The administration of atomoxetine during alcohol deprivation induces a time-limited increase in alcohol consumption after relapse

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Abstract
The administration of selective serotonin reuptake inhibitors (SSRIs) typically used as antidepressants increases alcohol consumption after an alcohol deprivation period in rats. However, the appearance of this effect after the treatment with selective noradrenaline reuptake inhibitors (SNRIs) has not been studied. In the present work we examined the effects of a 15-d treatment with the SNRI atomoxetine (1, 3 and 10 mg/kg, i.p.) in male rats trained to drink alcohol solutions in a 4-bottle choice test. The treatment with atomoxetine (10 mg/kg, i.p.) during an alcohol deprivation period increased alcohol consumption after relapse. This effect only lasted one week, disappearing thereafter. Treatment with atomoxetine did not cause a behavioral sensitized response to a challenge dose of amphetamine (1.5 mg/kg, i.p.), indicating the absence of a supersensitive dopaminergic transmission. This effect is markedly different from that of SSRI antidepressants that produced both long-lasting increases in alcohol consumption and behavioral sensitization. Clinical implications are discussed.

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Introduction
Alcoholism is a chronic relapsing disorder associated with multiple medical consequences, including the addictive disorders (Kessler et al., 1996). Depressive symptoms can be observed in approximately 80% of alcoholic, of which a 30% meet criteria for major depression (Kessler et al., 1996; Torrens et al., 2005). Thus, a large number of alcoholic patients have been prescribed with antidepressants, despite the lack of information on the potential long-term effect of such therapy and the scarcity of conclusive studies on the efficacy of antidepressants for comorbid alcoholic/depressive patients (Pettinati, 2004; Torrens et al., 2005). Among the different antidepressants, selective serotonin reuptake inhibitors (SSRIs) are the most frequently prescribed. Recently, concerns about the use of SSRIs have been raised because of: (1) the potential side effects (Coupland et al., 1996; Ashton and Young, 1999; Blier and Tremblay, 2006); (2) the low efficacy on preventing relapse (Pettinati, 2004; Torrens et al., 2005); and (3) lack of effectiveness of antidepressants, especially SSRIs, for the treatment of clinical depression in alcoholic patients (Kranzler et al., 2006; Nunes et al., 2006). A recent clinical report suggests that SSRIs are less effective than selective noradrenaline reuptake inhibitors (SNRIs) for the treatment of alcoholism associated with psychiatric comorbidity (Petrakis et al., 2012). Although these clinical observations remain to be firmly confirmed, preclinical studies found a similar efficacy of either SSRIs or SNRIs in different models of alcoholism (De Vry et al., 1999).

The serotonin and noradrenaline transmission systems are key elements in the neuroadaptions associated with loss of control of drug use. Both serotonin and noradrenergic deficits have been linked to alcoholism and studies using animal models have demonstrated the efficacy of the administration of either SSRIs or SNRIs for alcoholism associated with stress and depression (Higley et al., 1998; Getachew et al., 2010). The normal serotonergic function needs to be balanced with respect to other modulatory systems (i.e. dopaminergic and noradrenergic) and it
appears that the most abused drugs, including alcohol, disrupt this equilibrium when consumed chronically (Tassin, 2008). Thus, it is feasible that the induction of an imbalanced serotonergic transmission, as a result of chronic administration of SSRIs, might have an impact on addictive behaviors. This assumption is supported by the induction of behavioral sensitization observed in animals treated with fluoxetine (Collu et al., 1997). If this is proven, such a side effect might limit the use of SSRI antidepressants as a therapy for alcoholism-associated affective disorders.

This hypothesis has been recently addressed in an experimental approach using an animal model of alcoholism (Alén et al., 2013). In that study, the sub-chronic administration of SSRIs (fluoxetine or the noradrenaline/serotonin reuptake inhibitor venlafaxine) during a period of alcohol deprivation enhanced the alcohol deprivation effect (ADE), leading to a sustained increase in alcohol consumption that lasted at least 5 wk. In addition, fluoxetine-treated animals showed an enhanced response to amphetamine, indicating a sensitized state of the dopaminergic reward system, as previously described (Collu et al., 1997). This sensitization was not observed in venlafaxine-treated animals. This study opens the debate on the adequacy of SSRI-based therapy for depression associated with alcoholism (see Nunes and Levin, 2004), in the absence of longitudinal studies on the efficacy of SSRIs in the relapse rates of alcoholics affected of major depression (Pettinati, 2004; Petrakis et al., 2012). This is especially relevant if we consider the need to address the treatment of depression without promoting relapse to alcohol consumption. As an alternative to SSRI, the use of SNRIs can be considered on the basis of the preclinical studies described above. However, there is no information on the effects of chronic SNRI treatment on alcohol consumption after relapse with the exception of the cited report of Petrakis et al. (2012) in veterans suffering comorbid alcoholism and affective disorders. In order to explore this hypothesis, the present study was designed to address the effects of the sub-chronic treatment with a SNRI during a period of alcohol deprivation on the levels of alcohol consumption after relapse. We have selected atomoxetine, a SNRI widely used for attention-deficit/hyperactivity disorder (Newman et al., 2008; Robinson et al., 2008). Atomoxetine, an orally active propylamine derivative, was initially designed as an antidepressant that was proven to have clinical efficacy (Chouinard et al., 1984) with a clinical pharmacology profile in humans compatible with that of a selective noradrenergic uptake blocker (Zerbe et al., 1985). However, it is currently approved only for attention-deficit/hyperactivity disorder (Newman et al., 2008). Its unique pharmacological profile allow us to test whether or not the enhancement of noradrenergic transmission will increase alcohol consumption when given along an alcohol-deprivation period, as observed after SSRI administration (Alén et al., 2013).

**Methods**

**Animals**

Thirty-two adult male Wistar rats (8 per group) (Harlan, Barcelona, Spain) weighing 375–425 g at the beginning of the experiments were housed individually under a 12 h light/dark cycle (lights off at 00:00 hours) in a room at constant temperature (23 ± 1°C). Standard food and tap water were available ad libitum at the home-cage. The animals were allowed to accustom to the housing facilities for 2 wk before the beginning of the alcohol self-administration protocol.

All experimental animal procedures were performed in compliance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and with Spanish regulations (RD 53/2013 and 178/2004). All protocols were approved by the Ethics Committee of the Complutense University of Madrid. Special care was taken to minimize the suffering and number of animals to achieve our research goals.

**Drugs**

Atomoxetine was purchased from Tocris-Bioscience (BiogenCientífica, Madrid, Spain). A fresh solution was prepared daily (before injection) by dissolving atomoxetine hydrochloride in the vehicle (0.9% saline) solution. Atomoxetine was injected at different doses (1, 3 or 10 mg/kg, i.p.) in a volume of 2 ml/kg. The doses were chosen based on the literature reporting behavioral characterization of atomoxetine (Robinson et al., 2008; Economidou et al., 2011; Janak et al., 2012). Three alcohol solutions were prepared daily: 5, 10 and 20% ethanol in water (w/v). Amphetamine was obtained from Sigma-Aldrich (Spain), and administered i.p. to the animals prior to the locomotor sensitization test, in a dose of 1.5 mg/kg.

**Non-operative alcohol self-administration and relapse model**

We used the 4-bottle non-operative alcohol self-administration model (Hölter and Spanagel, 1999; Spanagel and Hölter, 1999) for training the animals to drink alcohol. After 1 wk of habituation to the animal room, all rats were given continuous access to tap water and to 5, 10 and 20% alcohol solutions in their home cages.

All drinking solutions were renewed weekly and at that time the positions of the four bottles were changed to avoid location preferences. Once stable baseline was achieved, alcohol intake was monitored for 1 week and then ethanol-containing bottles were removed for 15 d. During this period of time, animals received daily injections of either vehicle or atomoxetine (1, 3 or 10 mg/kg, i.p.). After this period, the treatments with atomoxetine or vehicle were interrupted and bottles containing alcohol were made available again. Weekly alcohol consumption
was monitored for three consecutive weeks, and changes in the patterns of alcohol consumption were monitored. To this end, alcohol consumption was measured on the 2nd, 4th and 7th day of each week.

**Behavioral sensitization experiment**

The same experimental conditions, previously described with atomoxetine and vehicle, were reproduced in another set of Wistar rats to establish their potential for inducing sensitization to the psychostimulant effects of the amphetamine.

Animals were trained to consume alcohol and subsequently injected with the doses of atomoxetine and vehicle for 15 d, similarly to the alcohol self-administration experiment. After treatment, locomotor activity was monitored for 30 min and then, the rats were returned to their home cages. The amphetamine challenge (1.5 mg/kg, i.p.) was administered on the 16th day for all treatment groups, and the differences in locomotion among the experimental groups were monitored for the 30 min-post-injection interval. The testing and the sensitization phase were performed keeping the context constant for each animal (e.g. using the same chamber for each animal, procedure time, light intensity and so on).

Behavioral testing was performed within activity monitoring chambers (35 × 35 cm) equipped with 8 photobeams evenly distributed in two rows of four cells at 5 and 10 cm from the floor. The number of times that each photo beam was broken was registered by a computer program devised for this purpose, and used as measure of locomotion activity.

**Data analysis**

Data are expressed as means±S.E.M. The statistical analysis of the results was performed using GraphPad Prism version 5.04 (GraphPad Software Inc, USA). The non-operant alcohol self-administration results were evaluated by two-way analysis of variance (ANOVA) with repeated measures (factors: the treatment and time). Regarding locomotor activity, within- and between-groups analysis were performed by either one-way or two-way ANOVA (factors: the treatment and amphetamine challenge), respectively. In addition, multiple comparison post-hoc tests were performed [Dunnett’s test (one-way ANOVA) and Bonferroni’s test (two-way ANOVA)]. A two-tailed p value less than 0.05 was considered to be statistically significant.

**Results**

**Effects of atomoxetine on weekly alcohol consumption (Fig. 1a–c)**

Following the 15-d period of alcohol deprivation, the ethanol consumption was increased during the relapse in the first week in all groups of treatment (51% [vehicle group], 97% [atomoxetine 3 mg/kg] and 416% [atomoxetine 10 mg/kg] of increase, approximately), with the exception of animals receiving 1 mg/kg of atomoxetine, which showed a slight increase of 6%.

The weekly pattern of alcohol consumption was different in each week. During the first week (Fig. 1a), a two-way ANOVA indicated that only the treatment had a significant primary effect on ethanol intake [$F_{3,84}=21.44$, $p<0.001$], with no interaction between treatment and time [$F_{6,84}=0.6726$, n.s.]. In fact, alcohol consumption was greater in the first 2 d for atomoxetine 3 and 10 mg/kg (**p<0.01** than vehicle. In the following days, only the group treated with atomoxetine 10 mg/kg...
(**p<0.01) remained high. During the second week (Fig. 1b), the time had a significant primary effect on ethanol intake \( \left[ F_{2,84}=7.605, p=0.0009 \right] \), with no interaction between treatment and time \( \left[ F_{6,84}=0.6259, \text{n.s} \right] \). However, during the third week (Fig. 1c), the ethanol intake was found to be similar in all groups with no significant effects or interaction between the time and treatment. These results suggest that the effects observed for atomoxetine were time-limited.

**Effects of atomoxetine treatment on locomotor sensitization (Fig. 2)**

One-way ANOVA indicated that sub-chronic treatment with atomoxetine had a significant primary effect on locomotion \( \left[ F_{3,31}=3.945, p=0.0182 \right] \). Thus, the post-hoc analysis showed that the atomoxetine induced a mild decrease in locomotion resulting significant at the doses of 3 and 10 mg/kg \( (\#p<0.05) \). However, the sub-chronic treatment with atomoxetine did not affect the psychostimulant response to amphetamine (1.5 mg/kg) \( \left[ F_{3,31}=0.1379, \text{n.s} \right] \).

A two-way ANOVA indicated that only amphetamine had a significant main effect on locomotion \( \left[ F_{1,36}=158.6, p<0.001 \right] \), with no interaction between amphetamine and atomoxetine treatment \( \left[ F_{3,36}=0.1418, \text{n.s} \right] \). In fact, animals with amphetamine displayed an increased locomotion compared with the control groups \( (**p<0.001 \text{ and } *p<0.01) \). This result indicated that chronic treatment with atomoxetine does not result in sensitization of the reward system, an effect distinct to that described for the SSRI in a previous report (Alén et al., 2013).

**Discussion**

The main finding of this study is that atomoxetine, given during a period of abstinence from alcohol, produced time-limited effects on alcohol consumption after relapse. Moreover, atomoxetine did not result in behavioral sensitization in the context of alcohol consumption, suggesting a preservation of the mesolimbic dopaminergic reward system. This profile is very different when compared to that described for SSRIs such as fluoxetine (Alén et al., 2013). SSRI administration during alcohol deprivation produced increased alcohol consumption after relapse, an effect that lasted more than 5 wk, whereas atomoxetine effects disappeared within a week. This profile is much more favorable, considering the proposed role of noradrenaline in facilitating the extinction of contextual memories associated with the self-administration of abused drugs (Economidou et al., 2011; Janak et al., 2012).

The increase in alcohol consumption was observed at the highest atomoxetine dose administered (10 mg/kg), that it is known to block the dopamine transporter (Turner et al., 2013). However, the effect on increased alcohol consumption observed seems to be independent of the dopaminergic transmission, as it did not result in behavioral sensitization. Supporting this hypothesis, methylphenidate, a drug used also for attention-deficit/hyperactivity disorder, did not affect alcohol consumption (Soeters et al., 2008). Methylphenidate is a potent inhibitor of the dopamine transporter, and a mild inhibitor of noradrenaline uptake, a pharmacological profile symmetrical to that of atomoxetine. Whether the short-term effect of the high dose of atomoxetine is related to a loss of selectivity towards the noradrenaline transporter, or to the interaction of the drug with other targets such as the N-methyl-D-aspartate (NMDA) receptor (Udvardi et al., 2013), or the serotonin transporter (Ding et al., 2014) remains to be studied. This is an important limitation on the clinical translation of the present observations, as Atomoxetine has been found to display differential selectivity in rats, non-human primates and clinical patients (Zerbe et al., 1985; Ding et al., 2014). In any case, and in rodents, the lack of effect of the intermediate dose on the ADE and the suppression of the ADE by the low dose are of a great interest. The low dose of atomoxetine used in this study, 1 mg/kg, is sufficient to block completely the noradrenaline transporter. Similar doses have been used in different tests of either impulsivity (Robinson et al., 2008; Ansquer et al., 2013) or extinction of conditioned responses in the context of addiction (Economidou et al., 2011; Janak et al., 2012). Considering the study of the effects of atomoxetine in drug-seeking behaviors, the administration of 1 mg/kg of atomoxetine facilitated the extinction of cocaine-seeking behavior, in a similar way to that observed after multiple, but not discrete, stimuli associated with drug presentation (Janak et al., 2012). The authors of these
studies considered that the enhanced arousal derived from the increased noradrenergic transmission produced by atomoxetine strengthened the extinction of drug-paired stimuli. In our study we did not control for conditioned responses, since the animals resumed alcohol self-administration after the three bottles containing different percentages of ethanol were presented again. However, the absence of the ADE observed in the group treated with atomoxetine 1 mg/kg, suggests that similar effects to that described in the above-cited studies, might account for the reduction in alcohol consumption. Further studies, using operant models of alcohol self-administration, will help to confirm that the extinction of conditional cues associated with alcohol is sensitive to the effects of atomoxetine, the same as it has been described for cocaine and heroin (Economidou et al., 2011).

In summary, we have observed that the administration of a noradrenaline reuptake blocker during alcohol deprivation produced time-limited effects on the consumption of alcohol after relapse. The effects were short lasting and not associated with behavioral sensitization, indicating a better pharmacological profile with respect to the commonly prescribed SSRIs. Careful analysis of in-between species differences in the pharmacological profile of atomoxetine have to be considered for a potential clinical translation of the present studies. Because atomoxetine is not used as an antidepressant, further studies using other SNRI antidepressants such as reboxetine or desipramine, should be addressed in order to confirm or not the existence of better clinical outcomes in alcoholics suffering depression when treated with these SNRIs.

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Statements of Interest
There are no actual or potential conflicts of interest.

References


