Short communication

Naloxone-induced taste aversions in opiate-naïve Lewis and Fischer 344 rat strains

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ABSTRACT

Background: The Lewis (LEW) and Fischer (F344) rat strains appear differentially sensitive to the aversive effects of several used and abused drugs. Naloxone, a mu opioid receptor antagonist that induces aversions in outbred rats but has no abuse potential, was assessed to determine the characteristics of compounds for which the strains differ.

Methods: Opioid-naïve male LEW and F344 rats were given access to saccharin followed by low (Experiment 1) and high (Experiment 2) doses of naloxone every 4th day for five pairings. Aversions were assessed in both one-bottle and two-bottle tests.

Results: In Experiment 1, aversions were evident at 10 mg/kg (one-bottle) and at 5.6 and 10 mg/kg (two-bottle) with no apparent strain difference for either assessment. In Experiment 2, aversions were evident for LEW animals (but not F344) at 18 and 32 mg/kg (one-bottle). LEW animals injected with 32 mg/kg displayed greater aversions than F344 animals receiving the same dose. Both strains displayed aversions at all doses in the two-bottle test with no strain difference.

Conclusions: Naloxone induced aversions that were strain dependent only at specific doses and under the one-bottle testing condition. These results parallel those of several other used and abused drugs but differ dramatically from those seen with morphine in the two strains (F344 > LEW). Further assessments utilizing the LEW–F344 model should investigate other drugs to establish the set of compounds for which the strains differ and to characterize the mechanism underlying the observed differences.

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1. Introduction

Although the Lewis (LEW) and Fischer (F344) inbred rat strains are well characterized in terms of their relative sensitivity to the rewarding effects of a variety of drugs of abuse (LEW > F344; see Kosten and Ambrosio, 2002; Riley et al., 2009), they also differ in their sensitivity to their aversive effects (see Riley et al., 2009). For example, the F344 strain displays stronger morphine-, nicotine- and ethanol-induced aversions (Lancelotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006), while the LEW strain displays greater aversions induced by cocaine and caffeine (Glowa et al., 1994; Vishwanath et al., 2011). Given that drug use and abuse vulnerability is a function of the balance between the drug's rewarding and aversive effects (Riley et al., 2009; Riley, 2011), strain differences in the aversive properties of drugs may provide insight into the role of genotype in such effects.

The LEW and F344 strains have been reported to have basal neurochemical and neuroanatomical differences in brain systems mediating responsivity to the opioids, particularly in the nucleus accumbens (NAc) and locus coeruleus (LC) (Guitart et al., 1992, 1993). For example, the LEW strain displays lower basal levels of proenkephalin mRNA in the ventro- and dorsolateral caudate putamen (Sánchez-Cardoso et al., 2007), as well as lower levels of dynorphin A and B and leu-enkephalin Arg6 in the striatum (Nylander et al., 1995). Further, the LEW and F344 strains have differential basal expression of intracellular G-proteins (F344 > LEW) and cAMP-dependent signaling (LEW > F344; Guitart et al., 1993) as well as differential levels of tyrosine hydroxylase (TH) in the LC (LEW > F344; Guitart et al., 1992). Given that these brain areas and systems may mediate the affective response to both natural and non-natural reinforcers (Koob and Nestler, 1997), these areas may play a role in the differential reactivity of the LEW and F344 strains in response to opioid administration. It then might be expected that the behavior of the two strains might also differ when these systems are blocked or deactivated in opiate-naïve animals. Although such an assessment has not been made in the LEW and F344 strains, administration of the opioid antagonist naloxone to opioid-naive outbred rats induces a dose-dependent conditioned taste aversion at doses as low as 1, 3.2 and 10 mg/kg IP (Stoelerman et al., 1978; see also Freng and Rogers, 1979; Leshem, 1984), presumably as a result of functional alterations in endogenous opioid systems (Mucha and Walker, 1987; Mucha and Herz, 1985; Mucha et al., 1985).
Interestingly, the classical emetic LiCl induces comparable dose-dependent aversions in the two strains (see Foynes and Riley, 2004), suggesting that some feature of drugs that are used and/or abused may be important in these strain differences. To address this possibility, the present studies examined the ability of the mu opioid antagonist naloxone to induce strain-dependent aversions. Naloxone induces aversions in outbred rats (Frenk and Rogers, 1979; Hunt et al., 1983; Leshem, 1984; Stolerman et al., 1978) and like LiCl, has little abuse liability and has emetic effects (see Crampton and Daunton, 1983; Bhargava et al., 1981). Given this, naloxone allows for the characterization of the nature of compounds for which the LEW and F344 strains differ. Accordingly, opiate-naïve LEW and F344 rats were given saccharin and then injected with various doses of naloxone (0, 3.2, 5.6, 10 mg/kg: Experiment 1; 0, 10, 18, 32 mg/kg: Experiment 2) to assess strain differences in its aversive effects.

2. Methods

2.1. Experiment 1: low-dose naloxone

2.1.1. Subjects. Subjects in Experiment 1 were 71 experimentally naive LEW (n = 36) and F344 (n = 35) male rats (Harlan, Indianapolis), 90 days old and 247 g (LEW) and 185 g (F344). They were run in two replicates (n = 15 and 14: Replicate 1; n = 21 and 21: Replicate 2). Subjects in the replicates did not differ [F(1,63) = 0.283, p = 0.597], and data were pooled for analysis and presentation. Animals were housed in individual wire-mesh cages and maintained on a 12:12 cycle (lights on at 08:00 h) and at an ambient temperature of 23 °C. Except where noted, food and water were provided ad libitum. Fluids were presented in 50 ml graduated Nalgene tubes. Animals were handled 2 weeks prior to the initiation of experimental manipulations to minimize handling stress. All manipulations were performed in the light phase of the light/dark cycle and were in compliance with the National Research Council (1996) and approved by the Institutional Animal Care and Use Committee at American University.

2.1.2. Drugs and solutions. Naloxone HCl (supplied by NDA) was dissolved in isotonic saline (1.0 mg/ml; Sigma) and administered intraperitoneally (ip). Sodium saccharin (0.1%; Sigma) was prepared as a 1 g/l solution in tap water.

2.1.3. Conditioned taste aversion.

2.1.3.1. Phase I: habituation. Following 23[12] h water deprivation, rats were given 20 min water access. This was repeated daily until consumption stabilized.

2.1.3.2. Phase II: conditioning. On Day 1 of this phase, subjects were given 20 min access to saccharin. Immediately following, subjects within each strain were assigned to four groups such that saccharin consumption was comparable and given an ip injection of 0, 3.2, 5.6 or 10.0 mg/kg naloxone, yielding Groups I0 (n = 8), L3.2 (n = 8), L5.6 (n = 8), L10 (n = 8) and F0 (n = 8), F3.2 (n = 8), F5.6 (n = 8) and F10 (n = 8). The letter denotes the strain; the number indicates the dose of naloxone. On the following 3 days, subjects were given 20 min water access. This cycle was repeated five times. The fifth saccharin trial served as a one-bottle test of the acquisition of the aversion to saccharin.

2.1.3.3. Phase III: two-bottle aversion test. On the day following the last cycle of Phase II, subjects were given 20 min access to water and saccharin in a two-bottle test of the aversion. The location of the bottles was counterbalanced within each group. Saccharin was initially presented for 5 s. Then, saccharin was removed and water was presented for 5 s. Both bottles were then presented for the remainder of the session. Saccharin preference was calculated as the percent saccharin of total consumption.

2.2. Experiment 2: high-dose naloxone

2.2.1. Subjects. Subjects in Experiment 2 were 66 experimentally naive LEW (n = 34) and F344 (n = 32) subjects of the same sex, strain and age and maintained under the same conditions as in Experiment 1. Subjects were run in two replicates (n = 17 and 17: Replicate 1; n = 17 and 17: Replicate 2). Subjects in the replicates did not differ [F(1,58) = 5.049, p = 0.056], and data were pooled for analysis and presentation. The procedures in this phase were identical to Experiment 1 with the following exception: during conditioning, subjects in each strain were injected with 0, 10, 18 or 32 mg/kg naloxone, yielding Groups L0 (n = 8), L10 (n = 8), L18 (n = 8), L32 (n = 8), F0 (n = 7), F10 (n = 7), F18 (n = 8) and F32 (n = 9).

2.3. Analysis

For each experiment, differences in mean saccharin consumption on the one-bottle aversion test (Trial 5) were analyzed using a 2 × 4 univariate ANOVA with between-subjects factors of Strain (LEW: F344) and Dose (0, 3.2, 5.6, 10 mg/kg: Experiment 1; 0, 10, 18, 32 mg/kg: Experiment 2). Differences in mean saccharin preference on the two-bottle aversion test were compared using a 2 × 4 univariate ANOVA with between-subjects factors of Strain and Dose. All pair-wise comparisons were made using one-way ANOVAs followed by Tukey’s HSD post hoc tests; significance was assessed at α < 0.05.

3. Results

3.1. Experiment 1: low-dose naloxone

3.1.1. Mean saccharin consumption – one-bottle aversion test. The 2 × 4 univariate ANOVA on the one-bottle test (Trial 5) revealed a significant effect of Dose [F(3,63) = 15.790, p < 0.01].collapsed across strain, animals injected with 10 mg/kg naloxone consumed significantly less saccharin than animals injected with vehicle ($p < 0.01$; see Fig. 1; top panel). There was no significant effect of Strain or a Strain × Dose interaction.
3.1.2. Saccharin preference – two-bottle aversion test. The 2 x 4 univariate ANOVA on the two-bottle test revealed significant effects of Strain [F(1,63) = 4.182, p < 0.05] and Dose [F(3,63) = 4.567, p < 0.01] but no Strain x Dose interaction [F(3,63) = 1.209, p = 0.314]. Collapsed across dose, the percent saccharin consumed by LEW animals was significantly less than that of the F344 animals. Collapsed across strain, the percent saccharin consumed by animals injected with 5.6 and 10 mg/kg naloxone was significantly less than that of vehicle-injected animals (p’s < 0.05; see Fig. 1; bottom panel).

3.2. Experiment 2: high dose naloxone

3.2.1. Mean saccharin consumption – one-bottle aversion test. The 2 x 4 univariate ANOVA on the one-bottle aversion test (Trial 5) revealed significant effects of Strain [F(1,58) = 8.644, p < 0.01] and Dose [F(3,58) = 14.355, p < 0.001] as well as a significant Strain x Dose interaction [F(3,58) = 3.084, p < 0.05]. LEW animals injected with 18 and 32 mg/kg naloxone consumed significantly less saccharin than vehicle-injected animals of their own strain (both p’s ≤ 0.01), indicative of the development of aversions at the higher doses of naloxone relative to vehicle-injected controls. F344 animals did not differ from vehicle-injected controls at any dose tested. At 32 mg/kg, LEW animals consumed significantly less saccharin than F344 animals (p < 0.01; see Fig. 2; top panel).

3.2.2. Saccharin preference – two-bottle aversion test. The 2 x 4 univariate ANOVA revealed an effect of Dose [F(3,58) = 19.173, p < 0.001]. There was no effect of Strain or Strain x Dose interaction [F(3,58) = 0.284, p = 0.837]. Collapsed across strain, the percent saccharin consumed by animals injected with 10, 18 and 32 mg/kg naloxone was significantly less than that of vehicle-injected animals (p’s < 0.0001; see Fig. 2; bottom panel).

4. Discussion

A variety of used and abused compounds differentially induce taste aversions in the LEW and F344 rat strains, the exception being LiCl (see Foynes and Riley, 2004). Given that naloxone induces taste aversions and has no abuse potential, it provides an assessment of whether use and abuse are necessary conditions for such differences. In the one-bottle aversion test in Experiment 1, naloxone induced aversions at the 10 mg/kg dose relative to vehicle controls with no effect of strain. Aversions were evident at both 5.6 and 10 mg/kg on the more sensitive two-bottle test (see Batzell and Best, 1993), but again there were no strain differences. In the one-bottle test in Experiment 2, naloxone induced aversions at 18 and 32 mg/kg in the LEW strain (relative to LEW animals administered vehicle). No dose of naloxone induced aversions in the F344 strain in this assessment. Additionally, the amount consumed by the LEW subjects injected with 32 mg/kg naloxone was significantly less than that of F344 subjects injected at this dose. All of the higher doses of naloxone induced aversions in the two-bottle test, but there were no strain differences in the degree of the aversion.

Although the two strains did differ in naloxone-induced taste aversions, these differences were modest and evident only under specific parametric conditions, e.g., at the highest dose and only in the one-bottle test. Such small, dose-dependent differences have been reported with a number of other compounds, including cocaine, nicotine, alcohol and caffeine (Glowa et al., 1994; Lancellotti et al., 2001; Pescatore et al., 2005; Roma et al., 2006; Vishwanath et al., 2011; for similar effects in other behavioral preparations, see Kosten and Ambrosio, 2002). That the strain difference was not evident on the two-bottle aversion test is likely due to the fact that the sensitivity of this test often precludes observing differences among groups, i.e., it can detect aversions not evident in less sensitive designs, but is too sensitive to reveal graded effects (see Rinker et al., 2011 for a recent discussion of this issue; see also Batzell and Best, 1993).

Although the effects with naloxone parallel those reported for a variety of other compounds (see above), the direction of the difference is highly drug-dependent. For example, the F344 strain shows greater aversions than the LEW strain with nicotine and alcohol, whereas the LEW strain displays greater aversions than the F344 strain with cocaine and caffeine. In relation to the current effects with naloxone, it is interesting that the effects reported here (LEW > F344) are opposite to those reported with morphine (F344 > LEW; see Lancellotti et al., 2001; Davis et al., 2009). In attempting to understand the basis for such effects, it is important to note that the LEW and F344 rat strains differ significantly in the neurochemical systems mediating opiate-induced effects (both endogenous and exogenous). For example, the LEW strain has lower levels of proenkephalin mRNA in the ventro- and dorsolateral caudate putamen (Sánchez-Cardoso et al., 2007), dynorphin A and B and leu-enkephalin Arg⁶ in the striatum (Nylander et al., 1995) as well as levels of intracellular G-proteins relative to F344 animals (Guitart et al., 1993). LEW animals also display higher levels of TH, cAMP-dependent protein kinase signaling and adenylyl cyclase in
the LC than F344 animals (Guitart et al., 1992, 1993). These basal differences have been discussed relative to their role in a variety of behavioral effects reported in the F344 and LEW strains (see Kosten and Ambrosio, 2002; Sánchez-Cardoso et al., 2007); however, their role in the differential aversions induced by naloxone is less clear. It has been argued (see Mucha et al., 1985; Stolerman, 1985) that the effects of naloxone administration in opiate-naïve animals is a function of homeostatic dysregulation in basal endorphin activity (i.e., basal opioid tone; see also Eisenberg, 1980: Frenk and Rogers, 1979) that produces an aversive state. Given this and the differing basal opioid activity of the two strains, it is possible that the antagonism of the different neurochemical systems mediating opioid activity in the two strains mediates their different putative responsiveness to naloxone (as indexed in the taste aversion design). What would remain to be explained is why the differences between the strains vary with agonist or antagonist administration. As noted, morphine is more aversive in the F344 than the LEW strain, whereas naloxone is more aversive in the LEW than the F344 strain. These differences suggest that the basal opioid conditions that mediate the sensitivity to the agonist may impact antagonist sensitivity, although the mechanism for such an effect remains unknown. In the absence of opioid binding profiles in the two strains, it is unknown if the affinity of morphine and naloxone in the two strains differ and if such differences (if they do exist) mediate the differential reactivity seen in the aversion design.

The purpose of the present series of experiments was to assess aversions induced by naloxone in the two strains and provide additional characterization of compounds on which the LEW and F344 strains may differ. As noted above, only compounds used and/or abused in humans and animal models have been reported to differentially induce aversions in these strains (the only exception to these differential effects being LiCl). The fact that strain differences were evident with naloxone suggests that the differential effects of drugs in these strains are not limited to drugs with rewarding properties, although the basis for the effects reported here are unknown. The present findings argue for assessments with other drugs to establish the range of compounds for which the strains differ and to characterize the basis for these differences. Additionally, neurobiological assessments of the reactivity of the LEW and F344 opioidergic systems are necessary in order to determine what role, if any, the reported basal differences and differential reactivity of these systems play in their response to naloxone. Given the role inbred strains play in isolating potential genetic factors in behavior, such information may be useful in determining if and to what extent genetic differences in sensitivity to the effects of drugs contribute to behavioral differences.

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Contributors

A.G.D., J.L.C and A.L.R. participated in the design and coordination of the study, performed the analysis and drafted up the manuscript. A.G.D. performed all data collection. All authors contributed to and have approved the final manuscript.

Conflict of interest

No conflict declared.