ORIGINAL ARTICLE

Different contribution of the lateral bed nucleus of the stria terminalis in intrahippocampal neostigmine-induced elevation of plasma glucose and adrenocorticotropic hormone in free moving rats

Waner Zhu [1,2], Jiucai Cui [1], Wenqian Wang [1], Rong Zhang [1], Hiroyuki Umegaki [2]

[1] Department of Medical Psychology, Zhejiang University Graduate School of Medicine, Hangzhou, 310058, China.
[2] Department of Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, 466-8550, Japan.

Correspondence to: Waner Zhu, PHONE: +86-571-88208291; FAX: +86-571-88208119
E-MAIL: waner_zhu@yahoo.com.cn

Submitted: 2009-06-20 Accepted: 2009-08-16 Published online: 2009-09-20

Key words: **glucose; ACTH; BNSTL; hippocampus; rat**

Neuroendocrinol Lett 2009; **30**(4): 511–514 **PMID:** 20010499 NEL300409A22

Abstract

OBJECTIVE: In our previous study, a lesion in the lateral bed nucleus of the stria terminalis (BNSTL) was found to significantly attenuate the elevation of adrenocorticotropic hormone (ACTH) in plasma during microinjectin of neostigmine, an inhibitor of acetylcholine esterase, into the rat hippocampus. The current study was designed to examine the role of the BNSTL in regulation of blood glucose elevation induced by hippocampal neostigmine injection.
MATERIALS AND METHODS: Ibotenic acid (15 μg/μl) was stereotaxically bilaterally injected into the BNSTL of rats. Two weeks after the injections, neostigmine methylsulfate (sigma, 5×10–8 mol) was microinjected into the rat hippocampus in a volume of 1 μl for 1 min using a CMA/100 microinjection pump. Plasma ACTH and glucose concentrations were examined using radioimmunoassay and immobilized enzyme casing/H_2O_2 method techniques, respectively.
RESULTS: Compared with sham-operated control rats, rats with BNSTL lesions produced by ibotenic acid showed significantly attenuated the elevations of plasma ACTH evoked by the microinjection of neostigmine into the hippocampus. However, no significant difference of blood glucose in response to the injection was observed between the BNSTL-lesioned rats and controls.
CONCLUSION: The results of the present study indicate that the BNSTL plays a role in ACTH regulation and not in blood glucose regulation when the hippocampal cholinergic system is activated.

INTRODUCTION

Stimulation of the brain cholinoceptive neurons has been reported to produce hyperglycemia (Iguchi *et al.* 1988,1990). Evidence from previous studies of our laboratory have indicated that microinjection of neostigmine, an acetylcholinesterase inhibitor, into the dorsal hippocampus, which should increase extracellular acetylcholine levels, produced marked hyperglycemia associated with the secretion of plasma epinephrine and norepinephrine in anesthetized rats (Uemura *et al.* 1989; Iguchi *et al.* 1991a,1992). We recently demonstrated that the activation of the cholinergic system in the hippocampus induces stress-like responses in free moving rats. Injection of the neostigmine into the rat hippocampus elicits an elevation of adrenocorticotropic hormone (ACTH) levels in

Abbreviations:
ACTH – adrenocorticotropic hormone
BNST – bed nucleus of the stria terminalis
BNSTL – lateral bed nucleus of the stria terminalis
HPA – hypothalamic-pituitary-adrenal

plasma and activation of sympathetic nervous system (zhu *et al.* 2001a). In this animal model the lateral bed nucleus of the stria terminalis (BNSTL) relays signals of hippocampal cholinergic system-mediated activation of the HPA axis. Lesions in this area by ibotenic acid significantly attenuated expression of Fos-ir in the PVN and elevation of plasma ACTH levels caused by hippocampal microinjection of neostigmine (zhu *et al.* 2001b). However, the relationship of blood glucose level and activation of hippocampal cholinergic system as well as the relative contribution of BNSTL to the net hyperglycemic response in free moving rats remains unknown. In this study, we investigated the importance of the BNSTL in generating HPA axis responses and glucoregulatory functions after stimulation of the hippocampal cholinergic system in free moving rats.

MATERIALS AND METHODS

Subjects

All experiments were conducted on adult male Wistar rats (250–300g), with the animals housed individually under standard laboratory conditions in temperature-controlled rooms (25°C). The rats were maintained on a 12h light/dark cycle (light on at 06.00) with food pellets and water available ad libitum. The rat studies were approved by the Animal Care and Use Committee of Nagoya University. The rats were randomly separated into the following four groups. Group 1, which hippocampal microinjected with saline, was used as the control (n=4). Groups 2 to 4 were microinjected with neostigmine at concentrations of 5×10^{-8}mol without BNSTL injection (n=5), BNSTL injected with saline (n=4), and BNSTL injected with ibotenic acid (n=6), respectively.

Surgery

Rats, which in group 4, were anaesthetized with an intraperitoneal injection of a mixture of ketamine (40 mg/kg) and xylazine (20 mg/kg). The rats were positioned in a stereotaxic apparatus (Narishige Scientific Instrument Laboratory, Tokyo, Japan), and guided into the BNSTL. Ibotenic acid was injected through a stainless steel needle (outside tip diameter 28μm), which was connected to a 1.0μl syringe via a tubing. The coordinates for the BNSTL were calculated relative to the bregma with the incisor bar set at –3.3mm. The coordinates used were AP –0.3mm, ML ±1.7mm, and DV 6.4mm from the skull surface in accordance with the Paxinos and Watson atlas (Paxinos and Watson,1986). The BNSTL lesions were produced by pressure-injecting 0.1μl of ibotenic acid (15μg/μl in 0.9% NaCl with Trypan Blue 0.2%, Sigma Chemical Co., St. Louis, Mo) bilaterally during a 5 min period. The tip was allowed to remain in the brain for 5 min after the injection in order to minimize the dorsal diffusion of the drug along the needle. Sham-operated rats, which in group 3, were treated in an identical manner to the ibotenic acid-lesioned rats but were injected with the same volume of 0.9% NaCl with 0.2% Trypan Blue without ibotenic acid. After a recovery period of approximately seven days, the rats were anaesthetized once again in order to stereotaxically implant a guide cannula (BAS, TOKYO, Japan) into the left dorsal hippocampus at the following coordinates: AP –2.0mm, ML 1.5mm, and DV 3.5mm in accordance with the Paxinos and Watson atlas 1 week before the experiments. Rats, which in groups 1 and 2, were hippocampally cannulated in an identical manner to the groups 3 and 4. The day before the experiments, each hippocampally cannulated rats were anaesthetized with diethyl ether (Kanto Chemical Co. Inc, Tokyo, Japan) and the jugular vein cannula for repeated blood sampling was inserted. A 2-cm longitudinal incision was made in the neck directly over the trachea. The underlying muscles were separated using blunt dissection and the right jugular vein was catheterized with Silastic tubing (Shiniest Polymer, Nagoya, Japan) filled with heparinized saline (10U/ml). The catheter was threaded through the vein for a distance of 2.5-cm, which allowed the tip of the cannula to rest in or near the atrium. The free end of the catheter was plugged with a knot, exteriorized and secured at the back of the neck with a special cap. Rats were kept in individual cages with free access to water and food.

Procedures

On the day of the experiment, saline or saline containing neostigmine methylsulfate (Sigma, 5×10^{-8} mol) was microinjected in a volume of 1μl for 1 min using a CMA/100 Microinjection Pump (BSA, Tokyo, Japan) through the guide cannula into the hippocampus of the free moving rats. In order to determine plasma ACTH levels, blood was sampled (0.8 ml) intermittently, starting at time 0, just prior to microinjection, and at 10, 30, 60, and 120 min after the microinjection. To minimize the effect of volume loss, an equal volume of heparinized saline was returned to the general circulation at each sampling. Blood sampled were kept on ice, centrifuged, and plasma was taken and then stored at –20°C in aliquots of approximately 400 μl for subsequent determination of ACTH by radioimmunoassay (sensitivity > 5pg/ml, intra-assay variation coefficient 4.3%) (Pignatelli *et al.* 1996). Plasma glucose concentrations were determined by the immobilized enzyme casing/H_2O_2 method with a compact glucose analyzer Antsense II (Bayer Medical Co.Ltd, Tokyo, Japan) (Tabata *et al.* 1998). All experiments were completed between 10.00–13.00 to minimize variability resulting from circadian rhythm differences.

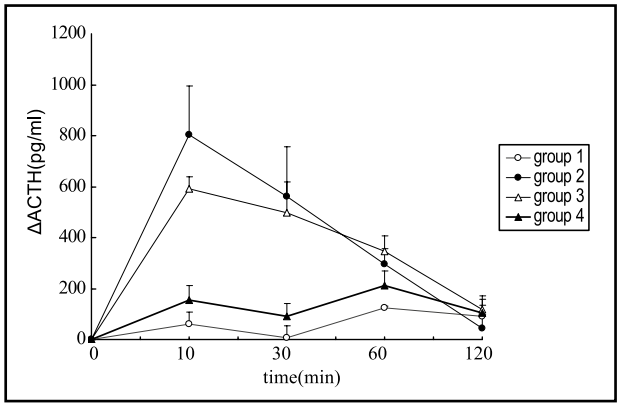

FIG. 1. Changes of plasma ACTH concentration in four groups after intrahippocampal microinjection. Group 1, saline injection, -o-, n=4; Group 2, neostigmine injection, -●-, n=5; Group 3, neostigmine injection, BNSTL injected with saline, -Δ-, n=4; Group 4, neostigmine injection, BNSTL lesioned with ibotenic acid -▲-, n=6. Results are expressed as means±S.E. Plasma ACTH levels are expressed as change from basal values. Δ, change; $p < 0.01$ in four groups by ANOVA, and $p < 0.01$ between group 1 and group 2, or group 2 and group 4 by Fisher's PLSD.

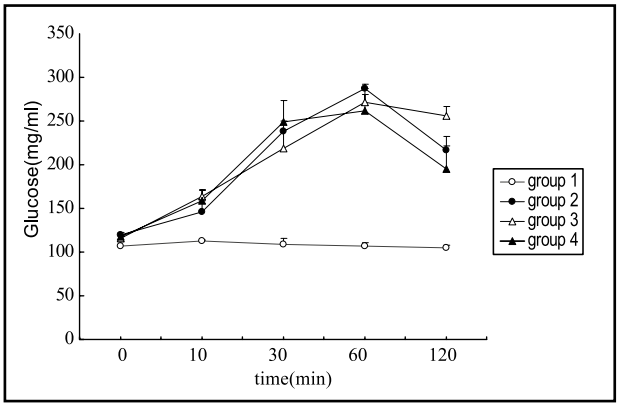

FIG. 2. Blood glucose concentration in four groups after intrahippocampal microinjection. Group 1, saline injection, -o-, n=4; Group 2, neostigmine injection, -●-, n=5; Group 3, neostigmine injection, BNSTL injected with saline, -Δ-, n=4; Group 4, neostigmine injection, BNSTL lesioned with ibotenic acid -▲-, n=6. Results are expressed as means±S.E; Repeated ANOVA showed no significant difference between group 2 and group 4, while group 2 had significantly higher concentration of blood glucose than group 1 ($p < 0.01$).

Statistical analysis

Plasma ACTH and glucose concentrations were expressed as mean ± S.D. Data were analyzed by repeated-measure one-factor ANOVA. A level of $p<0.01$ was accepted as statistically significant.

RESULTS

Figure 1 shows changes of plasma ACTH concentration in all experimented rats after hippocampal microinjection. Plasma ACTH concentration (ΔACTH) increased 10 min after neostigmine injection and continued to increase for 120 min. The baseline levels of ACTH, which expressed by the mean ± S.D. in groups 1 to 4, are 93 ± 21, 128 ± 15, 55 ± 18, and 91 ± 42, respectively. There was no significant difference in four groups (p=0.4633). Repeated-measures one-factor ANOVA indicated a significant difference on the changes of ΔACTH in four groups (p=0.0008). In post hocs, Fisher's Protected Least Significant Difference (Fisher's PLSD) showed that intrahippocampal injection of neostigmine induced significantly more ACTH secretions than saline (group 1 vs group 2, p=0.0005). Increased ACTH secretion was significantly suppressed by bilateral BNSTL lesion with Ibotenic acid (group 2 vs group 4, P=0.0014). No significant difference between bilateral BNSTL injected with saline (group 3) and group 2 (p=0.6437).

Figure 2 shows blood glucose concentration in all experimented rats after hippocampal microinjection. Blood glucose concentration increased 10 min after neostigmine injection and remained elevated for 120 min above that seen in control-treated rats. Repeated-measures one-factor ANOVA indicated a significant difference on the concentration of blood glucose in four groups (p=0.0002). In post hocs, Fisher's PLSD showed that intrahippocampal injection of neostigmine induced significantly more blood glucose secretions than saline (group 1 vs group 2, p<0.0001), although no significant difference between group 2 and bilateral BNSTL injected with or without Ibotenic acid (group 2 vs group 3, p=0.84; group 2 vs group 4, p=0.74).

DISCUSSION

The bed nucleus of the stria terminalis (BNST) is located in the rostral forebrain, ventral and adjacent to the septum. A lateral and medial BNST have been described that they contain many cytoarchitecturally distinct subdivisions (Moga *et al.* 1989). The BNST receives inputs directly from the ventral hippocampal/subiculum area (Cullinan *et al.* 1993). The lateral BNST (BNSTL) sends a heavy axonal projection into neuroendocrine cell regions of the hypothalamic paraventricular nucleus (PVN) (Sawchenko and Swanson, 1983). The BNST occupies a central position in pathways regulating hypothalamo-pituitary-adrenocortical (HPA) stress regulation (Herman and Cullinan, 1997; Choi *et al.* 2007). The BNSTL is believed to be involved in mediating the endocrine, autonomic, and behavioral effects in response to stress (Cecchi *et al.* 2002; Casada and Dafny, 1991). Stimulation of the BNSTL alters plasma corticosterone secretion and causes stress-like behaviors in rats. Lesions in the BNSTL reduce expression of corticotropin-releasing hormone (CRH) mRNA across the rostrocaudal extent of the medial parvocellular PVN (Herman *et al.* 1994), and inhibit increases in plasma adrenocorticotropic hormone (ACTH) secretion caused by conditioned stress (Gray *et al.* 1993).

We previously demonstrated that microinjection of neostigmine, an acetylcholinesterase inhibitor, into the hippocampus caused an elevation of plasma ACTH

and expression of c-Fos in the PVN in rats (zhu *et al.* 2001a). We also found that the BNSTL is involved in the regulation of ACTH release in response to hippocampal neostigmine injection (Zhu *et al.* 2001b). In the current study, we have discovered that the BNSTL is involved in the regulation of stress-like responses induced by hippocampal neostigmine injection. The lesions in this area significantly attenuated the ACTH elevation but did not affect blood glucose secretion.

Microinjections of neostigmine into the hippocampus produce hyperglycemia associated with the secretion of plasma cathecholamines, which showed similarities to stress responses. Regarding the mechanism responsible for the neostigmine-induced elevation of plasma glucose, at least four pathways had been hypothesized: (1) secreted epinephrine may directly act on the hepatic release of glucose; (2) epinephrine may induce the release of glucagons; (3) direct neuronal control in the pancreas causes glucagon secretion; (4) direct innervation in the liver induces glucose release (Iguchi *et al.* 1991b). We previously found that the entorhinal cortex is involved in the regulation of stress-like responses induced by hippocampal neostigmine injection (Shadi *et al.* 2005). Lesions in this area significantly attenuated the blood glucose elevation but did not affect ACTH secretion. And we have reported that the entorhinal cortex is involved in the stress response to immobilization but not to insulin-induced hypoglycaemia (Umegaki *et al.* 2003). Lesions in this area attenuate ACTH release induced by immobilization but have no effect on the blood glucose response. These findings suggest that glucose and ACTH are regulated differently within the brain. Accordingly, we hypothesized that there is a brain structure specific pathway in the glucose and ACTH regulation. However, the exact neuronal transpathway involved in this system needs further investigations.

In conclusion, we have shown that the lesion of the BNSTL attenuated ACTH elevation but did not affect the blood glucose response caused by hippocampal microinjection of neostigmine in the rat. These results suggest that the BNSTL plays role in ACTH regulation and not in blood glucose regulation when the hippocampal cholinergic system is activated.

Acknowledgements. This study was supported by the National Natural Science Foundation of China (No 30870772).

REFERENCES

1 Casada JH and Dafny N (1991). Restraint and stimulation of bed nucleus of the stria terminalis produce similar stress-like behaviors. Brain Res. Bull. **27:** 207–212.
2 Cecchi M, Khoshbouei H, Javors M, Morilak DA (2002). Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuroscience. **112**(1): 13–21.
3 Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM, Herman JP (2007). Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: implications for the integration of limbic inputs. J Neurosci. **27**(8): 2025–34.
4 Cullinan WE, Herman JP, Watson SJ (1993). Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis, J. Comp. Neurol. **332:** 1–20.
5 Gray TS, Piechowski RA, Yracheta JM, Rittenhouse PA, Bethea CL, Van de Kar LD (1993). Ibotenic acid lesions in the bed nucleus of the stria terminalis attenuate conditioned stress-induced increases in prolactin, ACTH and corticosterone. Neuroendocrinol. **57**: 517–524.
6 Herman JP, Cullinan WE, Watson SJ (1994). Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. J. Neuroendocrinol. **6**: 433–442.
7 Herman JP and Cullinan WE (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci. **20**: 78–84.
8 Iguchi A, Gotoh M, Matsunaga H, Yatomi A, Honmura A, Yanase M, et al (1988). Relative contributions of the nervous system and hormones to CNS-mediated hyperglycemia. Am. J. Physiol. **255** (Endocrinol. Metab. 18): E920–E926.
9 Iguchi A, Yatomi A, Gotoh M, Matsunaga H, Uemura K, Miura H, et al (1990). Neostigmine-induced hyperglycemia is mediated by central muscarinic receptor in fed rats. Brain Res. **507**: 295–300.
10 Iguchi A , Uemura K , Kunoh Y, Miura H, Ishiguro T, Nonogaki K, et al (1991a). Hyperglycemia induced by hippocampal administration of neostigmine is suppressed by intrahypothalamic atropine. Neuropharmacology. **30**(10): 1129–1131.
11 Iguchi A, Kunoh Y, Gotoh M, Miura H, Uemura K, Tamagawa T, et al (1991b). Relative contribution of nervous system and hormones to CNS-mediated hyperglycemia is determined by the neurochemical specificity in the brain. Physiol Behav. **50**: 1019–1025.
12 Iguchi A, Uemura K, Miura H, Ishiguro T, Nonogaki K, Tamagawa T, et al (1992). Mechanism of intrahippocampal neostigmine-induced hyperglycemia in fed rats. J. Neuroendocrinol. **55** : 44–50.
13 Moga MM, Saper CB, Gray TS (1989). Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. J. Comp. Neurol. **283**: 315–332.
14 Paxinos G. and Watson C (1986). The rat brain in stereotaxic coordinates, Academic Press, San Diego, 2nd Ed.
15 Pignatelli D, Pinto P, Azevedo M, Magalhaes M (1996). Acute stress effects on the adrenal cortexs in rat. A biochemical and immunohistochemical study. Endocr Res. **22**: 445–451.
16 Sawchenko PE and Swanson LW (1983). The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J. Comp. Neurol. **218**: 121–144.
17 Shadi AR, Umegaki H, Zhu W, Suzuki Y, Shinobu KO, Satsuki I, et al (2005). The entorhinal cortex regulates blood glucose level in response to microinjection of neostigmine into the hippocampus. Neuroendocrinol Lett. **26** (3): 225–230.
18 Tabata H, Kitamura T, Nagamats N (1998). Comparison of effects of restraint, cage transportation, anesthesia, and repeated bleeding on plasma glucose levels between mice and rats. Lab Animals. **32**: 143–148.
19 Uemura K, Iguchi A, Yatomi A, Miura H, Honmura A, Yanase M, et al (1989). Involvement of the hippocampus in central nervous system-mediated glucoregulation in rats. Endocrinology. **124**: 2449–2455.
20 Umegaki H, Zhu W, Nakamura A, Suzuki Y, Takada M, Endo H, et al (2003). Involvement of the Entorhinal Cortex in the Stress Response to Immobilization, But not to Insulin-Induced Hyperglycemia. J Neuroendocrinol. **15**: 237–241.
21 Zhu W, Umegaki H, Yoshimura J, Tamaya N, Suzuki Y, Miura H, et al (2001a). The elevation of plasma adrenocorticotrophic hormone and expression of c-Fos in hypothalamic paraventricular nucleus by microinjection of neostigmine into the hippocampus in rats: comparison with acute stress responses. Brain Res. **892**: 391–395.
22 Zhu W, Umegaki H, Suzuki Y, Miura H, Iguchi A (2001b). Involvement of the bed nucleus of the stria terminalis in hippocampal cholinergic system-mediated activation of the hypothalamo-pituitary-adrenocortical axis in rats. Brain Res. **916**: 101–106.