Possible involvement of integrin signaling pathway in the process of recovery from restraint stress in rats

Yu-Zhen GAO^{1,2,3}, Shi-Yu GUO^{1,2,3}, Qi-Zhang YIN^{1,2}, Xiang-Qin CUI⁴, Tadashi HISAMITSU³, Xing-Hong JIANG^{1,2}

¹Department of Neurobiology, ²Laboratory of Aging and Nervous Diseases, School of Medicine, Soochow University, Suzhou 215123, China

³Department of Physiology, School of Medicine, Showa University, Tokyo 142, Japan

⁴Department of Biostatistics, University of Alabama at Birmingham, Alabama 35294-0022, USA

Abstract: Objective To search novel genes or pathways involved in the recovery process after restraint stress in rats.

Methods We compared the hypothalamus transcriptional profiles of two different recovery patterns (fast recovery vs slow recovery) from restraint stress in rats using oligonucleotide microarray, the recovery pattern was determined by the decrement of plasma adrenocorticotrophic-hormone (ACTH) and corticosterone levels during one hour recovery period after stress. A real-time quantitative RT-PCR was applied to validate the differential expressed genes. **Results** Analysis of the microarray data showed that most of genes were not differentially expressed between fast recovery group and slow recovery group. Among the differentially expressed genes we found that talin, together with serine/threonine protein phosphatase PP1-beta catalytic subunit (PP-1B) and integrin α -6 precursor (VLA-6) genes, were at least 1.5 fold up-regulated in the fast recovery group, while junctional adhesion molecule 1 (*F11r*) was 1.5 fold down-regulated in the fast recovery group. **Conclusion** The results implied that integrin signaling pathway may be involved in the recovery from restraint stress in rats. The present study provided a global overview of hypothalamus transcriptional profiles during the process of recovery from the restraint stress in rats. The integrin signaling pathway seems to be involved in the recovery process, which deserves further study to clarify the integrin-mediated recovery mechanism after restraint stress.

Keywords: physical restraint; adrenocorticotrophic hormone; corticosterone; post-stress recovery; oligonucleotide microarray

1 Introduction

Although large inter-individual differences in stress response are found in many animal or human population, the detailed mechanism is poorly understood^[1,2]. These differences are critical as regards each individual's adaptive capacity, not only for adequate responses to challenging situations but also for variability in stress-induced susceptibility^[3,4]. Understanding the causes of individual differ-

ences and their consequences in terms of adaptive capacity and vulnerability to disease is a major challenge for modern life sciences. To date, the neurobiological bases of individual differences in rodents have been mainly focused on the hypothalamic-pituitary-adrenal (HPA) axis, one of the central components of the body's neuroendocrine response to stress^[5]. Although most studies on HPA response to stress have focused on the immediate response to the stressful situation, the importance of the capability of organisms recovering normal HPA function and so reducing the possible pathological impact of exposure to stressful situations is now well-recognized^[6]. The differential pattern of HPA recovery during the post-stress period might be important to explain individual differences in the pathological consequences of exposure to stressors^[7]. However, there are only a few studies specifically aiming at characterizing the individual differences in the recovery pattern in

Corresponding author: Xing-Hong JIANG

Tel: 86-512-65880126

E-mail: jiangxinghong@suda.edu.cn

Tadashi HISAMITSU

Tel: 81-3-37848110

Fax: 81-3-37845368

E-mail: tadashi@med.showa-u.ac.jp

Article ID: 1673-7067 (2007)04-0229-07

CLC number: R335; R338.2

Document code: A

Received date: 2007-03-05

rats^[7,8].

Despite some studies on stress response^[9,10], there has been no data known to screen and discover novel genes related to recovery speed after stress. As the hypothalamus is the center of stress response, as well as a region that integrates different stress pathways in the brain, comprehensive analysis of gene expression profile in the hypothalamus of restraint-stressed rats by using oligonucleotide microarray will be one of the most efficient approaches to identify the core genes that modulate recovery speed after stress. In this study, to search the novel genes that were up- or down-regulated in different recovery patterns in rat hypothalamus, the transcriptional activity of the genes expressed in rat hypothalamus was quantitatively analyzed by oligonucleotide microarray.

2 Materials and methods

2.1 Animals Fourty Adult outbred male Sprague-Dawley rats, weighing (280±10) g, were supplied by the Experimental Animal Center of Showa University, Japan. Animals were housed two per cage under standard conditions of temperature [(22±1)°C] and maintained on a 12/12 h light-dark schedule (lights on at 07:00 a.m.) with *ad libitum* access to food and water. In all experiments, rats were petted briefly for 3-5 min on each of two days prior to experimentation. Animals were allowed to acclimatize to the housing conditions at least one week before the experiments (which started in the morning).

2.2 Restraint stress and blood sampling For restraint stress (RES), 30 rats were placed in a plexiglass tube (4.5 cm diameter, 13 cm length) for 2 h. Rats were prevented from turning around by an adjustable plastic plug that was secured behind the rat once it was positioned in the tube. Holes were drilled in the sides and end of the plexiglass tube to allow fresh air in. Food and water were not provided during the restraint period. After the RES experiment, rats were released from the tube and returned to the original cage for recovery. Blood samples (300 µL) were taken at three time points: within 2 min just before the exposure to RES, the termination of the RES, and 1 h after the RES, by using tail-nick method which did not confound the profile of plasma ACTH and corticosterone (CORT)^[11]. After the last blood sample being taken, rats were immediately killed by decapitation within 2 min. The control rats without suffering the restraint stress ($n = 10$) were sampled following the same schedule with RES rats. The experimental proce-

dures were approved by the Ethical Committee for Animal Experimentation of Showa University.

2.3 Tissue preparation Following decapitation, the brains were removed and dissected as previously described^[12]. Briefly, a series of six cuts were performed using a razor blade. For viewing the ventral surface of the brain, two coronal cuts were made to isolate a hypothalamic block using the apex of the optic chiasm and the rostral margin of the mammillary bodies as landmarks. This slab was then placed flat and the first two cuts were placed on either side of the chiasm. The third cut was placed just dorsal to the third ventricle. The isolated hypothalamus was then immersed in RNAlater (Ambion Inc.) and stored at 4 °C until use.

Total RNA from the hypothalamus was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. RNA was quantified with the Nanodrop ND-1000 spectrophotometer (Nano-Drop Technologies, Rockland, DE, USA), and RNA integrity was assessed with RNA 6000 Nano LabChip kit by using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Only RNA with an RNA integrity number (RIN) higher than 9.6 was processed for labeling (2100 Expert software, Agilent Technologies).

2.4 Biochemical assays and grouping Plasma CORT was measured by Corticosterone Colorimetric (EIA) Kits (Assay Designs Inc., USA). Plasma ACTH concentration was measured with a conventional radioimmunoassay (RIA) kit supplied by SRL Inc., Tokyo, Japan. The fast recovery group (FR) and slow recovery group (SR) were determined by the decrement of plasma CORT and ACTH during the one hour recovery period after the stress. Each group contains 6 rats for further microarray analysis, and the rest of animals were discarded.

2.5 Microarray hybridization and scanning A half dye-swap design was applied so that half of the slow-to-fast recovery comparisons were cyanine-3 (Cy3) to (cyanine-5) Cy5 and the other half were Cy5 to Cy3. This design has been shown to be a good and efficient strategy^[13].

RNA samples were labeled by using the Agilent Low RNA Input Fluorescent Linear Amplification kit (5184-3523) according to the manufacturer's instructions. Briefly, hybridized targets consisting of amplified and fluorescent-labeled cRNA were obtained by reverse transcription, starting with 500 ng total RNA, and then concomitant amplification and labeling involving T7-polymerase transcription.

The test samples and reference samples were labeled with Cy5 and Cy3 labeled CTP, respectively. Cy5 and Cy3 labeling was monitored with the Nanodrop ND-1000 spectrophotometer, and the results were between 1.0 and 1.4 pmol/ μ L in all cases.

Hybridization was performed by using the Agilent oligonucleotide microarray *in situ* Hybridization Plus kit (5184-5340), following the manufacturer's recommendations. Briefly, 750 ng of fast recovery sample cRNA were mixed with 750 ng of SR sample cRNA in the presence of a control target. The final solution was subjected to fragmentation (30 min at 60 °C in the dark) and hybridized onto a 44K 60-mer whole rat genome oligonucleotide microarray (G4131A, Agilent Technologies) in a rotation oven (60 °C for 17 h, 4 r/min) in the dark. Slides were disassembled and washed in solutions I and II and dried using a nitrogen-filled air gun. The slides were then scanned according to Agilent's instructions. Microarrays were scanned with a dynamic autofocus microarray scanner (G2565BA, Agilent Technologies) using the Agilent parameters. Feature Extraction 8.5 software was used to extract data and to analyze the signal.

2.6 Analysis of microarray data Due to the fact that these arrays were special (endosperm) arrays, regular lowess normalization based on the assumption that most of the genes are not differentially expressed and there equal number of up and down regulated genes is not applicable. A special variation customized for this data set was applied here. Specifically, intensity lowess was conducted by fitting a smooth curve to the spots with log ratios within the range of ± 0.8 and then all spots were adjusted according to this

curve. The data were then normalized by the log ratio of the spiking controls. Finally, the channel mean of each channel on each slide was subtracted.

Because the replicated spots for the same gene are highly correlated, after normalization, the median of the replicated spots for each probe was used to fitted to a fixed effect ANOVA model one gene at a time, $Y_{ijk} = \mu + A_i + D_j + T_{k(ij)} + \varepsilon_{ij}$. The μ term is the gene mean. The A_i and D_j represent the array and dye effects, respectively. The $T_{k(ij)}$ represents the tissue effect, which is our interest. The ε_{ij} represents the residual measurement error. A shrinkage-based t test is used to identify genes that are differently expressed for each pair-wise tissue comparisons^[14]. The P values were obtained through sample permutation and pooling the permuted t statistics across genes. Adaptive false discovery rate was used to control for multiple testing^[15].

2.7 Real-time RT-PCR validation Total RNAs were used for quantitative RT-PCR. The SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Invitrogen) was used for quantitative PCR. Gene expression was analyzed with the ABI Prism 7000 sequence detection system, and the results were handled with the associated software (version 1.2; Applied Biosystems). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal reference for all analyses. The following forward and reverse primers were designed using the Primer Express software (version 2.0, PE Applied Biosystems) (Tab. 1). Primer specificity was assessed from the monophasic dissociation curves, and all were similarly efficient (data not shown). The threshold cycle (Ct) for the endogenous control GAPDH mRNA and the target signals was determined, and the relative RNA quantifica-

Tab. 1 Primers used for quantitative RT-PCR

Gene symbol	Primer sequence (5'→3')	
	Forward	Reverse
GAPDH	ATCCCGCTAACATCAAATGG	GTGGTTCACACCCATCACAA
Tln	GCAACCACAGAGCACATACG	CCCTTGGTCAATCGGATGAA
RT1-Bb	GTCTGCCCAGAGCAAGATGC	GAGGTCCTTTCTGACGCTTGTC
Gramd1a	TGGAGCAGTGACAGCAAGTG	GCGTCTGTGTCTCCACCACA
F11r	ACAGTTCCTATGCAGACCG	CACAGTGAGGTGGATGCTGA
PP-1B	AGAAATGACCTGAGAGATCG	GCAGAATTGACATGACTACCT
VLA-6	CCCTAGTTTCTCTCAGTCCA	CTCTAGTCTCTCCAGAATCG
Nfkb β	GCAGGCCACCAACTACAACG	ACATCAGACCCAAAGTCACC
Myosin-9b	GGCAAGCCTCACAGTAATGTG	CCGGCAACCCAGTGAGGTAT
Vamp5	TTCGACAAGGTCCTGGAACG	GCTTCTGCTGGGCTAAAGTC

tion was calculated using the comparative $2^{-\Delta\Delta C_T}$ method where $\Delta\Delta C_T = (C_{T,Target} - C_{T,GAPDH})_{FR} - (C_{T,Target} - C_{T,GAPDH})_{SR}$ [16]. Experiments were performed in triplicate.

2.8 Statistical analysis For plasma CORT and ACTH concentrations, statistical analysis was carried out with one-way ANOVAs. Data were expressed as mean $\pm$ SEM. Differences of RT-PCR were analyzed with Student's *t* test. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Distinct individual variation in the recovery rate after RES

Animals exposed to RES showed a high variability

in plasma ACTH and CORT levels at the termination of 2-h RES and at 1 h after RES. Our purpose is to compare the different patterns of recovery from RES, so according to these observations we selected the rats with similar plasma CORT levels at the termination of 2-h continuous exposure to RES, but with diversity of CORT levels at 1 h after RES for further analysis, thus forming FR and SR, with six rats in each group. Because adrenocortical response to circulating ACTH becomes saturated with intermediate levels of plasma ACTH, which are already achieved by exposure to moderate intensity stressors, CORT response may be not a completely reliable index of

Fig. 1 Plasma corticosterone and ACTH levels in control and RES stressed rats at different times. The stressed rats were classified as follows based on the plasma corticosterone levels at 1 h after RES: fast recovery (FR) and slow recovery (SR). The period of blood sampling with respect to stress exposure and time hours is indicated under the bars. Data were expressed as mean $\pm$ SEM ($n = 6$ per group). * $P < 0.01$ vs control, # $P < 0.01$ vs both FR and Control.

Tab. 2 List of genes differentially expressed between fast recovery group and slow recovery group

Gene symbol	Name	Fold change ^a	Fold change ^b	FDR ^c	Accession No.
RT1-Bb	RT1 class II, locus Bb	1.550 $\uparrow^d$	1.621 $\pm$ 0.124 $\uparrow$	0.007	NM_001004084
Grand1a	GRAM domain containing 1A	1.611 $\uparrow$	1.587 $\pm$ 0.137 $\uparrow$	0.052	NM_001014160
Tln	Talin	1.668 $\uparrow$	1.721 $\pm$ 0.203 $\uparrow$	0.084	NM_001039025
PP-1B	Serine/threonine protein phosphatase PP1-beta catalytic subunit	1.631 $\uparrow$	1.836 $\pm$ 0.223 $\uparrow$	0.007	XM_217507
VLA-6	Integrin α -6 precursor	1.661 $\uparrow$	1.645 $\pm$ 0.192 $\uparrow$	0.008	XM_346121
Myosin-9b	Myosin IXb (Unconventional myosin-9b)	1.901 $\uparrow$	2.168 $\pm$ 0.221 $\uparrow$	0.003	XM_346173
Nfkbia	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, α	1.331 $\uparrow$	1.523 $\pm$ 0.163 $\uparrow$	0.043	XM_343065
dlg	Discs, large homolog 5 (Placenta and prostate DLG)	1.757 $\uparrow$	—	0.007	XM_343681
F11r	Junctional adhesion molecule 1	1.495 $\downarrow$	1.575 $\pm$ 0.119 $\downarrow$	0.199	NM_053796
Vamp5	Vesicle-associated membrane protein 5	1.439 $\downarrow$	1.589 $\pm$ 0.194 $\downarrow$	0.194	NM_053555
Brwd1_predicted	Bromodomain and WD repeat domain containing 1	1.527 $\downarrow$	—	0.129	XM_221627

^athe fold change revealed by microarray analysis; ^bthe fold change revealed by quantitative RT-PCR; ^cfalse discovery rate; ^d $\uparrow$ and $\downarrow$ indicate the up- and down-regulation of gene expression, respectively.

adaptation to intermediate or severe stressful situations^[17]. By using the criteria of the decrement of ACTH levels from the termination of 2-h RES to 1 h post-RES to classify the animals, we got the similar groups as defined by CORT levels.

No differences in pre-stress CORT and ACTH levels were observed among FR, SR and control. At the termination of RES, the CORT levels between FR and SR had no significant difference. At 1 h after RES, the CORT level was significantly different between the two groups ($P < 0.006$) as revealed by non-parametric ANOVA (Fig. 1A). The level of plasma CORT at 1 h after RES was significantly higher in SR than that in control and FR, while there was no significant difference between FR and control rats, indicating that FR group has returned to the basal level after 1-h recovery. As for ACTH level, the result was found similar to that of CORT: at 1 h post-RES, significant differences were found between FR and SR ($P < 0.007$), while there was no significant difference between FR and control groups (Fig. 1B).

3.2 Identification of differentially expressed genes Analysis of the microarray data showed that most of genes were not differentially expressed between FR group and SR group. The variation range of over 1.5 fold in mRNA expression level was adopted in this study since over 1.5 fold fluctuation in gene expression level was considered to be sufficient to induce significant biochemical and physiological changes in living cells. As a result, 10 probes displayed differences in expression between two groups with a 1.5-fold change (Tab. 2).

To confirm the gene expression variations identified by the microarray experiments, the selected differentially expressed genes were tested by quantitative RT-PCR. The selected genes displayed similar gene expression differences as assessed by real-time RT-PCR (Tab. 2). In conclusion, quantitative RT-PCR results confirmed the microarray data.

4 Discussion

A tightly controlled termination of the stress response with a re-balance of homeostasis after a particular stressor has ceased is quite important for individuals, because chronic hyperactivation of HPA axis may comprise the maladaptive states that can be harmful^[18]. It is well-accepted that there are major individual differences in susceptibility to psychiatric pathologies and stress-related pathological processes in humans. However, our knowledge of the bio-

logical bases of such susceptibility is encumbered by obvious limitations of the research in humans. Thus, characterization of the individual differences in animals (e.g. rodents) may help to find the origin of susceptibility in humans. To date, neurobiological bases of individual differences in rodents have been mainly focused on the HPA axis and its relationship to the behavioral traits such as fear/anxiety and novelty-seeking (attraction for novelty)^[19]. In this study, we compared the hypothalamus (the upstream of HPA axis) transcriptional profiles of two different patterns of recovery (fast recovery *vs* slow recovery) from RES in rats by using oligonucleotide microarray and provided a global transcriptional profile of hypothalamus in the recovery process.

Compared with Garcia's^[7] result, we did not find strong CORT and ACTH levels as in the immobilization stress. Based on the plasma CORT and ACTH levels, the most possibility of this phenomenon is the intensity of stressor itself. Immobilization is a more severe stressor than restraint. This may explain why most of genes were not differentially expressed between FR and SR group. The reason is that RES may not so strong enough to activate related genes in the hypothalamus. Compared with SR group, talin, together with serine/threonine protein phosphatase PP1- β catalytic subunit (PP1B) and integrin α -6 precursor (VLA-6) was at least 1.5 fold up-regulated in FR group. Integrin transmembrane receptors have a unique property that distinguishes them from other signaling receptors. Their affinity for ligands in response to intracellular signals generated by non-integrin receptors can be modulated from the inside out. Recent findings provided novel mechanistic insights into this process by demonstrating that talin, a protein that links integrins to actin, was necessary for the inside-out activation of integrins. Talin is an important focal adhesion component that binds to multiple adhesion molecules including integrins, vinculin, focal adhesion kinase (FAK) and actin. It provides a vital regulatory switch that allows integrins to respond rapidly to physiological stimuli^[20]. Also, talin is a crucial downstream target of a variety of transmembrane signaling receptors and intracellular signaling pathways^[21]. As talin is one of the most important components in the integrin signaling pathway, its upregulation along with VLA-6 in the FR group implied its involvement in the process of recovery from RES in rats.

Protein phosphatases regulate numerous cellular functions and signal transduction pathways in cooperation with

protein kinases. Protein phosphatase types 1, known as PP1, are one of four major protein serine/threonine phosphatases that regulate diverse cellular events such as cell division, transcription, translation, muscle contraction, glycogen synthesis, and neuronal signaling^[22]. As a positive regulator in cell growth regulation, increased PP1 in hepatomas plays a role in their rapid growth rate^[23]. One possible assumption is that the upregulation of PP-1B promotes the cell growth in the hypothalamus which in turn contributed to the fast recovery from RES. Mitsuhashi *et al.* have demonstrated that PP1 was present in a multicomplex with Raf-1 and positively regulates Raf-1 activity. Myosin-binding subunit (M110), a PP1 regulatory protein, was reported to interact with Raf-1 *in vivo* and to be phosphorylated by Raf-1 *in vitro*^[23]. In the present study, Myosin-9b was also up-regulated in FR group.

It is interesting to note that *F11r*, also called junctional adhesion molecule-1 (*JAM1*) was down-regulated in FR group. Recent studies implied that neuronal cell adhesion molecules of the immunoglobulin superfamily (neural cell adhesion molecule, NCAM and L1) were important mediators of the effects of stress on the brain^[24]. *JAM1* is the first found immunoglobulin superfamily protein in the tight junctions (TJs) of epithelial cells and endothelial cells as well as on the surface of hematopoietic cells, such as platelets and leukocytes^[25]. The key role of *JAM1* in the TJ assembly, epithelial barrier function, and a variety of other cellular processes, including platelet aggregation, leukocyte transmigration, and angiogenesis have been reported^[26]. *JAM1* also participates in heterotypic interactions, namely with integrins^[27]. Thus, here is another reason which supports the involvement of integrin signaling pathway in the process of recovery from RES in rats. In addition, *nfkbia* (nuclear factor of kappa light chain gene enhancer in B-cells inhibitor α), which can inhibit NF- κ B by complexing with and trapping it in the cytoplasm, was found to be up-regulated in the FR group. *Nfkbia* may be involved in regulation of the transcriptional responses to NF- κ B, including cell adhesion, immune and proinflammatory responses, apoptosis, and differentiation.

Taken together, this research suggest that the integrin signaling pathway may be involved in the recovery from RES stress in rats, and cell adhesion function seems to be influenced in two different recovery patterns. These findings may lead to an improved understanding of the process of recovery from RES. Also, it may provide potential

therapeutic targets to treat stress-related disorders.

References:

- [1] Guimont S, Wynne-Edwards K. Individual variation in cortisol responses to acute "on-back" restraint in an outbred hamster. *Horm Behav* 2006, 50: 252-260.
- [2] Roy MP, Kirschbaum C, Steptoe A. Psychological, cardiovascular, and metabolic correlates of individual differences in cortisol stress recovery in young men. *Psychoneuroendocrinology* 2001, 26: 375-391.
- [3] de Kloet ER. Hormones, brain and stress. *Endocr Reg* 2003, 37: 51-68.
- [4] Lundberg U. Stress hormones in health and illness: The roles of work and gender. *Psychoneuroendocrinology* 2005, 30: 1017-1021.
- [5] de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005, 6: 463-475.
- [6] Linden W, Earle TL, Gerin W, Christenfeld N. Physiological stress reactivity and recovery: conceptual siblings separated at birth? *J Psychosom Res* 1997, 42: 117-135.
- [7] Garcia A, Armario A. Individual differences in the recovery of the hypothalamic-pituitary-adrenal axis after termination of exposure to a severe stressor in outbred male Sprague-Dawley rats. *Psychoneuroendocrinology* 2001, 26: 363-374.
- [8] Garcia A, Marti O, Valles A, Dal-Zotto S, Armario A. Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. *Neuroendocrinology* 2000, 72: 114-125.
- [9] Lee HC, Chang DE, Yeom M, Kim GH, Choi KD, Shim I, *et al.* Gene expression profiling in hypothalamus of immobilization-stressed mouse using cDNA microarray. *Brain Res Mol Brain Res* 2005, 135: 293-300.
- [10] Mizuno S, Kato K, Asai S, Takahashi Y, Nagata T, Komuro S, *et al.* Gene expression analysis in the stomachs of water immersion-restraint stress rats using high-density oligonucleotide array. *J Gastroenterol Hepatol* 2004, 19: 1264-1269.
- [11] Vahl TP, Ulrich-Lai YM, Ostrander MM, Dolgas CM, Elfers EE, Seeley RJ, *et al.* Comparative analysis of ACTH and corticosterone sampling methods in rats. *Am J Physiol Endocrinol Metab* 2005, 289: E823-828.
- [12] Reyes TM, Walker JR, DeCino C, Hogenesch JB, Sawchenko PE. Categorically distinct acute stressors elicit dissimilar transcriptional profiles in the paraventricular nucleus of the hypothalamus. *J Neurosci* 2003, 23: 5607-5616.
- [13] Bueno Filho JS, Gilmour SG, Rosa GJ. Design of microarray experiments for genetical genomics studies. *Genetics* 2006, 174: 45-57.
- [14] Cui X, Churchill GA. Statistical tests for differential expression in cDNA microarray experiments. *Genome Biol* 2003, 4: 210.
- [15] Cui X, Hwang JT, Qiu J, Blades NJ, Churchill GA. Improved statistical tests for differential gene expression by shrinking

- variance components estimates. *Biostatistics* 2005, 6: 59-75.
- [16] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* 2001, 25: 402-408.
- [17] Keller-Wood ME, Shinsako J, Keil LC, Dallman MF. Insulin-induced hypoglycemia in conscious dogs: I. dose-related pituitary and adrenal responses. *Endocrinology* 1981, 109: 818-824.
- [18] Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 1992, 267: 1244-1252.
- [19] Steimer T, Driscoll P. Divergent stress responses and coping styles in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. *Stress* 2003, 6: 87-100.
- [20] Nayal A, Webb DJ, Horwitz AF. Talin: an emerging focal point of adhesion dynamics. *Curr Opin Cell Biol* 2004, 16: 94-98.
- [21] Tadokoro S, Shattil SJ, Eto K, Tai V, Liddington RC, de Pereda JM, *et al*. Talin binding to integrin beta tails: a final common step in integrin activation. *Science* 2003, 302: 103-106.
- [22] Shenolikar S. Protein serine/threonine phosphatases-new avenues for cell regulation. *Annu Rev Cell Biol* 1994, 10: 55-86.
- [23] Broustas CG, Grammatikakis N, Eto M, Dent P, Brautigan DL, Kasid U. Phosphorylation of the myosin-binding subunit of myosin phosphatase by Raf-1 and inhibition of phosphatase activity. *J Biol Chem* 2002, 277: 3053-3059.
- [24] Sandi C. Stress, cognitive impairment and cell adhesion molecules. *Nat Rev Neurosci* 2004, 12: 917-930.
- [25] Mandell KJ, McCall IC, Parkos CA. Involvement of the junctional adhesion molecule-1 (JAM1) homodimer interface in regulation of epithelial barrier function. *J Biol Chem* 2004, 279: 16254-16262.
- [26] Mandell KJ, Babbin BA, Nusrat A, Parkos CA. Junctional adhesion molecule 1 regulates epithelial cell morphology through effects on beta1 integrins and Rap1 activity. *J Biol Chem* 2005, 280: 11665-11674.
- [27] Ostermann G, Weber KS, Zerneck A, Schroder A, Weber C. JAM-1 is a ligand of the β_2 integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat Immunol* 2002, 2: 151-158.

整合素信号通路可能参与大鼠束缚应激后的恢复过程

高玉振^{1,2,3}, 郭试瑜^{1,2,3}, 印其章^{1,2}, Xiang-Qin CUI⁴, 久光正³, 蒋星红^{1,2}

¹ 苏州大学医学院神经生物学教研室, ² 苏州大学衰老与神经疾病实验室, 苏州 215123

³ 昭和大学医学部第一生理学教室, 东京 142855, 日本

⁴ 阿拉巴马大学伯明翰分校生物统计学系, 阿拉巴马 35294-0022, 美国

摘要: **目的** 筛选与束缚应激大鼠应激反应恢复速度相关的基因, 探讨应激反应个体恢复的分子机制。 **方法** 观察大鼠 2 小时束缚应激后血浆促肾上腺皮质激素(adrenocorticotrophic-hormone, ACTH)及皮质酮的变化规律, 根据应激结束后1小时的血浆ACTH及皮质酮下降程度将大鼠分为快速恢复组与慢速恢复组。采用微阵列技术检测快速恢复组与慢速恢复组下丘脑组织基因表达的差异, 用实时逆转录-聚合酶链反应验证部分基因的表达差异。 **结果** 基因芯片分析结果显示: 大部分基因在两组间并无差异表达, 在 11 个差异表达的基因中, 快速恢复组中参与整合素信号通路的踝蛋白(talin)、整合素 α_6 和丝/苏氨酸蛋白磷酸酶 PP1 β 催化亚基(serine/threonine protein phosphatase PP1-beta catalytic subunit, PP1B)基因有 1.5 倍上调, 而结合粘附分子 1(junctional adhesion molecule 1, *F11r*)基因有 1.5 倍下调。实时逆转录-聚合酶链反应结果与芯片分析结果一致。 **结论** 本研究构建了束缚应激大鼠恢复过程的下丘脑基因表达谱, 结果提示整合素信号通路可能参与应激的恢复过程。

关键词: 束缚应激; 促肾上腺皮质激素; 皮质酮; 应激后恢复; 基因芯片