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Separation-induced body weight loss, impairment in alternation behavior, and autonomic tone: effects of tyrosine

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Abstract

We have investigated the effects of tyrosine on alternation behavior and hippocampal adrenergic and cholinergic tone in a model of self-induced weight loss caused by separation stress. Separation decreased body weight in mice ($P<.001$) and spontaneous alternations in the T-maze ($P<.001$). This impairment was associated with depletion of both norepinephrine (NE, $P<.001$) and dopamine ($P<.01$) while increasing MHPG ($P<.05$) and the ratio of MHPG/NE ($P<.05$). Increasing tyrosine availability restored performance to control levels ($P<.001$) and repleted dopamine ($P<.05$) and presumably also NE (indicated by increases in both MHPG, $P<.001$, and MHPG/NE, $P<.05$). Stress increased adrenergic α_2-receptor density ($P<.001$) without changing its K_d and the B_{max} and K_d of β-receptors, suggesting that it decreased NE transmission through action on α_2-receptors. The balance between β- and α_2-receptors appeared to be related to alternation behavior as shown by the decrease ($P<.01$) and increase ($P<.05$) in their ratios induced by stress and tyrosine, respectively. With regard to cholinergic tone, separation stress increased M1 receptor density ($P<.05$) and its mRNA signal ($P<.001$). Tyrosine further increased M1 receptor density of stressed mice ($P<.05$). Tyrosine might be a potential therapy for cognitive and mood problems associated with the maintenance of a reduced body weight in the treatment of obesity and in the extreme case of anorexia nervosa.

Keywords: Separation stress; Weight loss; T-maze; Tyrosine; Hippocampus; Adrenergic and cholinergic receptors

1. Introduction

Chronic voluntary and involuntary weight loss may lead to changes in autonomic tone (Lansberg and Young, 1978) and brain neurotransmitters (Avraham et al., 1996; Hao et al., 2000; Kaye et al., 1984; Schweiger et al., 1985). These may lead to reciprocal endocrine and neurobehavioral changes as found in eating disorders (anorexia nervosa) and the so-called reduced obese syndrome (Berry, 1999). In order to try and understand the underlying pathophysiology of these conditions, we have studied an animal model of self-induced weight loss based on chronic separation stress (Van Leeuwen et al., 1997) and also the effects of diet restriction (DR) (Avraham et al., 1996). In the former, mice are placed in cages with Perspex partitions such that they can see but not touch one another. This leads to anorexia and weight loss (Van Leeuwen et al., 1997). We believe that this paradigm may represent some of the chronic stress associated with human eating disorders and have now studied its effects on alternation behavior and autonomic tone.

Stress-induced behavioral deficits might related to norepinephrine (NE) depletion, and this has been reported in the brain area of hippocampus (Hellriegel and D'Mello, 1997; Lehnert et al., 1984; Nakagawa et al., 1981; Reinstein et al., 1984; Swenson and Vogel, 1983). Tyrosine, the precursor for catecholamine synthesis, alleviated mood and cognitive dysfunction under stress situations in both animals (Avraham et al., 1996; Lehnert et al., 1984; Rauch and Lieberman, 1990; Reinstein et al., 1984; Shurtleff et al., 1993) and man (Banderet and Lieberman, 1989; Beijen and Orlebeke, 1994; Owasoyo et al., 1992; Shurtleff et al., 1994; Thomas et al., 1999). However, little has been reported on its effects on adrenergic and cholinergic receptors in such stress situations. Stress has been reported to affect high

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affinity choline uptake and muscarinic receptor density (Gilad et al., 1983).

The hippocampus is a brain area innervated by both noradrenergic nerve terminals projecting from cell bodies in the locus coeruleus (Pickel et al., 1974) and a rich cholinergic innervation both from nuclei in the septal–diagonal band area and intrinsic cholinergic neurons (Lewis et al., 1967; Wainer et al., 1985). Alternation behavior in the T-maze is considered to be a test of hippocampal function (Isseroff, 1979; Maier and Isaacson, 1994). The functions of catecholaminergic (Low et al., 1984; Molino et al., 1989) and cholinergic (Hatcher et al., 1998) pathways might relate to alternation behavior.

We now report the effects of separation stress and weight loss on sympathetic and parasympathetic tone in the hippocampus and on a test of alternation behavior. We report the beneficial effects of tyrosine on this model of stress.

2. Materials and methods

In all experiments, the principles of laboratory animal care were followed, and the protocols were authorized by the Institutional Review Committee of the local animal care facility board.

2.1. Animal model of separation

As described previously (Van Leeuwen et al., 1997), 2-month female Sabra mice were kept in a temperature-controlled room at 22°C on a 12-h light/dark cycle (light on at 7:00 a.m.). Mice in the separation group were housed in a cage fitted with six individual Plexiglas partitions of size $11 \times 10 \times 12$ cm. They could smell each other and see their neighbors without physical contact except when transferred to regular cages for the 2-h feeding schedule. Control groups were housed 6 to the same cage without partitions. Water was provided ad libitum. Experiments lasted 2 weeks and weights of the mice were monitored between 9:00 and 10:00 a.m. before food intake.

2.2. Spontaneous alternation

Spontaneous alternation is a 'two-trial' phenomena in which an animal is said to alternate if its choice on the second trial of testing is opposite from that of the first trial. The method was based on that described by Henderson (1970) with modifications (Zahalka et al., 1995). The apparatus consisted of a T-shaped maze made of opaque Plexiglas and a transparent cover. The width and the height of the T-maze were 4×4 cm. Mice were randomized into each treatment group. From the second week, saline and L-tyrosine (100 mg/kg, dissolved in saline with pH 7.0) were administered intraperitoneally between 9:00 and 10:00 a.m. each day for 1 week. Maze testing was carried out on the second week between 9:00 and 12:00 a.m. before food intake, and saline and tyrosine were injected 1 h before the test (Wurtman et al., 1974). The mouse was placed in the entrance arm and the dividing door was open. Immediately after the mouse entered one of the horizontal arms, it was returned to the entrance arm for a 20-s delay before the next trial. Each mouse was allowed a maximum of four alternations (five entrances) (Zahalka et al., 1995).

2.3. Measurement of catecholamines

Mice were sacrificed by decapitation between 9:00 and 12:00 a.m. 1 h after saline and tyrosine treatment (Avraham et al., 1996). The hippocampus was immediately dissected out and kept at -70°C for all the measurements. Assays for NE, MHPG, and dopamine were performed by HPLC/ECD (high-performance liquid chromatography/electrochemical detector) using the same procedure reported previously (Avraham et al., 1996; Hao et al., 2000). Protein was determined using a commercial protein assay kit, based on the method of Bradford (Sigma).

2.4. Adrenergic receptors

Tissue preparation and the procedure of binding to adrenergic α_2- and β-receptors were similar as we reported previously (Avraham et al., 1996). Binding to the α_2-adrenergic receptors was studied using phenyl-4-[^{3}H]clonidine hydrochloride (Amersham, specific activity = 22 Ci/mmol) and nonspecific binding was measured by using phentolamine. β-Adrenergic receptors were evaluated using the antagonist [^{3}H]dihydro-alprenolol (Amersham, specific activity = 59 Ci/mmol) and nonspecific binding was measured by using alprenolol. The saturation binding data were plotted by Scatchard analysis, and the maximal number of binding sites (B_{max}) and the dissociation constant (K_d) were calculated.

2.5. Measurement of the binding to hemicholinium-3 (Hc-3), muscarinic M1 receptor and the activities of choline acetyltransferase (ChAT), and acetylcholinesterase (AChE)

2.5.1. Binding to the high affinity choline transporter Hc-3 and M1

Scatchard analyses for Hc-3 and M1 were performed with six ligand concentrations. [^{3}H]Hc-3 binding was determined as described by Vickroy et al. (1984) with modifications. Specific binding were performed by using [^{3}H]Hc-3 (specific activity = 124.4–144.4 Ci/mmol) ranging from 0.25 to 8.0 nM, and nonspecific binding was defined in the presence of an excess concentration (10 mM) of unlabeled Hc-3. Specific binding of [^{3}H]pirenzepine (specific activity = 81.3 Ci/mmol) to muscarinic M1 receptors was carried out in 100-μl aliquots of the homogenates essentially as described by Watson et al. (1982), and nonspecific binding was determined with the addition of 1 mM Atropine sulfate. The reaction mixtures of Hc-3 and M1 were stopped

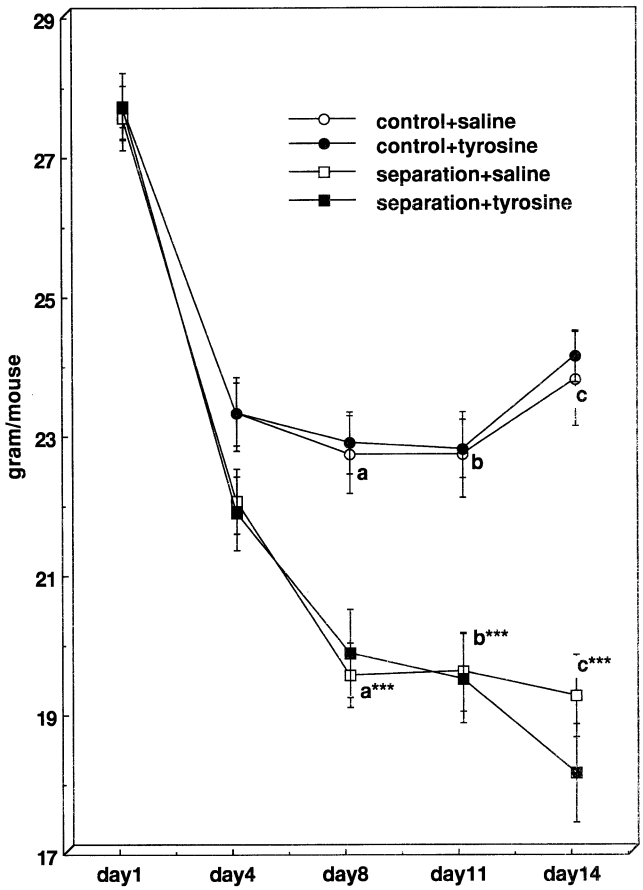

Fig. 1. Effects of separation and tyrosine on weight (mean ± S.E.), *** $P < .001$, letters represent pairs that are statistically different, 16–18 mice in each group.

by 5 ml of ice cold buffer after 20- and 60-min incubation, respectively. Labeled membranes were then trapped by rapid vacuum filtration onto Whatman GFC filters presoaked for 30 min with polyethyleneimine in buffer.

2.5.2. ChAT activity

Assays were conducted essentially as described by Lau et al. (1987), using 30 μl of diluted homogenate in a total volume of 60 μl containing final concentration of 0.4 mM [^{3}H]acetyl-coenzyme A. Samples were preincubated for 15 min on ice and then transferred to a 37°C water bath for 30 min, then terminated by placing samples on ice, and 0.3 ml of sodium tetraphenylboron (50 mg/ml in 3-heptanone) was added. The samples were mixed vigorously and 250 μl of organic phase was withdrawn and treated with 170 μl of 50 mM sodium phosphate (pH 7.3). A 200-μl aliquot of the organic phase was then transferred to scintillation fluid and counted.

2.5.3. AChE activity

Tissue (1% w/v) was homogenized in ice-cold 40 mM phosphate buffer (pH 7.4) containing 0.5% w/v Triton X-100. Enzyme activity was measured in the supernatant after centrifugation at 14,000 rpm by a modification of the method of Ellman et al. (1961) using acetylthiocholine as substrate. Each sample was incubated with the substrate alone and plus 1.5×10^{-6} M BW 284C51 dibromide (1.5-bis-/4-allyldimethyl-ammoniumphenyl/pentan-3-one dibromide) — the inhibitor of AChE. AChE activity was calculated from the difference in absorbance between the two tubes. Activity was expressed as micromoles of acetylthiocholine hydrolyzed per milligram protein per hour.

2.6. M1 receptor messenger RNA by Northern blotting

The procedure was based on that of Pinkas-Kramarski et al. (1989) with modifications. Total RNA was isolated using EZ-RNA kit (Sigma), fractionated on a 1% agarose gel (25 μg/lane) in the presence of formaldehyde, and then transferred to Nytran 0.45 membrane (Tamar). Membrane was subsequently hybridized to the probe of muscarinic M1 receptor (kindly supplied by Pro. M. Sokolovsky, Tel Aviv University). The level of M1 receptor mRNA signal was quantified by comparing to the amount of glyceraldehyde-3-phosphate (GAP) signal.

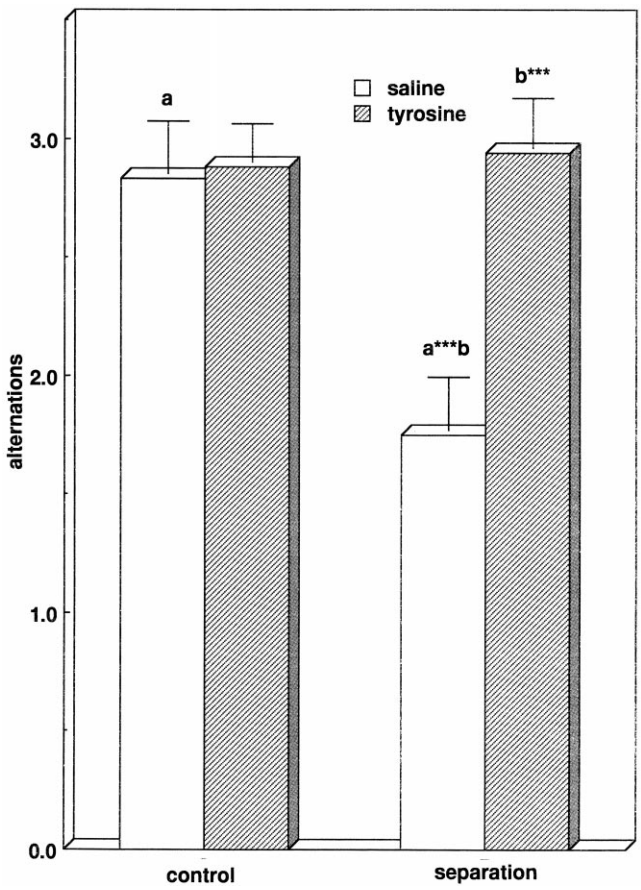

Fig. 2. Effects of separation and tyrosine on the alternations of mice in the T-maze (mean ± S.E.), *** $P < .001$, letters represent pairs that are statistically different, 16–18 mice in each group.

3. Statistical analysis

Data were evaluated first by analysis of variance with multiple levels. Homogeneity of variances of the different groups was then assessed by Bartlett's test. Post-hoc testing was only performed if the overall P value was less than .05 and was carried out using the Tukey–Kramer multiple comparisons procedure. Nonparametric Mann–Whitney U test was used to compare the difference between two groups when appropriate and significant level was based on $P \leq .05$ (two-tailed).

4. Results

4.1. Weight change

Separation stress significantly decreased the weight of the mice (Fig. 1, $P < .001$). Tyrosine did not affect the weights of either the control and separation groups.

4.2. Alternation behavior

Separation stress reduced the ability of mice to spontaneously alternate in the T-maze when compared to control groups (Fig. 2, $P < .001$a) while tyrosine normalized it ($P < .001$b).

4.3. Catecholamines

Separation stress decreased the level of NE (Fig. 3a, $P < .001$a) while it increased both MHPG (Fig. 3b, $P < .05$b) and the MHPG/NE ratio (Fig. 3c, $P < .05$d). It also decreased the concentration of dopamine (Fig. 3d, $P < .01$f). Tyrosine administration to stressed mice increased the levels of MHPG (Fig. 3b, $P < .001$c), MHPG/NE (Fig. 3c, $P < .05$e), and dopamine (Fig. 3d, $P < .05$g).

4.4. Adrenergic α_2- and β-receptors

Separation increased the B_{max} (Table 1, $P < .001$a) of α_2-receptors, while tyrosine administration normalized it to the

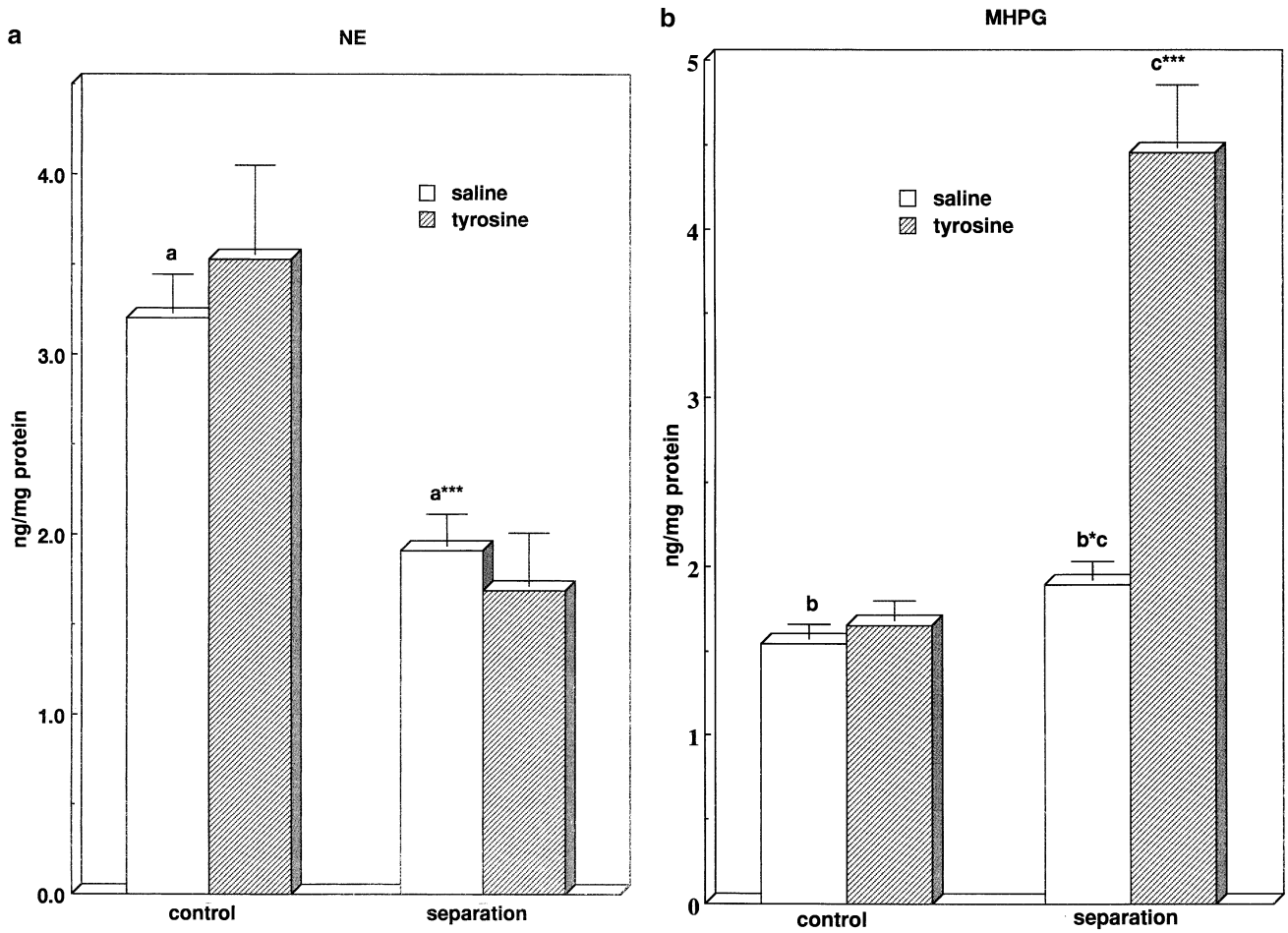

Fig. 3. * $P < .05$, ** $P < .01$, *** $P < .001$, letters represent values that are statistically different. (a) Effects of separation and tyrosine on the levels of NE (mean ± S.E.); 6–7 mice in each group. (b) Effects of separation and tyrosine on the levels of MHPG (mean ± S.E.); 6–7 mice in each group. (c) Effects of separation and tyrosine on the levels of MHPG/NE (mean ± S.E.); 5–7 mice in each group. (d) Effects of separation and tyrosine on the levels of dopamine (mean ± S.E.); 5–6 mice in each group.

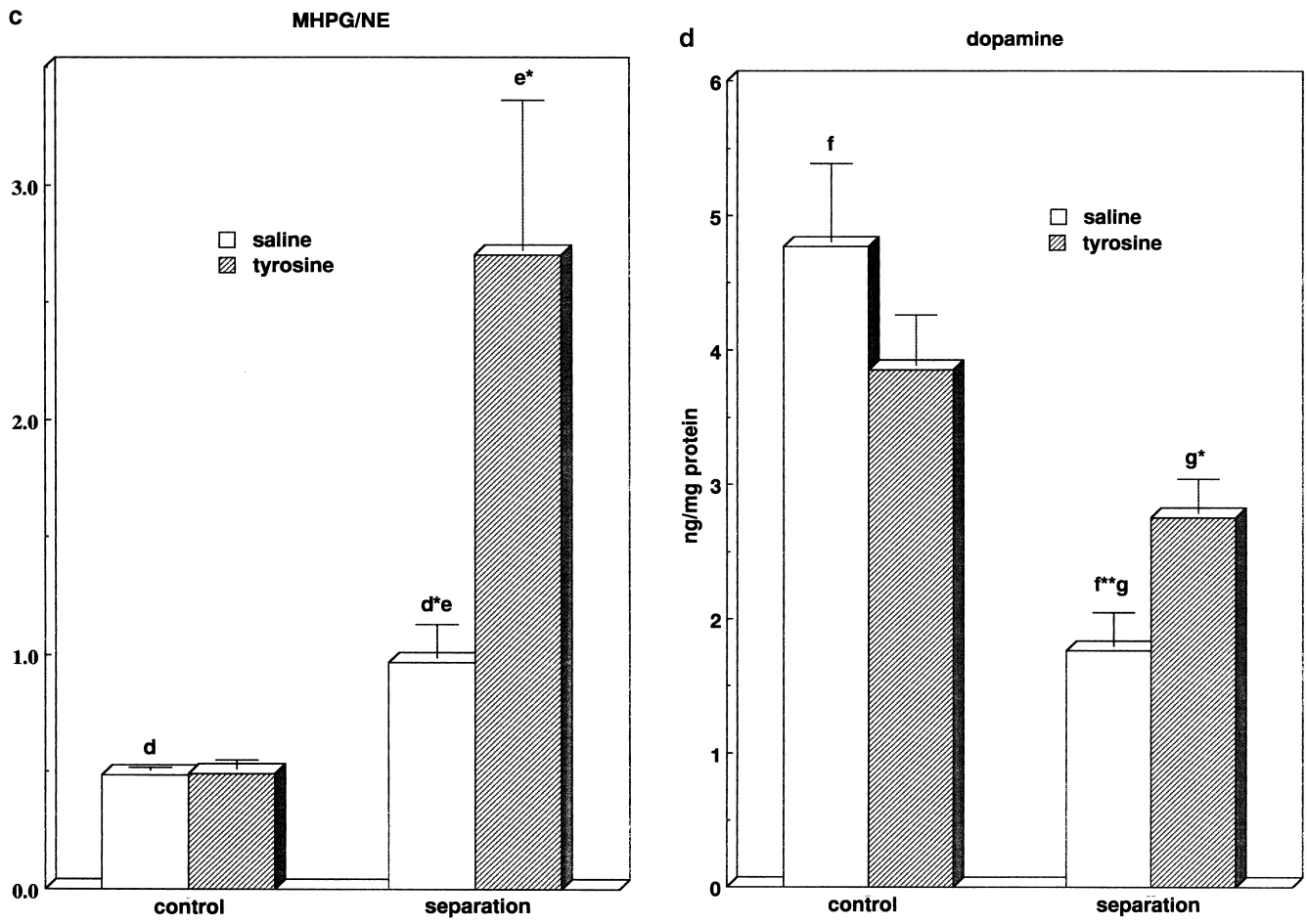

Fig. 3 (*continued*).

control levels ($P<.01$b). Tyrosine decreased β-adrenergic receptors in the separation group ($P<.05$c). Both separation and tyrosine did not affect the K_d's of the α_2- and β-receptors. Separation stress decreased the ratio of the B_{max} of β:α_2-receptors ($P<.01$d) while tyrosine administration increased it ($P<.05$e).

4.5. Cholinergic pathways

Separation stress increased the B_{max} of M1 receptors (Table 2, $P<.01$a) and tyrosine administration further increased it ($P<.05$b) without changing the K_d. Both separation stress and tyrosine did not change the activities of ChAT and AChE and the B_{max} and K_d of Hc-3. Northern blotting showed that separation stress increased M1 receptor mRNA signal (Fig. 4, $P<.001$a).

5. Discussion

These results are part of on-going studies on the interrelations between nutrition and brain function as part of a

Table 1
The effects of separation stress and tyrosine on the B_{max} (mean ± S.E. fmol/mg protein) and K_d (mean ± S.E. nmol/mg protein) of adrenergic α_2- and β-receptors

	α_2		β		β:α_2
Group	B_{max}	K_d	B_{max}	K_d	B_{max}
Control + saline	74.6 ± 17.6a	3.83 ± 1.73	323.6 ± 30.7	4.13 ± 0.30	4.32 ± 0.41d
Control + tyrosine	63.9 ± 8.9	3.46 ± 1.14	288.8 ± 55.8	4.32 ± 1.37	4.51 ± 0.87
Separation + saline	194.1 ± 22.3a,b ***	5.38 ± 2.45	377.7 ± 44.1c	4.71 ± 0.65	1.66 ± 0.19d,e **
Separation + tyrosine	86.9 ± 14.8b **	3.09 ± 1.42	235.2 ± 25.9c *	3.40 ± 1.04	2.71 ± 0.30e *

Letters represent pairs that are significantly different. All values from five experiments except β-receptor of control + tyrosine group, where $n=4$.
* $P<.05$.
** $P<.01$.
*** $P<.001$.

Table 2
The effects of separation stress and tyrosine on the B_{max} (mean ± S.E. fmol/mg protein) and K_d (mean ± S.E. nmol/mg protein) of muscarinic M1 receptors and high affinity choline transporter Hc-3, the activities of ChAT (mean ± S.E. nmol/mg protein/h), and AChE (mean ± S.E. μmol/mg protein/h)

	M1		Hc-3			
Group	B_{max}	K_d	B_{max}	K_d	ChAT	AChE
Control + saline	467.5 ± 49.9a	1.50 ± 0.22	489.4 ± 87.5	5.45 ± 1.09	48.06 ± 1.82	2.79 ± 0.31
	4	4	5	5	14	6
Control + tyrosine	399.3 ± 19.9	0.84 ± 0.13	458.1 ± 50.9	7.74 ± 1.58	43.78 ± 2.26	2.76 ± 0.38
	4	4	5	5	14	6
Separation + saline	755.7 ± 21.9a,b **	1.64 ± 0.25	399.3 ± 60.8	7.84 ± 1.54	42.96 ± 3.12	2.73 ± 0.32
	7	7	6	6	14	7
Separation + tyrosine	882.5 ± 27.8b *	1.65 ± 0.32	319.4 ± 65.9	6.00 ± 1.72	40.92 ± 3.24	2.91 ± 0.34
	7	7	5	5	14	6

Letters represent pairs that are significantly different.
* $P < .05$.
** $P < .01$.

psychobiological explanation for the enigma of the human disease of anorexia nervosa. According to this hypothesis malnutrition leads to depletion of precursors of brain neurotransmitters (e.g., tyrosine and tryptophan) (Gerner et al., 1984; Schreiber et al., 1991; Schweiger et al., 1985) with consequent effects on physiology and behavior. Decreased concentrations of NE, dopamine, homovanillic acid (HVA), and MHPG have been found in the CNS and urine of patients with anorexia nervosa (Gross et al., 1979; Kaye et al., 1984) and in the brains of diet-restricted animals (Avraham et al., 1996; Hao et al., 2000; Schweiger et al., 1985). These changes form a vicious cycle reenforcing the weight loss and behavior deficits such as delusions concerning body image.

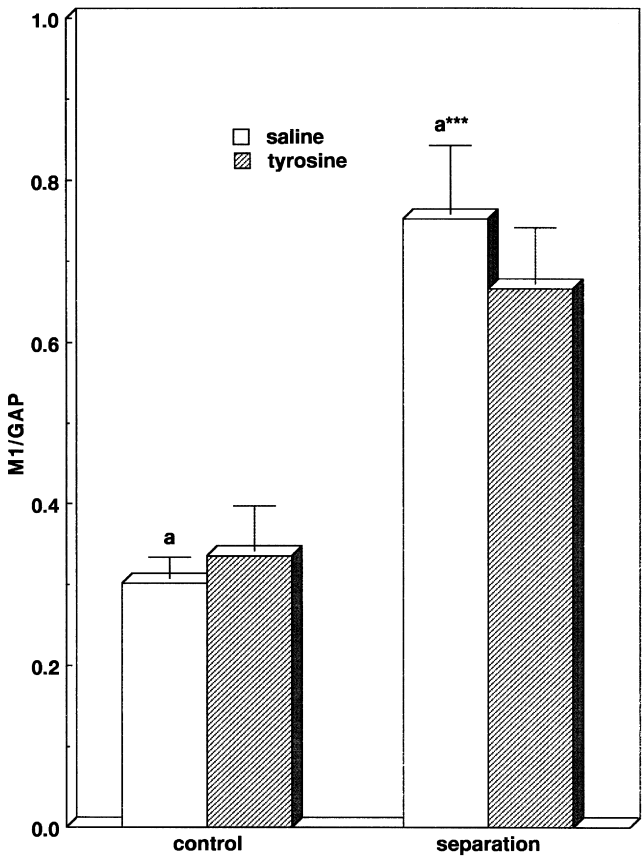

Fig. 4. Effects of separation and tyrosine on M1 receptor mRNA (mean ± S.E.), *** $P < .001$, letters represent pairs that are statistically different, 9–10 mice in each group.

5.1. Stress and body weight

Separation stress significantly decreased the weight of the mice. However, the weight changes cannot explain entirely the changes in behavior and the related neurochemical changes. Although the weight change was the same as that induced by 60% DR, its effects on neurochemistry are different (Avraham et al., 1996). The decreased NE and dopamine levels, and increased B_{max} of α_2-receptors induced by separation stress are more similar to the effects of the more serious undernutrition associated with 40% DR (Avraham et al., 1996). This suggests the additive effects of the separation stress over and above that of weight reduction.

5.2. Stress and alternation behavior

5.2.1. Norepinephrine

Our results showed that separation stress impaired performance in the T-maze task (Fig. 2) and caused marked reduction of NE concentration (Fig. 3a). Studies suggested that NE facilitates performance of rats in the T-maze (Low et al., 1984). Stress increased the density of adrenergic α_2-receptors while it did not change that of β-receptors (Table 1). Electric shock treatment also indicated that adrenergic β-receptors in the hippocampus are not sensitive to the stress response (Nomura et al., 1981). This implies that stress impaired alternation behavior is correlated with the decreased NE adrenergic α_2-receptor transmission.

This study agrees with many previous reports that stress in animals depletes NE levels (Hellriegel and D'Mello, 1997; Lehnert et al., 1984; Nakagawa et al., 1981; Reinstein et al., 1984; Swenson and Vogel, 1983) in the hippocampus

and that tyrosine reverses this and improves behavioral consequences (Lehnert et al., 1984; Rauch and Lieberman, 1990; Reinstein et al., 1984; Shurtleff et al., 1993). Although tyrosine administration did not change the NE levels in the separation model, our results showed that stress increased the levels of MHPG and MHPG/NE in the hippocampus. This is circumstantial evidence that stress probably increased the firing frequency of noradrenergic neurons (Abercrombie and Jacobs, 1987), enhancing NE release (Rosario and Abercrombie, 1999; Zhang et al., 1995), and eventually leading to NE depletion in the hippocampus in our study and others (Hellriegel and D'Mello, 1997; Lehnert et al., 1984; Nakagawa et al., 1981; Reinstein et al., 1984; Swenson and Vogel, 1983). The accelerated firing activated tyrosine hydroxylase — the rate-limiting step in the catecholamine synthesis (Stone, 1983), making it more sensitive to tyrosine. Thus, tyrosine administration to stressed mice may increase NE synthesis and its release, as measured by the further increased MHPG and MHPG/NE levels seen both in our results (Figs. 3b,c) and in other studies (Reinstein et al., 1984).

The decreased B_{max} of both α_2- and β-receptors after tyrosine administration (downregulation) is again circumstantial evidence for the presumably increased synaptic NE level, thus enhancing NE transmission and improving alternation behavior. Studies suggest that α_2-receptor agonists might be candidates for enhancing alternation behavior in the T-maze (Tanila et al., 1999) and delayed response task (Arsten and Goldman-Rakic, 1985; Arnsten et al., 1988), and the later might through the effect of NE on postsynaptic α_2-receptor (Arsten and Goldman-Rakic, 1985; Arnsten et al., 1988). Our present study also supports this hypothesis that the depletion of NE by stress upregulated postsynaptic receptor density, thus decreasing NE transmission and impairing alternation behavior. The relationship between alternation behavior and the receptor density ratio of β:α_2 is in agreement with our previous findings using the eight-arm maze (Avraham et al., 1996). Together, they indicate that better maze performance was correlated with an increased ratio of β:α_2, suggesting that this balance might be important in animal alternation behavior and spatial memory.

As mentioned above, separation stress impaired alternation behavior with increased MHPG and MHPG/NE levels. Tyrosine administration to the stressed mice further increased both to values much higher than the control group, while alternation behavior was improved to that of the controls (Figs. 2 and 3c). The relationship between MHPG, MHPG/NE, and alternation behavior has not been discussed in the literature. Our results suggest that both of them may not be related to such behavior in this stress model.

In man, tyrosine is reported to alleviate cold, high altitude, fatigue, and military training characterized by psychosocial and physical stress-associated memory impairment (Banderet and Lieberman, 1989; Beijen and Orlebeke, 1994; Owasoyo et al., 1992; Pickel et al., 1974; Thomas et al., 1999). This might be due to NE depletion in the hippocampus suggested by animal studies (Hellriegel and D'Mello, 1997; Lehnert et al., 1984; Nakagawa et al., 1981; Reinstein et al., 1984; Swenson and Vogel, 1983). Thus, tyrosine might be of potential beneficial to counter the cognitive function impairment related to decreased NE transmission and effects on α_2-receptors (Arnsten et al., 1988) as in stress accompanying normal situations such as aging (Arnsten et al., 1988) and military operations (Owasoyo et al., 1992) and those associated with disease as in depression (Gelenberg et al., 1982/1983), as well as in the maintenance of a reduced body weight in the treatment of obesity (Leibel et al., 1991) and in the extreme case of anorexia nervosa (Berry, 1999).

5.2.2. Dopamine

Our results indicate that dopamine depletion induced by stress and its repletion after tyrosine administration correlate with the impaired and improved alternation behavior. Cortical DA has been shown to facilitate alternation behavior (Sahakian et al., 1985). However, dopaminergic drugs such as SKF 38393, amphetamine, apomorphine, and quinpirole have been reported to reduce alternations (Molino et al., 1989; McFarland, 1989; Einat and Szechtman, 1995). This maybe due to multiple affects in different brain regions (Levin et al., 1997) and interactions with other pathways (Kokkinidis and Anisman, 1976).

5.3. Stress and the cholinergic system

Stress increased the B_{max} of M1 receptors (Table 2) and this was coupled to the enhancement of M1 receptor mRNA. Tyrosine further increased the muscarinic M1 receptor density after separation without changing the levels of mRNA, Hc-3, ChAT, and AChE. This might be due to the increased synthesis and release of NE decreasing the synaptic levels of acetylcholine (Birch and Fillenz, 1986; Moroni et al., 1983), thus upregulating receptor numbers. Stress has been reported to increase [^{3}H]quinuclidinylbenzilate binding (receptors M1–M5) in the hippocampus (Gilad et al., 1983). M1 receptor has been related to the alternation behavior by its partial agonist study (Hatcher et al., 1998). However, the function of M1 receptor in the alternation behavior in this stress model is not clear since impaired alternation was coupled to upregulation of M1 receptor density; furthermore, tyrosine administration reversed the alternation behavior but did not downregulate receptor density as might have been expected.

6. Conclusions

Separation stress depleted both NE and dopamine in the hippocampus leading to impaired alternation behavior in the T-maze. Stress upregulated α_2-receptor density without changing β-receptor, suggesting it decreased NE transmis-

sion through action on α_2-receptors. Tyrosine administration increased dopamine and presumably NE concentrations, downregulated both α_2- and β-receptor numbers and improved alternation behavior. The balance between the B_{max} ratio of β:α_2-receptor may also play a role in alternation behavior. The ameliorating effects of tyrosine on alternation behavior and adrenergic function induced by stress and weight loss, suggests a possible therapeutic role for psychobiological problems related to decreased NE transmission as in the maintenance of a reduced body weight in the treatment of obesity (Leibel et al., 1991), and in the extreme case of anorexia nervosa (Berry, 1999).

Acknowledgments

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