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The effects of lesions in the nucleus basalis of Meynert and physostigmine on rat frontal cortex acetylcholine level

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To clarify the relationship between acetylcholine (ACh) level in the frontal cortex and the area of lesion in the nucleus basalis of Meynert (nbM), we used ibotenic acid to bilaterally lesion the substantia innominata (SI), including the nbM, in rats and measured extracellular ACh by microdialysis. Baseline level of ACh (ACh-baseline) averaged 0.14 ± 0.07 ng/40 µl/20 min (n=13) in the steady state. We also measured the size of the lesioned areas in the SI (nbM-lesion) by using a computed image analysis system. Both ACh level and nbM-lesion varied widely among animals. The bilateral nbM-lesion averaged 28 ± 30 % (n=13). The correlation coefficient between ACh-baseline and bilateral nbM-lesion was -0.871 ($p<0.01$). After intraperitoneal injection of physostigmine, maximum ACh levels increased to 0.18 ± 0.06 ng/40 µl/20 min, and ΔACh (ACh-max/ACh-baseline) averaged 132 ± 19 % (n=8). There was no apparent correlation between ΔACh and bilateral nbM-lesion. These results show that the size of lesioned area in the bilateral SI was strongly correlated with the baseline ACh level in the frontal cortex, and indicate that the nbM is the major functional source of ACh in the frontal cortex. Our results also suggests that physostigmine possibly affects not only spared nbM-originated cholinergic fibers but also non-nbM-originated cholinergic fibers in the rat with the bilateral nbM lesion.

Key words : Alzheimer's disease, nucleus basalis of Meynert, acetylcholine, physostigmine, microdialysis

INTRODUCTION

Clinically, degeneration of the nucleus basalis of Meynert (nbM), from which cholinergic fibers project to the cerebral cortex, leads to the development of dementia of Alzheimer-type (DAT) [1, 2]. Activity of choline acetyltransferase (CAT) also decreases in the cerebral cortex in DAT [2, 3, 4]. However, the precise relationship between acetylcholine (ACh) release in the cortex and the extent of degeneration of the nbM is unclear. Experimentally, a decrease in CAT activity in the cerebral cortex [5] and disorder of learning and memory [6, 7] were induced by lesioning of the nbM in the rat. We have reported that ACh in the frontal cortex was decreased in the rat with bilateral nbM lesion [8], suggesting that ACh release in the cerebral cortex is regulated by the nbM. Therefore, to clarify the relationship between the decrease in ACh level in the frontal cortex and the area of lesion in the nbM, we measured the concentration of extracellular ACh and the size of the lesioned area in the substantia innominata (SI), including the nbM, in rats with bilateral nbM lesions induced with ibotenic acid injection.

MATERIAL AND METHODS

Animal model

To obtain more selective lesion in the SI, we employed ibotenic acid injection rather than kainic acid injection or electro-coagulation techniques [9, 10].

Thirteen male Wistar rats (9 weeks old; 290～320 g) were used for the lesioned group. The rats were anesthetized with 2.5 % halothane, and a 1-µl Hamilton microsy-

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ringe was inserted stereotaxically through a burr hole in the skull into the area of the substantia innominata (SI), including the nbM. According to the atlas of Paxinos and Watson [11], bilateral lesions of the SI were produced by injection of 1 μl of ibotenic acid (5 μg/μl of Hank's solution, pH 7.4) through a microsyringe. The stereotaxic coordinates of the SI were 0.8 mm posterior to the bregma, 2.5 mm lateral to the midline and 8.0 mm from the surface of the dura.

Another seven rats were used for the control group without the above surgery.

Measurement of extracellular acetylcholine

After lesioning of the nbM, rats of the lesioned group were kept in animal quarters with food and water ad libitum for 7 days. Then, 13 rats of the lesioned group and 7 rats of the control group were anesthetized with 2.5 % halothane, and a microdialysis probe (dialysis membrane of 3.0 mm long and 0.75 mm in diameter; Eicom Co., Ltd., Japan) was placed stereotaxically through another burr hole into the right frontal cortex, 2.5 mm anterior to the bregma, 2.5 mm lateral to the midline, and 4.0 mm deep at an oblique angle of 30 degrees from the surface of the dura, according to the atlas [11]. The probe was fixed with dental cement. All skin wounds were infiltrated with 1 % xylocaine. Twenty-four hours after fixation of the probe, the flow of dialysate was started at 2 μl/min. The dialysate was composed of normal saline with 10^{-5}M of physostigmine. From 60 minutes thereafter, fractions of the dialysate were collected from the probe at 20-minute intervals. The concentration of extracellular ACh in each fraction of the dialysate was determined by high-performance liquid chromatography with an electrochemical detector (HPLC-ECD system; Eicom Co., Ltd., Japan). The ACh response was calibrated with internal and external standards. Peak areas of ACh were determined using a computer (D-2500 Chromato-Integrator; Hitachi Co., Ltd., Japan). Rats were not anesthetized and were permitted to move freely in a cage (30 cm × 30 cm) during collection of the dialysate.

In all rats, the average concentration of ACh in the first three dialysate fractions was taken as "ACh-baseline".

Eight rats of the lesioned group and seven rats of the control group were injected with physostigmine (0.3 mg/kg, IP) after the first three dialysate fractions had been collected. The maximal concentration of ACh in the dialysate fractions seen within 120 minutes of the initiation of physostigmine injection was taken as "ACh-max".

Measurement of the size of the lesioned areas in the SI

At the end of the experiments, all rats of the lesioned group were perfused with phosphate-buffered saline (PBS) through the abdominal artery, and then with 4% paraformaldehyde in PBS. The brain was removed, cut and immersed in 4% paraformaldehyde. Each tissue block was dehydrated, embedded in paraffin, sectioned and double-stained with Luxol fast blue and Cresyl violet.

According to the atlas [11], we measured the percentage of lesioned areas in the SI, by using a computed image analysis system (MCID; Imaging Research Inc., Canada) [12]. The average percentage of lesioned areas in the anterior part of the SI in three sections (0.8-, 0.9- and 1.3-mm posterior to bregma) on each side was taken as right nbM-lesion or left nbM-lesion. The average of right and left nbM-lesions was taken as "bilateral nbM-lesion".

Statistical analysis

The statistical significance of the differences between right and left nbM-lesion and between ACh-baseline in the control group and in the lesioned group was analyzed by using the Mann-Whitney's U test. The correlation among ACh-baseline, ACh-max and nbM-lesion were analyzed by the Spearman's rank correlation and one-way ANOVA. The statistical significance of the differences between ACh-baseline and ACh-max was analyzed by application of the Wilcoxon signed-ranks test. For statistical analysis, all data in percentage were converted by inverse sine transformation. All values were expressed as mean ± SD.

RESULTS

ACh-baseline was 0.31 ± 0.11 ng/40 μl/20 min in the control group (n=7), and was 0.14 ± 0.07 ng/40 μl/20 min in the lesioned group (n=13). ACh-baseline in the lesioned group was significantly decreased in compared with the control group ($p<0.05$).

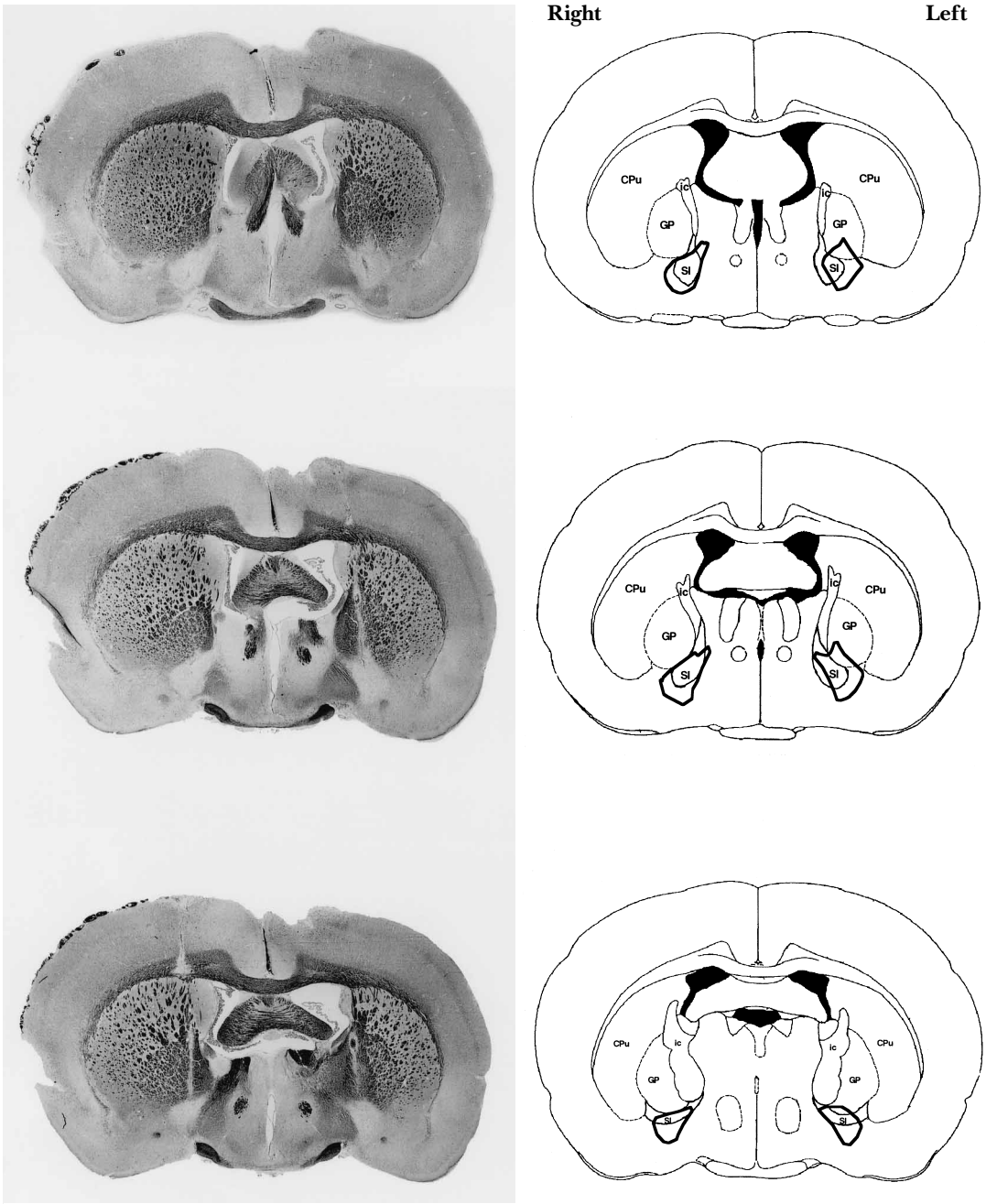

Fig. 1 Typical bilateral lesions of the substantia innominata induced with ibotenic acid injection. The lesion includes the nucleus basalis of Meynert. Stereotaxic coordinates of the top, middle and bottom are 0.8-, 0.9- and 1.3-mm posterior to the bregma. In this rat, the size of nbM lesion at 0.8-, 0.9- and 1.3-mm posterior to the bregma are 87, 63 and 48 %, respectively, on the right side and 48, 57 and 42 %, respectively, on the left side (Luxol fast blue and Cresyl violet double stain, ×7). The areas surrounded by thick lines on the diagrams indicate lesioned areas. SI: substantia innominata, GP: globus pallidus, CPu: caudate putamen, Ic: internal capsule.

The extent of lesioned areas as a percentage of the SI in each slice (0.8-, 0.9- and 1.3-mm posterior to bregma) amounted to 34 ± 38, 26 ± 30 and 23 ± 29, respectively, on the right side and 33 ± 37, 31 ± 34 and 23 ± 28, respectively, on the left side. The size of the lesion in the SI varied widely among animals. The right nbM-lesion was 28 ± 31 %, the left nbM-lesion was 29 ± 31 % and the bilateral nbM-lesion was 28 ± 30 %. There was no statistically significant difference between the right and the left nbM-lesion. Typical lesions in the SI are shown in Fig. 1. The correlation between ACh-baseline and bilateral nbM-lesion is shown in Fig. 2. The correlation coefficient (R) between ACh-baseline and bilateral nbM-lesion was −0.871 ($p<0.01$), indicating that there was a significant negative correlation between ACh-baseline and bilateral nbM-lesion.

After physostigmine injection, in the control group, ACh-max was 0.52 ± 0.22 ng/40 μl/20 min, and the magnitude of physostigmine-induced changes in ACh level (ΔACh) (ACh-max/ACh-baseline) was 167 ± 25 % (n=7). In the lesioned group, ACh-max was 0.18 ± 0.06 ng/40 μl/20 min, and ΔACh was 132 ± 19 % (n=8). The ΔACh in the lesioned group seemed to be lower than that in the control group. In both groups, ACh-max was significantly increased compared with ACh-baseline ($p<0.05$). In the lesioned group, the right nbM-lesion was 20 ± 24 %, the left nbM-lesion was 24 ± 23 % and the bilateral nbM-lesion was 22 ± 22 % (n=8). In this lesioned group, both ACh-max and the bilateral nbM-lesion also varied widely among animals. There was no statistically significant difference between the right and the left nbM-lesions (n=8). The correlation between ACh-max and bilateral nbM-lesion is shown in Fig. 3. The R between ACh-max and bilateral nbM-lesion was −0.711 ($p=0.06$). The relation between ΔACh and bilateral nbM-lesion is shown in Fig. 4. There was no apparent correlation between ΔACh and bilateral nbM-lesion.

DISCUSSION

Most of the fibers of the cholinergic projection to the cerebral cortex originate from the ipsilateral nbM, which is distributed over the SI in the basal forebrain, and the anterior part of the nbM provides cholinergic fibers to the frontal cortex [5]. A previous report showed that some cholinergic fibers are projected from the contralateral nbM [13]. Since the cholinergic fibers originated from the contralateral nbM may potentially compensate ACh level in the frontal cortex, we employed bilateral lesioning of the nbM.

In our previous [8] and present studies,

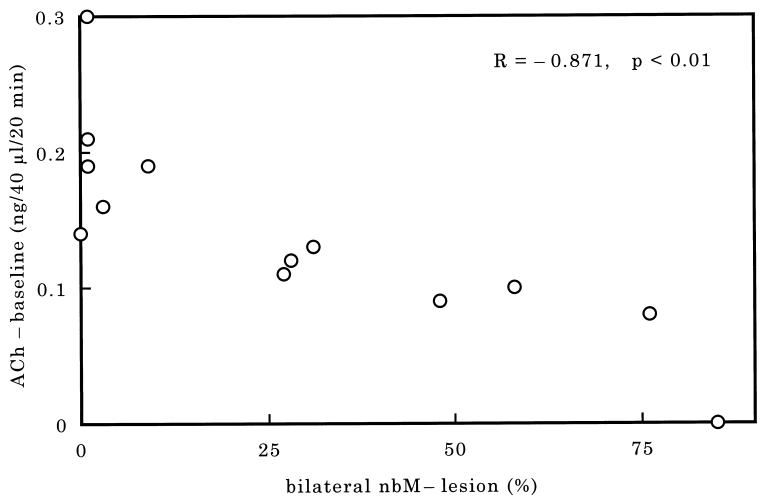

Fig. 2 Correlation between the baseline ACh levels (ng/40 μl/20 min) and the areas of bilateral nbM-lesion (%) (n=13). The level of ACh-baseline is plotted with (○) against the area of nbM-lesion for each rat. The x-axis is the average size of bilateral nbM-lesion. There is a significant negative correlation between the baseline ACh levels and the areas of bilateral nbM-lesion.

the baseline ACh level in the frontal cortex was decreased by lesioning of the bilateral nbM. These results coincide with the previous finding that lesioning of the anterior part of the nbM induces a decrease in CAT activity [5]. Since the size of ibotenic acid-induced lesion in the rat SI, which includes the nbM, varied widely among animals, we further analyzed the relationship between the ACh level and the size of the lesion in the SI. We found that the size of lesioned area in the bilateral SI was strongly correlated with the baseline ACh level. This result indicates that the nbM is the major functional source of ACh in the rat frontal cortex, as has been suggested by morphological studies [5, 13].

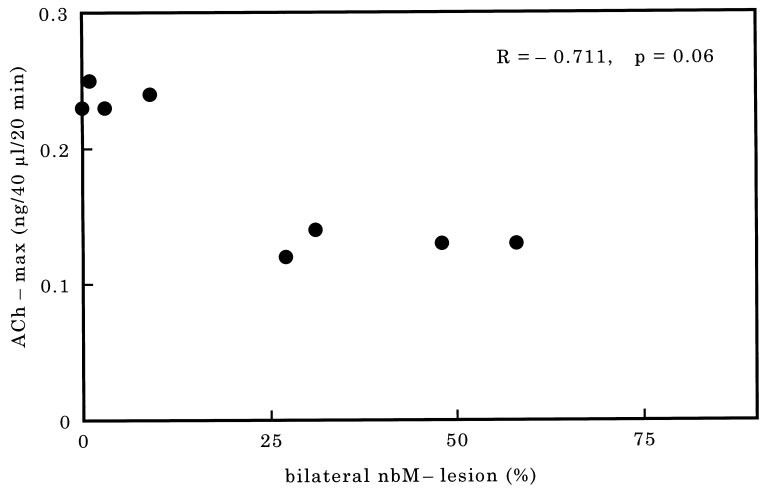

Fig. 3 Correlation between the maximum ACh levels (ng/40 μl/20 min) and the areas of bilateral nbM-lesion (%) (n=8). The level of ACh-max after physostigmine injection is plotted with (●) against the area of nbM-lesion for each rat. The x-axis is the average size of bilateral nbM-lesion.

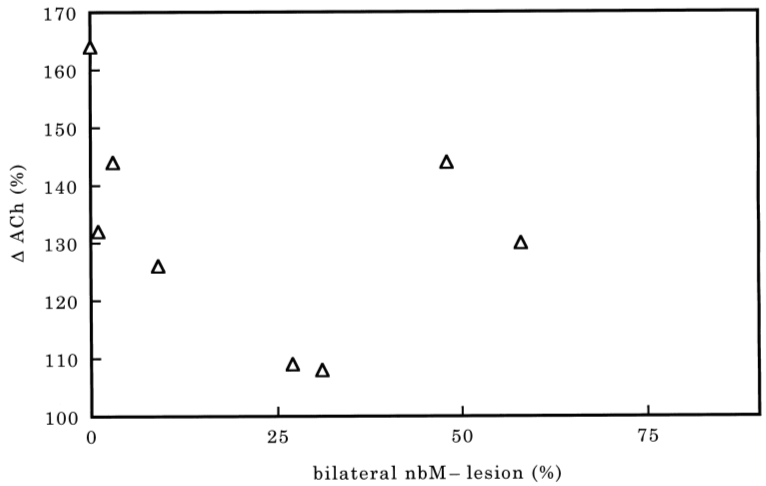

Fig. 4 Correlation between the magnitude of physostigmine-induced changes in ACh level (ΔACh) (ACh-max/ACh-baseline) and the areas of bilateral nbM-lesion (%) (n=8). The value of ΔACh is plotted with (△) against the size of nbM-lesion for each rat. The x-axis is the average size of bilateral nbM-lesion. There is no significant correlation between ΔACh and the areas of bilateral nbM-lesion.

The learning and memory disorder of nbM-lesioned rat was improved by physostigmine [6, 7]. If a choline esterase inhibitor acts only on nbM-originated cholinergic fibers, the increase in ACh release induced in the frontal cortex by a choline esterase inhibitor is also expected to correlate with the lesioning of nbM. However, our results showed only a very weak correlation between the increased ACh level by physostigmine injection and the size of the lesioned areas in the bilateral SI. Also the Δ ACh seen after physostigmine injection did not correlate with the size of lesioned area in the SI. This discrepancy between the physostigmine-induced increase in ACh level and the lesioning of the nbM may partly be due to the involvement of other cholinergic fibers present in the frontal cortex that do not originate from the nbM. These non-nbM-originated cholinergic fibers may derive from intracortical cholinergic neurons and provide cholinergic input from the brain regions that are not influenced by the lesioning of the nbM [5, 14]. Our results suggest that physostigmine possibly affects not only the spared nbM-originated cholinergic fibers but also the non-nbM-originated cholinergic fibers, thereby increasing the extracellular ACh level in the frontal cortex in the rat with the bilateral nbM lesion. Thus, the non-nbM-originated cholinergic neurons in the frontal cortex may have some role in learning and memory.

Two problems remained to be solved in this study. Firstly, it is not known why the ibotenic acid-induced lesion varied greatly in size among rats. Secondly, functional involvement of contralateral nbM in the ACh level in the frontal cortex is to be determined. Further study is necessary to clarify these issues.

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