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Antidepressant-like activity of the melatonin receptor antagonist, luzindole (N-0774), in the mouse behavioral despair test

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The anti-immobility effect of the selective melatonin receptor antagonist, luzindole, was investigated in the behavioral despair test using three different strains (C3H/HeN, C57BL/6J and albino ND/4) of mice. The time of immobility of the C3H/HeN during the 240 s swimming period measured at noon (12 : 00 to 14 : 00 h) was 47.8 ± 3.0 s (n = 63) and at midnight (00 : 00 to 02 : 00 h) was 67.7 ± 2.8 s (n = 68) ($P < 0.001$, when compared with the noon value), when the levels of endogenous melatonin are presumably low and high, respectively. Melatonin (30 mg/kg) given i.p. did not modify the time of immobility at either time of measurement. Luzindole (30 mg/kg i.p.) reduced the time of immobility in a dose-dependent manner, the effect being more pronounced at midnight (60% reduction) than at noon (39% reduction). The effect of luzindole was time-dependent, showing a maximal effect at 60 min. The anti-immobility effect of luzindole (10 mg/kg i.p.) was prevented by the administration of melatonin (30 mg/kg i.p.). Luzindole (30 mg/kg i.p.) did not modify the time of immobility either at noon or midnight in the albino ND/4 mouse, or in the C57BL/6J mouse, which does not produce melatonin. Our results suggest that endogenous melatonin plays a role during swimming in the C3H/HeN mouse behavioral despair test. We conclude that luzindole may exert antidepressant-like activity in the C3H/HeN mouse by antagonizing the action of endogenous hormone.

Luzindole (N-0774); Melatonin receptor antagonists; C3H/HeN mouse; ND/4 mouse; C57BL/6J mouse; Forced swimming test

1. Introduction

Melatonin (5-methoxy-N-acetyltryptamine), the major hormone of the pineal gland, is synthesized and secreted at night following a circadian rhythm resulting from both endogenous mechanisms and environmental cues (Axelrod, 1974; Klein, 1979; Tamarkin et al., 1985; Arendt, 1988). This hormone transduces photoperiodic information and appears to be involved in the regulation of a variety of endocrinological, neurophysiological and behavioral functions (Cardinali, 1981; Tamarkin et al., 1985; Dubocovich, 1983; 1988a,b; Datta and King, 1980). Endogenous melatonin regulates reproduction, body weight, metabolism and coat color in photoperiodic mammals (Darrow and Goldman, 1985; Tamarkin et al., 1985; Arendt, 1988) and circadian rhythms in birds and reptiles (Menaker, 1982; Underwood and Harless, 1985). Recent evidence suggests that administration of exogenous melatonin may be effective in entraining and synchronizing disturbed circadian rhythms in mammals including man (Redman et al., 1983; Cassone et al., 1986; Arendt et al., 1986; Arendt, 1988). Melatonin has been implicated in the etiology of some chronobiological disorders of mood

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and sleep (Rosenthal et al., 1984; Wehr et al., 1986; Lewy et al., 1987).

Behavioral effects in mammals produced by administration of exogenous melatonin include decreased locomotor activity and rearing, increased grooming, and sniffing (Kovács et al., 1974; Wong and Whiteside, 1968; Gaffori and Van Ree, 1985), and the induction of sleep (Hishikawa et al., 1969; Marczynski et al., 1964; Holmes and Sugden, 1982; Sugden, 1983). Forced swimming has been shown to decrease the levels of pineal melatonin and to cause increases in plasma melatonin in rats (Trioani et al., 1988) which presumably lead to activation of melatonin receptor sites in the central nervous system (Cardinali, 1981; Duncan et al., 1989; Dubocovich, 1988a; Siuciak et al., 1990).

The physiological effects of endogenous melatonin can be antagonized by exposure of animals to long photoperiods thus inhibiting the synthesis of this hormone, by removal of the pineal gland (Tamarkin et al., 1985; Darrow and Goldman, 1985) and by blocking of its action at melatonin receptor sites in target tissues (Dubocovich, 1985; 1988a,b). In the present study, we used luzindole, the first competitive melatonin receptor antagonist discovered to date (Dubocovich, 1988a,b), to investigate the physiological role of endogenous melatonin in the mouse during forced swimming. Forced swimming of mice produces a state referred to as 'behavioral despair' which is sensitive to antidepressant drugs (Porsolt et al., 1977; 1978a; Troiani et al., 1988). Clinically effective antidepressants, both typical and atypical, have been shown to reduce the time during which a mouse remains immobile during a session of forced swimming (Porsolt et al., 1977; 1978a). Because melatonin has been implicated in the etiology of certain depressive disorders (Rosenthal et al., 1984; Wehr et al., 1986), it was of interest to study the effect of luzindole in the behavioral despair test and to compare its effects with that of the typical antidepressant, desipramine. Three strains of mice: one albino, the ND/4 mouse, and two strains of black, the C3H/HeN and the C57BL/6J mouse were used in this test. The strains of black mice were selected because the C3H/HeN mouse, but not the C57BL/6J mouse, is known to produce melatonin in the pineal gland with high levels at night (Hotz et al., 1985; Ebihara et al., 1986; Goto et al., 1989). Testing was carried out in the middle of the light and of the dark cycle, i.e., when the levels of endogenous melatonin are low and high, respectively (Tamarkin et al., 1985; Darrow and Goldman, 1985; Hotz et al., 1985; Ebihara et al., 1986; Goto et al., 1989). We demonstrated that endogenous melatonin plays a role in responses in the behavioral despair test and that luzindole may reduce immobility during the test by blocking the in vivo effects of endogenous melatonin.

2. Materials and methods

2.1. Mouse behavioral despair test

Male black C3H/HeN mice, albino ND/4 mice and C57BL/6J mice (4-5 weeks old; 20-25 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Upon arrival in the laboratory, groups of eight mice were housed in plastic cages with free access to food and water. The mice were kept under a 14/10 h light/dark cycle (lights on at 04 : 00 and off at 18 : 00), and a room temperature of 22 ± 1° C. During the light period the light intensity at the level of the cage was about 250-300 lux. The animals were taken into an experimental room 6 h before the experiment and kept under the appropriate light/dark conditions. Experiments conducted between 12 : 00 and 14 : 00 h are referred to as 'noon' experiments and were performed with lights on unless indicated otherwise. Experiments conducted between 00 : 00 and 02 : 00 h are referred to as 'midnight' experiments and were performed in the dark unless indicated otherwise. Experiments in the dark were conducted under red dim light (Kodak, filter A).

The swimming test was conducted as described by Porsolt et al. (1977; 1978a). Forced swimming in a glass cylinder (11.5 cm diameter, containing 6 cm of water maintained at 23 ± 1° C) induced a 'depressed state' characterized by a readily identifiable immobile posture. In each experiment, the duration of immobility was measured in a maximum of four groups of mice (eight mice per group) receiving different treatments. One group was al-

ways treated with vehicle (control). The time of immobility was recorded simultaneously in two mice receiving different treatments. Each mouse swam only once during a period of 6 min. Following the first 2 min of adaptation, the time of immobility was recorded using two stop watches during the last 4 min. The mice were injected with vehicle or with drugs dissolved in vehicle.

Melatonin and luzindole were dissolved in 1% Tween 80, and desipramine was dissolved in water. Drugs or vehicle were administered by intraperitoneal injection (i.p.). Each animal received a volume of liquid equivalent to 10 ml/kg. All the controls received the same volume of vehicle. There were eight animals in each experimental group.

2.2. Release of [^{3}H]norepinephrine from C3H / HeN mouse cortex slices

Male black C3H/HeN mice (20-25 g) maintained on a 14-10-h light-dark cycle were killed by cervical dislocation during the light cycle. Brain cortical slices were prepared as previously described (Pelayo et al., 1980; Maurin et al., 1982). Slices were incubated for 20 min at 37°C in the presence of 0.1 μM D,L-[7-^{3}H]norepinephrine (specific activity, 12.7 Ci/mmol; Amersham Corporation, Arlington Heights, IL). Thereafter, the slices were washed in Krebs solution and were placed on the bottom of cylindrical plastic tubes with a thin nylon mesh on the bottom. The plastic tubes containing two cortical slices were transferred to individual glass superfusion chambers, each of which contained a pair of platinum electrodes 30 mm apart. The slices were superfused at a rate of 1 ml/min with Krebs solution at 37°C, until the spontaneous outflow of radioactivity had stabilized. Samples of the superfusate were collected by means of a fraction collector at 4-min intervals. The composition of the Krebs solution was mM): NaCl 108; KCl 4.7; glucose 11.1; $NaHCO_3$ 25.0; $MgCl_2$ 1.2; NaH_2PO_4 1.0; $CaCl_2$ 1.3; ascorbic acid 0.11; and disodium EDTA 0.004.

Tritium release was elicited by field stimulation using 3 Hz, 20 mA, 2-ms duration pulses that were delivered from the electrode by a Grass Stimulator, model S44. During stimulation, the pulses were monitored on an oscilloscope. In each experiment two periods of field stimulation were applied at 60 (S_1) and 100 (S_2) min after the end of the incubation with [^{3}H]norepinephrine. Samples of superfusate were collected before, during and after the period of stimulation. Desipramine (1 nM-10 μM) or luzindole (0.1-10 μM) were added to the perfusion medium 20 min before S_2 and were present throughout the remainder of the experiment. At the end of the experiment, the slices were solubilized in 0.5 ml of TS-1 (Research Products International, Elk Grove, IL) and the tritium content was determined by liquid scintillation counting.

Luzindole (N-0774; 2-benzyl-N-acetyltryptamine) was obtained from Nelson Research (Irvine, CA). All other chemicals and drugs were obtained from Sigma Chemical Company (St. Louis, MO). Luzindole was dissolved in a minimum amount of ethanol (95%) to give a stock solution of 10 mM; additional dilutions were done in distilled water. All solutions were freshly prepared the day of the experiment. The maximal concentration of ethanol in the superfusion medium for the highest concentration (10 μM) of luzindole was below 22 mM. This concentration of ethanol, when added alone before the second period of stimulation, did not significantly modify the calcium-dependent release of [^{3}H]norepinephrine.

2.3. Data analysis

The results are expressed as means ± S.E.M. Data were analyzed by means of the two-tailed unpaired Student's t-test when only two groups were to be compared. Comparisons of control with the results of different treatments were assessed by means of Dunnett's multiple comparisons procedure (Dunnett, 1955; Hochberg and Tamhane, 1987).

3. Results

3.1. Behavioral despair test

The duration of immobility during swimming was recorded during the last 240 s of a 360-s session with the three different strains of mice:

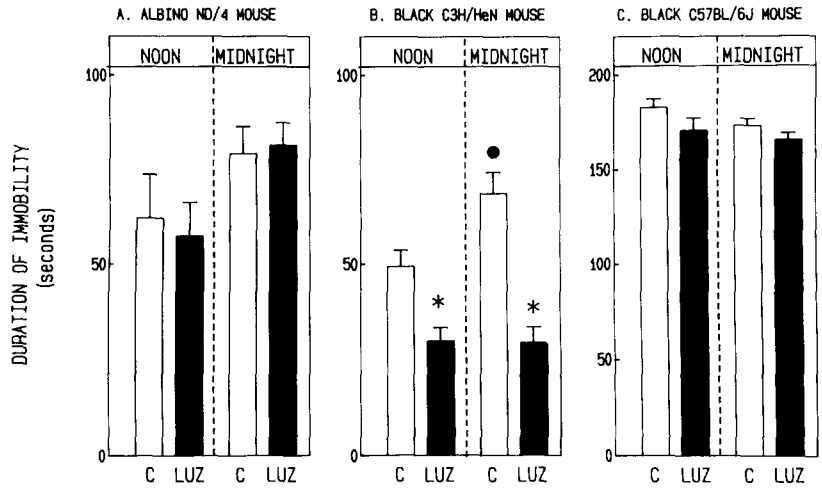

Fig. 1. Effect of luzindole on the duration of immobility in the forced swimming test with three strains of mice at noon and at midnight. The swimming test was conducte as described in Methods. Mice from three different strains (A: albino ND/4; B: C3H/HeN; C: C57BL/6J) were kept on a 14:10 h light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (NOON) and 00:00 and 02:00 h (MIDNIGHT). Vehicle (C) (□) or luzindole (LUZ) (■, 30 mg/kg i.p.) was administered 30 min before the test. The duration of immobility in vehicle- or desipramine-treated mice within each strain was determined in the same experiment in eight mice per group. Mean ± S.E.M. values pooled from different experiments are shown. * $P < 0.001$ when compared with the corresponding control (C) (Student's t-test). • $P < 0.001$ when compared with control (C) at noon (Student's t-test).

albino ND/4, C57BL/6J and C3H/HeN. The time during which a mouse remained immobile during the 240-s measurement period in control experiments conducted at noon was 72.4 ± 5.0 s (n = 40) for albino ND/4, 183.6 ± 4.4 s (n = 22) for C57BL/6J and 47.8 ± 3.0 s (n = 63) for C3H/HeN mice. C3H/HeN mice remained immobile in the water for a significantly longer period of time at midnight than at noon (67.7 ± 2.8 s, n = 68, $P < 0.001$ when compared with noon). The time of immobility of both the albino ND/4 and the C57BL/6J mice, during immersion at midnight was identical to that at noon.

TABLE 1

Dose-dependent effect of luzindole on the duration of immobility of the C3H/HeN mouse in the forced swimming test.

Treatment [b]	Duration of immobility (s) [a]	
	Noon	Midnight
Vehicle	43.2 ± 8.3 (8)	61.7 ± 7.3 (8)
Luzindole, 1 mg/kg	34.1 ± 11.7 (8)	47.7 ± 4.7 (8)
Luzindole, 10 mg/kg	28.2 ± 6.9 (8)	16.1 ± 5.2 (7) [c]
Luzindole, 30 mg/kg	44.7 ± 9.4 (7)	13.1 ± 3.6 (8) [c]

[a] The swimming test was conducted as described in Methods. The mice were kept on a 14:10 h light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (noon) and 00:00-02:00 h (midnight). [b] Vehicle or luzindole was administered 1 h before the test. Mean ± S.E.M. values are shown. The numbers in brackets represent the number of mice per group. Statistical differences between treated and control groups were assessed using Dunnett's multiple comparisons procedure. [c] $P < 0.01$ when compared with the corresponding control.

3.2. Effect of the melatonin receptor antagonist, luzindole, on the duration of immobility in the three strains of mice

The competitive melatonin receptor antagonist, luzindole (30 mg/kg, 30 min prior to testing, i.p.), did not affect the duration of immobility during immersion of the albino ND/4 or the C57BL/6J mice either at noon or midnight (fig. 1A,C). In contrast, the C3H/HeN mice, known to synthesize melatonin in the pineal glands following a circadian rhythm with higher levels at night (Hotz et al., 1985; Goto et al., 1989), had a significantly decreased duration of immobility (fig. 1) with luzindole (30 mg/kg, 30 min, i.p.). This effect of luzindole was more pronounced at midnight (60% reduction) than at noon (39% reduction) (fig. 1).

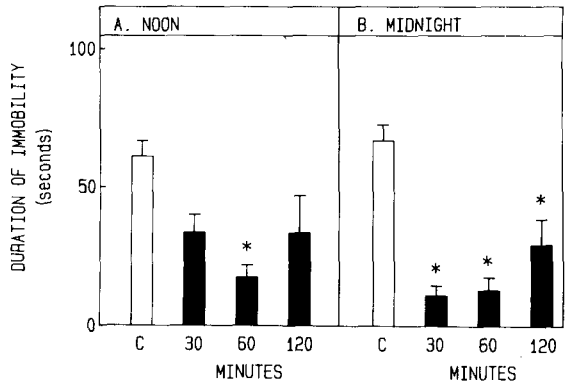

Fig. 2. Time-dependence of luzindole on the duration of immobility of the C3H/HeN mouse at noon and at midnight. The swimming test was conducted as described in Methods. C3H/HeN mice were kept on a 14:10 h light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (NOON) and 24:00 and 02:00 h (MIDNIGHT). Vehicle (C) (□) or luzindole (■, 30 mg/kg i.p.) was injected at 30, 60 and 120 min before the test. The duration of immobility in vehicle- or luzindole-treated mice, at noon or at midnight, was determined in the same experiment in eight animals per group. Mean ± S.E.M. values are shown. Statistical differences between treated and control groups were assessed using Dunnett's multiple comparisons procedure. * $P < 0.001$ when compared with the corresponding control.

The effect of luzindole (30 mg/kg) on the duration of immobility in the C3H/HeN mouse was time-dependent, both at noon and at midnight (fig. 2). Luzindole was effective in reducing immobility after 60 min of administration at noon, and after 30 and 60 min of administration at midnight (fig. 2). In subsequent experiments the effect of different doses of luzindole was studied 60 min after i.p. administration. Table 1 shows the time of immobility of the C3H/HeN mouse following i.p. administration of luzindole, 1, 10 and 30 mg/kg. The 10 mg/kg dose of luzindole elicited a maximal reduction in immobility of 35% at noon and of 74% at midnight. Overall, the effect of luzindole at all doses and conditions tested was significantly more pronounced at midnight (tables 1 and 2).

3.3. Effect of luzindole on the time of immobility of C3H/HeN mice during immersion following exposure to different light/dark regimens

In order to exclude the possibility that the more pronounced effect of luzindole at midnight was related to the darkness, the melatonin receptor antagonist was tested in the dark at noon when the levels of melatonin in the pineal gland of the C3H/HeN mouse are presumably low (Hotz et al., 1985; Goto et al., 1989). Figure 3A shows results of three experiments in which luzindole (10 mg/kg, 60 min, i.p.) reduced immobility (43%) at noon in the dark to the same extent as in the light (39%) (fig. 1). Conversely, exposure to light at midnight did not affect the duration of immobility either in controls or in luzindole (10 mg/kg, 60 min)-treated C3H/HeN mice (fig. 3B). Again, the effect of luzindole in experiments at midnight in the light was more pronounced (62% reduction) than that at noon in the dark (43%) (compare fig.

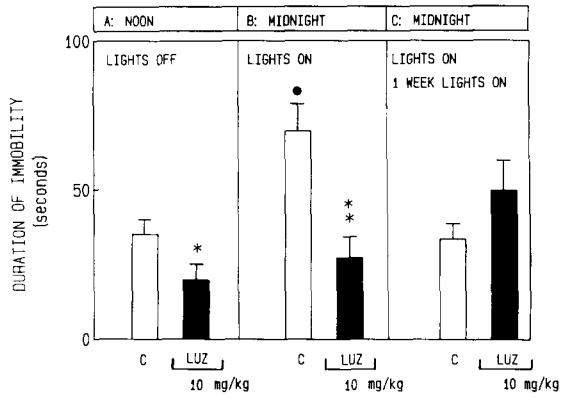

Fig. 3. Effect of luzindole on the duration of immobility of C3H/HeN mice during the forced swimming test performed under different light/dark regimens. The swimming test was conducted in C3H/HeN mice as described in Methods. (A) Noon (lights off): C3H/HeN mice kept on a 14:10 h light:dark cycle were kept in the dark on the day of the experiment. Experiments were conducted in the dark between 12:00 and 14:00 h. (B) MIDNIGHT (lights on): C3H/HeN mice kept on a 14:10 h light:dark cycle were kept with lights on the night of the experiment (lights on after 18:00 h). Experiments were conducted with the lights on beteen 24:00 and 02:00 h. (C) MIDNIGHT (1 week lights on): C3H/HeN mice were kept under constant light during 1 week and experiments were conducted in the light between 24:00 and 02:00 h. Luzindole (LUZ) (■, 10 mg/kg i.p.) was injected 60 min before the test. The duration of immobility of vehicle- or luzindole-treated mice within A, B or C was determined in the same experiment in eight animals per group. Mean ± S.E.M. values pooled from different experiments are shown. * $P < 0.05$; ** $P < 0.01$ when compared with the corresponding control (Student's t-test). • $P < 0.005$ when compared with the control at noon (Student's t-test).

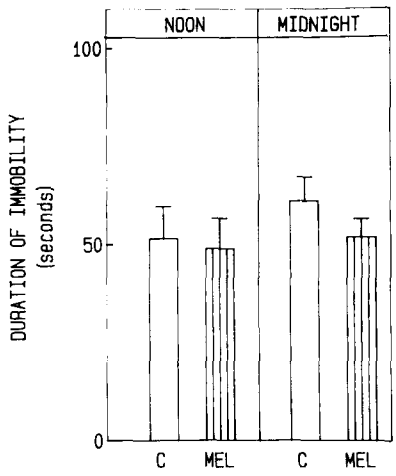

Fig. 4. Effect of melatonin on the duration of immobility of the C3H/HeN mouse. The swimming test was conducted as described in Methods. C3H/HeN mice were kept on a 14:10 h light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (NOON) and 00:00 and 02:00 h (MIDNIGHT). Vehicle (C) (□) or melatonin (MEL) (▥, 30 mg/kg i.p.) was administered 30 min before the test. The duration of immobility in vehicle or melatonin treated mice either at noon or midnight was determined in the same experiment in eight animals per group. Mean ± S.E.M. values are shown.

3A and 3B). Exposure to constant light for one week which presumably inhibits the production of melatonin by the pineal gland (Heydorn et al., 1983) reduced the time of immobility in control C3H/HeN mice to the noon values and prevented the effects of the melatonin receptor antagonist, luzindole (fig. 3C).

3.4. Effect of melatonin on the duration of immobility

The effect of exogenous melatonin in the behavioral despair test in the C3H/HeN mice was tested at noon when the levels of melatonin are presumably low and at midnight when the levels of melatonin are high (Hotz et al., 1985; Goto et al., 1989; Tamarkin et al., 1985; Arendt, 1988). Melatonin (30 mg/kg) administered i.p. 30 min prior to testing did not affect significantly the duration of immobility of the C3H/HeN mouse either at noon or at midnight (fig. 4). Similarly, at noon with the lights off, melatonin (30 mg/kg, 30 min, i.p.) only slightly increase the duration of

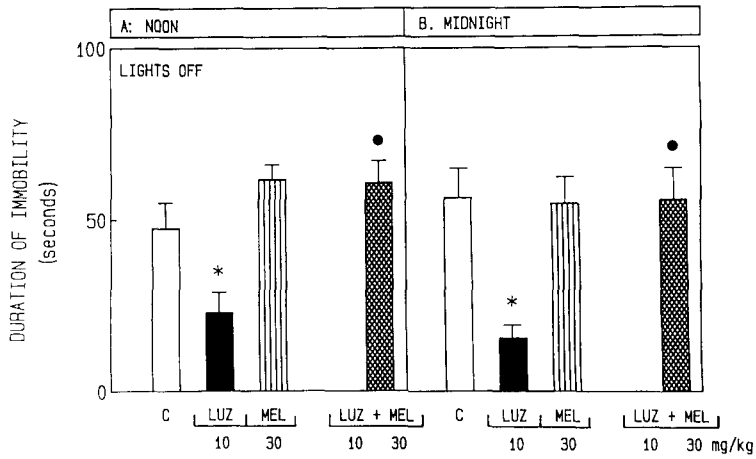

Fig. 5. Melatonin reversed the luzindole-induced decrease in duration of immobility of C3H/HeN mice in the forced swimming test. The swimming test was conducted as described in Methods. NOON (lights off): C3H/HeN mice kept on a 14:01 h light:dark cycle were kept in the dark on the day of the experiment. Experiments were conducted in the dark between 12:00 and 14:00 h. MIDNIGHT: C3H/HeN mice kept on a 14:10 h light:dark cycle were kept in the dark the day of the experiment (after 18:00 h). Experiments were conducted between 00:00 and 02:00 h. Vehicle (C) (□) luzindole (LUZ) (■, 10 mg/kg 60 min), or melatonin (MEL) (▥, 30 mg/kg 30 min) were administered alone or in combination (▩ LUZ plus MEL). The duration of immobility in vehicle- or drug-treated mice either at noon or midnight determined in the same experiment in eight animals per group. The values shown are means ± S.E.M. * $P < 0.05$; $P < 0.01$ when compared with the corresponding control (C). • $P < 0.01$ when compared with luzindole alone (Student's t-test).

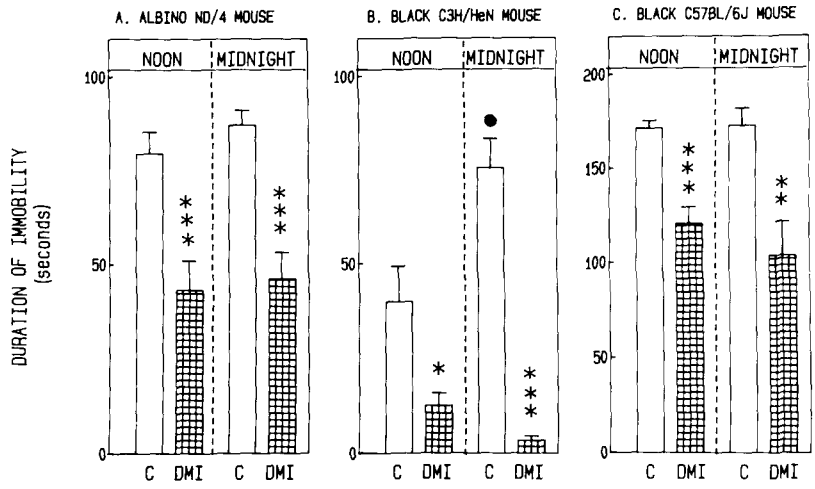

Fig. 6. Effect of desipramine on the duration of immobility in three strains of mice at noon and midnight. The swimming test was conducted as described in Methods. Mice from three different strains (A: albino ND/4; B: C3H/HeN; C: C57BL/6J) were kept on a 14:10 light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (NOON) and 00:00-02:00 h (MIDNIGHT). Vehicle (C) (□) or desipramine (DMI) (■, 30 mg/kg i.p.) was administered 1 h before the test. The duration of immobility in vehicle- or desipramine-treated mice, within each strain was determined in the same experiment in eight animals per group. Mean ± S.E.M. values are shown. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$ when compared with the corresponding control (Student's t-test). • $P < 0.05$ when compared with control at noon (Student's t-test).

immobility (41.3 ± 4.6, n = 24) when compared with the control (34.4 ± 4.4 s, n = 24).

3.5. Exogenous melatonin antagonized the reduction of immobility elicited by luzindole in the C3H / HeN mouse

Figure 5 shows experiments performed in C3H/HeN mice at noon and midnight in the dark, in which the effects of luzindole (10 mg/kg, 60 min) and melatonin (30 mg/kg, 30 min) alone and in combination were tested. Melatonin administered alone did not affect the duration of immobility when compared with the control but antagonized the reduction of immobility elicited by luzindole both at noon and at midnight (fig. 5). Together, these results suggest that luzindole may affect the duration of immobility of C3H/HeN mice in the behavioral despair test by antagonizing the action of melatonin in vivo.

3.6. Effect of the tricyclic antidepressant, desipramine, on the duration of immobility

Desipramine (30 mg/kg i.p.) significantly reduced the time of immobility in the albino ND/4, C3H/HeN and C57BL/6J mice in experiments conducted at noon and at midnight. The strains differed in their response to desipramine as a dose of 30 mg/kg reduced the time of immobility at noon by 45% in the albino ND/4 mouse, by 30% in the C57BL/6J mouse and by 70% in the C3H/HeN mouse (fig. 6). The anti-immobility effect of desipramine was the same at noon and at midnight in the albino ND/4 and C57BL/6J mouse, while it was significantly more pronounced in the C3H/HeN mouse at midnight (fig. 6, table 2).

The dose-dependent effect of desipramine (0.3-30 mg/kg) on the duration of immobility of the C3H/HeN mouse was more pronounced at midnight. The minimal dose of desipramine reducing immobility at noon was 3 mg/kg and at midnight was 0.3 mg/kg (table 2).

3.7. Effect of desipramine and luzindole on the calcium-dependent release of [^{3}H]norepinephrine from C3H / HeN mouse cortical slices

These experiments were undertaken to compare the effect of luzindole and desipramine on the

calcium-dependent release of norepinephrine. The spontaneous and calcium-dependent release of [^{3}H]norepinephrine from C3H/HeN cortical slices labeled with [^{3}H]norepinephrine was measured. The release of [^{3}H]norepinephrine induced by field stimulation at 3 Hz during 2 min (20 mA, 2 ms) was completely calcium-dependent. The percent of total tissue radioactivity released above the spontaneous release ($S_{p1} = 1.46 \pm 0.11\%$, n = 4) during the first period of stimulation (S_1) was $2.06 \pm 0.22\%$ (n = 4), and during the second period of stimulation (S_2), $2.20 \pm 0.26\%$ (n = 4). The ratio of the overflow in the controls between the second (S_2) and first (S_1) periods of stimulation was $S_2/S_1 = 1.07 \pm 0.03$ (n = 4).

The tricyclic antidepressant, desipramine (1 nM-10 μM), added before the second period of field stimulation increased the calcium-dependent release of [^{3}H]norepinephrine in a concentration-dependent manner (fig. 7). The concentration of desipramine increasing the stimulation-evoked release of [^{3}H]norepinephrine by 50% (EC_{50}) was 26.2 nM, and the maximal increase was obtained with 1 μM desipramine (fig. 7). Desipramine, 10 μM, significantly increased the spontaneous outflow of [^{3}H]norepinephrine. Luzindole added in concentrations up to 1 μM before the second period of stimulation did not affect either the spontaneous or stimulated overflow of tritium (fig. 7). Luzindole, 10 μM, slightly increased the calcium-dependent release of radioactivity, without affecting the spontaneous outflow.

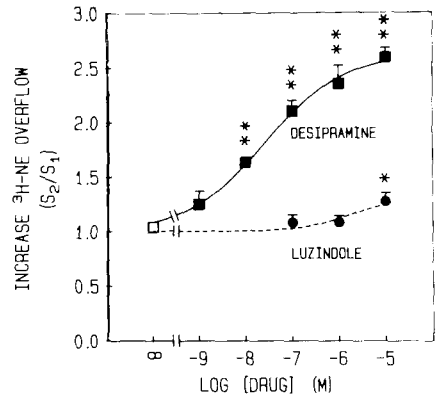

Fig. 7. Effect of desipramine and luzindole on the calcium-dependent release of [^{3}H]norepinephrine from C3H/HeN mouse cortical slices. Cortical slices from C3H/HeN mice were labeled with [^{3}H]norepinephrine and superfused as described in Methods. Ordinate: increase [^{3}H]NE ([^{3}H]norepinephrine) overflow, is the percentage of total tissue radioactivity released by field stimulation (3 Hz, 2 min, 20 mA, 2 ms) above the spontaneous levels of release. The results are expressed as the ratio obtained between the second (S_2) and the first (S_1) stimulation periods within the same experiment. The spontaneous outflow of radioactivity in the controls before the first period of stimulation (S_{p1}) was: $1.45 \pm 0.08\%$ (n = 6) and before the second (S_{p2}) was: $1.34 \pm 0.11\%$ (n = 6). The ratio S_{p2}/S_{p1} was: 0.92 ± 0.03 (n = 6). The radioactivity retained by the control slices at the end of the experiment was: 29.5 ± 2.7 nCi (n = 6). Abcissa: the molar concentration of desipramine or luzindole (logarithmic scale) added 20 min before the second period of stimulation (S_2) (closed symbols). Control, □; desipramine, ■; luzindole, ●. The values shown are means ± S.E.M. of three to seven experiments per group. Statistical differences between treated and control ratios were assessed using Dunnett's multiple comparisons procedure. * $P < 0.05$; ** $P < 0.01$ when compared with the control ratio.

TABLE 2

Dose-dependent effect of desipramine on the duration of immobility of the C3H/HeN mouse in the forced swimming test.

Treatment [b]	Duration of immobility (s) [a]	
	Noon	Midnight
Vehicle	48.5 ± 7.2 (8)	66.4 ± 9 (8)
Desipramine, 0.3 mg/kg	46.2 ± 12.9 (8)	33.2 ± 7.3 (8) [d]
Desipramine, 3 mg/kg	20.7 ± 3.7 (8) [c]	16.1 ± 6.0 (8) [d]
Desipramine, 10 mg/kg	20.7 ± 2.0 (8) [c]	13.2 ± 7.2 (8) [d]
Desipramine, 30 mg/kg	12.5 ± 6.4 (8) [d]	5.2 ± 2.1 (8) [d]

[a] The swimming test was conducted as described in Methods. The mice were kept on a 14:10 h light:dark cycle and the experiments were conducted between 12:00 and 14:00 h (noon) and 00:00-02:00 h (midnight). [b] Vehicle or desipramine was administered i.p., 1 h prior to the test. Mean ± S.E.M. values are shown. The numbers in parentheses represent the number of mice per group. Statistical differences between treated and control group were assessed using Dunnett's multiple comparisons procedure. [c] $P < 0.05$; [d] $P < 0.01$ when compared with the corresponding control.

4. Discussion

We now present evidence suggesting that the endogenous levels of melatonin in the C3H/HeN mouse play a role in responses in the behavioral despair test during forced swimming by prolonging the time of immobility. At midnight when the

levels of melatonin are high, the C3H/HeN mice remained immobile during the test for a longer period of time than at noon. This effect of endogenous melatonin was blocked by the melatonin receptor antagonist, luzindole. Furthermore, exposure of C3H/HeN mice to constant light for one week, which presumably inhibits the production of melatonin by the pineal gland, reduced the time of immobility at midnight to noon values. The longer time of immobility we now observe in the C3H/HeN mouse at midnight may be attributed to the secretion of endogenous melatonin by the pineal gland since this difference in time of immobility was not observed in the C57BL/6J mouse (Hotz et al., 1986; Ebihara et al., 1986; Goto et al., 1989) which does not synthesize melatonin.

The lack of a consistent and significant effect of exogenous melatonin on the duration of immobility during the test in the C3H/HeN mouse in difficult to explain. The short half life (25 min) of melatonin after i.p. administration (Clemens and Flaugh, 1985) and its rapid metabolism to less potent compounds (i.e., N-acetyl-5-methoxykynurenamine and 6-hydroxymelatonin) in brain and liver (Dubocovich, 1985; 1986b; Dubocovich and Takahashi, 1987; Hirata et al., 1974; Kopin et al., 1961), may contribute to the lack of a measurable response to melatonin in the forced swimming test. For these reasons, the use of a melatonin receptor antagonist was considered appropriate to demonstrate the physiological role of endogenous melatonin in the behavioral despair test.

Luzindole is a competitive melatonin receptor antagonist which shows high selectivity for melatonin receptor sites in the mammalian central nervous system (Dubocovich, 1988a,b). The development of luzindole (Dubocovich, 1988b), which blocks melatonin receptors at target sites within the central nervous system and may thus mimic the effects of pinealectomy or suppression of melatonin synthesis, led us to investigate the role of endogenous melatonin in the mouse behavioral despair test. This test is used to screen for drugs with antidepressant-like activity (Porsolt et al., 1977; 1978a). The luzindole-induced reduction in the time of immobility of C3H/HeN mice during immersion correlated with the levels of endogenous melatonin, i.e., immobility was more pronounced at midnight when the levels of endogenous melatonin are high, than at noon (Hotz et al., 1985; Goto et al., 1989). The effect of luzindole at midnight cannot be attributed to the darkness since the C3H/HeN mice in experiments conducted in the dark at noon or with lights at midnight gave results identical to those obtained with the normal photoperiod. The C3H/HeN mouse has been reported to have an unusually high threshold to light for entrainment of circadian rhythms (Ebihara and Tsuji, 1980), which presumably also affects the suppression of melatonin secretion by low intensity light (250-300 lux). Exposure to constant light for one week, which presumably inhibits the production of melatonin by the pineal gland (Heydorn et al., 1983) reduced the time of immobility in control C3H/HeN mice to noon values and prevented the effects of the melatonin receptor antagonist, luzindole. Although, administration of exogenous melatonin to the C3H/HeN mouse only slightly increased the duration of immobility, it impaired the reduction of immobility elicited by luzindole. These data further support the possibility of an interaction of luzindole with a melatonin receptor site in the C3H/HeN mouse brain. Taken together, these results suggest that luzindole may reduce immobility in the black C3H/HeN mouse by antagonizing the action of endogenous melatonin in vivo. The finding that a dose of luzindole (30 mg/kg) three times higher than the dose effective in the C3H/HeN mouse did not modify the time of immobility at noon or at midnight in the C57BL/6J, a species which does not synthesize melatonin in the pineal gland (Ebihara et al., 1986) further supports this proposal. In conclusion, luzindole may reduce the time of immobility in the behavioral despair test by blocking melatonin receptor sites in the central nervous system of the C3H/HeN mouse and thus impairing the effect of endogenous melatonin.

In the C3H/HeN mouse brain, activation of melatonin receptor sites appears to be involved in the regulation of responses in the forced swimming test (present data) and in the modulation of noradrenergic activity (Fang and Dubocovich, in press). Forced swimming has been shown to de-

crease the levels of pineal melatonin and to cause increases in plasma melatonin which presumably leads to activation of melatonin receptor sites in the central nervous system (Troiani et al., 1988). Recently, high affinity melatonin binding sites have been identified and localized in brain sections using 2-[^{125}I]iodomelatonin in various hypothalamic and thalamic regions of rodent brain (Duncan et al., 1988; 1989; Zisapel et al., 1987; Vanecek et al., 1987; Siuciak et al., 1990). In the C3H/HeN mouse specific 2-[^{125}I]iodomelatonin binding sites are found in the suprachiasmatic nucleus (SCN), the paraventricular nucleus (PVN) of the thalamus, and the median eminence/pars tuberalis (ME/PT) region of the brain (Siuciak et al., 1990). Some of these sites may be the target sites for endogenous melatonin. Although, melatonin binding sites are also discretely localized in various areas of the C57BL/6J mouse brain (Siuciak et al., 1990), the lack of melatonin production by its pineal gland (Ebihara et al., 1986; Goto et al., 1989), may have impaired the effect of luzindole on the duration of immobility.

It is unlikely that the decrease in the time of immobility induced by luzindole in the C3H/HeN mouse is mediated by an effect at histamine, muscarinic, serotonin, dopamine receptors or α- and β-adrenoceptors (Porsolt et al., 1977; DeGraaf et al., 1985; Browne, 1979; Wallach and Hedley, 1979; O'Neill and Gertner, 1986) because the same behavioral effects should have been observed in the albino ND/4 or C57BL/6J mouse. Luzindole shows selectivity for melatonin receptor sites since it does not interact with the binding of specific radioligands to α_1- and α_2-adrenoceptors, β_1- and β_2-adrenoceptors, 5HT-1 and 5HT-2, muscarinic, adenosine-1, benzodiazepine and histamine receptors in brain membranes (Krause, D.N. and Dubocovich, M.L., unpublished). A non-specific effect of luzindole on the duration of immobility can be excluded because the reduction in immobility elicited in the C3H/HeN mouse by the melatonin receptor antagonist was more pronounced at midnight. An effect of luzindole on the neuronal uptake of norepinephrine can also be ruled out, since in contrast with desipramine, luzindole did not modify the calcium-dependent release of total tritium from C3H/HeN mouse cortical slices. Norepinephrine uptake inhibitors potentiate the calcium-dependent overflow of total tritium from brain slices labeled with [^{3}H]norepinephrine (Pelayo et al., 1980), in tissues where the neuronal uptake of norepinephrine and subsequent storage in synaptic vesicles is the main mechanism of amine inactivation. These experiments suggest that luzindole did not affect the neuronal uptake of norepinephrine.

We have recently demonstrated that luzindole blocks melatonin receptor sites involved in the inhibition of noradrenergic activity in the hypothalamus of the C3H/HeN mouse that are activated by administration of exogenous 6-chloromelatonin at noon or by the secretion of endogenous melatonin at midnight (Fang and Dubocovich, in press). It is tempting to suggest that luzindole exerts its antidepressant-like activity in the C3H/HeN mouse by blocking the effects of endogenous melatonin and therefore by increasing noradrenergic activity.

The tricyclic antidepressant, desipramine, reduced immobility during immersion in the three strains of mice tested. The effect of desipramine was significantly more pronounced in the C3H/HeN mouse than in the albino ND/4 or C57BL/6J mouse. The mechanism by which desipramine reduced immobility in the forced swimming test cannot be attributed only to the inhibition of norepinephrine uptake, since both typical and atypical antidepressants are effective in this test (Porsolt et al., 1977; 1978a; Willner, 1984). The difference in immobility times of the albino ND/4, C3H/HeN and C57BL/6J mice in response to desipramine in the behavioral despair test can be attributed to inherent differences among strains (Porsolt et al., 1978b), and to the differences in melatonin levels (Ebihara et al., 1986; Hotz et al., 1985). Clinically effective antidepressants, i.e., desipramine, injected into the nucleus accumbens completely inhibits the melatonin-induced behavioral responses in the rat (e.g., decreased locomotor activity and rearing, and increased grooming and sniffing behavior) (Gaffori and Van Ree, 1985). Thus, we can speculate that the higher sensitivity of the C3H/HeN mouse to desipramine at midnight may be due to the antagonism of the endogenous melatonin-in-

duced increase in immobility time. Duncan et al. (1985) demonstrated a rapid down-regulation of β-adrenoceptors in forebrain of rats that were treated with tricyclic antidepressants and were forced to swim. It is likely that the down-regulation of β-adrenoceptors in the pineal gland of C3H/HeN mice treated with desipramine during forced swimming at midnight decreases melatonin synthesis and secretion, which may lead to a reduction in immobility time. These results suggest that the C3H/HeN mouse is a suitable strain of mouse to screen for antidepressant-like activity due to a sensitivity to the tricyclic antidepressant desipramine higher than that of other strains.

In conclusion, luzindole showed antidepressant-like activity in the C3H/HeN mouse behavioral despair test in vivo, possible by antagonizing the effects of endogenous melatonin. Alterations of melatonin levels and rhythmicity in humans have been related to psychiatric and sleep disorders due to depression, sexual maturation, shift work and jet lag (Tamarkin et al., 1985; Lewy and Sack, 1986; Lewy et al., 1987; Arendt et al., 1986; Arendt, 1988). Administration of melatonin at scheduled times appears to be effective in synchronizing circadian rhythms (Redman et al., 1983) and is used to treat sleep disturbances due to jet lag (Arendt et al., 1986; Arendt, 1988). Conversely, exposure to bright light is now being used experimentally to treat chronobiological mood and sleep disorders which are characterized by altered circadian patterns of melatonin secretion (Lewy et al., 1987; Rosenthal et al., 1984; Wehr et al., 1986). Whether or not this therapeutic effect of bright light is related to the suppression of melatonin is still an open question. It is suggested that luzindole, by antagonizing the physiological effects of melatonin at target receptor sites within the central nervous system, may be effective in synchronizing circadian rhythms and in treating chronobiological disorders involving abnormal patterns of melatonin secretion in humans.

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