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PRE-TREATMENT WITH TYROSINE REVERSES HYPOTHERMIA
INDUCED BEHAVIORAL DEPRESSION

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ABSTRACT

RAUCH, T.M. AND H.R. LIEBERMAN. Pre-treatment with tyrosine reverses hypothermia induced behavioral depression. BRAIN RES BULL. Cold exposure accelerates the firing frequency of norepinephrine (NA) neurons, enhancing NA release and leading to NA depletion in specific regions of the brain. The accelerated firing activates the enzyme tyrosine-hydroxylase, making it more tyrosine sensitive. The reduction of brain NA 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 NA 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 containing water. 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.

Key words: hypothermia, stress, tyrosine, norepinephrine, catecholamines, learned helplessness.

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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 noradrenaline (NA) levels in the hypothalamus and brain stem including the locus coeruleus (9,15,10). 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 NA depletion in the locus coeruleus (16) and the whole brain (14).

One of the dietary precursors of NA is the aromatic amino acid tyrosine. The effect of increasing brain tyrosine concentrations on brain catecholamine synthesis or release is minimal in normal 'non-stressed' animals. However, certain forms of acute stress, such as exposure to an ambient cold environment (4) accelerate the firing frequency of NA neurons, enhancing NA release and eventually leading to NA 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 with tyrosine or antidepressants have often confounded a hypothermic response with the forced-swim procedure (4,11,12). However, the reduction of brain NA that occurs after 30 min of swimming rats in cold water and the subsequent behavioral depression on the open field test are associated with the substantial hypothermia produced by the procedure (14). Therefore, hypothermia, swim stress and exercise have always been confounded with the forced swimming procedure. 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 second experiment is designed to

determine if treatment with the NA precursor tyrosine prevents hypothermic-induced behavioral despair.

Experiment 1

Materials and Methods:

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

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). T_e 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_e of 30° C 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_e of 37° C. T_e was recorded every two minutes for all treatment conditions until the criterion T_e was achieved. Two control days, during which each animal remained in their 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_e lowered to 30° C, 33° C or stabilized at 37° C. Behavioral despair was then immediately assessed by placing the rat in a Plexiglass cylinder (height 40 cm, diameter 25 cm) containing 30 cm water at 17° C. - as described by Porrolt et al. (11,12). All rats swam vigorously, attempting to escape for approximately 60 secs, 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_e (3 levels: 30, 33° and 37° C T_e). Lowering T_e significantly increased immobility in the swim test, $F(2,16) = 17.59$, $p < .001$. Post hoc Tukey ($p < .05$; two-tailed) comparisons showed that immobility times for rats cooled to a T_e 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 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 NA, and increased retention of H^3 -NA occur after 30 minutes of swimming rats in cold water, 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 NA and the increased retention of H^3 -NA. In the following study, we determined if treatment with the NA precursor tyrosine would prevent hypothermia induced behavioral despair.

Materials and Methods:

Subjects. Twelve male Charles River strain 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 hours.

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 $30^\circ T_e$ 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 ($17^\circ C$) for 25 - 30 min until a T_e of $30^\circ C$ was obtained. T_e was recorded every two minutes for saline and tyrosine conditions until the criterion T_e 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 animal received every treatment once and each animal received only one treatment per test day.

Behavioral Measurements. After lowering T_e 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, $F(1,8) = 50.37$, $p < .001$. Figure 2 shows the effect of tyrosine on immobility in the swim test. A post hoc Scheffe ($p < .05$) 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 ($T_e = 37^\circ\text{C}$) (mean $\pm$ SEM, 18.16 ± 2.57) and the hypothermic condition ($T_e = 30^\circ\text{C}$) when pre-treatment with tyrosine was administered (18.62 ± 3.03). Furthermore, both the normothermic control and tyrosine pre-treatment means significantly differed from both levels of hypothermia ($T_e = 30^\circ$ and 33°C) and the hypothermic condition ($T_e = 30^\circ\text{C}$) pre-treated with saline (see Table 1). Finally, there was no significant difference in immobility among the two levels of hypothermia ($T_e = 30^\circ$ and 33°C) and the hypothermic condition ($T_e = 30^\circ\text{C}$) pre-treated with saline. Tyrosine did not significantly influence the rate of deep body cooling to reach the criterion T_e during immersion.

Discussion

The results of the present study are best interpreted within the context of several well-documented findings. First, depletion in brain NA results from exposure to cold stress (4,14). Second, acute administration of l-tyrosine, the precursor to NA, prevents both the behavioral inactivity (as measured in an open field testing paradigm) and the regional NA 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_e will result in depletion of brain NA. Our findings also show that the behavioral depression induced by hypothermia is reversed by pre-treatment with l-tyrosine - presumably as the consequence of increased synthesis of NA 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° to 25° C water, in a restricted space with no escape (10,11), is amplified as a result of lowering T_e . It has previously been observed that the behavioral inactivity and the reduction of brain NA 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 NA. 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 pre-treatment with tyrosine, the precursor to NA, reverses the behavioral depression produced by hypothermia, possibly by preventing depletion of brain NA. The behavioral effect of pre-treatment 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 NA. In humans treatment with tyrosine protects against the adverse behavioral effects of acute exposure to the combination of cold and hypoxia perhaps acting via the same catecholaminergic mechanisms responsible for the effects in rodents (2).

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Table 1

Effects of hypothermia on the total duration of immobility over a 3 min trial. Pre-treatment with tyrosine reverses hypothermia induced behavioral depression.

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

* Any two means that do have a common subscript do not differ significantly from each other at $p < .05$.

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

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



