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# Tyrosine Pretreatment Reverses Hypothermia-Induced Behavioral Depression

T. MICHAEL RAUCH\* AND HARRIS R. LIEBERMAN†

\*U.S. Army Research Institute of Environmental Medicine, Kansas Street, Natick, MA 01760

†Department of Brain and Cognitive Sciences

Massachusetts Institute of Technology, Cambridge, MA 02139

Received 2 October 1989

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AD-A219 086

RAUCH, T. M. AND H. R. LIEBERMAN. *Tyrosine pretreatment reverses hypothermia-induced behavioral depression*. BRAIN RES BULL 24(1) 147-150, 1990. — Cold exposure accelerates the firing frequency of norepinephrine (NE) neurons, enhancing NE release and leading to NE depletion in specific regions of the brain. The accelerated firing activates the enzyme tyrosine-hydroxylase, making it more tyrosine sensitive. The reduction of brain NE is accompanied by a behavioral depression on the open field test. Two experiments were performed on adult male rats. First, it was determined whether systematic lowering of core body temperature produced behavioral depression in the swim test. Second, treatment with the NE precursor tyrosine was employed in an attempt to prevent hypothermia-induced behavioral depression. In Experiment 1, two levels of hypothermia were highly effective in producing behavioral depression in rats forced to swim in a narrow cylinder. In Experiment 2, treatment with tyrosine (400 mg/kg, IP) thirty minutes prior to the hypothermia procedure completely reversed the behavioral depression found in Experiment 1. Tyrosine administration did not significantly influence the rate of deep body cooling during the hypothermia treatment.

Hypothermia    Stress    Tyrosine    Norepinephrine    Catecholamines    Learned helplessness

A cascade of neurochemical and behavioral changes occurs in animals after exposure to an inescapable, acute stress. These changes have been attributed to neurotransmitter-based motor deficits (16) or explained in part by the paradigm of learned helplessness (8,13). The neurochemical alteration reported most consistently following acute stress and produced by a wide variety of stressors has been a reduction of norepinephrine (NE) levels in the hypothalamus and brain stem including the locus coeruleus (9, 10, 15). Behavioral alterations observed in animals subjected to various types of inescapable acute stress, include deficits in exploratory behavior (14), avoidance/escape behavior (1), spontaneous motor activity (15), aggressive behavior (3) and swimming (16). These behavioral abnormalities are correlated with NE depletion in the locus coeruleus (16) and the whole brain (14).

One of the dietary precursors of NE is the aromatic amino acid tyrosine. The effect of increasing brain tyrosine concentrations on brain catecholamine synthesis or release is minimal in normal "nonstressed" animals. However, certain forms of acute stress, such as exposure to an ambient cold environment (4) accelerate the firing frequency of NE neurons, enhancing NE release and eventually leading to NE depletion. The accelerated firing activates the enzyme tyrosine-hydroxylase (7), making it more tyrosine sensitive. It has been reported that tyrosine, administered for several weeks as part of the diet or given acutely, protects animals from some of the behavioral and neurochemical consequences of acute stress (3, 5, 6). One paradigm that has been employed to evaluate the behavioral effects of tyrosine as well as

other amino acids and is useful for distinguishing antidepressants from other psychoactive agents is a swim test (5). This test sometimes is termed the behavioral despair paradigm (11,12).

Behavioral despair, or depression, is induced by forcing a rat to swim in a narrow cylinder of water maintained at 25°C from which there is no escape. After an initial period of vigorous escape-directed behavior the rat adopts a characteristic immobile posture which is readily identifiable. This immobility reflects a state of depression which is selectively attenuated by antidepressant drugs at doses which do not increase spontaneous motor activity in an open field (12). Antidepressants can therefore be distinguished from psychostimulants because they decrease immobility at doses that do not increase general activity. Tyrosine appears to have a profile more similar to that of psychostimulants than antidepressants (5).

Previous studies of behavioral despair using the forced swim procedure have failed to differentiate hypothermic effects from those associated with the swim procedure itself (4, 11, 12). The reduction of brain NE that occurs after 30 min of forcing rats to swim in cold water and the subsequent behavioral depression on the open field test are clearly associated with the substantial hypothermia produced by the procedure (14). Therefore, hypothermia, swim stress and exercise have always been confounded when the forced swimming procedure is employed. The purpose of the present study is two-fold. The first experiment is designed to determine if hypothermia, without the confounding effects of swim stress and exercise, produces behavioral despair in rats. The

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second experiment is designed to determine if treatment with the NE precursor tyrosine prevents hypothermia-induced behavioral despair.

## EXPERIMENT 1

### METHOD

#### Subjects

Twelve male Charles River strain (Sprague-Dawley) rats ranging in age from 100 to 115 days, and a mean weight of 300 g (SD = 7.23) were used in this experiment. All rats were housed individually in hanging wire mesh cages and exposed to a 12-hr light-dark schedule with ad lib access to food and water. Behavioral testing was conducted between 0900 and 1500 hr.

#### Hypothermia Treatment

Hypothermia treatment consisted of restraining the rat in an acrylic rat restrainer and immersion up to the neck in a 17°C water bath (Lauda, RC 20). Core body temperature ( $T_c$ ) was monitored with a Yellow Springs series 400 temperature probe, inserted 2.5 cm in the rectum, and a Beckman 450 Digital Thermometer. On treatment days the animals were restrained and immersed in the water bath (17°C) for 25–30 min until a  $T_c$  of 30 or 33°C was obtained. The control treatment consisted of restraint and immersion in the water bath at 36°C for 30 min which allowed the animal to stabilize at a  $T_c$  of 37°C.  $T_c$  was recorded every two minutes for all treatment conditions until the criterion  $T_c$  was achieved. Two control days, during which each animal remained in its home cage with food and water ad lib, were inserted between treatment days. The treatment order was counterbalanced according to a Latin-square design. Every animal received every treatment once and each animal received only one treatment per test day.

#### Behavioral Measurements

Each animal was restrained and  $T_c$  lowered to 30°C, 33°C or stabilized at 37°C. Behavioral despair was then immediately assessed by placing the rat in a Plexiglas cylinder (height 40 cm, diameter 25 cm) containing 30 cm water at 17°C—as described by Porsolt *et al.* (11,12). All rats swam vigorously, attempting to escape for approximately 60 sec, after which they maintained a characteristic immobile posture with occasional episodes of activity. A rat was judged to be immobile when it ceased to struggle and was floating motionless as assessed by a trained rater. The total immobility time was recorded over a three minute period. The rater remained blind to the treatment.

### RESULTS

Immobility in the swim test, over a three minute trial, was analyzed using a repeated measures analysis of variance. The single factor in the analysis was  $T_c$  (3 levels: 30, 33 and 37°C  $T_c$ ). Lowering  $T_c$  significantly increased immobility in the swim test,  $F(2,16) = 17.59$ ,  $p < 0.001$ . Post hoc Tukey ( $p < 0.05$ ; two-tailed) comparisons showed that immobility times for rats cooled to a  $T_c$  of 30 or 33°C were significantly greater than the normothermic control. There was no significant difference in immobility, however, between the two levels of hypothermia. Figure 1 shows the effect of hypothermia on immobility in the swim test.

## EXPERIMENT 2

The results of the first experiment clearly demonstrate that

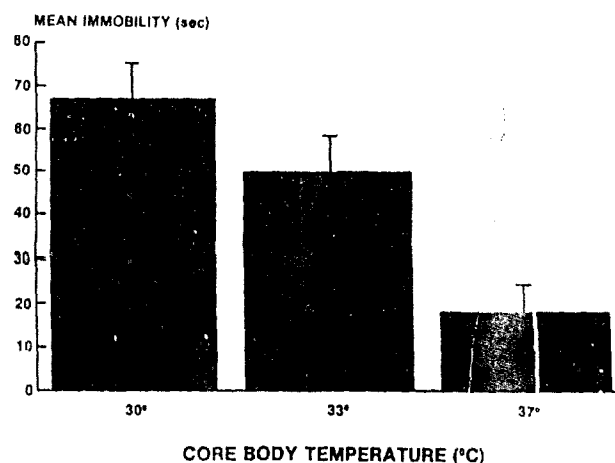


FIG. 1. The effect of two levels of hypothermia on behavior in the swim test. Results are expressed as mean  $\pm$  SEM.

hypothermia is an effective means of inducing behavioral despair in the rat. These results are consistent with a previous report that behavioral inactivity, reduction of brain NE, and increased retention of  $H^3$ -NE occur after 30 minutes of forcing rats to swim in cold water, and are associated with the severe hypothermia produced by this procedure (14). In the same study rapid rewarming of hypothermic rats resulted in a rapid return to control levels of exploratory behavior and reversed both the reduction of brain NE and the increased retention of  $H^3$ -NE. In the following study, we determined if treatment with the NE precursor tyrosine would prevent hypothermia-induced behavioral despair.

### METHOD

#### Subjects

Twelve male Charles River strain (Sprague-Dawley) rats used in Experiment 1 were used in Experiment 2. There was a two week rest period between experiments. All behavioral testing was conducted between 0900 and 1500 hr. All rats were continued on a diet which contained 0.83 percent tyrosine (Pro Lab RMH 3000, Agway Inc., Syracuse, NY).

#### Tyrosine Treatment

Saline or L-tyrosine (400 mg/kg) dissolved in saline were administered by intraperitoneal (IP) injection in a volume of 10 ml/kg, thirty minutes prior to the hypothermia treatment. This dose was the lowest dose of tyrosine shown to be effective at decreasing immobility in rats tested under normothermic conditions (4).

#### Hypothermia Treatment

The hypothermia procedure was identical to that described in Experiment 1 for the 30°  $T_c$  condition. All animals were injected with saline or L-tyrosine and returned to their home cage for thirty minutes. Then each animal was restrained and immersed in the water bath (17°C) for 25–30 min until a  $T_c$  of 30°C was obtained.  $T_c$  was recorded every two minutes for saline and tyrosine conditions until the criterion  $T_c$  was achieved. Two control days, during which each animal remained in their home cage with food and water ad lib was inserted between treatment days. The treatment order was counterbalanced in a cross-over design. Every

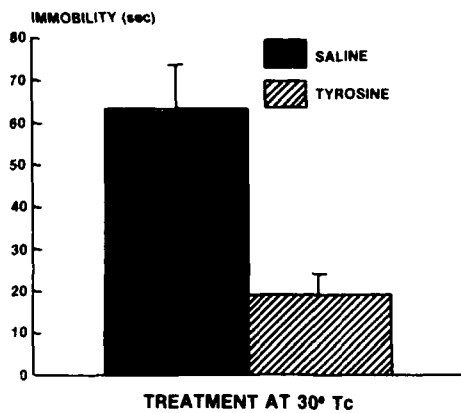


FIG. 2. The effect of tyrosine administration 30 minutes prior to hypothermia treatment on behavior in the swim test. Results are expressed as mean  $\pm$  SEM.

animal received every treatment once and each animal received only one treatment per test day.

#### Behavioral Measurements

After lowering  $T_c$  to 30°C each animal was immediately assessed for behavioral despair as in Experiment 1 (11,12). As in Experiment 1, the total immobility time was recorded over a three minute period. The rater was blind to the experimental treatment.

#### RESULTS

Tyrosine significantly decreased immobility in the swim test when compared to the saline control,  $F(1,8) = 50.37$ ,  $p < 0.001$ . Figure 2 shows the effect of tyrosine on immobility in the swim test. A post hoc Scheffe ( $p < 0.05$ ) test was performed on all possible combinations of treatment means from Experiments 1 and 2. The Scheffe test was necessary to allow comparisons across all possible combinations between Experiments 1 and 2. The results indicate that there was no significant difference in immobility between the normothermic control treatment ( $T_c = 37^\circ\text{C}$ ) (mean  $\pm$  SEM,  $18.16 \pm 2.57$ ) and the hypothermic condition ( $T_c = 30^\circ\text{C}$ ) when pretreatment with tyrosine was administered ( $18.62 \pm 3.03$ ). Furthermore, both the normothermic control and tyrosine pretreatment means significantly differed from both levels of hypothermia ( $T_c = 30$  and  $33^\circ\text{C}$ ) and the hypothermic condition ( $T_c = 30^\circ\text{C}$ ) pretreated with saline (see Table 1). Finally, there was no significant difference in immobility among the two levels of hypothermia ( $T_c = 30$  and  $33^\circ\text{C}$ ) and the hypothermic condition ( $T_c = 30^\circ\text{C}$ ) pretreated with saline. Tyrosine did not significantly influence the rate of deep body cooling to reach the criterion  $T_c$  during immersion.

#### GENERAL DISCUSSION

The results of the present study are best interpreted within the context of several well-documented findings. First, depletion in brain NE results from exposure to cold stress (4,14). Second, acute administration of L-tyrosine, the precursor to NE, prevents both the behavioral inactivity (as measured in an open field testing paradigm) and the regional NE depletion that follows exposure to an acute, uncontrollable stressor (tail shock) (6). Clearly, our findings indicate that hypothermia is an effective method for inducing behavioral depression in rats. We suggest, based upon previous research (4,14), that lowering  $T_c$  will result in depletion of brain NE. Our findings also show that the behavioral depression

TABLE 1  
EFFECTS OF HYPOTHERMIA ON THE TOTAL DURATION OF IMMOBILITY OVER A 3 MIN TRIAL.

Treatment (Experiment 1)	Duration of Immobility (sec) Mean $\pm$ SEM
Normothermia $T_c = 37^\circ\text{C}$	$18.16^* \pm 2.57$
Hypothermia $T_c = 33^\circ\text{C}$	$49.79^+ \pm 6.21$
Hypothermia $T_c = 30^\circ\text{C}$	$66.78^+ \pm 7.25$
Treatment (Experiment 2)	
Tyrosine + Hypothermia $T_c = 30^\circ\text{C}$	$18.89^* \pm 3.03$
Saline + Hypothermia $T_c = 30^\circ\text{C}$	$63.38^+ \pm 6.21$

Any two means that do have a common symbol do not differ significantly from each other at  $p < 0.05$ .

Pretreatment with tyrosine reverses hypothermia-induced behavioral depression.

induced by hypothermia is reversed by pretreatment with L-tyrosine—presumably as the consequence of increased synthesis of NE in key brain regions such as the locus coeruleus. Taken together, the data demonstrate a number of important points. First, the behavioral depression induced by forced swimming in 21–25°C water, in a restricted space with no escape (10,11), is amplified as a result of lowering  $T_c$ . It has previously been observed that the behavioral inactivity and the reduction of brain NE that occur after 30 minutes of swimming rats in 14.5°C water are associated with hypothermia (14) and that rapid rewarming of swim-stressed rats produces a return to control levels of exploratory behavior and reversal of the reduction of brain NE. Therefore, the behavioral depression induced by forced swimming in cold to ambient temperature water is significantly influenced by the hypothermic response to the procedure.

This study has also established that pretreatment with tyrosine, the precursor to NE, reverses the behavioral depression produced by hypothermia, possibly by preventing depletion of brain NE. The behavioral effect of pretreatment with tyrosine observed is similar to that reported by Gibson *et al.* (5) in mice swim-stressed in 23°C water without any pretest cooling. However, the magnitude of the effect we observed was substantially greater. The difference may be attributed to the profound hypothermia we induced and presumably therefore, increased depletion of NE. In humans treatment with tyrosine protects against the adverse behavioral effects of acute exposure to the combination of cold and hypoxia (2). The catecholaminergic mechanisms apparently responsible for the effects in rodents may also account for the comparable effects reported in humans.

#### ACKNOWLEDGEMENTS

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other official documentation.

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