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Intra- and interstrain differences in models of "behavioral despair"

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Abstract

In the present studies, base line and drug-induced performance of two mouse strains (C57Bl/6 and NIH-Swiss) was evaluated in the forced swim test (FST) and tail suspension test (TST). Intra- and interstrain comparisons indicate that the biological substrates mediating performance in these behavioral procedures are not identical. For example, in NIH-Swiss mice, a sevenfold difference in base line immobility was observed between the FST and TST. By contrast, the base line immobility in C57Bl/6 mice was similar in both procedures. Further, in C57Bl/6 mice, imipramine produced a "U-shaped" dose–response curve in the FST, whilst no evidence of a biphasic response was present in the TST at doses up to 45 mg/kg. In the FST, the AMPA receptor potentiator LY451646 produced a similar dose–response relationship in C57Bl/6 and NIH-Swiss mice, but the minimum effect dose (MED) was fivefold higher in NIH-Swiss mice. This potency difference appears due to both pharmacokinetic and pharmacodynamic factors. These intra- and interstrain differences in performance indicate that despite a face value similarity, the neurochemical pathways involved in mediating performance in these two widely used tests are not identical. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Forced swim test; Tail suspension test; Imipramine; LY451646; Antidepressants

1. Introduction

The forced swim test (FST) (Porsolt et al., 1977a,b, 1978a) and tail suspension test (TST) (Steru et al., 1985) remain widely used to predict antidepressant efficacy (reviewed in Borsini and Meli, 1988; Porsolt and Lenegre, 1992). Whilst many variations of the FST have been reported during the past two decades, as originally described, a mouse or rat is placed in a cylinder (diameter: 10 and 18 cm for mice and rats, respectively) containing 25 (mice) or 40 cm (rats) of water held between 22–25°C. After a period of time, the animal becomes immobile, or floats passively (Porsolt et al., 1977a, 1978a). As originally described, the TST requires a mouse to be suspended by the tail approximately 35 cm above the floor. The subject immediately engages in struggling or escape-like behaviors, which gradually abates to immobility (Steru et al., 1985). The behavioral phenomena observed in both procedures appear to result from exposure to inescapable aversive situations, and an animal alternates between periods of vigorous activity (searching behavior) and immobility (waiting behavior) (Steru et al., 1985). At face value, these two tests appear very similar, and a range of antidepressants decrease immobility following acute or subchronic (two to three doses) treatment. Although these behavioral despair procedures lack sufficient face and construct validity to be considered models of depression, both the FST and TST have good predictive validity. For example, it has been estimated that the "hit" rate (i.e., the ability to predict clinically effective antidepressants) of the FST ranges from 87% to 94% (in rats and mice, respectively) across a range of compounds that are structurally and mechanistically diverse (reviewed in Borsini and Meli, 1988; Porsolt and Lenegre, 1992).

Despite the face value similarity of these behavioral despair procedures, the spectrum of compounds active in the FST and TST overlaps, but is not identical. For example, tricyclic antidepressants, MAOIs, some "atypical" antidepressants (such as mianserin) (Steru et al., 1985; Borsini and Meli, 1988; Dalvi and Lucki, 1999),

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functional NMDA antagonists (Trullas and Skolnick, 1990; Maj et al., 1992), and AMPA receptor potentiators (Li et al., 2001) have been reported active in both tests. However, SSRIs, which are generally reported as active in the TST, fail to consistently reduce immobility in the FST at pharmacologically relevant doses (reviewed in Porsolt and Lenegre, 1992; but see Detke et al., 1995). In contrast, other "atypical" agents such as the rolipram and levoprotiline reduce immobility in the FST but have been reported inactive in the TST (Porsolt and Lenegre, 1992).

Overall, these findings provide pharmacological evidence supporting the hypothesis that the neurochemical pathways mediating behavior in the FST and TST are not identical. However, such pharmacological differences could also involve pharmacokinetic factors (e.g., differences in the rates at which various drugs are metabolized) that can confound interpretation of these data. The presence of significant differences in base line immobilities in the FST and TST within the same mouse strain would provide an independent line of evidence supporting this hypothesis. Whilst such comparisons can be made by comparing literature data across laboratories (Porsolt et al., 1978b; van der Heyden et al., 1987; Trullas et al., 1989; Lopez-Rubalcava and Lucki, 2000), subtle methodological differences (e.g., lighting level, the distance a subject is suspended from the ground in TST) and genetic "drift" among inbred strains obtained from different suppliers make such comparisons less than ideal.

In the present studies, we evaluated base line and drug-induced performance of two mouse strains (C57Bl/6 and NIH-Swiss) in the FST and TST. Two drugs were selected for this study: the prototypic tricyclic (imipramine) and a biarylpropylsulfonamide AMPA receptor potentiator (LY 451646) (Baumbarger et al., 2001; Miu et al., 2001). The former compound increases extracellular concentrations of norepinephrine and serotonin in prefrontal cortex at doses active in behavioral despair procedures (Jordan et al., 1994) whilst the latter group of compounds do not affect exreacellular concentration of biogenic amines (Skolnick et al., 2001). A comparison of intra- and interstrain differences provides compelling evidence that despite a face value similarity, the pathways involved in mediating performance in these behavioral despair models are not identical.

2. Materials and methods

2.1. Animals

Male C57Bl/6 and NIH-Swiss mice ($\sim$25 g) were purchased from Harlan Sprague–Dawley (Indianapolis, IN). Animals were housed in a vivarium for at least 1 week prior to use. The vivarium was maintained on a 12-h light/dark cycle (lights on/off at 06:00 h) with free access to rat chow and water. Animals were transferred to the testing area in their home cages and allowed to adapt to the new environment for at least 1 h prior to testing.

2.2. Forced swim test (FST)

Mice were placed in clear plastic cylinders (diameter: 10 cm; height: 25 cm) filled to 6 cm with 22–25°C water for 6 min. The duration of immobility was recorded during the last 4 min of the 6-min trial. A mouse was regarded as immobile when floating motionless or making only those movements necessary to keep its head above the water.

2.3. Tail suspension test (TST)

Mice were suspended by their tails to the edge of a shelf 80 cm above the floor. The tail was secured to the shelf by adhesive tape placed approximately 1 cm from the tip of the tail. The trial was conducted for a period of 5 min, and the duration of immobility was recorded during this period. Mice were considered immobile only when they hung passively and completely motionless. In both FST and TST, immobility was measured using the program PORSOLT (Infallible Software, Rockville, MD) by an observer aware of the drug treatment protocol.

2.4. Determination of LY451646 concentrations in the brain

Whole mouse brains (0.4–0.5 g) were homogenized in 0.5 ml of acetonitrile containing a fluorinated analogue of LY451646 as internal standard. Samples of brain acetonitrile mixtures were then centrifuged in an Eppendorf 5415C tabletop centrifuge for 5 min. Aliquots (10 μl) of the supernatant were injected into a Waters 2790 liquid chromatograph using a mobile phase consisting of 40% 5 mM ammonium acetate/60% acetonitrile (v/v) at a flow rate of 0.2 ml/min. A 1-min gradient to 10% 5 mM ammonium acetate/90% acetonitrile was initiated upon injection, and held at 90% acetonitrile for 1 min before returning to initial conditions. A Micromass ZMD single quadrupole mass spectrometer operated in negative ion mode was used to detect and analyze the M–H signal. Quantitation of LY451646 was accomplished by comparing the LY451646/internal standard peak area ratio of the unknowns to the LY451646/internal standard peak area ratio of standard curves developed from control brain spiked with LY451646 into 0.5 g of tissue.

2.5. Drugs

Imipramine was obtained from Sigma (St. Louis, MO). LY451646 was synthesized at Lilly Research Laboratories (Indianapolis, IN). Imipramine (as the HCl salt) was dissolved in water. LY451646 (as free base) was dissolved in 10% diluted Emulphor. Compounds and vehicles were administered intraperitoneally in a volume of 0.1 ml either 15 min (imipramine) or 30 min (LY451646) prior to testing.

2.6. Statistical analysis

Data were analyzed by one-way ANOVA given that they met tests of homogeneity of variance. Significant ANOVAs were followed by post-hoc Dunnett's tests.

3. Results

3.1. Base line immobilities of NIH-Swiss and C57Bl/6 mice in the FST and TST

NIH-Swiss mice were immobile for 137.6 ± 10.9 s of the 240-s recording period (~57%) in the FST. By contrast, during the TST this strain of mouse was immobile for ~8% (25.1 ± 4.8 s) of the 300-s test period. C57Bl/6 mice were immobile for ~65% of the recording period in both the FST (152.7 ± 11.8 s) and TST (193.7 ± 6.3 s) (Fig. 1). In the TST, increasing the test time to 6 min (as originally described by Steru et al., 1985) did not significantly change the percentage immobile time in both NIH-Swiss (~10%) and C57Bl/6 (~61%) (data not shown).

3.2. Drug effects in the FST and TST are strain dependent

In C57Bl/6 mice, imipramine produced a dose-dependent decrease in immobility in both the FST and TST (Fig. 2A, B). Nonetheless, the characteristics of the dose–response curve produced by imipramine are different in this strain. In the FST, imipramine produced a ''U-shaped'' dose–response curve with a minimum effective dose (MED, the first dose producing a statistically significant effect) of 5 mg/kg. At doses >15 mg/kg, the duration of immobility returned to values not significantly different from vehicle treated animals (Fig. 2A). The MED of imipramine in the TST was 10 mg/kg, with no evidence of a return towards base line values at doses of up to 45 mg/kg (Fig. 2B). In

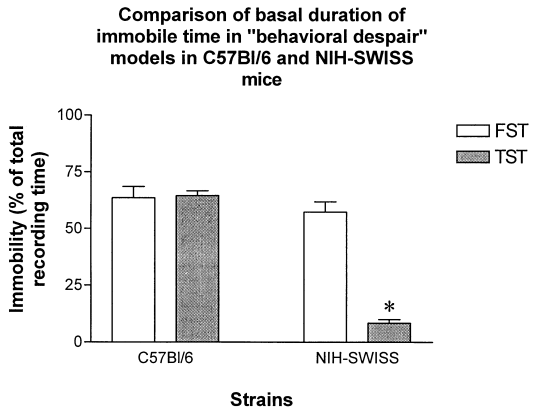

Fig. 1. Comparison of base line immobility in "behavioral despair" models in C57B1/6 and NIH-Swiss mice. Values are expressed as the % of total recording time, which are 300 s in the TST and 240 s in the FST respectively. Values represent mean ± SEM of 12-15 animals. * $P<0.05$, compared to the other behavioral despair model (Dunnett's test).

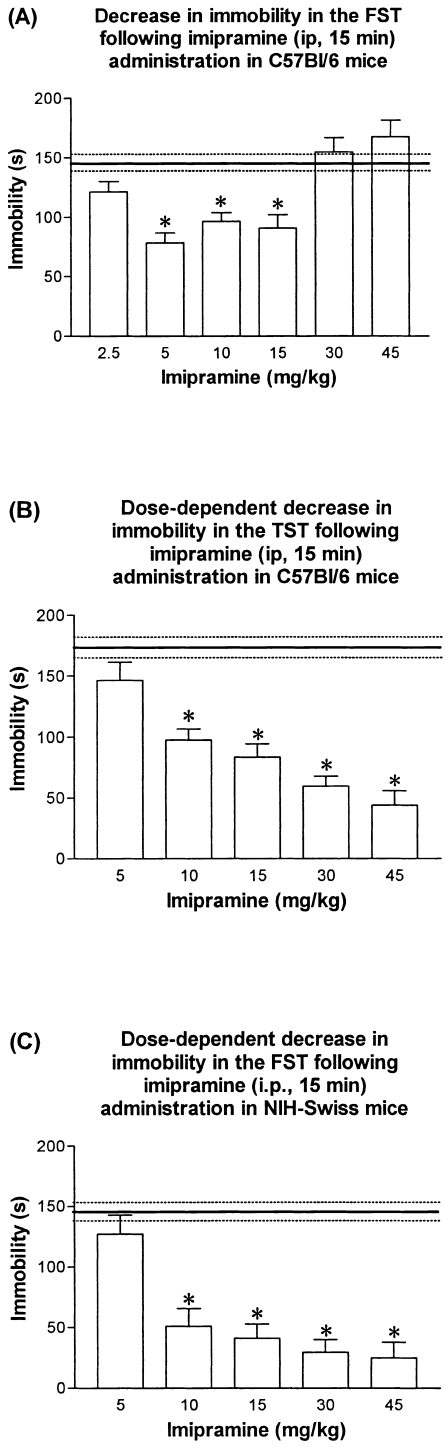

Fig. 2. Dose response curves of imipramine in the TST and FST in two mice strains. In C57B1/6 mice both FST (A) and TST (B) were performed. In NIH-Swiss mice, only FST was performed (C). Mice were administered imipramine or vehicle (i.p.) 15 min prior to testing as described in method. Values represent mean ± SEM of 6-18 animals. * $P<0.05$, compared to vehicle treated animals (Dunnett's test).

NIH-Swiss mice, imipramine also produced a dose-dependent reduction (MED, 10 mg/kg) in immobility in the FST. However, no evidence of a biphasic dose–response curve

was observed at doses of up to 45 mg/kg (Fig. 2C). Due to the low base line of immobility in the TST in NIH-Swiss mice, drug testing was not conducted.

Like imipramine, LY451646 reduced immobility in C57Bl/6 mice both in the FST (Fig. 3A) and TST (Fig. 3B) with a MED of 0.5 mg/kg. In NIH-Swiss mice, LY451646

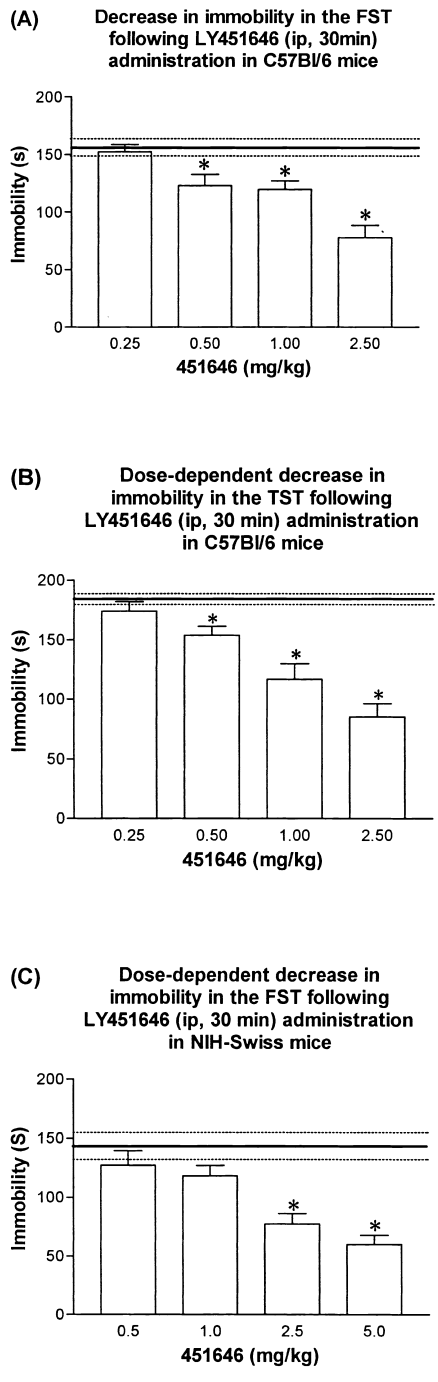

Fig. 3. Dose response curves of LY451646 in the TST and FST in two mice strains. In C57B1/6 mice both FST (A) and TST (B) were performed. In the NIH-Swiss mice, only FST was performed (C). Mice were administered LY451646 or vehicle (i.p.) 30 min prior to testing as described in method. Values represent mean ± SEM of 8-20 animals. * $P < 0.05$, compared to vehicle treated animals (Dunnett's test).

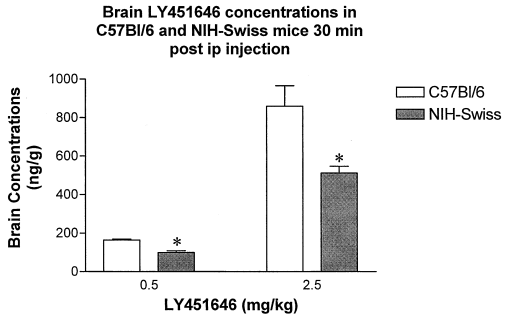

Fig. 4. Brain LY451646 concentrations in C57B1/6 and NIH-Swiss mice. Mice were administered LY451646 or vehicle (i.p.) 30 min prior to the brain tissue was collected. Values represent mean ± SEM of 5 animals. * $P < 0.05$, compared to another strain of animals (Dunnett's test).

also reduced immobility in the FST. However, the MED (2.5 mg/kg) in NIH-Swiss mice was five times higher than that in C57Bl/6 mice (Fig. 3C). Due to the low base line of immobility in the TST in NIH-Swiss mice, drug testing was not conducted.

3.3. Brain concentrations of LY451646 in C57Bl/6 and NIH-Swiss mice

A fivefold difference in the MED of LY451646 in the FST between these mouse strains led to an examination of brain concentrations of this compound. The doses of LY451646 used in this experiment corresponded to its MED in C57Bl/6 (0.5 mg/kg) and NIH-Swiss (2.5 mg/kg) mice. Brain concentrations of LY451646 were dose proportional (i.e., approximately fivefold higher at 2.5 than at 0.5 mg/kg, respectively) in both strains, but the absolute levels of this compound were $\sim$70% higher in C57Bl/6 than NIH-Swiss mice (Fig. 4).

4. Discussion

The present study was prompted by the very large difference in base line immobility of NIH-Swiss mice in the FST and TST (Fig. 1). Thus, NIH-Swiss mice (which have been used as test subjects for the FST [e.g., Trullas and Skolnick, 1990; Skolnick et al., 1992; Harkin et al., 1999; Li et al., 2001]) are immobile for $\sim$57% of the recording period in the former procedure, but spend only $\sim$8% of the trial immobile in the latter procedure. In the present study, a 5-min trial was performed in the TST, although the immobility is usually recorded for 6 min (Steru et al., 1985; van der Heyden et al., 1987). Considering the possibility that differences in the time parameters used in the TST and FST may affect the development of immobility, the TST was extended to a 6-min test. However, the percentage immobile time remained the same whether a 5- or a 6-min test was applied. Therefore the lack of immobility of NIH-Swiss mice in the TST was not due to the variation of the time parameters in

the present study. Consistent with these observations, there are indications from the literature of within-strain differences in base line immobility in these behavioral despair procedures. For example, Porsolt et al. (1978b) reported that NMRI mice are immobile in the FST for 40% of the recording period whilst van Der Heyden et al. (1987) reported these mice are immobile 22% of the test period in the TST. However, the contribution of interlaboratory variation (e.g., animal supplier, housing and testing environment, and experimentalist) may make such comparisons problematic. The present study is, to our knowledge, the first reported within-strain comparison of base line and drug-induced effects on performance in these behavioral despair procedures.

Because the FST and TST have considerable face value similarity, this approximately sevenfold difference in base line immobility can be considered *prima facie* evidence that the neurochemical determinants of performance in these tests need not be identical. Further, the base line values obtained in another intrastrain comparison using C57Bl/6 mice (Fig. 1) are consistent with reports indicating there is a substantial contribution of genetic factors in defining performance in these behavioral despair procedures (Trullas et al., 1989; Lopez-Rubalcava and Lucki, 2000). Both intra- and interstrain comparisons of drug effects provide convergent evidence that the neurochemical pathways mediating performance in the FST and TST are not identical. This is illustrated by the contrasting intra- and interstrain actions of imipramine. Thus, in C57Bl/6 mice imipramine produces a biphasic (U-shaped) dose–response curve in the FST whilst in NIH-Swiss mice, there is no evidence of a U-shape at doses of up to 45 mg/kg (Fig. 2). In this interstrain comparison, both pharmacokinetic (e.g., strain differences in the concentrations of imipramine and/or active metabolites) and pharmacodynamic (e.g., strain differences in transmitter, receptor, and signal transduction mechanisms) factors can be invoked to explain this interstrain difference in response to imipramine. In contrast, the qualitatively different response to imipramine in the intrastrain (C57Bl/6) comparison of performance in the FST and TST can clearly be attributed to inherent differences in the biology of these two procedures rather than pharmacokinetics or pharmacodynamics.

An additional intra- and interstrain comparison was made with an AMPA receptor potentiator, LY451646. The racemic form of LY451646, LY 404187, has been shown to positively modulate AMPA receptor response (Baumbarger et al., 2001; Miu et al., 2001). A structurally related biarylpropylsulfonamide, LY392098, was recently shown to reduce immobility in the both the FST and TST (Li et al., 2001). The observation that LY451646 also reduces immobility in these behavioral despair procedures (Fig. 3) indicates that these antidepressant-like actions may be a characteristic of this class of AMPA receptor potentiators. Moreover, LY451646, at doses up to 1 mg/kg, did not affect locomotor activity in rats (Tizzano, personal communication). In contrast to imipramine, LY451646 reduced immobility in C57Bl/6 mice in both the TST and FST with no evidence of a U-shape at doses up to fivefold higher than its MED (Fig. 3A, B). However, when an interstrain comparison was performed, LY451646 was found to be approximately fivefold more potent (based on MED) in C57Bl/6 compared to NIH-Swiss mice. The increased sensitivity of C57Bl/6 mice may be due, in part, to pharmacokinetic differences between the two strains. Thus, the brain concentrations of LY451646 are $\sim$70% higher in the C57Bl/6 mice than in NIH-Swiss mice (Fig. 4). Nonetheless, pharmacodynamic factors also appear to play a role in this strain difference because at 2.5 mg/kg (a dose active in both strains), the relative decrease in the immobility is very similar in both strains (Fig. 3A, C), although brain drug concentrations in the C57Bl/6 mice are 1.7-fold higher than detected in the NIH-Swiss mice (Fig. 4).

Behavioral despair procedures are highly predictive of antidepressant action (Borsini and Meli, 1988; Porsolt and Lenegre, 1992). One of the great advantages of these tests in the arena of drug discovery is that they are not based on preconceived notions of a drug's molecular mechanism of action (Porsolt and Lenegre, 1992). For example, many, but not all biogenic amine-based antidepressants are active in one or both these procedures, as are compounds with a presumed intracellular locus of action such as rolipram (a phosphodiesterase 4 inhibitor) (Porsolt and Lenegre, 1992; X.L. and P.S., unpublished observations) and the NO synthase inhibitor N^{G}-nitroarginine (Harkin et al., 1999). The intra- and interstrain comparisons of base line and drug-induced performance in the FST and TST reported here support the hypothesis that the neural circuitry mediating behavior in these tests is not identical. This lack of identity may, in fact, enhance the value of using these procedures to search for antidepressants with novel mechanisms of action.

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