Increase of glucocorticoids is not required for the acquisition, but hinders the extinction, of lithium-induced conditioned taste aversion.

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– ABSTRACT –

Increase of glucocorticoids is not required for the acquisition, but hinders the extinction, of lithium–induced conditioned taste aversion.

Lithium chloride at doses adequate to induce conditioned taste aversion (CTA) causes c–Fos expression in the paraventricular nucleus and increases the plasma level of corticosterone with activation of the hypothalamic pituitary adrenal axis. This study was examined to define the role of glucocorticoid in the acquisition and extinction of lithium–induced CTA. In experiment 1, Sprague–Dawley rats received dexamethasone (2mg/kg) or RU 486 (20mg/kg) immediately after 5% sucrose access, and then an intraperitoneal injection of isotonic lithium chloride (12ml/kg) was followed with 30 min interval. Rats had either 1 or 7 days of recovery period before the daily sucrose drinking tests. In experiment 2, rats were conditioned with the sucrose lithium pairing, and then received dexamethasone or vehicle at 30 min before each drinking test. In experiment 3, adrenalectomized (ADX or ADX + B) rats were subjected to sucrose drinking tests after the sucrose lithium pairing. Dexamethasone, but not RU486, pretreatment diminished the formation of lithium–induced CTA memory. Dexamethasone prior to each drinking test suppressed

sucrose consumption and prolonged the extinction of lithium-induced CTA. Sucrose consumption was significantly suppressed not only in ADX + B rats but also in ADX rats during the first drinking session; however, a significant decrease was found only in ADX rats on the fourth drinking session. These results reveal that glucocorticoid is not a necessary component in the acquisition, but an important player in the extinction, of lithium-induced CTA, and suggest that a pulse increase of glucocorticoid may hinder the extinction memory formation of lithium-induced CTA.

Keywords: Conditioned taste aversion, Lithium chloride, c-Fos, Hypothalamic pituitary adrenal axis

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I. INTRODUCTION

Conditioned taste aversion (CTA) is a robust form of associative learning with unique single-trial and long-delay characteristics. A single pairing of a novel taste with a toxic substance such as lithium chloride (LiCl) produces a strong and persistent avoidance of substances containing that taste (Nachman and Ashe, 1973). Intraperitoneal lithium chloride at large doses induces c-Fos expression in the brain regions, such as the hypothalamic paraventricular nucleus(PVN), the nucleus tractus of solitarius, and the central nucleus of amygdala, and c-Fos expression in these brain regions has been suggested to be correlated with CTA learning (Houpt et al., 1994; Lamprecht and Dudai, 1995; Sakaiand Yamamoto, 1997; Schafe and Bernstein,1996; Yamamoto et al., 1992). Of these brain regions, c-Fos expression in the PVN, the center of the hypothalamic pituitary adrenal (HPA) axis, is considered to refer the activation of the HPA axis by stressful stimuli (Briski and Gillen, 2001; Figueiredo et al., 2003); thus, c-Fos expression in the PVN by an intraperitoneal injection of lithium chloride implies lithium-induced activation of the HPA axis (Figueiredo et al., 2003). Indeed, studies have demonstrated that intraperitoneal injections of lithium chloride induce adrenocorticotrophic hormone (ACTH) release (Sugawara et al., 1988), activate the HPA axis (Hennessy et al., 1980) and increase

the plasma level of glucocorticoids (Spencer et al., 2005). Glucocorticoids are known to be involved in memory formation, such as enhancing the consolidation of hippocampus dependent spatial or contextual learning in rodents (Cordero and Sandi, 1998; Flood et al.,1978; Pugh et al., 1997). Although an effect of the hippocampus in lithium-induced CTA learning has been rarely reported, the lithium-induced activation of the HPA axis leading to a robust increase of the plasma glucocorticoids still suggests its putative implication. Hippocampus has a high density of glucocorticoid receptors(Reul and deKloet, 1985) and exerts a rapid glucocorticoid feedback inhibition of the HPA axis (Tasker and Herman, 2011). Also, glucocorticoids regulate several aspects of neuroplasticity in the hippocampus (Foyetal.,1987; Korz and Frey, 2003), and the treatment with ACTH or glucocorticoids controls the pattern of lithium-induced CTA learning (Hennessy et al.,1980; Revusky and Martin,1988). However, functional implication of the HPA axis activation in lithium-induced CTA learning has not been well understood yet. In this study, rats were pretreated with synthetic glucocorticoid dexamethasone or glucocorticoid receptor antagonist RU486 at the pairing of conditioned stimulus (CS; sucrose) and unconditioned stimulus (US; lithium chloride), in order to investigate if suppression of the HPA axis activation and blockage of the glucocorticoids action at the CS-US pairing differently affect the formation of lithium-induced CTA learning.

II. MATERIALS AND METHODS

A. Animals

Male Sprague-Dawley rats (200-250g, Samtako Bio, Osan, Korea) were individually housed and maintained in a specific pathogen free (SPF) barrier zone with the constantly controlled temperature (22 ± 1° C) and humidity (55%) on a 12h light-dark cycle (lights-on at 07:00h) in the Seoul National University Animal Facility Breeding Colony. Rats had ad libitum access to standard rodent chow (Purina Rodent Chow, Purina Co., Seoul, South Korea) and tap water, and were habituated in the animal colony at least for a week before experiments began. Animals were cared for according to The Guide for Animal Experiments, 2000, edited by the Korean Academy of Medical Sciences, which is consistent with the NIH Guide line for the Care and Use of Laboratory Animals, 1996 revised. All animal protocols were approved by the Committee for the Care and Use of Laboratory Animals at Seoul National University.

B. Experiment procedures

1. Dexamethasone or RU486 pretreatment on the conditioning day

The present experiment is schematically described in Fig. 1. Rats had free access to chow, but had only 5h of daily access to water (12:00–5:00 PM) as the only source of fluid for 5 days as training period. On the conditioning day, rats were allowed to drink 5% sucrose at 12:00 PM as the only source of fluid for 15min, and then received a subcutaneous injection of dexamethasone (2 mg/kg; Yuhan Co., Seoul, Korea), RU486 (20mg/kg; Sigma Chemical Co., St. Louis, MO, USA) or the same injection volume of vehicle (polyethylene glycol: saline = 7:3) followed by an intraperitoneal injection of isotonic LiCl (0.15M,12ml/kg; Sigma Chemical Co., St.Louis, MO, USA) with 30 min of interval (n=6 in each group; total 18 rats). The selected doses of dexamethasone and RU486 were based on our previous studies, in which the dose of dexamethasone effectively suppressed activation of the PVN neurons (Lee etal.,2003; Jahng et al.,2005) and RU486 blocked the corticosterone action (Kim et al., 2004). Water was supplied following the conditioning until 5:00 PM. After 1 day of recovery with 5h of water supply, rats had access to 5% sucrose for 15min daily at 12:00 PM and then water was offered until 5:00 PM. The weight of sucrose

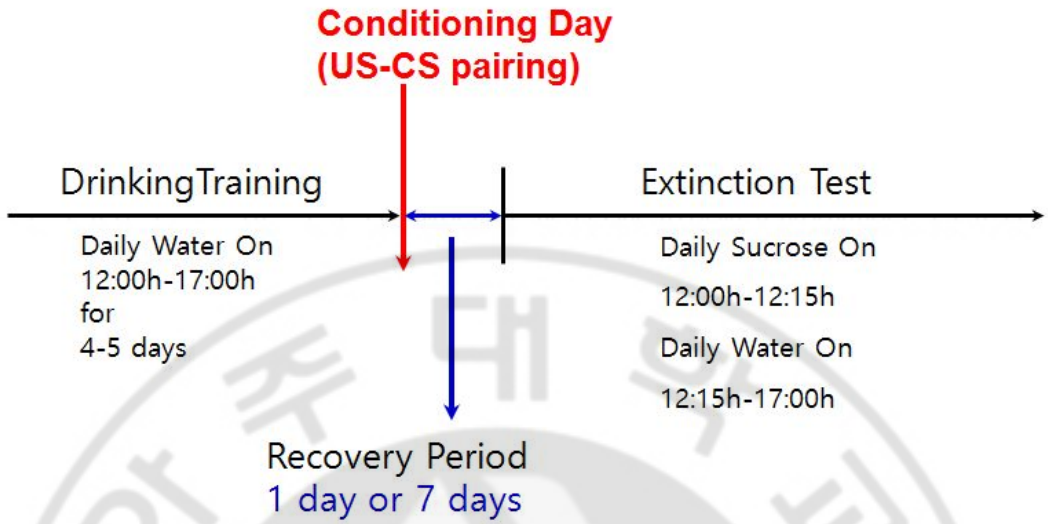

Fig. 1. Conditioned taste aversion protocol. Sprague-Dawley rats received dexamethasone (2mg/kg) or RU 486 (20mg/kg) immediately after 5% sucrose access, and then an intraperitoneal injection of isotonic lithium chloride (12ml/kg) was followed with 30 min interval. Rats had either 1 or 7 days of recovery period before the daily sucrose drinking tests. CS; conditioned stimulus (sucrose), US; unconditioned stimulus (lithium chloride),

solution consumed was recorded and used to quantify the CTA. Another groups of rats(n=6 in each group, total 18 rats) were processed with the same experimental schedule, except having 7 days of recovery period instead of 1 day after the conditioning (Fig 2).

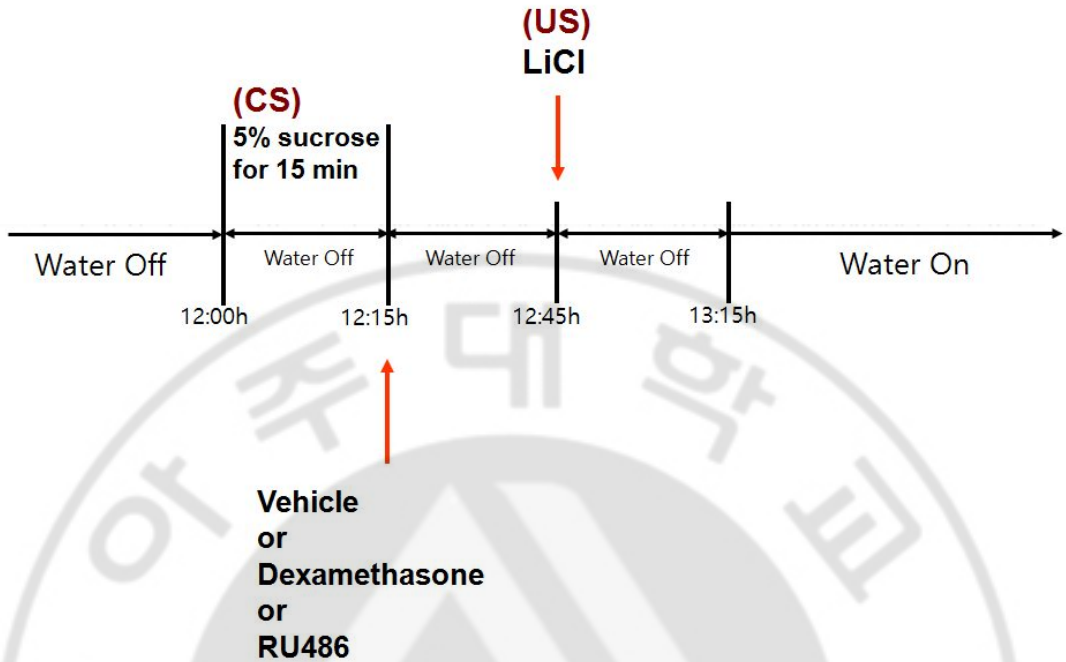

Fig. 2. Conditioned taste aversion protocol on conditioning day. On the conditioning day, rats were allowed to drink 5% sucrose at 12:00 PM as the only source of fluid for 15min, and then received a subcutaneous injection of dexamethasone, RU486 or the same injection volume of vehicle followed by an intraperitoneal injection of isotonic LiCl with 30 min of interval (n=6 in each group; total 18 rats). Water was supplied following the conditioning until 5:00 PM. CS; conditioned stimulus (sucrose), US; unconditioned stimulus (lithium chloride),

2. Dexamethasone pretreatment during the drinking test

Rats (n=6 in each group, total 12 rats) had free access to chow, but had only 5h of access to water daily(12:00-5:00PM) as the only source of fluid for 5 days as training period. On the conditioning day, rats were allowed to drink 5% sucrose as the only source of fluid for 15 min, and then received an intraperitoneal injection of isotonic LiCl (0.15M, 12ml/kg) at 12:15 PM. Water was supplied following the conditioning until 5:00 PM. After 1 day of recovery with 5h of water supply, rats had access to 5% sucrose for 15 min daily at 12:00 PM, and then water was offered until 5:00 PM. Rats received a subcutaneous injection of dexamethasone at a dose of 2mg/kg (n=6) or the same injection volume of polyethylene vehicle (n=6) at 30 min before each sucrose drinking test. The weight of sucrose solution consumed was recorded and used to quantify the CTA.

3. Lithium-induced CTA in adrenalectomized rats

Bilateral adrenalectomy was performed with dorsal approach as previously described (Waynforth and Flecknell, 1992). After surgery, rats received 0.9% saline instead of water to drink. After 1 week of postoperational recovery, adrenalectomized (ADX or ADX + B) and sham operated rats (Sham) (n=6 in each group, total 18 rats) underwent the sucrose lithium pairing (conditioning) following 5 days of water training, and then the

sucrose drinking test after 1 day of recovery from the conditioning, in the same schedule as described above in experiment 2. The weight of sucrose solution consumed was recorded and used to quantify the CTA. ADX-B rats received corticosterone replacement at a dose of 2mg/kg daily at 5:00 PM during the whole experiment period after surgery. Two days after the fourth drinking test, rats in each group were transcardially perfused with 4% paraformaldehyde (Merck Co., Damstadt, Germany) at 1h after an intraperitoneal injection of isotonic lithium chloride (12ml/kg) and the brain tissue sections of the paraventricular nucleus were processed for c-Fos immunohistochemistry, in order to confirm the lithium-induced HPA axis activation in ADX and ADX + B rats. Transcardiac perfusion and c-Fos immunohistochemistry were performed as described in the next section.

C. Measurement procedures

1. c-Fos immunohistochemistry

Rats received a subcutaneous injection of dexamethasone at 2 mg/kg dose (n=5) or the same injection volume of polyethylene vehicle (n=10), and then an intraperitoneal injection of isotonic LiCl at 12ml/kg dose (5DEX/LiCl rats and 5Veh/LiCl rats) or the same injection volume of physiologic saline (5Veh/NaCl rats) was followed with a 30 min interval. One hour after the intraperitoneal lithium or saline, rats were anesthetized with overdoses of sodium pentobarbital (Hallym Pharmaceutical Co., Seoul, Korea) and transcardially perfused first with heparinized isotonic saline, and then with 4% paraformaldehyde (Merck Co., Damstadt, Germany) in 0.1M sodium phosphate buffer. Brains were rapidly dissected out, blocked, post-fixed for 2h, and then transferred into 30% sucrose (Sigma Co., St.Louis, MO, USA) overnight for cryoprotection. Forty microncoronal sections were cut on a freezing, sliding microtome (HM440E, Microm Co., Germany). Alternate sections were collected throughout the rostro-caudal extent of the hypothalamic paraventricular nucleus (PVN; between bregma 1.3mm and 2.1mm; Paxinos and Watson, 2005). Immunohistochemistry was performed with standard DAB reaction using commercial ABC kit

(Vectastain Elite Kit, Vector Laboratories, Burlingame, CA, USA) as we previously described (Jahng et al., 2004). Polyclonal rabbit anti-c-Fos antibodies (1:20000 dilution, Calbiochem, Darmstadt, Germany) were used as primary antibodies, and biotinylated anti-rabbit IgG (1:200dilution, Vector Laboratories, Burlingame, CA, USA) as secondary. Immunostained sections were mounted in ananatomical order onto gelatin-coated slides from 0.05M phosphate buffer, air-dried, dehydrated through a graded ethanol to xylene, and cover-slipped with Permount.

2. Quantitative analysis

Cells expressing c-Fos in each brain region were hand-counted blind after digitizing 720_540 mm^2 images of all consecutive sections using an Olympus BX-50 microscope (Olympus Co., Tokyo, Japan) and MCID image analysis system (M2, Imaging Research Inc., Ont., Canada). Cells containing only distinct brown dot in the cytoplasm were counted as c-Fos positive cells. The number of cells in two sections from the PVN (closest sections to bregma 1.88mm) from each brain was averaged per section, and the individual mean counts for each brain were averaged across rats within experimental groups.

D. Statistical analyses

All data were analyzed by unpaired t-test, one- and two-way analysis of variance (ANOVA) and preplanned comparisons with the controls performed by post-hoc Fisher's Protected Least Significant Difference test using StatView software (Abacus, Berkeley, CA). Values are presented by means ± S.E.M. For all comparisons, the level of significance was set at $P \leq 0.05$.

III. RESULTS

A. c-Fos staining in the paraventricular nucleus

One hour of intraperitoneal lithium chloride at a dose of 12ml/kg markedly increased c-Fos expression in the paraventricular nucleus ($P < 0.05$, Veh/NaCl vs. Veh/LiCl), and dexamethasone pretreatment at a dose of 2mg/kg significantly attenuated the lithium-induced c-Fos expression ($P < 0.05$, Veh/LiCl vs. DEX/LiCl) (Fig.3).

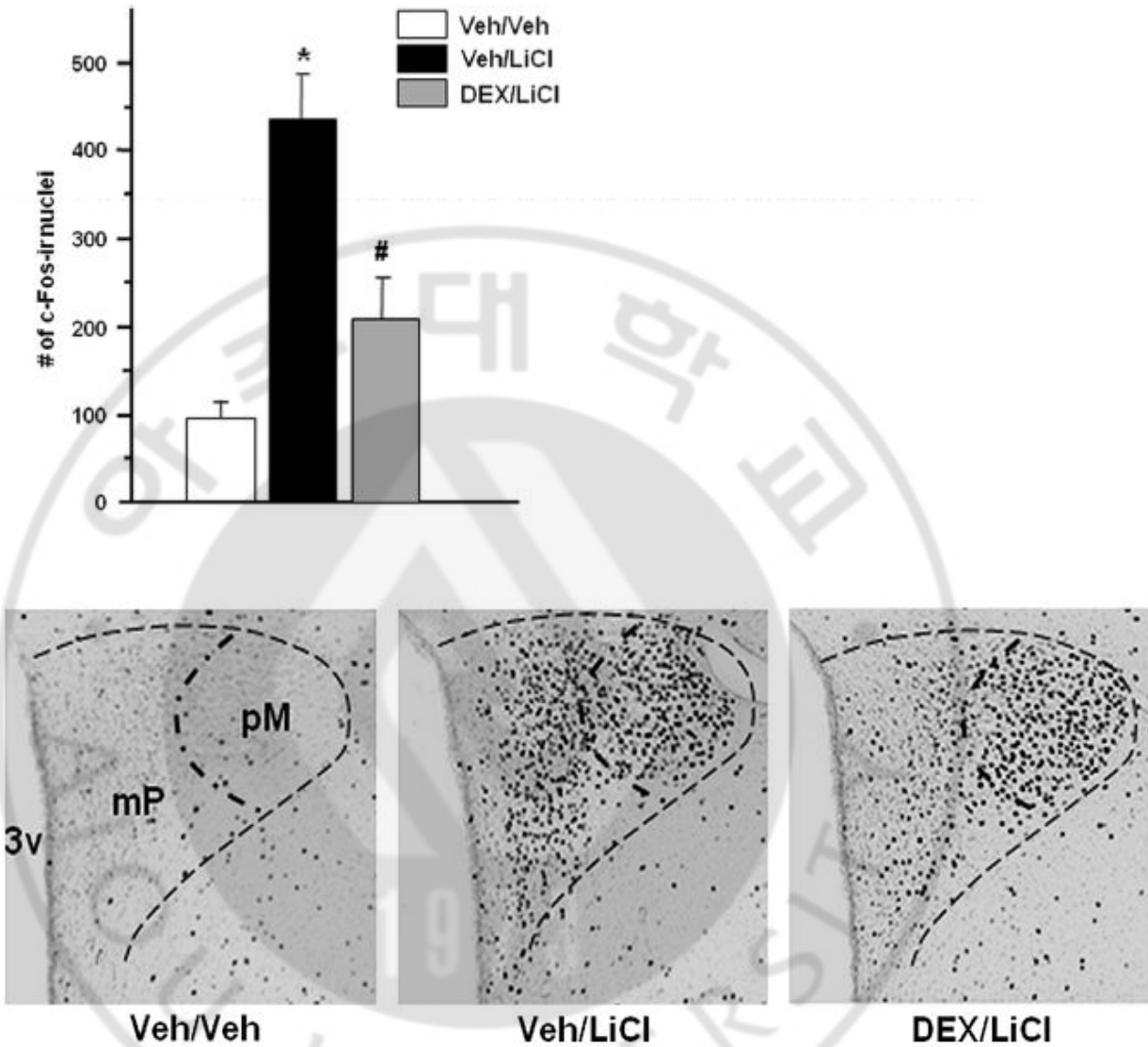

Fig. 3. c-Fos immunohistochemistry in the hypothalamic paraventricular nucleus. Rats received a subcutaneous injection of dexamethasone at 2mg/kg dose or the same injection volume of polyethylene vehicle, and then an intraperitoneal injection of isotonic LiCl at 12ml/kg dose (DEX/LiCl rats and Veh/LiCl rats) or the same injection volume of physiologic saline

(Veh/NaCl rats) was followed with 30 min interval. One hour after the intraperitoneal lithium or saline, rats were transcardially perfused with 4% paraformaldehyde, and then processed for c-Fos immunohistochemistry with standard DAB reaction using commercial ABC kit. 3v; third ventricle, mP; medial parvocellular subdivision of paraventricular nucleus, pM; posterior magnocellular subdivision of paraventricular nucleus, $^{*}P < 0.05$ vs. Veh/Veh, $^{\#}P < 0.05$ vs. Veh/LiCl, Data are presented by means ± S.E.M.

B. Dexamethasone or RU486 pretreatment on the conditioning day

Dexamethasone or RU486 prior to lithium chloride (US) did not affect the acquisition of lithium-induced CTA; however, dexamethasone delayed, while as RU486 facilitated the extinction of it in 1 day recovery paradigm (Fig. 4A). The amount of sucrose consumed on test day 5 did not differ from the conditioning day in Veh/LiCl rats, but was significantly decreased in DEX/LiCl rats (Po0.05, day 0 vs. day 5). Whilst the sucrose consumption on test day 4 was still significantly reduced in Veh/LiCl rats (P <0.05, day 0 vs.day4), it did not differ from the conditioning day in RU/LiCl rats. In 7 days recovery paradigm, dexamethasone prior to lithium chloride seemed to attenuate lithium-induced CTA learning (Fig. 4B). Sucrose consumption of DEX/LiCl rats showed a significant decrease only during the first drinking test (day 8) compared to the conditioning day, and thereafter no differences were found. Neither the acquisition nor the extinction of lithium-induced CTA appeared to be affected by RU486 pretreatment at the CS-US pairing in 7 days recovery paradigm. Two-way ANOVA revealed main effects of treatment (Veh, DEX or RU) both in 1day [$F(2,75)=5.460$, $P = 0.0061$] and 7 days recovery paradigm [$F(2,75)=27.864$, P <0.0001], and of experimental day both in 1 day [$F(4,75)=40.522$, P <0.0001] and 7 days recovery paradigm [$F(4,75)=28.576$, P <0.0001].

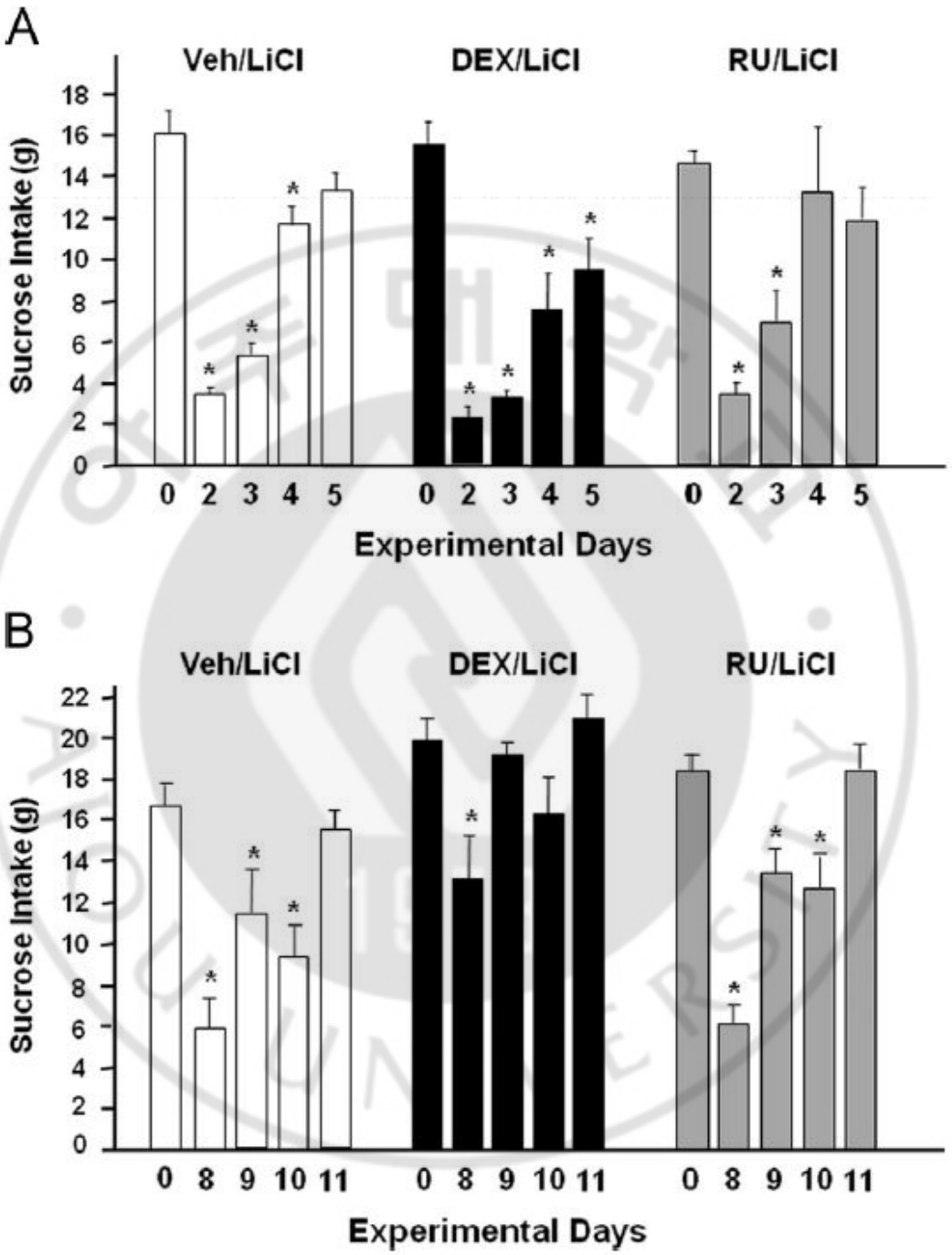
A
Veh/LiCl
DEX/LiCl
RU/LiCl
Sucrose Intake (g)
0 2 3 4 5
Experimental Days
B
Veh/LiCl
DEX/LiCl
RU/LiCl
Sucrose Intake (g)
0 8 9 10 11
Experimental Days

Fig. 4. Effects of dexamethasone or RU486 pretreatment on the formation of lithium-induced CTA memory. Rats received dexamethasone (2mg/kg; DEX) or RU486 (20mg/kg; RU) immediately after 5% sucrose access, and then an intraperitoneal injection of isotonic lithium chloride (12ml/kg) was followed with 30 min interval. Rats had either 1 (A) or 7 days (B) of recovery period before the daily sucrose drinking tests. $*P < 0.05$ vs. conditioning day in each group, data are presented by means ± S.E.M.

C. Dexamethasone pretreatment during the drinking test

Rats underwent the sucrose lithium pairing (conditioning), and then received a subcutaneous injection of dexamethasone at a dose of 2mg/kg or the same injection volume of polyethylene vehicle at 30 min each time before sucrose drinking test (Fig. 5). The amount of sucrose consumed by DEX rats during the first drinking test (day2) did not differ from Vehicle treated rats, but thereafter it was reduced significantly compared with Vehicle treated rats (P <0.05, Vehicle vs. DEX on each day) during the whole experimental period. Two-way ANOVA revealed main effects of treatment (Veh or DEX) [$F(1,70)=106.813$, P <0.0001] and experimental day [$F(6,70)=72.193$, P <0.0001], and significant interaction between treatment and day [$F(6,70)=10.616$, P <0.0001]. These results indicate that daily dexamethasone injection prior to sucrose drinking test suppressed the extinction of lithium-induced CTA memory.

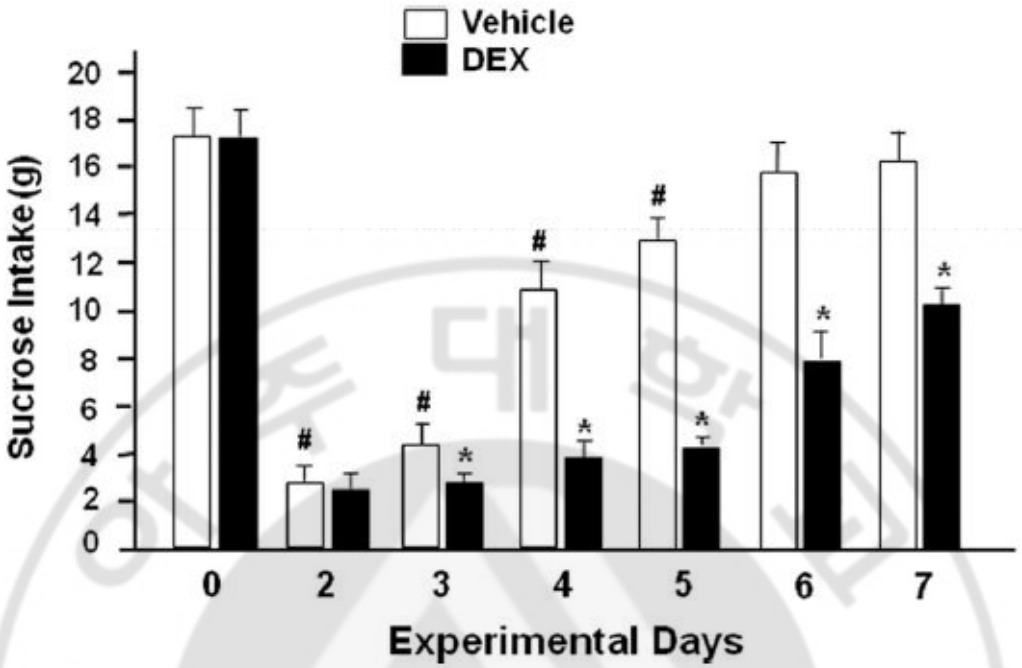
Vehicle
DEX
Sucrose Intake (g)
20
18
16
14
12
10
8
6
4
2
0
#
*
0
2
3
4
5
6
7
Experimental Days

Fig. 5. The effect of dexamethasone prior to each drinking test on the extinction of lithium-induced CTA. Rats were conditioned with the sucrose-lithium pairing, and then received dexamethasone (2mg/kg; DEX) or vehicle injections at 30 min before each drinking test. $*P$ <0.05 vs.Vehicle on each day, $\#P$ <0.05 vs. day 0 in vehicle injected rats, data are presented by means ± S.E.M.

D. Lithium-induced CTA in adrenalectomized rats

Adrenalectomized (ADX or ADX + B) and sham operated rats (Sham) underwent the sucrose lithium pairing (conditioning), and then daily sucrose drinking tests after 1 day of recovery from the conditioning (Fig. 6A). Sucrose consumption was significantly decreased in all groups during the first drinking session (day 2) compared to the conditioning day (day 0). However, during the fourth drinking session (day 5), a significant decrease (P <0.05) was found only in ADX rats. Analysis of the ADX effect with two-way ANOVA revealed main effects of treatment (Sham or ADX) [$F(1,30)=4.295$, $P = 0.0469$] and experimental day [$F(2,30)=88.155$, P <0.0001], and significant interaction between treatment and day [$F(2,30)=3.457$, $P = 0.0446$]. Analysis of the ADX+B effect with two-way ANOVA revealed no effect of treatment (Sham or ADX+B) and main effect of day [$F(2,30)=42.470$, P <0.0001]. The PVN-Fos expression was examined at 1h after an intraperitoneal injection of lithium chloride at the same dose as used for the conditioning (Fig. 6B). Number of c-Fos immunoreactive nuclei in the PVN of ADX or ADX+B rats did not differ from sham rats.

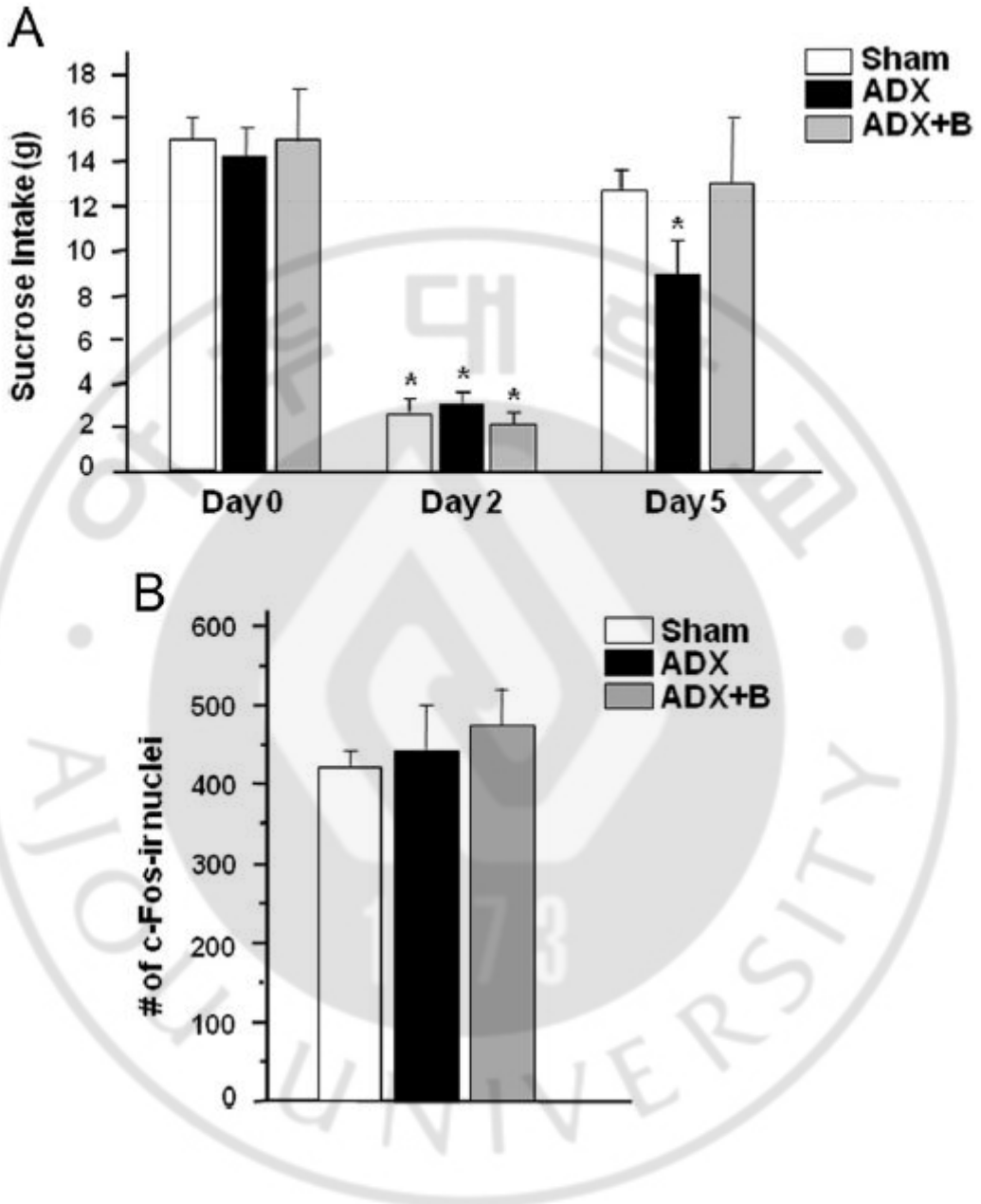
A
Sucrose Intake (g)
18
16
14
12
10
8
6
4
2
0
Sham
ADX
ADX+B
*
*
*
*
Day 0
Day 2
Day 5
B
# of c-Fos-ir nuclei
600
500
400
300
200
100
0
Sham
ADX
ADX+B

Fig. 6. Effects of adrenalectomy on the lithium-induced CTA formation (A) and c-Fos expression in the hypothalamic paraventricular nucleus (B). (A) A week after bilateral adrenalectomy or sham operation, rats underwent the sucrose-lithium pairing following 5 days of water training, and then were tested for sucrose drinking after 1 day of recovery from the conditioning (B). Two days after the fourth drinking test (on day 8) rats in each group were perfused with 4% paraformaldehyde at 1h after an intraperitoneal lithium chloride (0.15M, 12ml/kg), and the paraventricular nucleus sections were processed for c-Fos immunohistochemistry. ADX; adrenalectomized rats, ADX-B; rats received corticosterone replacement (2mg/kg daily) during the whole experiment period after surgery. * P <0.05 vs. conditioning day in each group, data are presented by means ± S.E.M.

IV. DISCUSSION

Intraperitoneal administration of lithium chloride activates the HPA axis in rats; i.e., increases plasma levels of ACTH and corticosterone (Hennessy et al., 1980; Spencer et al., 2005; Sugawara et al., 1988). Studies have suggested that c-Fos expression in the PVN refers the activation of the HPA axis by stressful stimuli (Briski and Gillen, 2001; Figueiredo et al., 2003). In this study, an intraperitoneal injection of lithium chloride markedly increased c-Fos expression in the PVN, and dexamethasone pretreatment significantly reduced it, suggesting that dexamethasone pretreatment at a dose used in this study attenuates lithium- induced activation of the HPA axis. The blunted c-Fos expression in the PVN by dexamethasone pretreatment suggests a tentative reduction in the lithium-induced increase of plasma ACTH, and ACTH is reported to be implicated in CTA formation (Smotherman, 1985). Thus, this result led us to hypothesize that lithium-induced CTA formation may be attenuated by dexamethasone pretreatment. However, lithium-induced CTA formation was not affected by dexamethasone prior to lithium chloride at the CS-US pairing in the protocol with 1 day of recovery from the toxin injection. Contrarily, lithium-induced CTA formation appeared to be potentiated by dexamethasone pretreatment with a delayed extinction. Interestingly, in the

protocol with 7 days of recovery, lithium-induced CTA formation was remarkably attenuated by dexamethasone pretreatment with markedly shortened extinction. This difference may be due to pharmacokinetic and pharmacodynamic profile of dexamethasone. Biological half-life of dexamethasone is 36-54 h (Schimmer and Parker, 2005). That is, the present results suggest that the delayed extinction in the protocol with 1 day of recovery may be due to the effect of residual dexamethasone during the extinction period, and that the HPA axis activation, most likely increased ACTH, plays an important role in the lithium-induced CTA formation. In fact, the tentative residual effect of dexamethasone on the extinction of lithium-induced CTA was confirmed by the current result showing that dexamethasone prior to each drinking test significantly delayed the extinction of lithium-induced CTA. Recent studies have demonstrated that extinction is a process of relearning (Berman and Dudai, 2001), resulting in the acquisition and consolidation of a new memory, the so-called extinction memory (Burgos-Robles et al., 2007; Sotres-Bayonetal., 2009). The retrieval of conditioned memory (the CS-US association) is blocked by the extinction memory, indicating that the CTA memory is not actually discarded from the brain by the extinction (Berman and Dudai, 2001; Bouton and King, 1983; Quirk,2002). The difference between the acquisition and retention processes of memory has been demonstrated by several reports using the local blockade of N-methyl-D-

aspartate (NMDA) receptor (Burgos-Robles et al., 2007; Sotres-Bayonetal., 2009) in brain regions related to CTA memory or the extinction memory. These reports have suggested that the extinction memory is an active learning process requiring NMDA receptors. Glucocorticoid has been reported to suppress the activity of NMDA receptors in cultured hippocampal neurons (Zhang et al., 2012). Therefore, it is suggested that the delayed extinction by dexamethasone in this study may reveal a tentative implication of the hippocampal function in the extinction memory formation of lithium-induced CTA, and its function may be affected by an increased glucocorticoid action due to the dexamethasone treatment. In this study, pretreatment with glucocorticoid receptor antagonist RU486 at the CS-US pairing did not affect the acquisition, but shortened the extinction of lithium-induced CTA in the 1 day of recovery paradigm. However, in the 7 days of recovery paradigm, neither the acquisition nor the extinction was affected by RU486 at the conditioning. The physical and biological half-life of RU486 are approximately 24h (Baulieu, 1989; Deraedt et al., 1985). Also, it was reported that when the CS to which a CTA has been developed is presented, reminiscent of that experience also increases the plasma levels of corticosterone (Smotherman e tal., 1976). Thus, it is plausible that the shortened extinction of lithium-induced CTA by RU486 in the 1 day of recovery paradigm may be a residual effect; i.e.,the residual RU486 might have blocked the glucocorticoid action at least during the first

drinking test, possibly by the increased plasma corticosterone due to aversive response to CS, and facilitated the extinction memory formation of lithium-induced CTA per se. Studies have reported that chronic stress is thought to affect memory through the actions of glucocorticoids on the hippocampus, such as decreasing cell density and corticosteroid receptor binding (Cordero et al., 2002; Leverenz et al.,1999; Sapolsky, 1999). Taken together, increased glucocorticoids action seems to be harmful for memory formation. In order to determine whether an increased glucocorticoid action by the HPA axis activation is necessary or just the basal level of corticosterone is sufficient for the acquisition and/or extinction of lithium-induced CTA memory, ADX and ADX+B rats were used for the study. In this study, sucrose consumption during the first drinking test was significantly suppressed not only in ADX+B rats but also in ADX rats, compared to each conditioning day. This result reveals that glucocorticoid is not a necessary component for the acquisition of lithium-induced CTA. Following adrenalectomy, there is a triphasic response of ACTH secretion characterized by an initial high rise immediately following the adrenalectomy, secondary to the stress of the procedure. There is then a fall to resting levels, which occurs between 2 and 6h after adrenalectomy. This low level persists for a short, variable period followed by a gradual increase, with significant elevations above normal (Dallman et al., 1972; Matsuyama et al., 1971; Yietal., 2010). In addition, constant

corticosterone replacement permit ssustained ACTH hypersecretion after stress in ADX rats (Akana et al.,1988). c-Fos is an immediate early gene, its expression in the brain regions is transiently increased by acute stimuli. In this study, intraperitoneal lithium (US) markedly induced c-Fos expression in the PVN not only of sham rats but also of ADX and ADX+B rats, suggesting the lithium-induced HPA activation, in turn ACTH increase in ADX and ADX+B rats as well. ACTH is involved in the acquisition of CTA (Smotherman, 1985). Thus, the present results suggest that ACTH rather than glucocorticoid may play a key role in the acquisition of lithium-induced CTA. However, the extinction was delayed in ADX, but not in ADX+B, rats, suggesting that glucocorticoid is an important component for the extinction of lithium-induced CTA memory, although its pulse increase by the HPA activation due to aversive response to CS (Smotherman et al.,1976) may not be required.

V. CONCLUSION

The present results reveal that glucocorticoid is not a necessary component in the acquisition, but an important player in the extinction, of lithium-induced CTA. A pulse increase of glucocorticoid due to aversive response to CS may hinder the extinction memory formation of lithium-induced CTA.

REFERENCES

1. Akana F, Jacobson L, Cascio CS, Shinsako J, Dallman MF: Constant corticosterone replacement normalizes basal adrenocorticotropin (ACTH) but permits sustained ACTH hypersecretion after stress in adrenalectomized rats. *Endocrinology* 122: 1337-1342, 1988

2. Baulieu EE: Contragestion and other clinical applications of RU486, an antiprogesterone at the receptor. *Science* 245: 1351-1357, 1989

3. Berman DE, Dudai Y: Memory extinction, learning a new, and learning the new : dissociations in the molecular machinery of learning in cortex. *Science* 291:2417-2419, 2001

4. Bouton ME, King DA: Contextual control of the extinction of conditioned fear: tests for the associative value of the context. *J Exp Psychol Anim Behav Process* 9:248-265, 1983

5. Briski K, Gillen E: Differential distribution of Fos expression with in the male rat preoptic area and hypothalamus in response to physical vs. psychological stress. *Brain Res Bull* 55:401-408, 2001

6. Burgos-Robles A,Vidal-Gonzalez I, Santini E, Quirk GJ: Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventro medial prefrontal cortex. *Neuron* 53:871-880, 2007

7. Cordero MI, Kruyt ND, Merino JJ, Sandi C: Glucocorticoid involvement in memory formation in a rat model for traumatic memory. *Stress* 5:73-79, 2002

8. Cordero MI, Sandi C: A role for brain glucocorticoid receptors in contextual fear conditioning: dependence upon training intensity. *Brain Res* 786:11-17, 1998

9. Dallman MF,Jones MT, Vernikos-Danellis J, Ganong WF: Corticosteroid feedback control of ACTH secretion: rapid effects of bilateral adrenalectomy on plasma ACTH in the rat. *Endocrinology* 91:961-968, 1972

10. Deraedt R, Bonnat C, Busigny M, Chatelet P, Cousty C, Mouren M, Philibert D, Pottier J, Salmon J: Pharmacokinetics of RU486, 1985.

11. The Antiprogestin Steroid RU486 and Human Fertility Control. *Reprod Biol* 103-122

12. Figueiredo HF, Bodie BL, Tauchi M, Dolgas C M, Herman JP: Stress integration after acute and chronic predator stress: differential activation of central stress circuitry and sensitization of the hypothalamo-pituitary-adrenocortical axis. *Endocrinology* 144: 5249-5258, 2003

13. Flood JF, Vidal D, Bennett EL, Orme AE, Vasquez S, Jarvik ME: Memory facilitating and anti-amnesic effects of corticosteroids. *Pharmacol Biochem Behav* 8:81-87, 1978

14. Foy MR, Stanton ME, Levine S, Thompson RF: Behavioral stress impairs long-term potentiation in rodent hippocampus. *Behav Neural Biol* 48: 138-149, 1987

15. Hennessy JW, Smotherman WP, Levine S: Investigations into the nature of the dexamethasone and ACTH effects upon learned taste aversion. *Physiol Behav* 24: 645-649, 1980

16. Houpt TA, Philopena JM, Wessel TC, Joh TH, Smith GP: Increased c-fos expression in nucleus of the solitary tract correlated with conditioned taste aversion to sucrose in rats. *Neurosci Lett* 172: 1-5,

1994

17. Jahng JW, Lee JH, Lee S, Lee JY, Kim GT, Houpt TA, Kim DG: N (omega)-nitro-L-arginine methylester attenuates lithium-induced c-Fos, but not conditioned taste aversion, in rats. *Neurosci Res* 50:485-492, 2004

18. Jahng JW, Lee JY, Yoo SB, Kim YM, Ryu V, Kang DW, Lee JH: Refeeding-induce dexpression of neuronal nitric oxide synthase in the rats paraventricular nucleus. *Brain Res* 1048: 185-192, 2005

19. Kim YM, Lee JY, Choi SH, Kim DG, Jahng JW: RU486 blocks fasting- induced decrease of neuronal nitric oxide synthase in the rat paraventricular nucleus. *Brain Res* 1018: 221-226, 2004

20. Korz V, Frey JU: Stress-related modulation of hippocampal long-term potentiation in rats: Involvement of adrenal steroid receptors. *J Neurosci* 23: 7281-7287, 2003

21. Lamprecht R, Dudai Y: Differential modulation of brain immediate early genes by intraperitoneal LiCl. *Neuroreport* 7:289-293, 1995

22. Leverenz JB, Wilkinson CW, Wamble M, Corbin S, Grabber JE, Raskind MA, Peskind ER: Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J Neurosci* 19: 2356-2361, 1999

23. Lee JY, Lee JH, Kim DG, Jahng JW: Dexamethasone blocks the refeeding- induced phosphorylation of cAMP response element-binding protein in the rat hypothalamus. *Neurosci Lett* 344: 107-111, 2003

24. Matsuyama H, Mims RB, Ruhmann-Wennhold A, Nelson DH: Bioassay and radioimmunoassay of plasma ACTH in adrenalectomized rats. *Endocrinology* 88: 696-701, 1971

25. Nachman M, Ashe JH: Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiol Behav* 10: 73-78, 1973

26. Pugh CR, Fleshner M, Rudy JW: Type II glucocorticoid receptor antagonists impair contextual but not auditory-cue fear conditioning in juvenile rats. *Neurobiol Learn Mem* 67: 75-79, 1997

27. Quirk GJ: Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. *Learn Mem* 9: 402-407, 2002

28. Reul JM, deKloet ER: Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* 117: 2505-2511, 1985

29. Revusky S, Martin GM: Glucocorticoids attenuate taste aversions produced by toxins in rats. *Psychopharmacology* 96:400-407, 1988

30. Sakai N, Yamamoto T: Conditioned taste aversion and c-fos expression in the rat brain stem after administration of various USs. *Neuroreport* 8: 2215-2220, 1997

31. Sapolsky RM: Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. *Exp Gerontol* 34: 721-732, 1999

32. Schafe GE, Bernstein IL: Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion: I.Theamygdala. *BrainRes* 741: 109-116, 1996

33. Schimmer BP, Parker KL: Adrenocorticotropic hormone: adrenocortical steroids and their synthetic analogs: inhibitors of the synthesis and actions of adrenocortical hormones. Goodman and Gilman's the pharmacological basis of therapeutics, 9 th edition 1459-1485, 2005, McGraw-Hill, NewYork p.1485

34. Smotherman WP: Glucocorticoid and other hormonal substrates of conditioned taste aversion. *Ann NY Acad Sci* 443: 126-144, 1985

35. Smotherman WP, Hennessy JW, Levine S: Plasma corticosterone levels during recovery from LiCl produced taste aversions. *Behav Biol* 16: 401-412, 1976

36. Sotres-Bayon F, Diaz-Mataix L, Bush DE, LeDoux JE: Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: NR2B contribution. *Cereb Cort* 19: 474-482, 2009

37. Spencer CM, Jahng JW, Ryu V, Houpt TA: Lithium-induced gene expression of inducible cyclic adenosine monophosphate early repressor in the rat adrenalgland. *J Neurosci Res* 82:273-282, 2005

38. Sugawara M, Hashimoto K, Hattori T, Takao T, Suemaru S, Ota Z: Effects of lithium on the hypothalamo-pituitary-adrenal axis.

Endocrinol Jpn 35: 655-663, 1988

39. Tasker JG, Herman JP: Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress* 14: 398-406, 2011

40. Waynforth HB, Flecknell PA: Experimental and surgical technique in the rat. Academic Press, NewYork 1992

41. Yamamoto T, Shimura T, Sako N, Azuma S, Bai WZ, Wakisaka S: C-fos expression in the rat brain after intraperitoneal injection of lithium chloride. *Neuroreport* 3:1049-1052, 1992

42. Yi, SS, Hwang IK, Shin JH, Choi JH, Lee CH, Kim IY, Kim YN, Won MH, Park IS, Seong JK, Yoon YS: Regulatory mechanism of hypothalamo- pituitary-adrenal (HPA) axis and neuronal changes after adrenalectomy in type 2 diabetes. *J Chem Neuroanat* 40: 130-139, 2010

43. Zhang Y, Sheng H, Qi J, Ma B, Sun J, Li S, Ni X: Glucocorticoid acts on a putative G protein-coupled receptor to rapidly regulate the activity of NMDA receptors in hippocampal neurons. *Am J Physiol*

Endocrinol Metab 302: E747-758, 2012

– 국문요약 –

리튬에 의한 조건적 미각혐오학습과정에서 당질코르티코이드의 혈중농도 증가의 역할 연구.

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리튬은 조건화된 미각 회피 형성을 위해 흔히 사용되는 물질로 동물의 복강내 주입시 뇌의 여러 영역에서 c-Fos를 발현시키며 c-Fos는 미각 회피 학습과 연관이 있는 것으로 알려져 있다. 또한 Hypothalamic-pituitary-adrenal (HPA) axis도 미각 회피 학습에 중요한 역할을 한다. 복강내 리튬 주입시 adrenocorticotrophic hormone (ACTH)호르몬이 분비되며 이는 HPA axis를 활성화시켜 혈중 glucocorticoids를 증가 시킨다. ACTH 나 glucocorticoids를 주면 리튬으로 인한 미각 회피 학습에 영향을 주는 것으로 보고되고 있다. 그러나, 리튬으로 인한 미각 회피 학습 형성에 있어 HPA axis와 미각 회피 학습 및 c-fos 발현과는 잘 알려져 있지 않다.

본 연구는 5% sucrose를 백서에 섭취시킨후 dexamethasone (2 mg/kg) 또는 RU486 (20 mg/kg)을 주었으며 이후 복강에 리튬을 주입함으로써 sucrose섭취에 대한 반응을 관찰하였다. 미각 회피 학습이 형성된 이후에 dexamethasone이나 vehicle를 sucrose섭취전 주입함으로써 sucrose섭취에 대한 반응도 관찰

하였다. 마지막으로 부신절제후 미각 회피 학습이 형성된 백서에서 sucrose섭취에 대한 반응을 관찰하였다. 그 결과 sucorose섭취전 dexamethasone투여는 리튬으로 형성된 미각 회피 학습의 소실이 지연됨을 확인하였고 부신이 절제된 백서에서는 생리적인 스테로이드 용량을 준 백서나 주지 않았던 백서 모두에서 sucrose섭취의 감소를 보였다. 그러나 sucrose섭취를 보는 4일째 실험에서는 부신을 절제한 백서(생리적 스테로이드를 주입받지 않은 백서)에서만 sucrose의 섭취 감소를 보였다. 이러한 결과들은 glucocorticoid는 리튬으로 인한 미각 회피 학습 형성에는 필수적인 요소는 아니지만 형성된 미각 회피 학습의 소멸에는 중요한 역할을 하고 있음을 의미한다. 또한 glucocorticoid의 급격한 증가(생리적 반응 이상의 증가)가 미각 회피 학습의 소멸을 방해할지 모른다는 것을 확인할 수 있었다.

핵심어: 시상하부-뇌하수체-부신 축, c-fos, 리튬, 조건화 미각 혐오