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Carnosine-induced antidepressant-like activity in rats

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ABSTRACT

Depression is a pathological state of mood and is considered as one of the major causes of disabilities. Thus, the prevention of depression and care for individuals with depression is important. In the present study, we examined whether a single oral dose of CBEX (chicken breast extract), or carnosine (one of the major components of CBEX) affects immobility time, an index of depressive-like behavior, in the forced swimming test in male Wistar rats. CBEX tended to (P=0.09) and carnosine significantly (P<0.05) decreased immobility time in the forced swimming test. In the hippocampus, both CBEX and carnosine significantly decreased 3-methoxy-4-hydroxyphenylglycol, a major metabolite of norepinephrine, indicating that CBEX and carnosine could reduce NE activity in the hippocampus in the forced swimming test. CBEX and carnosine did not affect total locomotive distance or rearing in the open field test, suggesting that the reductions of immobility time by both treatments in the forced swimming test were not merely due to the stimulation of general motor activity. Taken together, these results suggest that CBEX has an antidepressant-like effect, which may be due, in part, to the effect of carnosine.

1. Introduction

Depression is a pathological state of mood. A depressed individual sees everything (e.g., self, world, and future) through a dark prism. Feelings of helplessness, hopelessness, and worthlessness are common (Gelenberg and Hopkins, 2007). The World Health Organization estimated that unipolar depressive disorders were the fourth main cause of disability in 2002, and predicts that they will be the second main cause of disability in 2030 (Mathers and Loncar, 2006). Therefore, preventing depression and caring for individuals with depression is important. Antidepressants are recognized as effective treatments for individuals with depressive disorders (Gelenberg and Hopkins, 2007). One of most frequently used animal models for screening antidepressants for clinical use is a rat forced swim test (Borsini and Meli, 1988; Porsolt et al., 1978).

Carnosine (β-alanyl-L-histidine) and its derivative anserine (β-alanyl-1-methyl-L-histidine) are present at high levels in meat and fish; they are especially rich in the breast muscle of chickens (Aristoy and Toldra, 2004). These dipeptides have antioxidant activities (Kohen et al., 1988) and buffering capacities (Abe, 2000), and are putative neurotransmitters in the brain (Tomonaga et al., 2004, 2005).

CBEX (chicken breast extract) is a commercially available supplement rich in carnosine and anserine (Tomonaga et al., 2007). Long-term supplementation with CBEX has been shown to improve high-intensity exercise performance (Sato et al., 2003) as well as relatively high-intensity endurance performance (Maemura et al., 2006) in humans. We previously suggested that a single oral dose of CBEX (20 ml/kg) could increase carnosine and anserine levels and stimulate nitric oxide (NO) generation as measured by citrulline production in the hypothalamus and hippocampus of rats (Tomonaga et al., 2007). However, the effects of CBEX on the brain have not yet been fully investigated.

Taking previous studies into consideration, the present study examined whether orally administered CBEX or one of its major components, carnosine, influences depressive-like behavior in rats as measured by the forced swimming test. Brain monoamine levels were also analyzed to assess the influence of CBEX or carnosine on the brain functions. In the brain, we focused on the hypothalamus and hippocampus because these brain regions have been previously reported to have important roles in the pathophysiology of depression (Campbell and Macqueen, 2004; Ehlert et al., 2001).

2. Materials and methods

2.1. Animals and drugs

Male Wistar rats (six weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were kept in cages (four per cage) in a room at 25±1 °C on a 12:12 light–dark cycle (lights on at 08:00 h, lights off at 20:00 h), and given free access to a commercial diet (MF; Oriental Yeast, Tokyo, Japan) and water. They were allowed to habituate for one week before beginning the experiments. All

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experiments were conducted from 12:00 to 17:00 h. This study was performed according to the National Research Council publication, Guide for Care and Use of Laboratory Animals and guidance for Animal Experiments for the Faculty of Agriculture and for the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

CBEX was a gift from Nippon Meat Packers (Tsukuba, Japan). Triethylamine and sodium acetate trihydrate were purchased from Wako (Osaka, Japan). Sodium-1-octane sulfonate was purchased from Nacalai Tesque (Kyoto, Japan). Disodium ethylenediaminetetraacetic acid was purchased from Dojindo (Kumamoto, Japan). All other drugs for which no manufacturer is noted were purchased from Sigma (St. Louis, USA).

2.2. Forced swimming test

In Experiments 1 and 2, the effect CBEX or carnosine on depressive-like behavior was investigated using the forced swimming test as described by Porsolt et al. (1978) with some modifications. To confirm whether our modified experimental conditions were suitable for measuring antidepressant effect, the effects of imipramine, a tricyclic antidepressant, on the immobility time in the forced swimming test of rats were investigated in a preliminary experiment. Intraperitoneal (i.p.) injection of imipramine (30 mg/kg) 60 min before the main test significantly ($P<0.005$, t-test) reduced the immobility time (mean (second)±SEM: control, 101±14; imipramine treated, 44±6, respectively). The results obtained were consistent with the previous report (Araki et al., 1984). Therefore, we assumed that the present conditions of forced swimming test are valid. After a one-week habituation period, rats were randomly selected and divided into three groups of eight for Experiment 1 ($n=24$) and four groups of eight for Experiment 2 ($n=32$). Two swim sessions were conducted on two consecutive days (one test per day). In the first session (pretest), a rat was placed individually into a black bucket made of steel (height 31 cm, diameter 29 cm) filled with water (24±1 °C) to a depth of 20 cm for 15 min. After the session, the rat was wiped with a paper towel and returned to the home cage. The second session (main test) was conducted 24 h after the pretest session. In the second session, rats were orally administered CBEX (5 or 10 ml/kg) in Experiment 1 or carnosine (0.175, 0.7 or 1.4 mmol/10 ml/kg) in Experiment 2. The volume of CBEX 5 ml/kg was adjusted by adding distilled water to CBEX. Carnosine was also diluted with distilled water. Control groups were treated with distilled water (10 ml/kg). A total of 120 min after drug treatment, rats were again placed in a black bucket (the same as the pretest) for 5 min. The main test was monitored by an 8-mm video camera, recorded on PC where immobility was scored later. The immobility time is an index of depressive-like behavior in the forced swimming test (Porsolt et al., 1978). A rat was defined as immobile when floating motionless or making only those movements necessary to keep its head above water. Water in the bucket was changed after each session. As soon as the main test was finished, the rat was sacrificed by dislocation of the cervical vertebrae and the brain was removed. The hypothalamus and hippocampus were dissected from the brain, weighed, rapidly frozen with liquid nitrogen, and stored at −80 °C until analysis.

2.3. Analysis of monoamines in the brain

To investigate the influence of CBEX or carnosine on the brain functions in Experiments 1 and 2, brain monoamine levels were analyzed using a previously described method (Saito et al., 2004) with some modifications. Briefly, the brain was homogenized in 0.2 mol/l ice-cold perchloric acid and the homogenate was cooled by ice for 30 min for deproteinization. The homogenate was centrifuged at 20,000× g for 5 min at 4 °C. Next, the pH of the supernatant was adjusted to approximately 3.0 by adding 1 mol/l sodium acetate. The sample was filtered through a 0.45-μm filter (Millipore, Bedford, USA) at 20,000× g for 15 min at 0 °C. The 30-μl filtrate was applied into a high performance liquid chromatography (HPLC) system (Eicom, Kyoto, Japan) with a 150×2.1 mm octadecyl silane (ODS) column (SC-5ODS, Eicom) and electrochemical detector (ECD-300, Eicom) at an applied potential of +700 mV versus an Ag/AgCl reference analytical electrode. The changes in electric current (nA) were recorded in a computer using an interface system (Power Chrom ver 2.3.2.J; AD Instruments, Tokyo, Japan). The mobile phase was composed of aceto-citric acid buffer (pH 3.5, 0.1 mol/l), methanol, sodium-1-octane sulfonate (0.46 mol/l) and disodium ethylenediaminetetraacetic acid (0.015 mmol/l) (830:170:1.9:1) at a flow rate of 0.2 ml/min. The concentrations of serotonin (5-HT), 5-Hydroxyindoleacetic acid (5-HIAA), dopamine (DA), norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined, and their levels in the brain were calculated. The detection limits of the system for all monoamines were 0.1 pg/sample.

2.4. Open field test

In Experiment 3, in order to clarify any association of immobility in the forced swimming test with changes in motor activity, the activities of rats treated with the highest dose of CBEX (10 ml/kg) or carnosine (1.4 mmol/10 ml/kg) were tested in the open field test. Control groups were treated with distilled water (10 ml/kg). After a one-week habituation period, rats were randomly selected and divided into three groups, with eight rats per group ($n=24$). The open field consisted of a circular area (height, 35 cm; diameter, 60 cm) made from black takiflex. A total of 120 min after drug injection, the rat was placed at the center of the open field and allowed to explore freely for 5 min. The rat was then taken out of the apparatus and was returned to the home cage. The apparatus was cleaned with an ethanol–water solution after each test. The locomotive distance was analyzed automatically with a computer-based video-tracking system (AXIS-90, Neuroscience, Inc., Tokyo, Japan). The test was recorded on a PC connected to an 8-mm video camera, and the number of rearing incidents was counted later.

2.5. Analysis of dipeptides in the plasma and brain

In Experiment 4, to clarify the effects of CBEX on the brain, we examined whether a single oral dose of CBEX (10 ml/kg) affects brain carnosine and anserine levels in rats. After a one-week habituation period, rats were randomly selected and divided into five groups, with seven rats per group ($n=35$). Four of the five groups were orally administered CBEX (10 ml/kg). The hypothalamus and hippocampus were collected 30, 60, 120 and 240 min after treatment, using the same method as Experiments 1 and 2. Fifth group, the “initial group” was not given the study drug. Rats were decapitated and the blood was collected into heparinized tubes. Blood samples were centrifuged for 15 min at 4 °C at 6000 g, and the plasma was removed and stored at −80 °C until analysis. Hypothalamus and hippocampus were also collected, weighed, rapidly frozen with liquid nitrogen, and stored at −80 °C until analysis.

Carnosine and anserine were analyzed using a previously described method (Tomonaga et al., 2007), with some modifications. Briefly, brain was homogenized with distilled water. The homogenate was then centrifuged at 10,000× g for 20 min. The supernatant and plasma were deproteinized by filtration through a 10,000 dalton molecular weight cut-off filter (Millipore) via centrifugation at 10,000× g for 60 min. The samples (40 μl) were then completely dried under reduced pressure. Dried residues were resolved with 10 μl of a 1 mol/l sodium acetate–methanol–triethylamine (2:2:1) solution. The samples were re-dried, and resolved in 20 μl of derivatization solution (methanol–water–triethylamine–phenylisothiocyanate [7:1:1:1]]. The sample was maintained at room temperature for 20 min to allow phenylisothiocyanate to react with the amino groups to produce phenylthiocarbamyl amino acid residues. The sample was

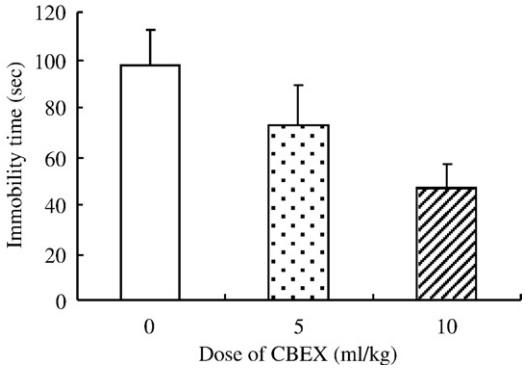

Fig. 1. Effects of oral administration of CBEX on immobility time of rats in the forced swimming test in Experiment 1. The numbers of rats used were: control eight; CBEX (5 ml/kg) eight; CBEX (10 ml/kg); six. Values are presented as means±SEM.

dried again, and was resolved with 100 µl of Pico-Tag Diluent (Waters, Milford, USA). This diluted sample was filtered through a 0.45-µm filter (Millipore). The same method was applied to standard solutions prepared by diluting carnosine and anserine nitrate salt with distilled water. These derivatized samples (plasma and brain: 20 µl; standard solution: 5 µl) were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column [3.9×300 mm], Alliance 2690 separation module, 2487 dual-wavelength UV detector, and Millennium 32 chromatography manager; Waters). The samples were equilibrated with buffer A (70 mmol/l 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 measured, and concentrations of carnosine and anserine were determined and their levels in the plasma and brain were calculated.

2.6. Statistical analysis

All analyses were conducted using a one-way analysis of variance. When significant ($P<0.05$) effects were detected, comparisons between means were carried out using the Tukey–Kramer test. These analyses were performed with StatView (version 5, SAS Institute, Cary, U.S.A. 1998). Outlying data were eliminated by Thompson's test criterion for outlying observations ($P<0.05$).

3. Results

3.1. Experiment 1: effect of orally administered CBEX on depressive-like behavior and levels of monoamines and their metabolites in the brain in the forced swimming test

At the beginning of the experiment, each group comprised eight rats. However, two rats in the CBEX (10 ml/kg) group were eliminated from the study: one rat jumped out of the apparatus during the main test, the other rat had an immobility time that was considered outlying data ($P<0.05$). Therefore, the final number of rats in the CBEX (10 ml/kg) group was six. Only in the analysis of levels of monoamines and their metabolites in the hippocampus, one sample in the control group was lost and the number of rats in the group was seven.

Fig. 1 shows effects of oral administration of CBEX on immobility time in the forced swimming test. The effect of CBEX was not significant but tendency was detected ($F(2, 19)=2.744$, $P=0.09$).

Table 1 shows effects of oral administration of CBEX on levels of monoamines and their metabolites in the hypothalamus in the forced swimming test. The effect of CBEX on MHPG level was significant ($F(2, 19)=5.075$, $P<0.05$). MHPG level in the CBEX (5 or 10 ml/kg) group was significantly ($P<0.05$) lower than that in the control group. There was no significant effect on 5-HT, 5-HIAA, DA, DOPAC, HVA or NE level.

Table 2 gives effects of oral administration of CBEX on levels of monoamines and their metabolites in the hippocampus in the forced swimming test. The effect of CBEX on MHPG level was significant ($F(2, 18)=4.873$, $P<0.05$). MHPG level in the CBEX (5 or 10 ml/kg) group was significantly ($P<0.05$) lower than that in the control group. There was no significant effect on 5-HT, 5-HIAA, DA or NE level. HVA and DOPAC were not detected.

Table 1
Effects of oral administration of CBEX on levels of monoamines and their metabolites in the hypothalamus in the forced swimming test

CBEX (ml/kg)	0	5	10	*F* value (2,19)	*P* value
MHPG	406±26	322±22*	307±21*	5.075	<0.05
NE	2642±120	2868±187	2269±410	1.507	0.247
DOPAC	391±33	386±29	529±92	2.284	0.129
DA	1586±136	1543±118	1822±315	0.591	0.564
5-HIAA	1442±59	1390±52	1402±34	0.281	0.758
HVA	85±7	81±6	130±31	2.769	0.088
5-HT	1092±106	1117±44	921±103	1.356	0.281

Values are means±SEM in pmol/g wet tissue. *Significant difference when compared with the control at $P<0.05$. The numbers of rats used were: control eight; CBEX (5 ml/kg) eight; CBEX (10 ml/kg); six.

Table 2
Effects of oral administration of CBEX on levels of monoamines and their metabolites in the hippocampus in the forced swimming test

CBEX (ml/kg)	0	5	10	*F* value (2,18)	*P* value
MHPG	429±25	345±23*	330±23*	4.873	<0.05
NE	515±22	505±43	453±32	0.821	0.456
DA	185±41	139±11	111±14	2.042	0.159
5-HIAA	97±21	73±6	58±7	2.042	0.159
5-HT	965±23	925±22	894±32	1.812	0.192

Values are means±SEM in pmol/g wet tissue. *Significant difference when compared with the control at $P<0.05$. The numbers of rats used were: control seven; CBEX (5 ml/kg) eight; CBEX (10 ml/kg); six.

3.2. Experiment 2: effect of orally administered carnosine on depressive-like behavior and levels of monoamines and their metabolites in the forced swimming test

At the beginning of the experiment, each group comprised eight rats. However, one rat in the carnosine (1.4 mmol/kg) group was eliminated after jumping out of the apparatus during the main test. Thus, the final number of rats in the carnosine (1.4 mmol/kg) group was seven.

Fig. 2 shows effects of oral administration of carnosine on immobility time in the forced swimming test. The effect of carnosine

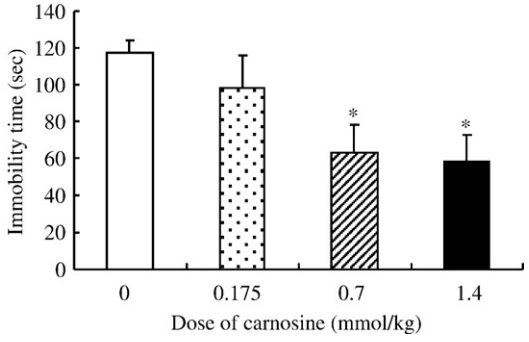

Fig. 2. Effects of oral administration of carnosine on immobility time of rats in the forced swimming test in Experiment 2. The numbers of rats used were: control eight; carnosine (0.175 mmol/kg) eight; carnosine (0.7 mmol/kg) eight; carnosine (1.4 mmol/kg) seven. Values are presented as means±SEM. *Significantly different from the control group ($P<0.05$).

Table 3
Effects of oral administration of carnosine on levels of monoamines and their metabolites in the hypothalamus in the forced swimming test

Carnosine (mmol/kg)	0	0.175	0.7	1.4	*F* value (3, 27)	*P* value
MHPG	362±16	362±17	338±17	317±12	1.792	0.172
NE	3182±144	3097±162	3280±135	2970±148	0.765	0.524
DOPAC	260±20	302±27	268±20	292±24	0.731	0.543
DA	1770±98	1953±118	1950±72	2014±117	1.067	0.38
5-HIAA	1049±32	1023±36	1020±38	991±23	0.494	0.69
HVA	65±4	72±4	66±2	76±3	2.369	0.093
5-HT	1245±79	1142±130	1299±107	1218±57	0.44	0.727

Values are means±SEM in pmol/g wet tissue. The numbers of rats used were: control eight; carnosine (0.175 mmol/kg) eight; carnosine (0.7 mmol/kg) eight; carnosine (1.4 mmol/kg) seven.

was significant ($F(3, 26)=5.644$, $P<0.005$). The immobility time of carnosine (0.7 mmol or 1.4 mmol/kg) group was significantly ($P<0.05$) shorter than that of control group.

Table 3 shows effects of oral administration of carnosine on levels of monoamines and their metabolites in the hypothalamus in the forced swimming test. There was no significant effect on MHPG, 5-HT, 5-HIAA, DA, DOPAC, HVA or NE level.

Table 4 gives effects of oral administration of carnosine on levels of monoamines and their metabolites in the hippocampus in the forced swimming test. The effect of carnosine on MHPG level was significant ($F(3, 27)=2.999$, $P<0.05$) and the value for the highest carnosine group was lowest. There was no significant effect on 5-HT, 5-HIAA, DA or NE level. HVA and DOPAC were not detected.

3.3. Experiment 3: effect of orally administered CBEX or carnosine on behaviors in the open field test

Fig. 3 shows the effects of orally administered CBEX (10 ml/kg) or carnosine (1.4 mmol/kg) on locomotive distance and the number of rearing incidents in the open field test. CBEX or carnosine did not significantly affect locomotive distance ($F(2, 21)=1.017$, $P=0.379$). The number of rearing incidents was fewer with carnosine or CBEX than with distilled water alone, but these differences were not significant ($F(2, 21)=2.584$, $P=0.099$).

3.4. Experiment 4: effect of orally administered CBEX on dipeptide levels in the plasma and brain

At the beginning of the experiment, each group comprised seven rats. However, data of two rats (one was in the initial group and the other was in the 240 min group) were eliminated because they were outlying ($P<0.05$) in anserine levels. Thus, in the results of plasma, the final number of rats in the initial group and 240 min group was six.

Table 4
Effects of oral administration of carnosine on levels of monoamines and their metabolites in the hippocampus in the forced swimming test

Carnosine (mmol/kg)	0	0.175	0.7	1.4	*F* value (3, 27)	*P* value
MHPG	375±14	375±10	351±13	331±10	2.999	<0.05
NE	489±37	516±27	546±43	490±32	0.579	0.634
DA	157±10	162±5	150±11	167±13	0.519	0.673
5-HIAA	925±50	928±34	887±34	932±22	0.319	0.811
5-HT	389±55	423±51	493±56	404±50	0.761	0.526

Values are means±SEM in pmol/g wet tissue. The numbers of rats used were: control eight; carnosine (0.175 mmol/kg) eight; carnosine (0.7 mmol/kg) eight; carnosine (1.4 mmol/kg) seven.

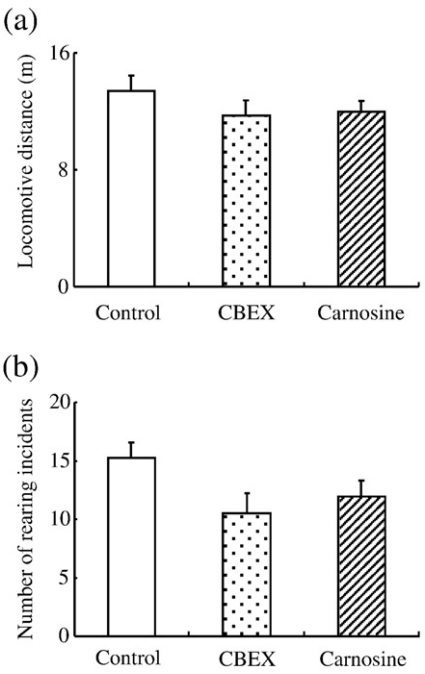

Fig. 3. Effects of oral administration of CBEX (10 ml/kg) or carnosine (1.4 mmol/kg) on (a) locomotive distance and (b) the number of rearing incidents in rats in the open field test in Experiment 3. The numbers of rats used were eight in all groups. Values are presented as means±SEM.

Fig. 4 shows effects of orally administered CBEX (10 ml/kg) on carnosine and anserine levels in the plasma. The effect on carnosine level was significant ($F(4, 28)=14.877$, $P<0.0001$). Carnosine level in 30, 60 or 120 min group was significantly increased compared to the

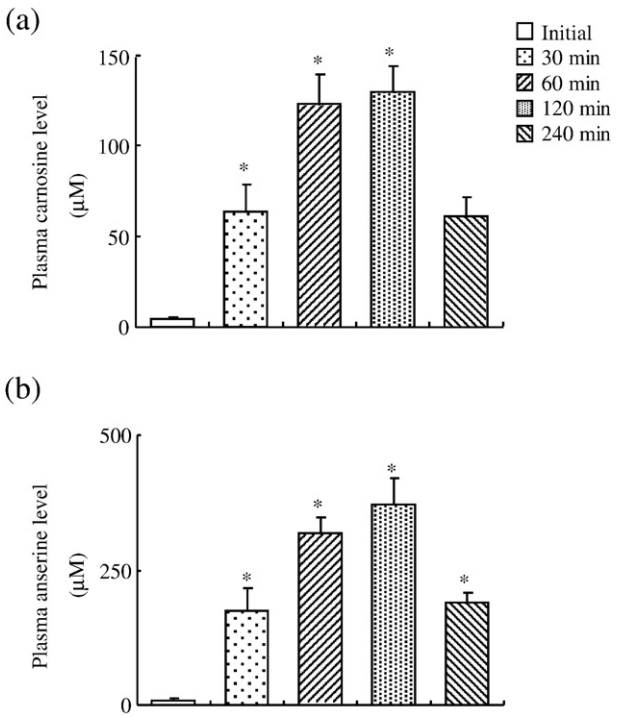

Fig. 4. Effects of oral administration of CBEX (10 ml/kg) on (a) carnosine and (b) anserine levels in the plasma of rats in Experiment 4. The numbers of rats used were: initial six; 30 min seven; 60 min seven; 120 min seven; 240 min six. Values are presented as means±SEM. *Significantly different from the initial group ($P<0.05$).

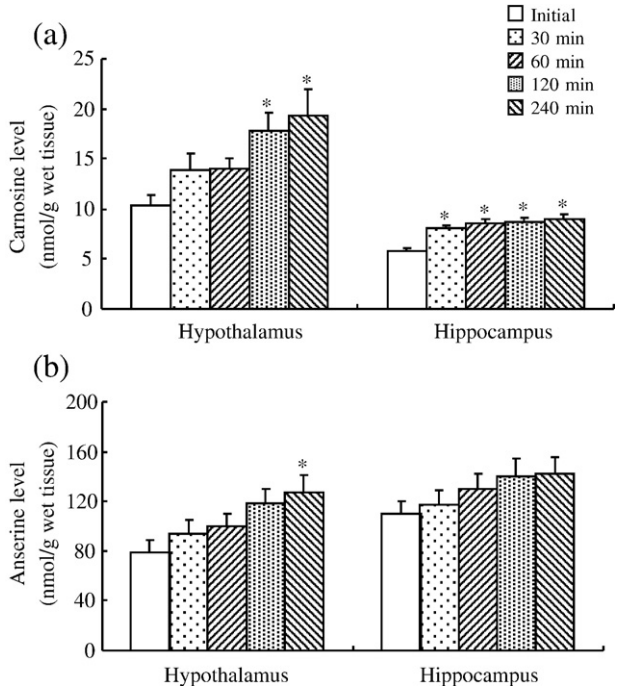

Fig. 5. Effects of oral administration of CBEX (10 ml/kg) on (a) carnosine and (b) anserine levels in the hypothalamus and hippocampus of rats in Experiment 4. The numbers of rats used were seven in all groups. Values are presented as means ± SEM. *Significantly different from the initial group in each brain region ($P<0.05$).

initial group ($P<0.05$). The effect on anserine level was also significant ($F(4, 28)=17.251$, $P<0.0001$). Anserine level in 30, 60, 120 or 240 min group was significantly increased compared to the initial group ($P<0.05$).

Fig. 5 shows effects of orally administered CBEX (10 ml/kg) on carnosine and anserine levels in the hypothalamus and hippocampus. Carnosine level was significantly increased in the hypothalamus ($F(4, 30)=4.278$, $P<0.001$) and hippocampus ($F(4, 30)=10.17$, $P<0.0001$). In the hypothalamus, carnosine level in 120 or 240 min group was significantly increased compared to the initial group ($P<0.05$). In the hippocampus, carnosine level in 30, 60, 120 or 240 min group was significantly increased compared to the initial group ($P<0.05$). Anserine level was also significantly increased in the hypothalamus ($F(4, 30)=2.96$, $P<0.05$) while there was no significant effect on anserine level in the hippocampus ($F(4, 30)=1.292$, $P=0.295$). In the hypothalamus, anserine level in 240 min group was significantly increased compared to the initial group ($P<0.05$).

4. Discussion

In the present study, CBEX tended to attenuate depressive-like behavior in the forced swimming test in rats. Carnosine, one of the major constituents of CBEX, significantly attenuated the behavior. The significant reduction was observed in the hypothalamus by CBEX treatment while the tendency was observed by carnosine treatment in the MHPG level. Furthermore, significant reductions of MHPG levels were observed in the hippocampus by both CBEX and carnosine treatments. MHPG is a major metabolite of NE in the mammalian brain. Its level in the brain is a reliable index of the central NE activity (Demet and Halaris, 1979). Therefore, CBEX and carnosine could reduce NE activity in the hypothalamus and hippocampus in the forced swimming test. In some stress conditions, brain MHPG levels have been shown to significantly increase and these increases could be treated as stress responses in norepinephrinergic neurons in the brain (Demet and Halaris, 1979). Accordingly, CBEX and carnosine may have the ability to attenuate stress induced by forced swimming. Because forced swim episode increased MHPG levels in the hypothalamus and hippocampus of rats (Miyauchi et al., 1981), the increase might be also observed in the present conditions. Therefore, to clarify central effects of CBEX and carnosine further, to investigate whether swim episode affect levels of MHPG, NE, other monoamines and their metabolites and whether their changes (if it is confirmed) were reversed by CBEX and carnosine may be effective. No significant effects on behaviors in the open field test were observed following treatment with CBEX or carnosine. These findings suggest that the antidepressant-like effects of both treatments were not merely due to stimulation of general motor activity. These results indicate that the effects of CBEX during this study were at least partly due to the effects of one of its major components, carnosine.

The neurotransmitter 5-HT has been given much attention as a treatment for depression. Neither CBEX nor carnosine modified brain 5-HT and its major metabolite 5-HIAA. However, in our preliminary experiment using the microdialysis technique, we demonstrated that 5-HIAA levels in the hypothalamus increased following oral administration of CBEX, suggesting that CBEX could stimulate 5-HT neurons (Tomonaga et al., unpublished data). Thus, further studies are necessary to clarify the mechanism by which CBEX and carnosine induce antidepressant-like effects.

The most effective dose of CBEX in the present study was 10 ml/kg, which has a carnosine concentration of approximately 1.4 mmol/10 ml (Tomonaga et al., 2007). Since the carnosine concentrations were identical with the most effective doses of both CBEX and carnosine treatments, it appears that carnosine is primarily involved in the antidepressant-like effect of CBEX. However, it remains possible that other substances are involved in the antidepressant-like effect of CBEX. In the present study, orally administered CBEX significantly increased not only carnosine level but also anserine level in the hypothalamus. Because these dipeptide levels also increased in the plasma, there remains a possibility that increases in the brain dipeptides reflected the increases in the brain vasculature, rather than in the parenchyma. However, 240 min after injection, these dipeptide levels in the plasma were decreased compared to those 120 min after injection while these dipeptide levels in the brain were still increased at the same time. Therefore, we speculated that these dipeptides could be at least in part absorbed into brain parenchyma. Further study was needed to clarify how much dipeptides could be absorbed into the brain parenchyma in the present conditions. We previously hypothesized that orally administered CBEX (20 ml/kg) has NO-generative action in the brain, possibly via an increase in carnosine and/or anserine (Tomonaga et al., 2007). Another previous report suggests that NO-generative effects have antidepressant-like properties in the forced swimming test in mice (Inan et al., 2004). Thus, the antidepressant-like effects of CBEX may be related to the NO-generative action induced by an increase in carnosine and/or anserine in the brain. Because carnosinase hydrolyzes carnosine and anserine to their constituent amino acids in the rat brain (Kunze et al., 1986), carnosine and anserine as well as the constituents derived from their degradation may influence the antidepressant-like effect of CBEX. I.p. injection of histidine, one of the constituents of carnosine, has an antidepressant-like effect through the stimulation of histaminergic neurons in mice (Lamberti et al., 1998). Therefore, histidine derived from the degradation of carnosine may contribute to the antidepressant-like effects of CBEX and carnosine.

In conclusion, CBEX had an antidepressant-like effect in the forced swimming test in rats. This effect may be due, in part, to one of its major components, carnosine. The possibility also remains that anserine, another major component of CBEX, influences the antidepressant-like action. Further study focusing on carnosine, anserine, and their constituents not only in rats but also in humans should be performed to evaluate the actions and mechanisms of supplemental CBEX in the brain.

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