receptors suggests the possible involvement of number of different transmitter systems in the behavioral effects of 5-HT3 antagonists. Below, we speculate on two potential neuronal mechanisms that may be involved in the effects of the 5-HT3 receptor antagonists on footshock-induced reinstatement of alcohol seeking.

One potential mechanism involves the role of 5-HT3 receptors in the release of 5-HT from dendrites of neurons in the raphe nuclei. In the dorsal raphe, stimulation of 5-HT3 receptors induces the local release of 5-HT (Bagdy et al. 1998). The 5-HT released in the raphe would stimulate inhibitory 5-HT1a autoreceptors located on the cell bodies of the 5-HT neurons, which, in turn, would

inhibit the release of 5-HT in the terminal areas of the raphe projections (Blier et al. 1998). We speculate, therefore, that injections of ondansetron and tropisetron inhibit the dendritic release of 5-HT in the raphe, thereby releasing 5-HT neurons from autoreceptor inhibition, resulting in increased 5-HT cell firing, and subsequently, increased release of 5-HT in terminal regions. Within this framework, the critical effect of 5-HT3 antagonists on footshock-induced reinstatement would involve their effect on 5-HT release, as is the case with dexfenfluramine and fluoxetine.

Another potential mechanism that could explain the blockade of footshock-induced reinstatement of alcohol seeking by ondansetron and tropisetron involves the effects of these 5-HT3 receptor antagonists on dopamine release. Systemic injections of 5-HT3 receptor antagonists reduce stress- and drug-induced dopamine release in the nucleus accumbens and frontal cortex (De Deurwaerdere et al. 2005; Wozniak et al. 1990; Yoshioka et al. 1995). At the parameters used in our studies, intermittent footshock increases dopamine release in both the nucleus accumbens (Shaham and Stewart 1995, 1996) and the ventral tegmental area (Wang et al. 2005). It is also likely that the intermittent footshock stress we employed increases dopamine release in the prefrontal cortex because this brain area is particularly susceptible to stress-induced dopamine release (Deutch and Roth 1990; Thierry et al. 1976). In addition, systemic injections of a nonselective dopamine receptor antagonist attenuate stress-induced reinstatement of heroin seeking (Shaham and Stewart 1996), and local injections of dopamine receptor antagonists into the medial prefrontal cortex attenuate stress-induced reinstatement of cocaine seeking (Capriles et al. 2003; McFarland et al. 2004; Sanchez et al. 2003). Thus, while the effect of dopamine receptor antagonists on footshock-induced reinstatement of alcohol seeking has not been determined, it is possible that the effect of ondansetron and tropisetron on footshock-induced reinstatement involves their effect on the release of mesocorticolimbic dopamine induced by this stressor.

Concluding remarks

We found that systemic injections of dexfenfluramine, and the 5-HT3 receptor antagonists, ondansetron, and tropisetron attenuate footshock stress-induced reinstatement of alcohol seeking. The results with dexfenfluramine extend our previous observation with the 5-HT reuptake blocker fluoxetine (Le et al. 1999), suggesting that increasing 5-HT transmission attenuates footshock-induced reinstatement of alcohol seeking. The neuronal mechanisms underlying the effect of ondansetron and tropisetron on footshock-induced reinstatement are less clear. One speculative possibility is that 5-HT3 receptor antagonists act in the cell body region of the raphe nuclei to decrease somatodendritic release of 5-HT, thereby releasing 5-HT neurons from autoreceptor inhibition, and causing increased release of 5-HT in terminal areas. Another possibility is that the 5-HT3 receptor antagonists attenuate footshock-induced dopamine release, and this inhibition of dopamine release in relevant brain areas such as the medial prefrontal cortex mediates the 5-HT3 antagonist effects on reinstatement.

Finally, results from several clinical trials suggest that ondansetron is moderately effective in the treatment of alcohol dependence (Johnson et al. 2000; Kranzler et al. 2003; Sellers et al. 1994). Thus, the present results on the effect of ondansetron on footshock-induced reinstatement of alcohol seeking, as measured in a preclinical model (Epstein and Preston 2003; Shaham et al. 2003; Stewart 2003), support the idea that the reinstatement model can be used to identify promising pharmacological agents for the prevention of relapse to alcohol use (Le and Shaham 2002; Weiss 2005).

Acknowledgement This work was supported by a grant from the NIAAA (AA13108) to A.D. Le.

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