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Comparison of the effects of desmethylinipramine on behavior in the forced swim test in peripubertal and adult rats

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Abstract

Although there are similarities in the clinical presentation of adolescent and adult depression, there are differences in the biological correlates and the responses to pharmacologic treatment. Selective serotonin reuptake inhibitor-type antidepressants are efficacious, but tricyclic antidepressants have no or limited efficacy in treating adolescent patients. The forced swim test (FST) is a widely accepted animal model used to screen drugs for antidepressant activity. It is not known whether tricyclic antidepressants produce differential effects in peripubertal and adult rats, as is found in adolescent and adult humans. The objective of the study was to test the hypothesis that the tricyclic antidepressant desmethylinipramine (DMI) would show efficacy in the FST in adult, but not in peripubertal, rats. Thirty-day-old (peripubertal) and 112-day-old (young adult) rats were pretreated with saline or DMI and subjected to the FST. DMI reduced the amount of floating behavior and increased the amount of climbing behavior in both peripubertal and adult rats. Thus, the tricyclic antidepressant DMI has antidepressant-like activity in peripubertal rats in the FST. Owing to the discrepancy between the preclinical and clinical data, the predictive validity of the FST might need to be reevaluated across different age groups.

Keywords

adolescence; animal models; antidepressant; depression; development; peripubertal; rat; tricyclic

Introduction

Adolescence is the period of highest risk for the onset of depression (Burke *et al.*, 1990). At any given time, 5–8% of adolescents meet the criteria for major depressive disorder (Lewinsohn *et al.*, 1994) and up to 25% have been affected by their late teens (Kessler *et al.*, 2001). Recognition of its prevalence has led to the increased use of antidepressants in this age group. As adolescent depression can mark the gateway into recurrent mood disorders in adulthood, effective treatment during the early phase of the illness can minimize the negative consequences of initial and repeated depressive episodes (Kovacs, 1996).

Similarities in the clinical presentation have been found, but there are differences in the responses to pharmacologic treatment between adolescent and adult depression (Kaufman *et al.*, 2001). Whereas numerous studies have shown the effectiveness of several classes of

antidepressant drugs in the treatment of depression in adults (Charney *et al.*, 1998), the efficacy of antidepressants in depressed adolescents is controversial. Selective serotonin reuptake inhibitor-type antidepressants are efficacious (Kaufman *et al.*, 2001; Keller *et al.*, 2001; Wagner *et al.*, 2003), but tricyclic antidepressants have no or limited efficacy in the treatment of depression in adolescents (Kaufman *et al.*, 2001; Hazell *et al.*, 2002; Mulder *et al.*, 2003). This is an important issue because treatment with antidepressants that have marginal efficacy can lead to serious consequences, including increased morbidity and mortality.

The forced swim test (FST) is a widely accepted animal model used to screen drugs for antidepressant activity (Porsolt *et al.*, 1977). Although different classes of antidepressants produce diverse neurochemical and neurobiological effects, the FST is a good predictor of the clinical efficacy of antidepressants (Willner, 1990; Detke *et al.*, 1995; Cryan *et al.*, 2002). It is not known whether tricyclic antidepressants produce differential effects in peripubertal and adult rats, as is found in adolescent and adult humans. The purpose of this study was to use the FST to test the hypothesis that the tricyclic antidepressant desmethylimipramine (DMI) would show efficacy in adult, but not in peripubertal, rats.

Methods

Subjects

Sprague–Dawley rats (Charles River, Hollister, California, USA) were received 7 days before testing. They were separated by age and group housed in a humidity-controlled and temperature-controlled (21–22°C) vivarium on a 12:12 h light/dark cycle (lights on: 07.00 h; lights off: 19.00 h) with free access to food and water. The behavioral testing was conducted between 09.00 and 13.00 h.

Forced swim test

The modified FST was conducted as described by Detke *et al.* (1995). The rats were placed into individual Plexiglass cylinders (46 cm tall and 20 cm in diameter) filled to a depth of 30 cm with room temperature water (23–25°C). Two swim sessions were conducted: a 15-min pretest, followed 24 h later by a 5-min test. The 5-min test sessions were videotaped and viewed at a later time by two raters blind to treatment (interrater reliability was determined to be 0.9). The raters scored the behavior for each 5-s period (60 times for the 5-min test) as one of the following: (i) immobility – making only those movements necessary to keep its head above water; (ii) swimming – making active swimming movements; and (iii) climbing – making vigorous movements with the forepaws in and out of the water, usually against the cylinder walls.

Drug administration

Separate groups of rats were tested at 30 or 112 days of age (± 1 day). These age groups include a time point when the rats would be expected to exhibit adolescent-typical neurobehavioral characteristics, and a time point when adult neurobehavioral characteristics should predominate, respectively. Although the temporal boundaries of adolescence can be difficult to define (Spear, 2000), and direct comparison between developmental age in rodents and humans is not clear-cut, based upon available data the peripubertal period in rats (postnatal days 30–37) corresponds to the periadolescent period in humans (approximately 10–14 years of age). Within each age group ($n = 10$ –12/group), the rats were randomly assigned to treatment with saline or DMI hydrochloride (Sigma; St Louis, Missouri, USA). The rats were given the pretest swim on day 1, immediately followed by a subcutaneous injection of saline or DMI dissolved in saline (equivalent to 10 mg/kg DMI base; 2.5 ml/kg). Two additional injections were given 12 and 1 h before the swim test on day 2.

Data analysis

The data were analyzed using StatView (SAS Institute, Cary, North Carolina, USA). The number of time points where climbing, swimming or immobility were scored over the 5-min experimental session were summed for each rat. The summed scores were analyzed by two-way analysis of variance (treatment $\times$ age treatment) for each behavior, followed by *t*-tests for comparing drug effects on each behavior within each age group. A criterion of $P < 0.05$ was used for the rejection of the null hypothesis.

Results

Statistically significant treatment and age effects for floating [treatment $F(1,51) = 9.8$; $P < 0.01$; age $F(1,51) = 5.0$; $P < 0.05$], climbing [treatment $F(1,51) = 23.3$; $P < 0.01$; age $F(1,51) = 53.4$; $P < 0.01$] and swimming [treatment $F(1,51) = 5.1$; $P < 0.01$; age $F(1,51) = 45.1$; $P < 0.01$] were found. Significant interaction effects for climbing [$F(1,1) = 4.1$; $P < 0.05$] and swimming [$F(1,1) = 7.1$; $P < 0.01$] were found, but not for floating.

In the adult rats, pretreatment with DMI decreased the amount of floating behavior (Fig. 1a) and increased the amount of climbing behavior (Fig. 1b), but did not affect the amount of swimming (Fig. 1c). In the peripubertal rats, pretreatment with DMI also decreased floating behavior (Fig. 1a) and increased climbing behavior (Fig. 1b), but there also were statistically significant reductions in swimming (Fig. 1c). Within the saline controls, the peripubertal rats showed more climbing (Fig. 1b) but less swimming behavior (Fig. 1c) compared to the adult rats.

Discussion

The results show that DMI produced antidepressant-like activity in peripubertal rats. On the basis of the predictive validity of the FST, this outcome suggests that DMI should have antidepressant activity in peripubertal humans; however, the clinical data show that DMI is not efficacious in this age group. The reason for the discrepancy between the preclinical and clinical data is not known. Although the predictive validity of the FST has been rigorously evaluated in adult animals, few studies have been carried out in young rodents. Abel (1993) found that the immobility response emerges at 21 days of age and stabilizes beginning at 26 days. Therefore, the immobility response is present in young rats. Nakamura and Tanaka (2001) found that 63-day-old rats showed reduced immobility in the FST in response to tricyclic antidepressants, and repeated treatment with DMI in this same age group causes downregulation of β -adrenergic receptors (Deupree *et al.*, 2007). This age corresponds to young adulthood in humans, around 20 years of age (Spear, 2000). To our knowledge the predictive validity of the FST has not been characterized in peripubertal rats. It is possible that the test does not have predictive validity in immature animals.

In humans, the reasons for differential efficacy of tricyclic antidepressants in childhood and adult depression are not clear. It has been suggested that the etiology of depression might be different in adolescents and adults (Kaufman *et al.*, 2001). Another possibility is that neural substrates on which antidepressants act are not mature during adolescence. The immaturity of these systems might not allow an adult-type response to occur. Preclinical data do show that there are developmentally related differences in the adaptive responses to repeated antidepressant administration (McCracken and Poland, 1995; Carrey *et al.*, 2002). Furthermore, Moll and colleagues (2000) found that the density of serotonergic and noradrenergic transporters display different developmental patterns. It is interesting to note that in this study the pattern of behavioral activity was not the same in the saline-treated peripubertal and adult rats. The peripubertal rats showed significantly more climbing and less

swimming behavior. Thus, the fundamental neurobehavioral mechanisms underlying these behaviors might differ across age groups.

Apart from the well-known side effects profile of tricyclic antidepressants, in adolescent patients they can produce activation effects (i.e. activation syndrome), such as anxiety, hostility, agitation and irritability. It is possible that tricyclic antidepressants do have antidepressant activity in adolescents, but only at higher doses. A similar DMI dosing procedure has been found to produce comparable brain levels of DMI in juvenile and adult rats (Kozisek *et al.*, 2007); however, because in this study only a single dose of antidepressant was tested, it is not possible to determine whether there was a difference in sensitivity to DMI between the peripubertal and adult rats. In adolescent patients, the tricyclic antidepressant imipramine produced a far greater incidence of adverse effects compared with the selective serotonin reuptake inhibitor paroxetine (Keller *et al.*, 2001). If adolescents can respond but are less sensitive to DMI, the adverse effect profile might preclude the ability to administer the drug at therapeutic doses.

In conclusion, although tricyclic antidepressants have no or limited efficacy in treating depression in adolescents, the results of this study demonstrate that the tricyclic antidepressant DMI has antidepressant-like activity in peripubertal rats in the FST. A limitation of this study is that the experimental design did not include a positive control, that is, a drug that produces some type of behavioral response, but does not have antidepressant activity. Moreover, there might be some assay conditions (e.g. water depth or tank size) under which tricyclic antidepressants do not produce antidepressant activity in the FST. The discrepancy between the results of this study and the clinical data, however, indicates that the predictive validity of the FST needs to be reevaluated across different age groups.

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Fig. 1.

The effects of pretreatment with saline or DMI on the forced swim test in peripubertal and adult rats. The rats were given the pretest swim on day 1, immediately followed by an injection of saline or DMI (10 mg/kg/subcutaneously). Two additional injections were given 12 and 1 h before the swim test on day 2. Floating (a), climbing (b) and swimming (c) behaviors were recorded and summed over the 5-min test session. The data represent means $\pm$ SEM ($n = 10-12$ /group). * $P < 0.05$ compared with the saline-treated rats in the same age group; † $P < 0.05$ compared with the saline-treated rats in the other age group.