Neonatal Administration of the Selective Serotonin Reuptake Inhibitor Lu 10–134-C Increases Forced Swimming-Induced Immobility in Adult Rats: A Putative Animal Model of Depression?

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ABSTRACT
Chronic administration of the tricyclic antidepressant clomipramine to neonatal rats from postnatal days 8 to 21 is reported to induce several behavioral changes in adult life, and it may serve as an animal model of human depressive disorder. Findings include increased immobility time in the forced swim test and locomotor hyperactivity in the open field test. Clomipramine is a serotonergic reuptake inhibitor, which suggests that altered development of the serotonergic system could account for the observed behavioral changes in the adult rat. The present study was carried out with a selective serotonin reuptake inhibitor (SSRI) to investigate whether the serotonin system, in particular, is involved in the neonatal animal model. The substance, Lu 10–134-C (LU), was characterized in monoamine reuptake and receptor binding assays and found to be an SSRI. Rats received LU during postnatal days 8 to 21 (2.5–15 mg/kg b.i.d.), and they were assessed in open field, forced swim and social interaction tests at the age of 4 months. Behavior of LU-treated rats and saline controls did not differ in the open field and social interaction tests. However, in the forced swim tests LU-treated neonates showed prolonged immobility time compared with saline controls. In conclusion, chronic LU treatment during neonatal life produces long-term changes in the forced swim test, but not in the open field and social interaction tests. The behavioral changes in the forced swim test suggest that the central serotonergic system may be involved in the putative neonatal animal model of depression.

The cause and pathogenesis of depressive disorder remains almost unknown. Multiple postmortem studies in brains from patients with depression suggest that serotonergic neurotransmission is attenuated in depressive patients and that antidepressant therapy reverses this state (Caldecott-Hazard and Schneider, 1992; Risch and Nemeroff, 1992). However, despite the effective treatment with antidepressants, many questions remain to be answered, in particular why only long-term antidepressant therapy is efficient in reducing depressive symptoms. In terms of animal models, models that show more pronounced phenomenological similarities to the affective disorder and in which the time course of antidepressant effect is mimicked are needed.

Chronic administration of the tricyclic antidepressant clomipramine in the rat during the neonatal state of life (postnatal days 8 to 21) has produced adult rats whose behavior mimics some of the core symptoms of major depressive disorders in humans. The original study by Mirmiran et al. (1981) emphasized the immediate deprivation of REM sleep during the treatment period. This was followed by a long-lasting increase of REM sleep in adult life (Mirmiran et al., 1981, 1983; Vogel et al., 1990a). In adult life, these rats also showed several behavioral abnormalities similar to those observed in patients with depression, including impaired masculine sexual activity (Mirmiran et al., 1981, 1983; Neill et al., 1990; Velasquez-Moctezuma et al., 1993a, b), diminished pleasure-seeking behavior (Vogel et al., 1990b) and reduced aggressiveness (Vogel et al., 1988). Also, locomotor hyperactivity in an open field test has been reported in the paradigm (Goodman et al., 1993; Hartley et al., 1990; Mirmiran et al., 1983). Finally, increased immobility time in the forced swimming test has been reported in several studies (Fernandez-Pardal and Hilakivi, 1989; Goodman et al., 1993; Velasquez-Moctezuma et al., 1992).

Clomipramine is preferentially a 5-HT reuptake inhibitor, relative to NA and DA reuptake inhibition (Hyttel, 1994).

ABBR EVIAT IONS: FST, forced swimming test; OFT, open field test; SIT, social interaction test; SSRI, selective serotonin reuptake inhibitor; 5-HT, serotonin (5-hydroxytryptamine); NA, noradrenaline; DA, dopamine; SAL1, saline control group 1; SAL2, saline control group 2; LU, Lu 10–134-C [5-chloro-1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-phthalan]; 8-OH-DPAT, [2,3(n)-3H]-2-(N,N-dipropylamino)-8-hydroxy-1,2,3,4-tetrahydrodronaphtalen; QNB, [3H]quinuclicinyl benzilate; REM, rapid eye movement; ANOVA, analysis of variance; l-5-HTP, l-5-hydroxytryptophan.
Consequently, current findings in the neonatal paradigm have led to the hypothesis that chronic neonatal antidepressant treatment mimics features of major depression with melancholic (“endogenous”) features, and that the serotonin component of clomipramine may, at least in part, account for the observed effects (Vogel et al., 1990c). This is supported by findings of an attenuated serotonergic electric activity in the rat serotoninic dorsal raphe nucleus and increased metabolism of brainstem 5-HT after chronic neonatal clomipramine administration (Hilakivi et al., 1995; Yavari et al., 1993).

Development of REM sleep is intimately related to the serotonergic system, and REM sleep patterns are established during the second and third postnatal week in the rat (Mirmiran et al., 1981, 1983). Furthermore, the neonatal treatment period coincides with the maturation of the monoaminergic system. Ontogenic studies of the rat brain 5-HT transporter and 5-HT receptors indicate a rapid increase during the perinatal and early postnatal period to reach adult levels by the end of third postnatal week. That time course is closely related to synaptogenesis (Igyv-May et al., 1994; Jacobs and Azmitia, 1992). 5-HT may show trophic activity in the brain, thereby enabling developing axons to find their synaptic targets and to permit early neurotransmission (Lauder, 1990). Hence, it has been hypothesized that sustained antidepressant administration during neonatal life may disrupt the maturation of serotonergic transmitter function in the rat central nervous system and that such alterations may account for the observed behavioral changes in the adult rat (Vogel et al., 1990c).

The principal metabolite of clomipramine, demethylclomipramine, is a potent NA reuptake inhibitor, whereas clomipramine itself is a fairly selective serotonin reuptake inhibitor (Hyttel, 1994). Thus, effects on central noradrenergic and/or serotonergic neurotransmission may be responsible for the observed effects in the neonatal clomipramine paradigm.

The present study investigated the role of the serotonergic system by means of neonatal treatment with a SSRI. The SSRI, Lu 10–134-C (LU) (fig. 1), has been characterized as a potent 5-HT reuptake in vivo and in vitro (Bigler et al., 1977). The present study presents evidence of a large selectivity ratio relative to catecholamine reuptake inhibition and affinities for several receptors. Therefore, if LU produces results similar to clomipramine when administered early in the postnatal period, it provides support for the hypothesis of serotonergic involvement in the neonatal paradigm.

**Methods**

**Functional in Vitro and in Vivo Characterization of Lu 10–134-C**

**Animals.** Male Wistar rats (Mol: Wist, SPF), 150 to 200 g, and male albino mice (NMRI/BOM, SPF), 20 to 25 g, were used. Rats (five animals/Macrolon type III cages) and mice (20 animals/plastic cage) were housed conventionally in animal rooms with automatic control of temperature (21 ± 2°C), relative humidity (55 ± 5%) and air exchanges (16 times per hour) and day/night cycle (light on from 6:00 A.M. to 6:00 P.M.). They had free access to commercial pellet diet and tap water.

**In vitro amine reuptake inhibition studies.** Inhibition of reuptake of 10 nM [3H]-5-HT into rat whole-brain (minus cerebellum) synaptosomes, 12.5 nM [3H]-DA into rat striatal synaptosomes and 10 nM [3H]-NA into synaptosomes from rat frontal plus temporal cortex were determined as described by Hyttel (1982a) and Hyttel and Larsen (1985). Synaptosomes were prepared as described by Hyttel (1978a). Samples were incubated for 30 min at 37°C and then filtered. At least two concentration-response experiments were performed for each drug, each experiment consisting of five concentrations in triplicate.

**In vitro receptor binding studies.** Except when specified, IC50 values were estimated with membranes from rat whole-brain (minus cerebellum) preparations. Except for 5-HT2C receptors, the general assay was as follows: Rats were sacrificed and brain tissue was quickly removed and placed on ice, weighed and homogenized in ice-cold 50 mM Tris buffer in an ethanol-rinsed glass/Teflon homogenizer. The homogenate was centrifuged twice at 20,000 g for 10 min at 4°C, with rehomogenization of the pellet in ice-cold buffer. The final pel homogenate was rehomogenized in ice-cold buffer, and aliquots of tissue were incubated with the radioactive ligand alone or in the presence of test compound. The binding experiments were initiated by addition of drug solution and by placing the tubes in a 37°C water bath. After incubation, the samples were filtered, and the content of radioactivity was estimated by liquid scintillation counting.

- **5-HT1A receptors:** Inhibition of binding of 1.0 nM [3H]-8-OH-DPAT was determined as described by Hyttel et al. (1988b). 5-HT1A receptors were determined as described by Hyttel (1987b).
- **5-HT2A receptors:** Inhibition of binding of 0.5 nM [3H]-ketanserin to rat cortical 5-HT2A receptors was determined as described by Hyttel (1987b).
- **5-HT2C receptors:** Inhibition of binding of 0.5 nM [3H]-mesulergine was assessed in membranes from NIH-3T3 cells expressing the cloned rat 5-HT2C receptor (Baggeson et al. (1995)). The 5-HT2C receptor expressing cell line was cultured for 6 to 7 days, harvested by scraping and centrifuged at 400 × g for 5 min. The resulting pellet was resuspended in Tris buffer and homogenized with 0.5 nM [3H]-mesulergine for 60 min at 37°C, including various concentrations of test compound. After filtration, the filters were dried and a solid scintillation (Meltron × 8/HS) was melted into the filters, whereafter the radioactivity was determined in a Beta-plate scintillation counter from Wallac.

**Alpha adrenoceptors.** Inhibition of binding of 0.25 nM [3H]-prazosin or 1.0 nM [3H]-diazoxan to alpha-1 and cortical alpha-2 adrenoceptors, respectively, was determined as described by Arnt et al. (1992) and Megens et al. (1986). Beta adrenoceptors: Inhibition of binding of 0.5 nM [3H]-dihydroalprenolol in rat cortical membranes was determined as described by Hyttel et al. (1984). Dopamine receptors: Inhibition of binding of 0.2 nM [3H]-SCH 23390 or 0.5 nM [3H]-spiperone to striatal dopamine D1 and dopamine D2 receptors, respectively, was determined as described by Hyttel (1982b, 1986, 1987c) and Hyttel and Arnt (1987). Histamine H1 receptors: Inhibition of the binding of 2.0 nM [3H]-nepyrine was determined as described by Hall and Ögren (1984). Muscarinic cholinergic receptors: Inhibition of the binding of 0.12 nM [3H]-QNB in rat brain homogenates was estimated as described by Arnt et al. (1992).

**In vivo amine reuptake inhibition.** All tests were performed in mice as described by Hyttel et al. (1988a). The injection volumes were
10 ml/kg body weight and route of administration was s.c. if nothing else is stated.

5-HT reuptake inhibition measured as potentiation of l-5-HTP-induced 5-HT syndrome: Mice were injected with l-5-HTP (100 mg/kg i.v.) 30 min after application of test drug. During the next 15 min, the animals were observed for the presence of the following symptoms: head weaving, tremor and abolition of the hind limbs. Each animal was given one point for each symptom present. Six to nine mice were used per dose. The dose that induced half-maximal score of the 5-HT syndrome was calculated (ED_{50}).

DA reuptake inhibition measured as antagonist of tetrabenazine-induced ptosis: Mice were given test compound 30 min before tetrabenazine (40 mg/kg i.p.) and tested 30 min after tetrabenazine. The animal boxes were tilted, and 30 sec later the animals were scored for the degree of ptosis, according to Rubin et al. (1957). ED_{50} values were calculated as the doses reducing the ptotic score to half of that of a control group receiving tetrabenazine alone. At least six mice were used at each dose level.

DA reuptake inhibition measured as potentiation of apomorphine-induced gnawing: Test drug was injected 15 min before apomorphine (10 mg/kg i.p.) and the mice were placed in pairs in Perspex observation cages. At least six pairs were used at each dose level. The cages were placed on corrugated paper (corrugating facing upward), and after 1 hr it was evident whether or not the mice had been biting the corrugated paper. Results were expressed as the fraction of pairs that were biting.

**Neonatal Treatment with Lu 10–134-C**

**Animals.** Female and male Wistar WU rats (Charles River, Hanover, Germany) were mated in metabolism cages of wire mesh with a plastic tray to collect plugs. One female and male were placed in each cage, and males were changed if plugs were not observed after 3 to 4 days. When plugs were observed, female rats were single-housed. During pregnancy and 3 weeks after giving birth, rats received breeding diet. Post weaning, diet to the offspring was changed to a conventional type. Throughout the experiment, tap water was freely available. Room temperature (21 ± 2°C), relative humidity (55 ± 5%) and air exchanges (16 times per hour) were systematically controlled. Rats were kept on a 12-hr light-dark cycle (lights on 6:00 a.m. to 6:00 p.m.) in Macrolon cages type III.

The day after giving birth, the male offspring were randomly cross-fostered by placing neonates with a different lactating dam. Within each litter, pups were subsequently randomly allocated to different treatment regimens. Each litter consisted of an equal representation of all drug regimens with a total of four pups per litter. To avoid any later hormonal interference because of estrous periods, female pups were eliminated from the experiment. When treatment was initiated, all pup tails were marked with different colors to identify each neonate in the litter. All rats were weighed daily before treatment and received either either saline (5 ml/kg i.p.).

Two studies were performed. In the first, LU doses were 2.5 (n = 16), 5.0 (n = 14) and 10 (n = 14) mg/kg b.i.d. at (8:00 a.m. and 8:00 p.m.) during postnatal days 8 to 21. Controls received saline injections b.i.d. In the second study, rats received LU 15 mg/kg b.i.d. (n = 11) or saline injections (n = 24). Post weaning at postnatal day 22, rats were separated for housing with age-matched rats that had received the same neonatal treatment in groups of three or four. All rats were weighed weekly during postnatal weeks 4 to 16 (postnatal days 28 to 112) to consider any long-term effects on body weight gain.

**Apparatus.** The open field test was conducted in an open arena (l, w, h cm): 150 × 100 × 40, with bottom and sides made of Plexiglas covered with black, nonreflecting material. The beding was sawdust exposed to other rats before testing to provide a constant odor level in the arena. The arena was placed in a quiet room. Behavior of the rat was recorded by a video camera (Cohou model 4722–2000 with Ernitec 6 mm/1.2 lens) placed above the arena and connected to a S-VHS video cassette recorder (JVC model HR-5000SH). Lighting in the room consisted of fluorescent lighting with high lighting intensity (300–420 lux). A screen was placed between the arena and the experimenter to avoid visual contact. Videotapes were analyzed off-line with a video cassette recorder (Panasonic model AG-7350), which was connected to an automated behavior recognition system by a software program (Ethovision v1.50, Noldus Information Technology Corp., Wageningen, The Netherlands) on a computer (Compaq 386 SX).

In the forced swim test, the rat was placed in a water-filled glass cylinder (l, d cm): 35, 24) with fluorescent lighting mounted above the top of the pail. Behavior was videotaped by a S-VHS video cassette recorder (Nicam model HR 54700EH), which was connected to a fixed positioned camera (Sony model CCD). Videotapes were analyzed manually by a single rater with a video cassette recorder (Panasonic model AG-7350).

The social interaction test was performed in the open field arena. Behavior was recorded in two successive tests and was recorded under both low-light (1.5 lux) and high-light (300–420 lux) conditions. Under low-light conditions (test I), the only light source was a dark-red 25 W light bulb and light was diffuse to minimize shadows in the arena. During the second test (test II) lighting was changed to high-light conditions with general fluorescent lighting (36 W). Videotapes were analyzed off-line with a video cassette recorder (Panasonic model AG-7350), connected to a software program (Ethovision v1.50 and The Observer v3.0, Noldus Information Technology Corp., Netherlands). An additional software program was used to analyze frequency of active social interaction.

**Experimental Procedures**

All rats were put through all behavioral tests. Tests were carried out in the following sequence with at least 1 week separating each test (age of rat in parentheses): OPT (17–19 weeks), FST (18–20 weeks) and SIT (20–22 weeks). Rats were placed in the testing room at least 1 day before testing.

The OPT was carried out for 10 min and took place between 10 and 16 hr under unfamiliar conditions, i.e., the rat was placed in the corner of the arena without any prior habituation. The ambulation parameters were per cent time spent and distance traveled in the peripheral and central arena.

The design of the FST was adapted from Porsolt et al. (1977). The rat was immerses in the water (25 ± 0.3°C) for 15 min (test I), and 24 hr later for 10 min (test II). Water level was adjusted to rat body weight: 350 to 375 g (19.6 cm), 375 to 400 g (20.6 cm), and >400 g (21.6 cm), ensuring that the rat was just able to touch the bottom of the cylinder with its hindpaws. To avoid confounding results because of possible alarm substances from urine, water was replaced by fresh water between every trial (Abel et al., 1990, 1991). After testing the rat was removed from the pail, dried and returned to a clean home cage. Behavioral categories were only observed to be immobile, swimming and climbing. A rat was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line (Porsolt et al., 1977). Swimming was scored when the rat made active swimming motions, i.e., moved around in the cylinder in a horizontal paddling position (Armario et al., 1988). Climbing was determined when the rat was making active movements with its forepaws in and out of the water, usually intense movements directed against the wall (Armario et al., 1988).

At least 7 days before introducing rats to the SIT, they were dyed with either black or beige hair color (Polyco, Henkel Cosmetics, Düsseldorf, Germany) to promote off-line recognition by the software programs. Color was applied to the head and shoulder, slightly below the forelimbs and on the back, leaving an uncolored middle body area of about 8 cm. After 45 to 60 min surplus color was washed out thoroughly, the rat was dried and returned to a clean home cage. Pairs of rats that had received identical neonatal treatment, but were unfamiliar to each other were tested. The SIT was performed in two consecutive trials under different light conditions. Pairs of a
dark and a beige rat were introduced to the arena and testing was carried out in the dark cycle between 20 and 03 hr (test I). At least 5 days later, unfamiliar pairs of rats were exposed to the second test (test II) in the light cycle between 10 and 16 hr. Under both conditions, trials were carried out for 10 min and rats were returned to their individual home cages. Behavioral parameters were quantified by the software programs, i.e., social interaction time (rats were less than 20 cm apart), active social interaction time (rats were in contact and moving) and passive social interaction time (rats were in contact but not moving) as described by Sams-Dodd (1995).

Data Analysis

Results obtained in the neonatal study were analyzed by a two-way (treatment x block) ANOVA. Post hoc testing was carried out by using Dunnett’s two-tailed test for comparison of Lu 10–134-C-treated groups to saline controls, and Neuman-Keuls test for comparison between drug-treated groups. Body weight data were analyzed by a three-way ANOVA (repeated measure analysis), and data of interest were compared post hoc via an independent t test. A P value less than .05 was considered statistically significant. An asterisk (*) indicates levels of statistical significance; *P < .05, **P < .01 and ***P < .001.

Drugs

The following drugs were dissolved in saline or water: Lu 10–134-C-HCl (H. Lundbeck A/S, Copenhagen, Denmark); l-5-HTP (Sigma, St. Louis, MO); tetrabenazine, HCl (synthesized at H. Lundbeck A/S). Apomorphine-HCl (Unichem, Copenhagen, Denmark) was dissolved in 0.02% ascorbic acid.

The following tritiated ligands were used: 1-[7,8-3H]NA (specific activity, 37–45 Ci/mmol); [G-3H]5-HT creatinine sulfate (18 Ci/mmol); [N-methyl-3H]SCH 23390 (72–80 Ci/mmol), [phenyl-4-3H]prazosin (16 Ci/mmol); 8-OH-DPAT (200 Ci/mmol); QNB (42 Ci/mmol); [3H]dioxazan (53 Ci/mmol) were obtained from Amersham International (Buckinghamshire, England). [furoyl-5-3H]prazosin (20 Ci/mmol), 3,4-[ring-2,5,6-3H]DA HCl (33 Ci/mmol); l-[propyl-1,2,3-3H]dihydroalprenolol HCl (101 Ci/mmol); [ethylene-3H]ketanserin HCl (77 Ci/mmol); [3H]Hompyrazine (22 Ci/mmol); [3H]mesulergine (81 Ci/mmol) were obtained from New England Nuclear ( Dreieich, FRG).

Results

Functional in Vivo and in Vitro Tests of Lu 10–134-C

LU was a potent inhibitor of the accumulation of 5-HT into rat synaptosomes. The effect on NA and DA was weak (table 1), showing ratios of NA/5-HT and DA/5-HT reuptake inhibition greater than 1,000 (approximately 1,100 and 3,100, respectively). The in vitro 5-HT reuptake profile of LU was much more selective than those of tricyclic antidepressants clomipramine and imipramine.

The very low inhibitory effect on NA and DA reuptake in rat synaptosomes was also reflected in the weak activity in functional in vivo tests (table 1). Ratios for tetrabenazine antagonism/5-HTP potentiation and apomorphine potentiation/5-HTP potentiation were equal, i.e., higher than 28, and these ratios were higher than observed for clomipramine, imipramine and fluoxetine.

In Vitro Receptor Binding Profile of Lu 10–134-C

LU had no affinity for serotoninergic, adrenergic, dopaminergic, histaminergic or muscarinic cholinergic receptors (table 2). The affinity for 5-HT1A receptors was in micromolar concentrations far higher than observed for clomipramine and imipramine. The affinity for 5-HT2A and 5-HT2C receptors was also low. LU had no affinity for alpha-1, alpha-2 and beta adrenoceptors or dopaminergic D1 and D2 receptors. Additionally, LU had low affinity for histaminergic H1 and muscarinic cholinergic receptors.

Neonatal Treatment with Lu 10–134-C

Body weight gain during development. Before treatment of neonatal rats, no difference in body weight was observed (data not shown). Neonatal treatment with Lu 10–134-C showed an overall influence on body weight gain during maturation compared with SAL (P < .001). Post hoc analysis revealed that LU doses ranging from 5.0 to 15 mg/kg all induced significant reductions of body weight gain during postnatal weeks 4 to 7, and the effect was persistent in 15 mg/kg LU rats throughout the experiment (fig. 2).

Open field activity. As shown in table 3, measurement of activity confined to the periphery and central arena did not differ between LU- and SAL-treated rats. Rats spent almost all the time in the periphery of the arena (95–96%), and the traveled distance of LU-treated rats did not differ from control levels, neither in the periphery nor in the central sections of the arena.

Social interaction activity. Levels of active and passive social interaction of SAL1 and SAL2 rats differed statistically (P = .007). Consequently, levels of active and passive social interaction of LU 15 mg/kg rats were compared with their respective controls (table 4). In general, there was no evidence of a differential social activity, either in test I or test II. The LU 2.5 mg/kg rats, however, showed a slightly increased duration of active social interaction under high-light conditions compared with SAL1 levels (P = .04).

Forced swimming activity. Levels of all behavioral parameters detected in the FST, i.e., immobility, swimming and climbing time, differed between SAL1 and SAL2 rats with P values ranging from .001 to .04. No inter-rater variability was found after reevaluation of both control groups. Hence, LU 15 mg/kg rats were compared with their respective controls.

### Table 1

**Functional in vivo and in vitro monoamine reuptake inhibitory potency of Lu 10–134-C**

For comparison, in vivo and in vitro data on fluoxetine, clomipramine and imipramine are inserted (from Hyttel, 1994).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Functional in Vivo Profile (ED&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>In Vitro Reuptake Inhibition (IC&lt;sub&gt;50&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-HTP potentiation</td>
<td>Tetrabenazine antagonism</td>
</tr>
<tr>
<td></td>
<td>μmol/kg s.c.</td>
<td>μmol/kg s.c.</td>
</tr>
<tr>
<td>Lu 10–134-C</td>
<td>3.9</td>
<td>&gt;110</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>10&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Imipramine</td>
<td>27&lt;sup&gt;*&lt;/sup&gt;</td>
<td>16&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The route of administration was i.p. in this study.
Immobility time in test I was significantly increased in 10 and 15 mg/kg LU rats ($P < .001$) compared with SAL1 ($P = .006$) and SAL2 ($P < .001$) rats, respectively (fig. 3). Retesting rats in test II revealed that neonatal doses ranging from LU 5.0 to 15 mg/kg produced a significant increase of immobility time compared with saline levels ($P < .001$, $P < .001$ and $P < .001$). However, no differential response between LU 5.0 mg/kg, LU 10 mg/kg and LU 15 mg/kg rats was detected ($P = .677$).

Swimming time was also influenced by neonatal treatment of Lu 10–134-C. As shown in figure 3, reduced levels of swimming time were apparent in 15 mg/kg LU rats in both test I and test II compared with SAL2 rats ($P < .001$ and $P = .013$, respectively). Further, 5.0 mg/kg LU rats also exhibited a reduction of swimming time ($P = .028$).

In contrast, no differential climbing response between LU and SAL rats was detected by the ANOVA (fig. 3). This was apparent in both test I ($P = .11$) and test II ($P = .29$).

No correlation was observed between body weight at test time and performance in the forced swim test ($r$ values ranged from 0.0 to 0.4).

**Discussion**

The present study indicates that chronic treatment with LU during neonatal life increases immobility time in the FST in adult life in a dose-dependent manner, with the dose of 15 mg/kg/b.i.d. showing the most consistent results in both test I and test II. LU was a more potent and selective 5-HT uptake inhibitor than the SSRI fluoxetine, and LU showed only negligible affinity for a range of receptors. Hence, LU was found to be a potent SSRI. Therefore, the observation that neonatal administration of LU affected the outcome of FST in adult rats indicates that the neonatal rat central nervous system was sensitive to selective serotonin uptake blockade and that the response was persistent.

The two control groups, SAL1 and SAL2, responded differently in the FST and SIT. Except for a slight change in the mating sessions, all parameters in the experimental procedures were held constant. We did not use the same female rats in both mating sessions, and females in the second mating session were younger and not experienced as mothers. Hypothetically, mothers may therefore have responded differently to the same environment during pregnancy and rearing of the litters, leading to differences in the behavior of the offspring. Furthermore, litters were delivered during the autumn and winter, respectively.

The elevated immobility time in the FST is in agreement with previous reports on neonatal clomipramine administration (Fernandez-Pardal and Hilakivi, 1989; Goodman et al., 1993; Velasquez-Moctezuma and Ruiz, 1992). Thus, the fact that clomipramine is preferentially a serotonergic reuptake inhibitor (Hyttel, 1994) suggests that the serotonergic component of clomipramine may, at least in part, account for the increased immobility time in the FST. This is also supported by the observation that neonates receiving the highest dose of LU had reduced swimming time during test I and test II, whereas climbing time was not clearly affected. Separation of specific behavioral categories other than immobility in the FST has not been used routinely in the FST procedure by others investigating the neonatal paradigm. However, the present results are in general agreement with tricyclic anti-
depressant and SSRI experiments performed in adult rats (Detke et al., 1995). The authors emphasized that acute SSRI treatment in adult rats affected immobility and swimming behavior, whereas tricyclic antidepressants affected immobility and climbing behavior in the FST.

It is well known that the 5-HT system is involved in appetite regulation and drugs that are able to increase extracellular 5-HT concentrations, e.g., dexfenfluramine and SSRIs, are effective anorectic agents (Leonard, 1994). Hence, the observation that treatment with LU 15 mg/kg caused a persistent reduction of weight gain, which was not related to the behavioral categories assessed in the FST, may indicate that permanent changes in 5-HT function were induced. This also agrees with observations of a lowered firing frequency and

**TABLE 3**
The open field test (mean values ± S.E.M.): Behavioral effects in adult male rats treated neonatally with Lu 10-134-C or saline

<table>
<thead>
<tr>
<th>Open Field Test</th>
<th>Saline</th>
<th>Lu 10-134-C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5 mg/kg</td>
<td>5.0 mg/kg</td>
</tr>
<tr>
<td>% time in peripheral arena</td>
<td>96.0 ± 1.0</td>
<td>96.0 ± 1.0</td>
</tr>
<tr>
<td>% time in central arena</td>
<td>4.0 ± 1.0</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>Traveled distance in peripheral arena (cm)</td>
<td>4600 ± 340</td>
<td>4200 ± 410</td>
</tr>
<tr>
<td>Traveled distance in central arena (cm)</td>
<td>330 ± 80</td>
<td>330 ± 91</td>
</tr>
</tbody>
</table>

**TABLE 4**
The social interaction test (mean values ± S.E.M.): Behavioral effects of adult male rats treated neonatally with Lu 10-134-C or saline

Test were conducted under low light levels (test I) and high light levels (test II).

<table>
<thead>
<tr>
<th>Social interaction test</th>
<th>SAL1</th>
<th>2.5 mg/kg LU</th>
<th>5.0 mg/kg LU</th>
<th>10 mg/kg LU</th>
<th>15 mg/kg LU</th>
<th>Sal2</th>
<th>15 mg/kg LU</th>
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**Test I: Low-light conditions**
Social interaction time (sec) | 172 ± 9 | 163 ± 8.0 | 183 ± 7.0 | 201 ± 10 | – | 153 ± 9.0 |
Active social interaction (sec) | 210 ± 9.0 | 180 ± 8.0 | 200 ± 7.0 | 220 ± 8.0 | 170 ± 7.0 | 170 ± 7.0* |
Passive social interaction (sec) | 4.4 ± 2.3 | 4.1 ± 1.6 | 10 ± 4.0 | 6.8 ± 2.3 | 0.2 ± 0.2 | 0.9 ± 0.6* |

**Test II: High-light conditions**
Social interaction time (sec) | 180 ± 15 | 200 ± 10 | 180 ± 8.0 | 200 ± 15 | – | 180 ± 23 |
Active social interaction (sec) | 160 ± 13 | 200 ± 7.0* | 170 ± 8.0 | 190 ± 14 | 200 ± 9 | 180 ± 21* |
Passive social interaction (sec) | 20 ± 7.0 | 16 ± 6 | 15 ± 4 | 17 ± 6 | 10 ± 4.0 | 14 ± 6* |

* LU 15 mg/kg compared with SAL2 levels; *P < .05.
Reduced 5-HT messenger RNA levels in the serotonergic dorsal raphe complex in adult rats after neonatal LU-treatment (Hansen, H. H. and Skarsfeldt, T., unpublished observations).

However, the involvement of 5-HT in the FST is poorly understood. Forced swimming has been shown to elevate in vivo 5-HT dialysate concentration in the rat brain (Kirby et al., 1995). Chronic but not acute administration of SSRIs in adult rats is reported to induce anti-immobility effects in the FST (Kelly and Leonard, 1994; Pucilowski and Overstreet, 1993). An overall increase of serotonergic neurotransmission caused by desensitization of 5-HT1A autoreceptors in the serotonergic dorsal raphe complex may be involved in this effect. Microdialysis studies in chronically SSRI-treated rats have demonstrated elevated extracellular 5-HT levels in terminal areas and attenuated ability of 5-HT1A receptors in the raphe nuclei to regulate terminal 5-HT release (Kreiss and Lucki, 1995). This is supported by findings of an anti-immobility effect after intraraphe administration of the 5-HT1A receptor agonist, 8-OH-DPAT (Borsini, 1995). Thus, changes in 5-HT1A autoreceptor function may be involved in the neonatal paradigm. However, the hypothermic responses to 8-OH-DPAT is blunted by chronic antidepressant treatment, which suggests desensitization of postsynaptic 5-HT1A receptors (Kelly and Leonard, 1994).

At present, a few neurochemical changes related to the serotonergic system of rats neonatally treated with antidepressants have been reported. Neonatal administration of the SSRI zimeldine produced a significant increase of 5-HT metabolism in the rat brainstem and cortex in adulthood (Hilakivi et al., 1995). Also, acute systemic administration of the SSRI citalopram in adult rats, neonatally treated with clomipramine, demonstrated that the firing frequency of the dorsal raphe neurons was less sensitive (Mauduit et al., 1995). The effect was interpreted by the authors to be mediated by an increased tolerance to tonic 5-HT stimulation of inhibitory 5-HT1A autoreceptors. However, we did not find any changes in 5-HT1A, 5-HT1B or 5-HT2C receptor messenger RNA content in the dorsal raphe in adult rats, previously neonatally treated with LU (Hansen, H. H. and Mikkelsen, J. D., unpublished observations). Thus, the possible involvement of 5-HT receptors in the neonatal animal model has yet to be further characterized.

The paradoxical increase in immobility behavior in the FST after neonatal LU treatment is in opposition to the usual findings of a reduction after long-term SSRI administration in adult rats (Porsolt et al., 1978; Borsini 1995). From a complementary angle, an increase of immobility time may then reflect a state of suppression of behavioral activity in the rat as proposed by Porsolt et al. (1977). However, the interpretation of the significance of immobile posture is a matter of great controversy and it is not known if such a simple behavior may be related to human depressive state, or just reflects a simple coping strategy of the adult rat (West, 1990). Consequently, to get further information about the relevance of the behavioral findings in the FST after neonatal LU treatment, rats were also assessed in the OFT and SIT.
Exposing rats to the OPT procedure, which is thought to provide information about locomotor dysfunction (Denenberg, 1969; Mirmiran et al., 1983; Walsh and Cummins, 1976) revealed no changes of locomotor behavior of LU-treated rats compared with saline-treated controls. The lack of an effect by LU in the OPT may indicate that the observed change in immobility and swimming time is not related to general motor disturbances of the LU-treated rats.

Our OPT experiment contrasts one of the most common findings in the neonatal paradigm. Neonatal clomipramine administration is reported to increase locomotor activity in the peripheral arena, interpreted as an index of increased fearfulness and motor restlessness to a specific open, exposed environment (Hartley et al., 1990; Hilakivi et al., 1984; Mirmiran et al., 1983). This has also been shown after neonatal treatment with another tricyclic antidepressant, desipramine (Dwyer and Roy, 1993). The lack of an effect cannot be explained by differential test principles because most previous reports used similar procedures. On a tentative basis we suggest that the lack of an effect of LU in the OPT may reflect the differences of the pharmacological profile of neonatally injected test substances. Clomipramine and desipramine are tricyclic antidepressants which also exhibit affinity for a range of, e.g., serotonergic, adrenergic and dopaminergic receptors (Hyttel, 1994). Moreover, the demethyl metabolite of clomipramine, demethyldesipramine, is a potent inhibitor of NA uptake (Hyttel, 1994). Thus, other aminergic mechanisms may contribute to OPT-induced effects in adult rats previously neonatally treated with clomipramine.

Additionally, LU-treated rats did not show any disturbances of social behavior in the SIT procedure. Change of lighting intensity between sessions did not reveal any effect. Such procedures are thought to be crucial to the test (File, 1980), however, and the mild stressor effect of the previous OPT and FST procedures might have reduced sensitivity to the SIT. Neither acute nor chronic administration of SSRIs in adult rats is sensitive to the SIT model (File, 1985, 1988), which indicates that effects of neonatal SSR1 treatment may also not necessarily be detectable in the SIT. This is in accordance with File and Tucker (1983), who also did not demonstrate a social dysfunction in rats neonatally treated with clomipramine.

In conclusion, administration of LU to neonatal rats during early development induces long-lasting behavioral abnormalities in the forced swim test. These may be related to the serotonergic system because LU has been proved to be a potent SSRI. However, the presence of many unknown factors in forced swimming-induced behavior makes the implications in the putative model of human depression unclear. Consequently, further initiatives should be taken to provide a further basis for understanding the effects of chronic SSR1 treatment in the putative neonatal model of depression.

References


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