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Up-Regulation of A-Kinase Anchoring Protein in the Nucleus Tractus of Solitarius of Rats by Intraperitoneal Lithium

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Abstract: We have examined the proteome patterns of the Nucleus Tractus of Solitarius (NTS) of rats shortly after an intraperitoneal injection of lithium chloride at a conventional dose widely used as unconditioned stimulus inducing the formation of conditioned taste aversion. The intraperitoneal lithium induced an up-regulation of A-Kinase Anchoring Protein (AKAP), which binds to cAMP-dependent protein kinase A (PKA) and regulates the phosphorylation of various proteins implicated in synaptic plasticity and memory consolidation. Expressions of cAMP/PKA signaling related genes and proteins in the NTS were analyzed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Western blot and phosphorylation of cAMP response element-binding protein (CREB) was increased in the brainstem NTS by the intraperitoneal lithium. These results suggest that up-regulation of AKAP and CREB activation may be involved in the lithium-induced c-fos expression in the rat NTS. In addition, tentative implications of tight junction protein, cadherin protein and acetyl-Co A carboxylase in the lithium-induced synaptic plasticity and memory formation are discussed.

Key words: Lithium, nucleus tractus of solitarius, proteomics, A-kinase anchoring protein, cAMP response element-binding protein

INTRODUCTION

Lithium chloride induces c-Fos expression in the brain regions involved in Conditioned Taste Aversion (CTA) learning such as the hypothalamic paraventricular nucleus, the Nucleus Tractus of Solitarius (NTS) and the central nucleus of amygdale (Houpt *et al*., 1994; Koh and Bernstein, 2005; Sakai and Yamamoto, 1997; Schafe and Bernstein, 1996; Yamamoto *et al*., 1992). Induction of c-Fos expression implicates that numerous genes are going to be expressed in the c-Fos expressing neurons (Lamprecht and Dudai, 1995; Schafe *et al*., 1995; Swank *et al*., 1996). Dimerization of c-Fos with Jun family is necessary to enable transcriptional efficacy at the AP-1 sequence of putative target gene promoter regions (Foletta, 1996; Karin *et al*., 1997). Swank (1999) has demonstrated a coordinate up-regulation of c-Fos, FosB and JunB by intraperitoneal LiCl in a majority of cells of NTS, parabrachial nucleus and central nucleus of amygdale regions. Although, the increase of c-Fos immunoreactivity by Conditioned stimulus (CS) Unconditioned stimulus (US) pairing in lateral and medial parabrachial nucleus and intermediate NTS regions has been reported (Berman and Dudai, 2001; Koh and Bernstein, 2005), little is known about the intercellular signaling pathways in the brainstem that regulate c-Fos expression during CTA. It has been reported that infusions of the Mitogen-Activated Protein (MAP) kinase inhibitor or antisense oligonucleotides to c-Fos into the fourth ventricle of mice potently blocks CTA acquisition (Swank *et al*., 1996; Swank, 2000) suggesting the implication of MAP kinase activity in c-Fos induction by CS-US pairing.

In order to identify the intercellular signaling pathways implicated in the lithium-induced CTA acquisition, we examined lithium-induced proteome patterns in the NTS region, where the gustatory information is firstly relayed to the brain during CTA acquisition. Peptide sequences of proteome spots were analyzed by MALDI-TOF peptide analysis system. Also, the lithium-induced expression of cAMP/PKA signaling related genes and proteins in the NTS were analyzed by RT-PCR and western blot, since up-regulation of A-Kinase Anchoring Protein (AKAP) was observed in the proteome pattern of lithium treated NTS.

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MATERIALS AND METHODS

Animals: Male Sprague-Dawley rats (200-250 g) were purchased (Orient, Co. Korea) and acclimated to the laboratory condition in a specific-pathogen-free barrier area where the temperature (22±1°C) and humidity (55%) were controlled constantly with a 12/12 h light/dark cycle (lights-on at 07:00 AM). Rats had *ad libitum* access to standard laboratory food (Purina Rodent Chow, Purina Co., Seoul, Korea) and membrane filtered purified water. Rats were cared according to the Guideline for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guidelines for the Care and Use of Laboratory Animals, revised 1996.

Drug treatment and tissue preparation: Lithium chloride (LiCl, Sigma Co., MO, USA) was dissolved in distilled water at a concentration of 0.15 M and autoclaved. Rats (300-350 g) received intraperitoneal injection of 0.15 M LiCl or aseptic saline (0.15 M NaCl) at a dose of 12 mL kg^{-1}. We previously showed that intraperitoneal LiCl at this dose induces c-Fos expression in the NTS of rats (Jahng *et al*., 2004). One hour after the injections, rats were briefly anesthetized by carbon dioxide gas and decapitated once unresponsive. Brains were removed immediately and NTS region was rapidly dissected on ice and frozen in liquid nitrogen until used.

Two-dimensional gel electrophoresis: NTS tissues were homogenized in R/S buffer {9 M Urea, 2% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate, 50 mM dithiothreitol (DTT), 0.4% ampholyte} containing protease and phosphatase inhibitor cocktail 0.5% and then ultracentrifuged at 100,000 g for 1 h at 20°C. Protein contents of the supernatants were determined using a protein assay kit (BioRad, Hercules, CA, USA) and then the protein samples were prepared at 1 mg/350 μL concentration with R/S buffer, stored at -80°C. One milli grams of protein samples was loaded on an immobilized pH gradient (IPG) strip (pH5-8) in PROTEAN IEF CELL (BioRad, Hercules, CA, USA) and the strip was covered with mineral oil and then rehydrated at 50 V for 12 h. Moist wicks were placed at both ends of the strip to avoid salt contamination while the protein samples were separated by their iso-electric points (IP) at 10 KV for 15 h. Then the IPG strip was soaked in equilibration buffer (375 mM Tris, 6 M Urea, 2% sodium dodecyl sulfate (SDS), 20% glycerol, 2.5% iodoacetamide) for 10 min and then the protein samples in the strip, which were separated by IP, were separated again on a 2-dimensional SDS-polyacrylamide gel (2D-PAG) [4.0 mL of 30% acrylamide/Bis, 2.5 mL of 1.5 M Tris-HCl (pH 8.8), 0.1 mL of 10% SDS (w/v), 50 μL of 10% ammonium persulfate, 5 μL of N,N,N'-tetramethylethylenediamine in 10 mL of 12% 2D-PAG] by their molecular weights at 20 mA/gel for 16 h. Protein spots on 2D-gel were stained with coomassie brilliant blue for 24-48 h (Matsui *et al*., 1999) de-stained in double distilled water and then the image of stained protein spots was analyzed using PDQuest software (BioRad, Hercules, CA, USA). The protein spots of lithium-treated samples that showed an increased staining density, at least, up to two fold compared with their saline controls were selected for further assay.

Digestion of proteins: Selected protein spots were individually excised into new tubes and the coomassie blue staining was destained with 50% acetonitrile/25 mM ammonium bicarbonate. Gel slices containing protein spots were treated with 10 mM DTT at 56°C for 30 min and with 55 mM iodoacetamide at room temperature for 25 min avoiding light source and then digested with trypsin at 37°C for 16 h with shaking. After pooling the supernatant into new tubes, peptide samples were dried completely using SpeedVac system for 4 h. Samples were dissolved in 50% acetonitrile/0.1% trifluoroacetic acid (TFA) and co-crystallized by mixing with matrix (α-cyano-4-hydroxy-cinnamic acid saturated with 0.1% TFA/50% acetonitrile) and loaded on the selicon-coated 96 well microtiter sample plate (Applied Biosystems, Foster City, USA).

Mass spectrometry and database searching: Peptides masses were analyzed using Voyager DE Matrix assisted laser desorption ionization-Time of flight mass spectrometry (MALDI-TOF) mass spectrometer (Applied Biosystems, Foster City, USA). Mass spectra were obtained by averaging 100 to 150 individual laser shots. Calibration of spectra was performed externally by two standard peptide, angiotensin 1 (m/z 1296.6853) and adrenocorticotrophic hormone (18-39 clip) (m/z 2465.1989). A database search for protein sequence for homology was performed using MS-Fit search algorithm (http://prospector.ucsf.edu/prospector/4.0.8/html/msfit.htm). Mass tolerance for the monoisotopic peptide masses were set to 50 ppm.

Proteomic analysis; i.e., two-dimensional gel electrophoresis and MALDI-TOF analysis, was carried out twice with different samples in each treatment group in order to confirm the peptide profiles analyzed.

Reverse transcription-polymerase chain reaction (RT-PCR): NTS tissue samples were collected at 1 h after

an intraperitoneal injection of LiCl. Total RNA was extracted from rat NTS tissues with TRIzol reagent(Invitrogen, Carsbad, California, USA) according to the manufacturer's instructions (n = 3). The yield of total RNA was determined by measuring the absorption at 260 nm. Reverse transcription of 1 μg of total RNA was performed in final volume of 20 μL using 200 μ of Superscript II reverse transcriptase, 40 μ of RNaseOUT, 0.5 μg oligo dT_{12-18} as a primer, 0.5 mM dNTP Mix, 10 mM DTT and First strand buffer (Invitrogen, Carsbad, California, USA). The mixture of oligo dT_{12-18} primer, dNTP mix, total RNA and dep C treated ddH_2O was heated at 70°C for 10 min first. Then, other components were added and incubated at 42°C for 1 h. Subsequent incubation at 70°C for 15 min was used to inactivate the reverse transcriptase.

To determine the relative levels of mRNAs, polymerase chain reaction of cDNA was carried out in a 20 μL mixture containing 1 μL cDNA, 10X reaction buffer, 2.5 mM dNTP mix, 6 pmol of each 5' and 3' primers and 1U of G-Taq DNA polymerase (COSMOGenetech, Seoul, Korea). The oligonucleotide-specific primer pairs for gene identification were designed based on Gen-Bank accession numbers BC087025 for STAT-3, X60002 for CREB and BC059110 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR specific primer sets used for the experiments were used as follows: for STAT-3 (forward: AAGGACATCAGTGGCAAG; reverse: ACAGGCGGACAGAACATAG), for CREB (forward: GTGACTAGATGACCATGGACTC; reverse: CTACGACATTCTCTTGCTGCTTCC), for GAPDH (forward: TGAAGGTCGGTGTGAACGGATTTGGC; reverse: CATGTAGGCCATGAGGTCCACCAC).

The reactions were started at 94°C for 5 min and amplified for 30 cycles of 45 sec at 94°C, 45 sec at 58°C and 1 min at 72°C. Final extension were continued for 10 min at 72°C to compete polymerization. The GAPDH was used an internal control to confirm equal loading of the samples. The PCR products were separated on 2% agarose gel and analyzed by a digital image analysis system.

Western blot: For the Western blot analysis, NTS tissue samples were collected one hour after the lithium i.p., homogenized in a single detergent lysis buffer (50 mM Tris, pH 8.0; 150 mM NaCl; 1% Triton X-100; protease and phosphatase inhibitor cocktail 0.5%) and then centrifuged at 13,000 g for 20 min at 4°C. The supernatants transferred into new tubes were measured for protein contents using a protein assay kit (BioRad, Hercules, CA, USA) aliquoted at an 80 μg/20 μL concentration in lysis buffer and stored at -80°C, otherwise used in the same day. The samples were mixed with loading buffer (100 mM Tris, pH 6.8; 200 mM DTT; 4% SDS; 20% glycerol; 0.2% bromophenol blue) at 1:1 dilution, boiled for 5 min, quickly chilled on ice and then electrophoresed on 12% SDS-polyacrylamide Tris-glycine gels. The proteins transferred onto nitrocellulose membranes (Hybond-C, Amersham, Bucks, UK) were treated with 5% nonfat dry milk in 1X phosphate buffered saline-Tween (PBST) (1.46 mM $NaH_2PO_4H_2O$; 8.05 mM Na_2HPO_4; 144.72 mM NaCl; 5% Tween 20) overnight at 4°C. The membranes were reacted with rabbit anti-pERK, anti-pSTAT 3 (Cell Signaling technology, Beverly, MA, USA) or anti-pCREB antibodies (Upstate biotechnology an Argonex Company, Lake Plasid, NY, USA) at 1:1000 dilution for 1 h and then reacted with HRP-conjugated goat anti-rabbit antibodies (Zymed Laboratories Inc., CA, USA) at 1:5000 dilution for 1 h at room temperature, respectively. The bound antibodies were detected with chemiluminescence according to the manufacturer's instructions (NEN Life Science Products, Inc., MA, USA) and quantified using a video densitometer (using Adobe PHOTOSHOP and MCID system).

RESULTS

Protein spots on the 2D gels of the NTS tissue samples collected 1 h after a single injection of lithium chloride or saline were stained with coomassie blue and the staining densities of the spots were analyzed with PDQuest software. Fourteen protein spots on the lithium gels, exhibiting increased staining densities, at least, two-fold higher than the saline controls, were selected for MALDI-TOF assay (Fig. 1a, b). Identified peptide sequences of the protein spots and its percent sequence coverage, the ratio of the portion of peptide sequence covered by matched protein to the whole length of peptide sequence of the spot, were as shown in Table 1. A-Kinase Anchoring Protein (AKAP) was identified from spot 4 (MW 25 kDa) with 57% of sequence coverage. Tight junction proteins were identified from spot 1 (MW 40 kDa) and spot 12 (MW 85 kDa) with 25 and 12% of sequence coverage, respectively. Cadherin protein was identified from spot 2 (MW 35) with 26% of sequence coverage. Enzymes such as DNA polymerases, acetyl-coenzyme A carboxylase β, proline 4-hydroxylase were identified from spot 3 (MW 26 kDa) and spot 14 (MW 100 kDa), spot 8 (MW 75 kDa) and spot 10 (MW 45 kDa) with 23, 4, 6 and 15% of sequence coverage, respectively. Tuberin-like protein 1 was identified from spot 5 (MW 32 kDa) with 29% of sequence coverage.

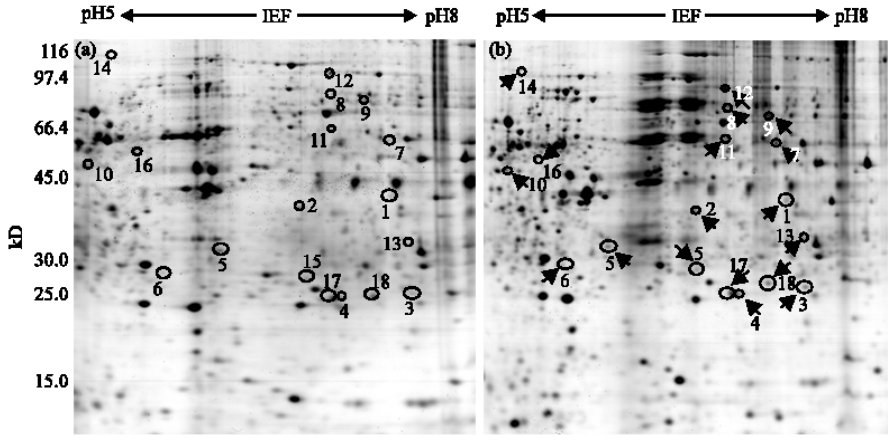

Fig. 1: The proteome patterns of the rat nucleus tractus of solitarius (NTS) 1 h after lithium treatment. (a) Saline-controlled NTS tissue; and (b) Lithium-treated NTS tissue. Fourteen protein spots on the lithium gel, exhibited two-fold higher staining densities than the saline control and four spots appeared two-fold lower staining density, were marked as circles and arrows. Numbered spots were excised from the lithium gels, in-gel digested with trypsin and identified by MALDI-TOF assay. The results are listed in Table 1

Table 1: Selected 18 protein spots were individually analyzed. The spot number (Spot No.) corresponds to the position marked on the gel in Fig. 1

			MW (kDa)		pI[2]			
Spot No.	Identified proteins	Accession No.	D[3]	E4	D	E	Sequence[5] coverage (%)	Density
1	Tight junction protein 2	NP_0552	105.0	40	6.0	7.2	25	Increase
2	Cadherin 23	AAK0767	366.6	35	4.5	6.8	26	Increase
3	DNA polymerase	BAA9076	355.1	26	9.0	7.3	23	Increase
4	A-kinase anchoring	AAD2276	417.1	25	5.0	6.9	57	Increase
5	Tuberin-like protein 1	NP_0644	84.73	32	5.6	6.1	29	Increase
6	Hypothetical protein	XP_1518	10.01	30	5.8	5.8	25	Increase
7	Ig heavy chain V region	AAC4883	12.74	55	7.9	7.1	44	Increase
8	Acetyl-coenzyme A	NM_0010	282.2	75	6.4	6.8	6	Increase
9	Similar to KIAA1662	XP_1394	111.6	73	7.6	7.0	12	Increase
10	Proline 4-hydroxylase	NM_0009	57.46	45	4.8	5.2	15	Increase
11	PDE10A3	BAA8899	90.81	58	6.1	6.8	13	Increase
12	Tight junction protein 3	NP_0552	105.0	85	6.0	6.8	12	Increase
13	Ig heavy chain V region	AAF0454	14.38	33	9.1	7.4	16	Increase
14	DNA polymerase	BAA9076	355.1	10	9.0	5.3	4	Increase
15	MHC protein-cotton-top	I68913	9.24	28	9.3	6.7	46	Decrease
16	DOC2/DAB2 interactive	NP_6197	111.1	50	6.7	5.5	8	Decrease
17	Ig kappa chain V region	AAA3875	10.27	25	5.5	6.8	24	Decrease
18	Similar to unknown	AAH2755	12.44	28	5.8	7.1	23	Decrease

Identified proteins and accession numbers were derived from the MS-Fit search algorithm. MW (kDa); Molecular weight of protein pI: Isoelectric point, D: The value obtained from database, E: The value estimated from spots on the SDS gel. Sequence coverage; the ratio of the portion of peptide sequence covered by matched protein to the whole length of peptide sequence of the spot

Immunoglobulin proteins were identified from spot 7 (MW 55 kDa), spot 13 (MW 33 kDa) and spot 17 (MW 25 kDa) with 44, 16 and 24% of sequence coverage. Additionally, four unknown proteins, hypothetical protein, KIAA1662 protein, PDE10A3 and unknown protein were identified from spot 6, spot 9, spot 11 and spot 18.

AKAP that identified from spot 4 is a family of scaffolding proteins interacting with cAMP-dependent protein kinase A (PKA); thus, the lithium-induced expression of cAMP/PKA signaling related genes in the NTS was examined by using RT-PCR and Western blot techniques (Fig. 2a, b). The mRNA expressions of cAMP response element-binding protein (CREB) and signal transducer and activator of transcription-3 (STAT3) in the lithium-injected NTS did not significantly differ from ones in the saline-injected NTS (Fig. 2a). Phosphorylated CREB (pCREB), but neither phosphorylated STAT3 (pSTAT3) nor phosphoylated extracellular signal-regulated kinase 1/2 (pERK1/2), was increased in the NTS at 1 h after the intraperitoneal lithium injection (Fig. 2b).

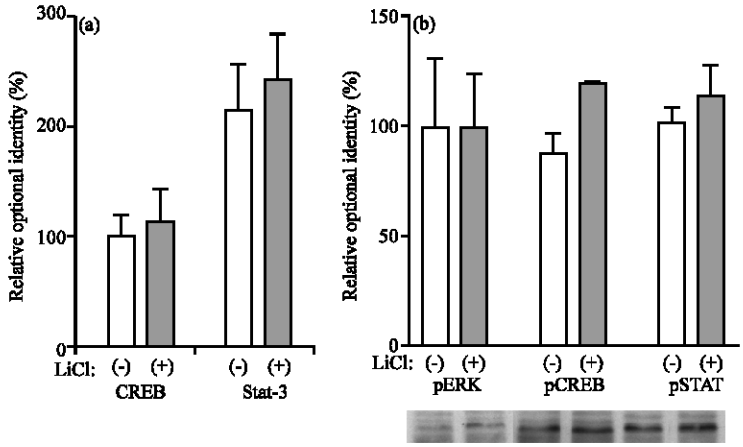

Fig. 2: Lithium-induced mRNA expressions of CREB and STAT-3 (a) and protein expressions of pERK, pCREB and pSTAT-3 (b) in the rat NTS determined by RT-PCR or western blot, respectively. Samples were collected at 1 h after an intraperitoneal injection with 12 mL kg^{-1} of 0.15 M lithium chloride or saline

DISCUSSION

Lithium dose used in this study (0.15 M, 12 mL kg^{-1}) was reported to induce c-Fos expression in the brainstem including NTS and parabrachial nucleus (Houpt *et al*., 1994; Jahng *et al*., 2004; Sakai and Yamamoto, 1997). The induction of immediate early gene c-fos by LiCl increases intracellular cAMP and activates cAMP-dependent PKA, which is widely implicated in synaptic plasticity and memory (Koh *et al*., 2003; Schafe and LeDoux, 2000). Four protein kinase cascades; i.e., Ca^{2+}/phospholipid-dependent protein kinase, Ca^{2+}/calmoduline-dependent protein kinase II, PKA and ERK, are known to serve an important role in the formation of long-term potentiation. Especially, PKA has been implicated in the protein-synthesis-dependent changes underlying the memory consolidation during CTA learning (Selcher *et al*., 2002). The CTA is well known to be a robust and long-lasting learning and memory established by a single pairing of gustatory conditioned stimulus and visceral malaise caused by US (Garcia *et al*., 1974). In this study, expression of AKAP was increased in the rat NTS by an intraperitoneal injection of lithium at a high dose, conventional US widely used. AKAP is a family of scaffolding protein that is consisted of two conserved structural modules: a targeting domain that serves as a scaffold and membrane anchor, a tethering domain that interacts with PKA (Newlon *et al*., 2001). Several researches have reported that AKAP binding to PKA regulates the phosphorylation of various proteins, some of which have been implicated in synaptic plasticity and memory consolidation (Colledge *et al*., 2000; Rosenmund *et al*., 1994; Westphal *et al*., 1999) and that inhibition of AKAP or PKA anchoring to AKAP impairs memory consolidation (Nijholt *et al*., 2008; Tunquist *et al*., 2008). It has been shown that AKAP is expressed in the brain regions involved in learning and memory (Ostroveanu *et al*., 2007). The brainstem NTS is where the gustatory information including visceral malaise is firstly relayed to brain. Taken all together, it is suggested that intraperitoneal lithium, as US, may trigger the interaction between AKAP and PKA in the brainstem NTS during CTA memory formation.

Previous study has demonstrated that infusion of a highly selective MAP kinase inhibitor into fourth ventricle blocked the acquisition of CTA learning by lithium (Swank, 2000), suggesting that lithium may produce a rapid and robust phosphorylation of MAP kinase in the brainstem. In the present results, CREB phosphorylation appeared to be increased in the NTS by 1 h of lithium. The CREB phosphorylation is likely accompanied with phosphorylation of ERK1/2 (MAP kinase); however, the activation of ERK1/2 in the NTS by lithium has been hardly reported and not observed in this study as well. The CREB activation results in the expression of many genes including other transcription factors such as c-Fos and intraperitoneal injections of lithium at high doses induce c-Fos expression in the brainstem NTS (Benbrook and Jones, 1990; Houpt *et al*., 1994; Jahng *et al*., 2004; Sakai and Yamamoto, 1997). Thus, the present result suggests that lithium-induced c-Fos expression in the NTS may be mediated by CREB activation.

In this study, we have demonstrated that an intraperitoneal lithium injection increases tight junction proteins in the rat NTS region. Tight junction proteins forming the major paracellular barrier in epithelial and

endothelial cells play a role to limit the movement of molecules through the intercellular space in epithelial and endothelial cells. It has been reported that the loss of tight junctions causes an impairment of the blood-brain barrier and deterioration of blood-retinal barrier function (Bolton *et al*., 1998; Chan-Ling *et al*., 2007; Sandoval and Witt, 2008). A recent study has demonstrated a reduced immunoreactivity of the tight junction protein in the brain of aged rats that showed a significant impairment in short-term memory (Chan-Ling *et al*., 2007), suggesting that tight junction proteins may be implicated in memory. Thus, the present result may support a tentative implication of tight junction protein in memory formation.

In this study, increased expression of cadherin protein was observed in the lithium-treated NTS. Classic type I cadherin, such as neuronal (N)-cadherin, plays an important role in the developmental organization of the brain and synapse formation in the adult central nervous system (Junghans *et al*., 2005; Redies, 2000). Although, a role of cadherins in memory is controversial yet, a role of N-cadherin in synaptic potentiation (Murase and Schuman, 1999) and a significant impairment of hippocampal long-term potentiation by blocking the dimerization of type I cadherin (Tang *et al*., 1998) have been reported. Also, increased synthesis of N-cadherin and its recruitment to newly formed synapses were observed during long-term potentiation (Bozdagi *et al*., 2000). Recent studies have suggested that cadherin may be implicated in the modulation of synaptic connectivity and activity (Salinas and Price, 2005; Takeichi and Abe, 2005). Taken together, it is suggested that cadherin expression may be involved in the synaptic modulation during lithium-induced CTA formation.

Acetyl-CoA Carboxylase (ACC) is the key regulatory enzyme of fatty acids biosynthesis. It catalyzes the formation of malonyl-CoA, the intermediate in the fatty acid synthetic pathway and a regulator of mitochondrial long-chain fatty acyl-CoA uptake (Beaty and Lane, 1985; Obici *et al*., 2003). A recent study has reported that inhibition of hypothalamic ACC blocks leptin-induced decreases of food intake, body weight and the hypothalamic neuropeptide Y (Gao *et al*., 2007), suggesting that the hypothalamic ACC activation may contribute to the anorectic effects of leptin. Systemic administration of LiCl not only produces CTA, but also suppresses food intake (Curtis *et al*., 1994; Ritter *et al*., 1980). It has been suggested that glucagon-like peptide-1, an anorectic peptide, may mediate the lithium-induced anorexia (Rinaman, 1999) and c-Fos expression in the NTS (Thiele *et al*., 1998). The brainstem NTS is generally assumed to respond to short-term signals that regulate meal initiation and termination (Ellacott and Cone, 2004). In this study, ACC peptide expression was increased in the NTS shortly after the systemic lithium treatment. Thus, it is hypothesized that ACC peptide expression in NTS may be involved in the lithium-induced anorectic pathway, perhaps, as a signaling molecule for meal termination.

We did not discuss the rest peptides found to be increased or decreased by lithium treatment, because some of them were unknown with their function in general and the others hard to draw a tentative implication in lithium's action in the boundary of our current knowledge.

Lithium chloride is usually administrated systemically in both clinical and experimental treatments. The acute or chronic effects of systemic lithium are generally assumed to result from a direct action of lithium on the central nervous system. For many effects, however, the site of lithium's action is unknown and the molecular base of lithium's action has been poorly understood, despite the long history of lithium usage both for therapeutic and toxic purposes. Although, the present study is limited to the acute effect of lithium at high dose, the proteomic analysis introduced would provide, at least, new insight to understand the molecular mechanism of lithium's action.

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