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Forced swimming and imipramine modify plasma and brain amino acid concentrations in mice

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ABSTRACT

The relationships between monoamine metabolism and forced swimming or antidepressants have been well studied, however information is lacking regarding amino acid metabolism under these conditions. Therefore, the aim of the present study was to investigate the effects of forced swimming and imipramine on amino acid concentrations in plasma, the cerebral cortex and the hypothalamus in mice. Forced swimming caused cerebral cortex concentrations of L-glutamine, L-alanine, and taurine to be increased, while imipramine treatment caused decreased concentrations of L-glutamate, L-alanine, L-tyrosine, L-methionine, and L-ornithine. In the hypothalamus, forced swimming decreased the concentration of L-serine while imipramine treatment caused increased concentration of β-alanine. Forced swimming caused increased plasma concentration of taurine, while concentrations of L-serine, L-asparagine, L-glutamine and β-alanine were decreased. Imipramine treatment caused increased plasma concentration of all amino acid, except for L-aspartate and taurine. In conclusion, forced swimming and imipramine treatment modify central and peripheral amino acid metabolism. These results may aid in the identification of amino acids that have antidepressant-like effects, or may help to refine the dosages of antidepressant drugs.

1. Introduction

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth and disturbed sleep or appetite. It has been primarily treated by antidepressants that are related to the monoaminergic neuron system. However, it is unclear whether nutrients cause an antidepressive effect. The identification of such nutrients may raise the possibility of treating or preventing depression by daily nutrient supplementation. Amino acids have several functions and some play important roles in the brain as neurotransmitters. For example, L-arginine, L-serine, and L-cysteine cause sedative effects (Suenaga et al., 2008; Asechi et al., 2006), and acute injection of L-tryptophan or L-arginine is associated with an antidepressant-like effect during forced swimming (Wong and Ong, 2001; da Silva et al., 2000).

The forced swimming test is one of the most common antidepressant screening tests. If a reagent under study reduces the amount of time an animal is immobile during forced swimming, that reagent likely caused an antidepressive effect. Excitatory amino acids are associated with antidepressive reagents. For example, antagonists of the *N*-methyl-D-aspartate receptor for glutamate are effective in the forced swimming test (Trullas and Skolnick, 1990). Imipramine, one of the tricyclic antidepressant drugs that is an inhibitor of noradrenaline and serotonin (5-HT) reuptake on presynaptic terminals, is used widely for the treatment of depression and other mental disorders.

Thus, changes in amino acid concentrations after forced swimming and imipramine treatment were examined in the present study. The elucidation of amino acid concentration changes under these conditions may help identify amino acids that cause antidepressant-like effects. Therefore, the aim of the present study was to investigate the effect of forced swimming and imipramine on amino acid concentrations in the plasma, cerebral cortex and hypothalamus of mice.

2. Materials and methods

2.1. Animals

Six-week-old male ICR mice were purchased from Charles River Japan (Kanagawa, Japan). Mice were housed 8 per cage under a light/dark cycle (lights on at 08:00, lights off at 20:00) at room temperature of 23±1 °C. Mice had ad libitum access to food and water for 1 week before the experiments. Mice were handled 1 day before the test. This study was performed according to the guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

2.2. Drugs and administration

Imipramine (Sigma, Japan) was freshly prepared in 0.9% saline. Mice were divided into 4 groups of 8 mice each. Two of the groups

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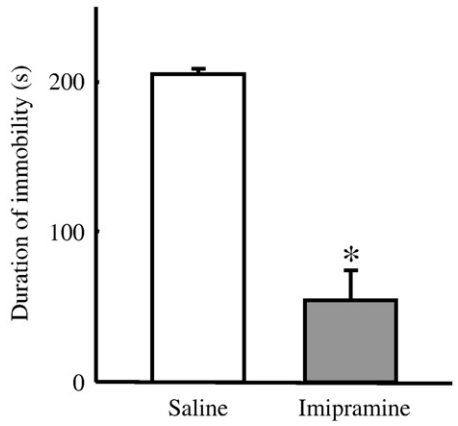

Fig. 1. The effect of imipramine (30 mg/10 ml/kg) on the duration of immobility in the forced swimming test. Data express mean ± S.E.M. of 8 mice per group. *, $P<0.01$ versus control (saline).

were administered saline (10 ml/kg, i.p.) and the other two groups were administered imipramine (30 mg/10 ml/kg, i.p.) 1 h prior to the test.

2.3. Forced swimming test

The experiment was carried out as previously described (Porsolt et al., 1977). Mice from one of the two groups administrated saline or imipramine were individually placed in a plastic bottle (22 cm high, 9.7 cm in diameter) for 6 min containing 16 cm of water that was maintained at 24–26 °C. The other groups were not subjected to the forced swimming test. A mouse was judged to be immobile when it remained floating in the water, making only small movements to keep its head above water. The total duration of immobility was recorded by video camera during the final 4 min after a 2-min habituation period. In each test, fresh water was used. After the test, mice were sacrificed by cervical dislocation and the trunk blood was collected. The cerebral cortex and hypothalamus were immediately dissected and weighed. The samples were frozen in liquid nitrogen, and stored at −80 °C until analyzed.

2.4. Analysis of free amino acids

The concentrations of 23 free amino acids were assayed by high-performance liquid chromatography (HPLC). The tissue samples were homogenized in ice-cold 0.2 M perchloric acid solution containing 0.01 mM EDTA·2Na and left for deproteinization in ice. After 30 min, the mixtures were centrifuged at 20,000 ×g for 15 min at 0 °C and the resultant supernatants then adjusted to pH 7 with 1 M sodium hydroxide. Plasma was prepared by centrifugation at 2500 rpm for 25 min at 4 °C, and was then filtered through an ultrafiltration tube. Each sample (20 μl) was then completely dried under reduced pressure. Dried residues were dissolved in 10 μl of 1 M sodium acetate–methanol–triethylamine (2:2:1) solution, re-dried, and dissolved in 20 μl of derivatization solution (methanol–water–triethylamine–phenylisothiocyanate (7:1:1:1)). After 20 min at room temperature phenylisothiocyanate was allowed to react with the amino groups and the samples were dried again and then dissolved in 100 μl of Pico-Tag Diluent (Waters, Milford, USA). These diluted samples were filtered through a 0.45-mm filter (Millipore, Bedford, USA). The same method was applied to standard solutions prepared by diluting a commercially available L-amino acid solution (type AN II and type B; Wako, Osaka, Japan) with distilled water. These derivatized samples were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column (3.9 mm×300 mm), Alliance 2690 separation module, 2487 dual-wavelength UV detector, and Millennium 32 chromatography manager; Waters, Milford, USA). They were equilibrated with buffer A (70 mM sodium acetate (pH 6.45 with 10% acetic acid)–acetonitrile (975:25)) and eluted with a linear gradient of buffer B (water–acetonitrile–methanol (40:45:15)) (0, 3, 6, 9, 40, and 100%) at a flow rate of 1 ml/min at 46 °C. The absorbance at 254 nm was applied to determine concentrations of free amino acids. Triethylamine and sodium acetate trihydrate were purchased from Wako (Osaka, Japan), while other drugs for which no manufacturer is noted were purchased from Sigma (St Louis, USA).

Table 1
Effect of FS[a] and IMI[b] on amino acid concentrations in the cerebral cortex

IMI (mg/10 ml/kg)	FS		Non-FS		*P*		
	0	30	0	30	FS	IMI	FS×IMI
l-Aspartate	2428±46	2370±39	2426±63	2329±80	NS[c]	NS	NS
l-Glutamate	9976±139	9353±187	9579±215	9200±225	NS	$P<0.05$	NS
l-Serine	772±21	779±17	790±21	747±15	NS	NS	NS
l-Asparagine	106±2.2	103±3	109±2.6	105±2.3	NS	NS	NS
Glycine	577±10	571±17	565±7.7	547±11	NS	NS	NS
l-Glutamine	4148±98	4171±132	3736±146	4014±95	$P<0.05$	NS	NS
B-Alanine	51±1.6	53±1.3	50±1.7	54±2.0	NS	NS	NS
Taurine	17,261±271	17,881±340	16,755±224	16,538±403	<0.01	NS	NS
l-Histidine	55±3.4	61±2.4	54±3.1	59±3.8	NS	NS	NS
GABA	1217±29	1193±19	1248±23	1214±27	NS	NS	NS
l-Threonine	265±14	256±11	280±15	265±16	NS	NS	NS
l-Alanine	634±23	602±26	573±12	502±19	$P<0.001$	$P<0.05$	NS
l-Arginine	85±3.3	72±7.2	83±4.5	78±4.1	NS	NS	NS
l-Proline	53±1.7	53±2.3	50±2.5	48±1.6	NS	NS	NS
l-Tyrosine	53±5.0	42±4.1	50±3.1	43±3.4	NS	$P<0.05$	NS
l-Valine	87±406	87±669	87±355	85±343	NS	NS	NS
l-Methionine	53±1.5	48±2.5	50±2.3	47±1.6	NS	$P<0.05$	NS
L-Isoleucine	25±1.1	25±0.84	26±0.86	25±0.86	NS	NS	NS
L-Leucine	31±1.5	31±0.57	33±0.65	32±0.76	NS	NS	NS
l-Phenylalanine	38±1.1	37±0.92	36±0.63	37±1.3	NS	NS	NS
l-Tryptophan	12±0.78	10±0.59	10±0.73	10±0.62	NS	NS	NS
l-Ornithine	7.4±0.45	5.3±0.34	7.9±0.41	6.3±0.48	NS	$P<0.001$	NS
l-Lysine	164±7.6	144±12	151±5.8	149±4.0	NS	NS	NS

The number of samples used for analysis was 7–8. All results are expressed as mean ± S.E.M. in pmol/mg tissue.
[a] Forced swimming.
[b] Imipramine.
[c] Not significant.

2.5. Statistical analysis

Data for the forced swimming test was analyzed by *t*-test, and amino acid concentrations were analyzed by two-way analysis of variance (ANOVA). Significance was set at $P<0.05$. Data are represented as mean±S.E.M. All analyses were performed with StatView (version 5, SAS Institute Cary, United States, SAS 1998).

3. Results

3.1. Immobility in the forced swimming test

The effect of imipramine on immobility in the forced swimming test is shown in Fig. 1. Imipramine significantly reduced the duration of immobility compared to saline.

3.2. Amino acid concentration in the brain

The concentrations of free amino acids in the cerebral cortex are shown in Table 1. Concentrations of L-glutamine, L-alanine, and taurine were significantly increased by forced swimming. Imipramine treatment caused the concentrations of L-glutamate, L-alanine, L-tyrosine, L-methionine, and L-ornithine to be decreased.

The concentrations of free amino acids in the hypothalamus are shown in Table 2. L-serine concentration was significantly decreased by forced swimming and β-alanine concentration was significantly increased by imipramine treatment.

The concentrations of free amino acids in the plasma are shown in Table 3. Taurine concentration was increased, while L-serine, L-asparagine, L-glutamine, and β-alanine concentrations were decreased by forced swimming. However, all amino acid concentrations, except for L-aspartate and taurine, were significantly decreased by imipramine treatment.

Table 2
Effect of FS[a] and IMI[b] on amino acid concentrations in the hypothalamus

IMI (mg/10 ml/kg)	FS		Non-FS		*P*		
	0	30	0	30	FS	IMI	FS×IMI
l-Aspartate	2045±120	2099±114	2262±63	2159±65	NS[c]	NS	NS
l-Glutamate	5653±296	5741±240	5898±193	5502±227	NS	NS	NS
l-Serine	494±29	535±26	614±26	559±24	$P<0.05$	NS	NS
l-Asparagine	98±6.6	100±4.8	113±3.9	102±2.7	NS	NS	NS
Glycine	939±51	991±51	1015±35	938±39	NS	NS	NS
l-Glutamine	4299±255	4578±196	4350±195	4378±122	NS	NS	NS
β-Alanine	75±4.8	87±3.8	77±5.2	83±3.0	NS	$P<0.05$	NS
Taurine	8149±596	8698±443	8629±317	8502±459	NS	NS	NS
l-Histidine	20±1.9	23±3.1	21±2.8	18±3.2	NS	NS	NS
GABA	3501±212	3086±474	3775±196	3566±76	NS	NS	NS
l-Threonine	230±19	250±16	273±16	250±11	NS	NS	NS
l-Alanine	194±13	206±14	209±18	162±13	NS	NS	NS
l-Arginine	106±6.7	100±9.5	115±10	107±7.4	NS	NS	NS
l-Proline	30±2.3	33±2.3	34±2.8	29±1.4	NS	NS	NS
l-Tyrosine	71±5.0	67±8.8	79±4.9	70±5.7	NS	NS	NS
l-Valine	81±7.5	75±12	93±3.4	81±3.5	NS	NS	NS
l-Methionine	452±30	461±24	488±25	448±15	NS	NS	NS
L-Isoleucine	27±2.1	29±1.4	33±2.4	28±1.5	NS	NS	NS
L-Leucine	45±4.8	45±2.2	56±4.3	45±3.0	NS	NS	NS
l - Phenylalanine	37±2.4	40±2.3	43±2.3	38±1.9	NS	NS	NS
l-Tryptophan	ND[d]	ND	ND	ND	ND	ND	ND
l-Ornithine	15±1.0	17±2.3	28±6.3	16±1.4	NS	NS	$P<0.05$
l-Lysine	221±14	211±18	221±7.9	232±15	NS	NS	NS

The number of samples used for analysis was 7–8. All results are expressed as mean±S.E.M. in pmol/mg tissue.
[a] Forced swimming.
[b] Imipramine.
[c] Not significant.
[d] Not detectable.

Table 3
Effect of FS[a] and IMI[b] on plasma amino acid concentrations

IMI (mg/10 ml/kg)	FS		Non-FS		*P*		
	0	30	0	30	FS	IMI	FS×IMI
l-Aspartate	15±2.1	12±0.38	17±2.9	13±0.76	NS[c]	NS	NS
l-Glutamate	84±8.6	60±3.4	97±12	67±2.2	NS	$P<0.005$	NS
l-Serine	109±9.6	55±4.7	128±15	93±9.4	$P<0.01$	$P<0.001$	NS
l-Asparagine	37±3.0	17±1.3	40±4.6	27±2.3	$P<0.05$	$P<0.001$	NS
Glycine	237±22	141±14	273±24	190±16	NS	$P<0.001$	NS
l-Glutamine	480±33	274±13	500±35	381±22	$P<0.05$	$P<0.001$	NS
β-Alanine	4.4±0.69	1.2±0.37	5.0±0.48	3.6±0.40	$P<0.01$	$P<0.001$	NS
Taurine	957±101	927±62	912±101	687±21	$P<0.05$	NS	NS
l-Histidine	2.4±0.44	1.9±0.36	3.7±0.95	1.8±0.26	NS	$P<0.005$	NS
GABA	ND[d]	ND	ND	ND	ND	ND	ND
l-Threonine	169±18	82±7.0	185±21	125±17	NS	$P<0.001$	NS
l-Alanine	364±44	204±25	351±35	245±31	NS	$P<0.001$	NS
l-Arginine	128±10	60±8.3	113±13	75±8.6	NS	$P<0.001$	NS
l-Proline	108±10	54±5.1	105±13	65±7.0	NS	$P<0.001$	NS
l-Tyrosine	68±1.9	30±2.2	57±4.8	38±3.1	NS	$P<0.001$	$P<0.01$
l-Valine	243±14	155±12	229±14	171±9.8	NS	$P<0.001$	NS
l-Methionine	45±5.1	15±1.6	41±5.7	22±2.9	NS	$P<0.001$	NS
L-Isoleucine	98±7.4	59±5.9	87±6.2	69±4.0	NS	$P<0.001$	NS
L-Leucine	111±6.9	72±7.7	100±7.0	83±4.3	NS	$P<0.001$	NS
l - Phenylalanine	75±3.3	52±4.6	62±3.9	51±3.4	NS	$P<0.001$	NS
l-Tryptophan	ND	ND	ND	ND	ND	ND	ND
l-Ornithine	69±5.5	42±5.8	68±12	44±6.3	NS	$P<0.005$	NS
l-Lysine	310±18	193±16	290±28	222±17	NS	$P<0.001$	NS

The number of samples used for analysis was 7–8. All results are expressed as mean±S.E.M. in μmol/l.
[a] Forced swimming.
[b] Imipramine.
[c] Not significant.
[d] Not detectable.

4. Discussion

Imipramine significantly reduced the duration of immobility in the present study, in accordance with previous observations (Porsolt et al., 1978), thereby again confirming the antidepressive effect of imipramine.

The changes observed in the concentrations of several amino acids in the cerebral cortex may be largely explained by L-glutamate metabolism. First, the concentration of L-glutamine was significantly increased by forced swimming. This change may have been caused by increased L-glutamate release, since acute stress increases L-glutamate release in the rat prefrontal cortex (Moghaddam, 1993). This may be related to stress modulation of the expression and functional properties of glutamate receptors (Kole et al., 2002) and transporters (Reagan et al., 2004). Therefore, the forced swimming may have induced increased L-glutamate concentration. However, because of the neurotoxicity of L-glutamate overflow, it is conceivable that L-glutamate was immediately metabolized to L-glutamine, resulting in an increase in L-glutamine concentration.

A significant increase in L-alanine was also observed after forced swimming. This was not likely caused by transit of L-alanine through the blood-brain barrier because L-alanine does not easily pass through this barrier (Oldendorf, 1971). Thus, the increase may have been due to either increased synthesis or decreased degradation of L-alanine. This increase may also be explained by metabolism of L-glutamate. Waagepetersen et al. (2000) hypothesized that L-glutamate is metabolized to α-ketoglutaric acid in glutamatergic neurons by alanine aminotransferase. This is accompanied by the production of L-alanine, and L-alanine is then changed to pyruvic acid by the same enzyme in astrocytes, accompanied by the production of

L-glutamate. Metabolism of L-glutamate or L-alanine increases the concentration of L-alanine or L-glutamate, respectively. Thus, the metabolism of excessive L-glutamate may have induced the synthesis of L-alanine, or the excessive L-glutamate reduced the degradation of L-alanine.

The metabolism of other amino acids, such as L-aspartate, L-ornithine, and L-tyrosine, are also related to glutamate synthesis. If the aforementioned hypotheses are correct, the reason for the increase in L-alanine concentration alone, as a result of overflow of L-glutamate, is unclear. One possible explanation arises from a previous study that suggested that L-alanine may be an important carrier for ammonia transfer between astrocytes and glutamatergic neurons (Waagepetersen et al., 2000). L-Glutamate is metabolized in both glutamatergic neurons and astrocytes involving ammonia metabolism, and L-alanine may act as an ammonia carrier between them. Due to this distinctive role, L-alanine may be preferentially involved in glutamate metabolism. Furthermore, central L-alanine has been shown to have a sedative effect (Kurauch et al., 2006).

The taurine concentration was also significantly increased by the forced swimming test. Taurine has been suggested as a neuromodulator that displays inhibitory effects and is involved in osmotic regulation and neuroprotection (Albrecht and Schousboe, 2005; Pasantes-Morales and Schousboe, 1997; Saransaari and Oja, 2000). Moreover, Kamisaki et al. (1993) demonstrated that taurine inhibits the evoked overflow of L-glutamate through taurine-specific sites. Thus, the synthesis of taurine may have been activated to inhibit excessive release of L-glutamate, resulting in an increase in taurine concentration.

The L-glutamate concentration was decreased by imipramine treatment, similar to a previous report (Michael-Titus et al., 2000), which also found that acute administration of antidepressants, including imipramine, inhibited glutamate release in the rat frontal cortex. Thus, amino acid overflows are possibly being modified by changes in amine concentration. Imipramine is a 5-HT and noradrenaline reuptake inhibitor and those monoamines may influence glutamate concentration. L-Glutamate release in the cerebral cortex synaptosomes is inhibited by 5-HT acting at 5-HT_{1D} receptors (Maura et al., 1998), and furthermore noradrenaline also inhibits veratrine-induced L-glutamate release in the cortical slices (Crowder and Bradford, 1987). Accordingly, the L-glutamate concentration may decrease.

The L-alanine concentration was also decreased by imipramine treatment. As described previously, L-alanine may be preferentially involved in L-glutamate metabolism. In short, decreased L-glutamate release may reduce the synthesis of L-alanine, or induce the degradation of L-alanine. Moreover, L-alanine is metabolized from L-tryptophan, which is a precursor of 5-HT. Therefore, increased 5-HT synthesis may decrease the L-tryptophan concentration and consequently the L-alanine concentration. Yet, the concentration of L-tryptophan was not decreased in the present study, although this may have been due to the greater ability of L-tryptophan to pass through the blood-brain barrier compared to L-alanine (Oldendorf, 1971).

The L-tyrosine concentration was also decreased by imipramine treatment. Similar to L-tryptophan, L-tyrosine is a precursor of noradrenaline and dopamine, so this reduction may have been due to the elevated noradrenaline synthesis caused by the imipramine administration.

As shown in Table 1, the concentration of L-methionine was reduced by imipramine treatment. This result may be related to the function of an L-methionine metabolite. First, L-methionine is metabolized to *S*-adenosyl-L-methionine (SAMe). This SAMe then donates methyl groups in the reactions involved in the synthesis of the neurotransmitters, 5-HT, noradrenaline and dopamine (Mischoulon and Fava, 2002). Since the synthesis of these monoamines was increased by imipramine, the metabolism of SAMe may have also increased, resulting in a decrease in L-methionine concentration upon imipramine administration.

The L-ornithine concentration was also reduced by imipramine treatment. This result might not be due to the ornithine cycle because ornithine transcarbamylase, a constituent of the ornithine cycle, is not present in the brain (Sadasivudu and Rao, 1976). Ammonia generated in the process of metabolism of L-glutamate is metabolized by aspartate transcarbamylase in the brain. Therefore, some other metabolic pathways of L-ornithine may be involved. Hietala et al. (1983) demonstrated that imipramine increases ornithine decarboxylase activity in the mouse brain, which synthesizes polyamine from L-ornithine. Therefore, the decreased L-ornithine concentration may have resulted from this imipramine action.

In the hypothalamus, alterations in only two amino acid concentrations were found; L-serine was decreased by the forced swimming and β-alanine was increased by imipramine treatment. L-Serine is an important amino acid because it provides a large fraction of the one carbon pool, used for synthesis of purine nucleotides and thymidylate, and it also supports a positive nitrogen balance. Moreover, L-serine has a sedative effect in chicks (Asechi et al., 2006). Due to this inhibitory effect, this amino acid might be involved in forced swimming. With regard to β-alanine, its release has been suggested to be enhanced by β-alanine itself and by the structural analogs taurine and GABA (Saransaari and Oja, 1999). Furthermore, β-alanine is the primary agonist at hippocampal glycine receptors (Mori et al., 2002). Accordingly, these amino acids might act as neurotransmitters involved in forced swimming and imipramine treatment.

These results raise the possibility of functional and metabolic differences of amino acids between the cerebral cortex and the hypothalamus. However, the reasons for such differences are unclear.

In plasma, several amino acid concentrations were also altered. Taurine was significantly increased, while L-serine, L-asparagine, L-glutamine and β-alanine were significantly decreased by forced swimming. Altamura et al. (1995) demonstrated that the plasma concentration of taurine is elevated in major depression, while those of L-serine and L-glutamine are unchanged and that of glycine is decreased. These changes in amino acid concentrations may be caused not only by acute stress but also by exercise. Matsuzaki et al. (2002) reported that the taurine concentration in skeletal muscle decreases after more than 30 min of exercise. Moreover, various plasma amino acid concentrations change after a marathon (Cuisinier et al., 2001). Thus, the changes in plasma amino acid concentrations in the present study are thought to be caused by the swimming exercise rather than acute stress, although the forced swimming lasted only 6 min.

Almost all the amino acid concentrations tested, except for L-aspartate and taurine, were significantly decreased by imipramine treatment. However, these two amino acids tended to decrease. This might be due to protein synthesis and/or amino acid degradation. Chronic imipramine treatment has been shown to decrease the corticotropin releasing factor levels in the rat brain (Fadda et al., 1995), and low levels of corticotropin releasing factor attenuate the release of corticosterone. As a result, protein degradation decreases, which might induce a decrease in plasma amino acids. In the present study, imipramine was acutely administered. A previous study demonstrated that imipramine stimulates the release of cortisol, growth hormone and prolactin in humans (Nutt et al., 1987), and that venlafaxine, a dual 5-HT noradrenaline reuptake inhibitor also increases the release of corticosterone and growth hormone in the rat (Piacentini et al., 2003). On the other hand, the plasma corticosterone level is unchanged by treatment with tricyclic antidepressant drugs in mice (Azpiroz et al., 1999; Weber et al., 2006). In addition, antihistamines, like tricyclic antidepressant drugs including imipramine, reduce histamine-induced adrenocorticotropic hormone release (Reilly and Sigg, 1982). Thus, the corticosterone level in plasma might be affected by differences between animal species and the drug involved. Moreover, Lambert and Lauder (1999) demonstrated that the 5-HT receptor activates the synthesis

of insulin-like growth factor. 5-HT neurons are stimulated by imipramine, and insulin-like growth factor is related to protein synthesis in bone and muscle, which may in turn lead to a decrease in plasma amino acid concentrations. However, the non-protein amino acids also decreased in the present study, and thus the reason for the reduction in amino acid concentrations is ultimately unclear. A further examination of the relationship between imipramine and plasma amino acids is required.

The present results demonstrate that the metabolism of several amino acids in the brain and plasma is altered, in different ways, by forced swimming and imipramine treatment. Clear explanations for these observations are difficult as the amino acid metabolic pathways and functions are complicated. However, the findings in the present study may aid the search for amino acids with antidepressant-like effects or perhaps help reduce the dosage of antidepressant drugs in the future.

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