Chronic Oral Treatment with 13-cis-Retinoic Acid (Isotretinoin) or all-trans-Retinoic Acid Does Not Alter Depression-Like Behaviors in Rats

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INTRODUCTION

Oral treatment with the anti-acne drug Accutane (isotretinoin, 13-cis-retinoic acid) has been associated with suicide ideation and depression. Here, depression-like behaviors (i.e., behavioral despair and anhedonia) were quantified in adult Sprague-Dawley rats gavaged daily beginning at postnatal day (PND) 82 with 13-cis-RA (7.5 or 22.5 mg/kg) or all-trans-retinoic acid (10 or 15 mg/kg). Tested at PND 130–131 in the Forced Swim Test, 7.5 mg/kg 13-cis-RA marginally decreased immobility and slightly increased climb/struggle durations whereas neither all-trans-retinoic acid group differed from controls. Voluntary saccharin solution (0.03%) intake at PND 102–104 and PND 151–153 was not different from controls in any treated group, although all RA-treated groups had lower intakes. Swim speed in a water maze at PND 180 was similar across groups, indicating no RA-induced differences in physical ability. Open field activity was mildly decreased at PND 91 in 7.5 mg/kg–treated males only, but it was within the control range at PND 119, 147, and 175. Thus, at serum levels similar to those in humans receiving the drug, chronic 13-cis-RA treatment did not severely affect depression-like behaviors in rats. These data do not substantiate the hypothesis of 13-cis-RA–induced depression.

Key Words: isotretinoin; 13-cis-retinoic acid; all-trans-retinoic acid; depression; anhedonia; locomotor activity; Forced Swim Test.
have been pharmacologically validated (Nakamura and Tanaka, 2001; Overstreet et al., 1995; Papp et al., 1996; Rex et al., 2004; Sanchez and Meier, 1997), and the end points are responsive to antidepressant treatment (Borsini and Meli, 1988; Lucki, 1997; Papp et al., 1996). More importantly, however, these tests have been shown to be sensitive to depression-inducing paradigms or treatments such as chronic mild stress (Duncko et al., 2001; Tannenbaum et al., 2002; Willner, 1997) and to rodent strains that are genetically prone to depression (Ayensu et al., 1995; Overstreet et al., 1995; Pucilowski et al., 1993; Yadid et al., 2000). Furthermore, increased immobility in the FST has been demonstrated in relation to human pharmacological treatments such as neonatal cocaine or clomipramine exposure or adult MDMA treatment (Hansen-Trench and Barron, 2005; Thompson et al., 2004; Vazquez-Palacios et al., 2005). Other behaviors that have been associated with depression in rodents include decreased locomotor activity (D’Aquila et al., 2000; Kopp et al., 1999) and loss of appetite and body weight (Weiss, 1968).

Measures of depression in nonhuman animals are limited, and the FST and voluntary saccharin intake are the most often used. Other measures include learned helplessness, anhedonia as measured by voluntary electrical brain stimulation, and tail suspension. Willner has suggested that the learned helplessness model may be more relevant to anxiety than to depression (Willner, 1995), whereas Nestler et al. (2002) describe the paradigm as more directly modeling posttraumatic stress disorder. Decreased response rates for voluntary electrical brain stimulation have been reported after exposure to either chronic mild stress or inescapable shock (Nielsen et al., 2000; Zacharko et al., 1983). However, there is some concern that increased response rates, rather than decreased rates, may be more reflective of anhedonia in that animals are responding more to achieve the same level of stimulation as prior to the depression-inducing paradigm (i.e., inescapable shock) (Anisman and Matheson, 2005). Tail suspension tests are not typically conducted with rats.

The doses of 13-cis-RA (7.5 and 22.5 mg/kg) chosen for the current study were based on previous research indicating that 7.5 mg/kg produces serum levels of 13-cis-RA comparable to those of human Accutane users (Ferguson et al., unpublished data). Groups treated with all-trans-RA were included because 13-cis-RA itself has low affinity for any of the RA receptors (Crettaz et al., 1990; Kim et al., 1994) and its dermatological effects are thought to be exerted via isomerization to all-trans-RA (Geiger et al., 1996). Doses for all-trans-RA were more difficult to determine, as levels of this isomer are generally not reported in studies of human Accutane users. However, previous research had indicated that there were no body weight or food/water intake alterations after a 7-day treatment period in rats treated with the doses (10 and 15 mg/kg) used in the present study.

Finally, both sexes were examined in our study to determine sex-specific effects. Although there is no indication that 13-cis-RA has such effects, there is ample evidence to suggest that depression is much more common in women than in men (Angst, 1998; Blazer et al., 1994; Ernst and Angst, 1992; Fennig et al., 1994).

### MATERIALS AND METHODS

#### Animals

Sixty weanling Sprague-Dawley rats (30 male and 30 female) were obtained from the breeding colony at the National Center for Toxicological Research/FDA and pair-housed with a same-sex rat in standard polycarbonate tub cages lined with wood chip bedding on postnatal day (PND) 21 until PND 52, at which time each was individually housed. Pelleted rat chow (NIH-31, Purina Mills, St. Louis, MO) and water were provided ad libitum in the home cage. Rats were maintained in vivariums at 22° ± 1°C (mean ± SEM) and 45–55% humidity on a 12:12 hour light–dark cycle (lights on at 0700, off at 1900). All animal procedures were approved by the NCTR Institutional Animal Care and Use Committee.

#### Animal Treatments

Capsules (40 mg each) of 13-cis-RA (Accutane, Roche Pharmaceuticals, Nutley, NJ) were obtained from Washington Wholesale Drug Exchange (Savage, MD). Capsules were opened and their contents diluted in soybean oil (ICN Biomedicals, Irvine, CA) to produce 5.25 and 15.75 mg/ml (7.5 and 22.5 mg/kg dose levels) suspensions. The all-trans-RA (Across Organics, Morris Plains, NJ) was similarly diluted to produce solutions to provide 7.0 and 10.5 mg/ml (10 and 15 mg/kg dose levels) suspensions. Suspensions were prepared every 2 days and maintained at room temperature in amber glass containers to prevent photoisomerization. All areas and rooms were fitted with UV filters over fluorescent lights.

On PND 80–81, each rat was gavaged with soybean oil only to facilitate adaptation to the gavage procedure. Rats were assigned to treatment groups such that there were six animals/sex/dose group and gavaged daily with either 0 (soybean oil only), 7.5 (low), or 22.5 (high) mg/kg of 13-cis-RA, or 10 (low) or 15 (high) mg/kg of all-trans-RA in a volume of 1.42 ml/kg beginning on PND 82. All gavages were done in the morning hours and always prior to behavioral testing.

#### Body Weight, and Food and Water Intake

Body weights and food intake were recorded daily beginning on PND 75 and continued until sacrifice (i.e., at PND 185–189). Body weights were averaged for each week. Food and water intake were standardized to body weight (i.e., g food/g body weight and ml water/g body weight) for each day and then averaged for each week, including the 5 days prior to RA treatment. The week prior to beginning RA treatment is expressed as baseline or week 0.

#### Behavioral Testing

**Forced Swim Test (FST): procedure.** Depression-like behaviors were assessed on PND 130–131 using methodology similar to that originally described by Borsok et al. (1977), but modified by Cryan et al. (2005). Rats were gavaged 30–100 min prior to testing and transported to the testing room immediately prior to testing. Order of testing was randomized with respect to treatment and sex. White noise of approximately 60 dB (AM radio set between stations) buffered extraneous noise. The apparatus was a clear Plexiglas cylinder (height = 61 cm; diameter = 29 cm) filled with 50 cm of fresh tap water, maintained at 25° ± 1°C. White noise masked background noise. Each rat was placed into the cylinder on 2 consecutive days for 10 min (PND 130) or 5 min (PND 131), after which time it was removed, gently towel dried, and returned in its cage to the housing room. Early removal from the swim test (i.e.,
RESULTS

Body Weight, Food and Water Intake, and General Health Observations

13-cis-RA. Although there was a significant interaction between sex × week (F(15,450) = 74.39, p < .0001) on body weight, post-hoc analyses simply indicated that males weighed more than females in all weeks. There was no main effect of treatment, nor were there any significant interactions with treatment. Food intake analyses indicated a significant interaction of sex × week (F(15,450) = 1.74, p < .05); however, females consumed more than males at each week as expressed as g food/g body weight. Water intake analyses were similar in that there was a significant sex × week interaction (F(15,449) = 4.15, p < .0001), and post-hoc analyses indicated that females drank more at each week as expressed as ml water/g body weight.

All-trans-RA. Although there was a significant interaction of treatment × sex × week (F(30,449) = 2.02, p < .002) on body weight, post-hoc analyses indicated no significant differences in any week between female controls and same-sex all-trans-RA groups; nor were there any significant differences between male controls and same-sex all-trans-RA groups. Food intake analyses indicated a significant interaction of sex and week (F(15,448) = 2.70, p < .0007); however, females consumed more than males in each week, as expressed as g food/g body weight. There was a significant interaction between sex and week and the analysis of water intake (F(15,448) = 2.02, p < .02), and post-hoc analyses indicated that females drank more than males during all weeks, except weeks 2, 4, and 11.

Home cage observations indicated that some all-trans-RA–treated rats had bone fractures, an observation that was later confirmed at sacrifice. Seven rats (one male and six females, of which three were treated with 10 mg/kg and four with 15 mg/kg) had fractures of the tibia or humerus. None of these rats had been previously rescued during the FST, and all had swim speed data within the range of controls. Similarly, locomotor activity in the open field appeared unaffected by these fractures. Full details of the fractures and the effect of all-trans-RA on bone metabolism and density will be available in a later publication (Hotchkiss et al., in press).

Forced Swim Test Behavior

13-cis-RA. Five male rats required rescue. These were two control animals on day 1, one 7.5 mg/kg 13-cis-RA on day 1, one control on day 2, and one 22.5 mg/kg 13-cis-RA on day 2. No females required rescue. Figure 1 illustrates durations of immobility and climb/struggle averaged over the two test days. There was a significant interaction between sex and day (F(1,25) = 8.99, p < .007) and a marginally significant effect of treatment (F(2,29) = 2.83, p < .08) on duration of immobility. Post-hoc tests indicated that both sexes were significantly less immobile on day 2 than on day 1 (p < .05).
Analysis of the duration of climb/struggle indicated a significant interaction between sex and day (F(1,25) = 8.64, p < .007). Post-hoc tests indicated that females performed significantly less climb/struggle on day 2 than on day 1 (p < .05), but the durations for males did not differ between days. A main effect of day on duration of swim (F(1,25) = 12.37, p < .002) indicated less swimming behavior on day 2 than on day 1. Analysis of frequency of dives indicated a significant interaction between treatment and day (F(2,25) = 3.42, p < .05) and although there was more diving on day 1 than on day 2, this was significant only for the 22.5 mg/kg 13-cis-RA group (p < .05). There were no significant effects on number of fecal boli.

All-trans-RA. One male rat treated with 10 mg/kg all-trans-RA required rescue on day 2. No females required rescue. There was an interaction between sex and day (F(1,24) = 6.39, p < .02) and a marginally significant effect of treatment (F(2,31) = 3.29, p = .06) on duration of immobility. Post-hoc tests indicated that both males and females exhibited less immobility on day 2 than on day 1 (p < .05). The treatment effect indicated that rats treated with 15 mg/kg all-trans-RA were somewhat more immobile than rats treated with 10 mg/kg. Analysis of duration of climb/struggle indicated a significant interaction between sex and day (F(1,24) = 7.19, p < .02), and post-hoc tests indicated that females exhibited much less climb/struggle behavior on day 2 than on day 1, and less than males on day 2. Main effects of sex (F(1,31) = 8.39, p < .007) and day (F(1,24) = 23.10, p < .0001) on duration of swim indicated that females swam longer than males and there was more swimming behavior exhibited on day 1 than on day 2. A significant interaction of sex × day on dive frequency (F(1,30) = 5.63, p < .03) indicated that males exhibited dives more frequently than females on day 1 (p < .05), and dive frequency for males was higher on day 1 than on day 2 (p < .05). The significant effect of sex on number of fecal boli (F(1,30) = 12.67, p < .002) indicated that females had higher numbers of fecal boli than males.

Saccharin Solution Intake

13-cis-RA. Figure 2 illustrates saccharin in solution intake by RA treatment and sex averaged over the two tests. There were no significant treatment effects on voluntary saccharin solution intake. A significant interaction between sex and day (F(2,147) = 3.89, p < .03) indicated that females consume more saccharin on the first day of each test than do males on the first day and that males consume more than females on the second and third days of each test (p < .05). Intake on the second day was also higher than intake on the third day for females only (p < .05); intake over the three days of each test did not differ for males.

All-trans-RA. A main effect of sex (F(1,30) = 13.54, p < .0009) indicated increased saccharin intake in females while a main effect of day (F(2,148) = 9.95, p < .0001) indicated higher intake on the first day than on the second and third days (p < .05).

Open Field Activity

13-cis-RA. Figure 3 illustrates open field activity by RA treatment, sex and age. There was a significant interaction of treatment × sex × age (F(6,88) = 3.71, p < .003) on total activity, and post-hoc tests indicated that control males were more active than 7.5 mg/kg 13-cis-RA males at PND 91 (p < .05). Additionally, activity of control males at PND 91 was higher than at PND 147 and 175 (p < .05). Analysis of freeze duration indicated a similar interaction of treatment × sex × age (F(6,89) = 3.48, p < .004), and post-hoc tests reflected similar results: lower durations of freezing behavior in control males at PND 91 than in 7.5 mg/kg 13-cis-RA males of the same age, as well as in control males at PND 147 and 175 (p < .05).
All-trans-RA. There was a marginal effect of treatment on total activity (F(2,32) = 2.63, p < .09) which indicated that the 10 and 15 mg/kg all-trans-RA groups had somewhat less activity overall than controls. A significant main effect of age (F(3,87) = 15.66, p < .0001) indicated higher levels of total activity at PND 91 in all groups and sexes than all other ages and higher levels at PND 119 than at PND 147 or 175 (p < .05). Post-hoc tests of the main effects of treatment (F(2,32) = 3.72, p < .04), sex (F(1,32) = 6.93, p < .02), and age (F(3,86) = 12.55, p < .0001) on freeze duration indicated several effects: no treatment group was significantly different from control, males had a lower duration of freezing than females (p < .05), and freeze duration at PNDs 91 and 119 was lower than at PNDs 147 and 175 (p < .05).

**Swim Speed**

13-cis-RA. There were no significant effects of 13-cis-RA treatment on swim speed (m/s), % time slow, or % thigmotaxis time (Table 1). There was a main effect of sex on % time slow (F(1,31) = 6.52, p < .02), which indicated increased duration of time spent in slow swimming in females relative to males.

All-trans-RA. A main effect of sex on swim speed (F(1,32) = 4.94, p < .04), % time slow (F(1,32) = 17.93, p < .0002), and % thigmotaxis time (F(1,32) = 4.33, p < .05) indicated slower swim speeds and more time in slow swimming in females while increased thigmotaxis time in males (Table 1).

**DISCUSSION**

Published reports suggesting the potential of Accutane to induce depression in humans led to the proposed hypothesis...
that 13-cis-RA treatment in rats would cause increased quantitative signs of depression in validated behavioral assessments. Contrary to that hypothesis, neither 7.5 nor 22.5 mg/kg 13-cis-RA treatment caused any significant signs of depression in either the forced swim test (FST) or the measure of anhedonia, voluntary saccharin intake. Groups treated with all-trans-RA also were relatively unaffected by treatment. Furthermore, neither RA-treated group exhibited alterations in body weight or food and water intake.

These results suggest that there are no severe effects of 13-cis-RA on behavioral indices of depression. The FST methodology used here was more complete than the typical single measure of immobility and followed the methodology proposed by Cryan et al. (2005) in that three behaviors (immobility, climb/struggle, and swim) were measured for each session. Measurement of behaviors other than immobility is particularly important because drugs with different mechanisms of action can differentially alter behaviors (Lucki, 1997). However, the direction of the effects observed were opposite those originally predicted in that if 13-cis-RA treatment were hypothesized to cause depression, then increased immobility and decreased climb/struggle durations should have been exhibited in the FST. Yet, this was not the case. Much has been made of the sensitivity of the FST and whether immobility represents an adaptive response to the environment (Hawkins et al., 1978; Nishimura et al., 1988; Thierry et al., 1984) and contributes to survival (Bruner and Vargas, 1994) or is a reflection of “behavioral despair” (Porsolt et al., 1977). Some investigators have questioned the ethological relevance of these standard measures of depression (Mitchell and Redfern, 2005), suggesting that other tests may be more appropriate. Regardless, neither RA-treated group exhibited severely altered FST behaviors. Furthermore, the lack of significant 13-cis-RA effects on forced swim behaviors has recently been replicated with rats treated with 7.5 and 30 mg/kg 13-cis-RA (Ferguson, unpublished data).

Like the results of the FST, anhedonia as measured by voluntary saccharin intake was not significantly affected by 13-cis-RA or all-trans-RA treatment, although all treated groups had lower intakes relative to controls. Locomotor open field activity was only marginally affected in that males treated with 7.5 mg/kg had lower levels of activity than did control males and only at the earliest test age (PND 91), a finding that is likely a spurious effect. Finally, swim speed was unaffected by RA treatment, even when such treatment (e.g., all-trans-RA) caused bone fractures.

One relevant question is whether the tests as used here would have been sensitive to RA treatment effects. Certainly, significant effects of sex and session were apparent and in the same direction as expected. For example, females are reported to struggle more and exhibit decreased immobility in the FST (Alonso et al., 1991; Barros and Ferigolo, 1998; Brotto et al., 2000), and relatively similar behaviors have been noted here. Dive frequency was less on the second FST session here as has been reported (Barros and Ferigolo, 1998; Hawkins et al., 1978). During the 3-day saccharin tests, all rats consumed more on the first day and females consumed more than males as previously described (Ferguson et al., 2000; Flynn et al., 2001). Finally, decreased open field activity with increasing age is a classical finding (Asano, 1986; Furchtgott et al., 1961). Thus, irrespective of RA treatment, the significant results are similar to those previously described.

The two measures of depression used here, FST and voluntary saccharin intake, are thought to model “behavioral despair” and anhedonia, respectively. How well these two measures model the human symptoms of major depressive disorder is intertwined with complex issues regarding the utility of animal models of depression. For example, Matthews et al. (2005) argued that nonhuman animals may be unable to model many of the symptoms of depression. Recognizing the complexity and co-morbidity associated with depression, Frazer and Morilak (2005) suggest that animal models would serve better to reflect three specific behavioral dimensions of human depression, rather than attempting to model the entire disorder. They propose that symptoms such as helplessness/worthlessness be categorized into a negative affect dimension while anhedonia be classified in the loss of positive affect dimension. One advantage of this system is that the dimensions are likely to be regulated by different neural systems, and pharmacological treatments may affect one dimension while having little or no effect on others. Similarly, treatments or paradigms that induce depression in animal models may increase symptomatology within one dimension only. If behavioral despair as experienced in the FST is reflective of

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**TABLE 1**

Swimming abilities in 13-cis-RA and all-trans-RA treated rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Swim speed (m/s)</th>
<th>% Time slow</th>
<th>% Thigmotaxis time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control males</td>
<td>0.2282 ± 0.0130</td>
<td>13.0 ± 1.6</td>
<td>70.2 ± 4.9</td>
</tr>
<tr>
<td>7.5 mg/kg 13-cis-RA</td>
<td>0.2482 ± 0.0115</td>
<td>14.1 ± 1.5</td>
<td>66.2 ± 2.6</td>
</tr>
<tr>
<td>males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.5 mg/kg 13-cis-RA</td>
<td>0.2552 ± 0.0163</td>
<td>11.4 ± 1.2</td>
<td>63.1 ± 7.3</td>
</tr>
<tr>
<td>males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg all-trans-RA</td>
<td>0.2400 ± 0.0083</td>
<td>13.6 ± 1.8</td>
<td>72.2 ± 6.3</td>
</tr>
<tr>
<td>males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 mg/kg all-trans-RA</td>
<td>0.2495 ± 0.0224</td>
<td>12.2 ± 1.3</td>
<td>65.5 ± 6.7</td>
</tr>
<tr>
<td>males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control females</td>
<td>0.2182 ± 0.0128</td>
<td>19.5 ± 2.7</td>
<td>52.3 ± 5.8</td>
</tr>
<tr>
<td>7.5 mg/kg 13-cis-RA</td>
<td>0.2424 ± 0.0195</td>
<td>14.7 ± 2.0</td>
<td>67.3 ± 4.8</td>
</tr>
<tr>
<td>females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.5 mg/kg 13-cis-RA</td>
<td>0.2483 ± 0.0116</td>
<td>17.2 ± 2.8</td>
<td>54.1 ± 5.9</td>
</tr>
<tr>
<td>females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg all-trans-RA</td>
<td>0.2095 ± 0.0216</td>
<td>21.3 ± 3.5</td>
<td>64.3 ± 7.5</td>
</tr>
<tr>
<td>females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 mg/kg all-trans-RA</td>
<td>0.1865 ± 0.0283</td>
<td>29.9 ± 5.5</td>
<td>57.5 ± 8.1</td>
</tr>
</tbody>
</table>
helplessness/worthlessness, then the current study measured two of the three dimensions proposed by Frazer and Morilak. Such relatively global assessments make the conclusions even more solid.

Despite the questions surrounding the validity of the model, the nature of the findings was somewhat surprising. Although there was not overwhelming evidence to suggest that 13-cis-RA–induced behavioral alterations would appear, the information certainly was suggestive. For example, aged mice exhibit reduced hippocampal expression of specific RA receptors mRNAs relative to adult mice as well as impairments in spatial learning and memory (Etchamendy et al., 2001). Further, RA treatment (0.15 mg/kg, injected subcutaneously) reverses the cognitive impairment those aged mice (Etchamendy et al., 2001). However, 13-cis-RA treatment (1 mg/kg, injected intraperitoneally) during young adulthood is associated with both suppressed hippocampal cell proliferation and decreased spatial learning and memory in mice (Crandall et al., 2004). Thus, the lack of behavioral alterations in the current study was surprising, particularly given the doses used here (7.5 and 22.5 mg/kg, oral gavage). The different routes of administration are of little concern since we have documented the serum levels achieved by these doses (Ferguson et al., unpublished data). It might be hypothesized that 13-cis-RA treatment specifically affects learning and memory while leaving depressive behaviors in rodents within control limits. However, we have recent data indicating that spatial learning and memory in 13-cis-RA treated rats is intact (Ferguson, unpublished data). Thus, at present, species differences in RA sensitivity appear to be the most likely candidate explanation, with rats much less sensitive than mice.

Oral treatment of rats with 13-cis-RA that produced serum levels similar to human therapeutic levels did not affect traditional measures of behavioral despair and anhedonia. Similarly, all-trans-RA had few effects on rat behavior, and neither 13-cis-RA nor all-trans-RA affected body weight or food and water intake. Open field locomotor activity was only marginally affected. Although other end points might reveal 13-cis-RA effects in rats, it seems clear that RA does not severely alter depression-like behaviors.

REFERENCES


