Beneficial Effects of DX-9386, a Traditional Chinese Prescription, on Memory Disorder Produced by Lesioning the Amygdala in Mice

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The amygdala is one of the key areas of the brain involved in learning and memory. Bilateral lesions of the amygdala in 9-week-old mice induced impairment of memory acquisition and retention. DX-9386, a traditional Chinese medicinal prescription consisting of ginseng, polygala, acorus and hoelen, was orally administered to the lesioned mice after the operation until all the experiments were completed. From 15 d after surgery, learning behavior in the step-down test was observed daily for 10 d. DX-9386 treatment ameliorated the memory acquisition deficit. The number of step-down events in the first testing trial was significantly decreased by administration of 250 mg/kg of the prescription to the lesioned group of mice. Choline acetyltransferase activity in the cerebral cortex of the lesioned mice was significantly decreased, while repeated administration of the prescription did not affect this biochemical parameter. These results indicate that the memory acquisition enhancing effect of DX-9386 may not be achieved by direct activation of cholinergic transmission in the brain but by some other mechanisms(s).

Keywords memory acquisition; cholinergic; ginseng; polygala; acorus; hoelen

DX-9386, a traditional Chinese medicinal prescription, consists of ginseng (Panax ginseng C. A. MEYER), polygala (Polygala tenuifolia WILDENEW), acorus (Acorus gramineus SOLAND) and hoelen (Poria cocos WOLF) in the ratio of 1:1:25:50 (dry weight). It has long been employed in the clinical treatment of mental disorders such as brain hypoxia and senile amnesia in China.1) However, little research into its pharmacological effects on the central nervous system has been carried out. In our previous studies, single oral administration of DX-9386 potentiated the evoked potential induced by subthreshold tetanic stimulation in the rat hippocampal dentate gyrus.2) Moreover, the prescription antagonized the blocking effect of ethanol on the generation of long-term potentiation (LTP) induced by suprathreshold tetanic stimulation. LTP in the hippocampus is supposed to be a form of activity-dependent synaptic plasticity that may underlie learning and memory. In addition, we recently found that the prescription improved the memory deficit induced by thymectomy in ddY mice.3) These results indicate that DX-9386 may also improve cognitive functions in a mouse memory deficit model mice induced by brain lesioning.

The amygdala is involved in a variety of emotional, autonomic, endocrine and behavioral responses.4)–6) Recently, a number of studies have revealed the involvement of the amygdala in the memory processes associated with various behavioral tasks using the technique of either lesioning the nucleus or giving an intra-amygdala injection of neurotransmitters or chemicals in monkeys or rats.7)–10) In this paper, we evaluated the effect of DX-9386 on the memory disorder induced by amygdala lesioning in mice.

MATERIALS AND METHODS

Animals Male ddY mice weighing 38–42 g, 9 weeks old at the beginning of the experiments, were used (SLC, Hamamatsu, Japan). Animals were housed in groups of 5 in metal cages (30 × 20 × 10 cm) under uniform condition of temperature (22 ± 1°C) and humidity (55 ± 5%). Food (CE-2, Clea Japan, Tokyo, Japan) and water were available ad libiturum.

Amygdala Lesioning Under anesthesia produced with a mixture of ketamine and xylasine (80 and 7 mg/kg, respectively, i.m.), mice received bilateral amygdala lesions by passing a radiofrequency current (Lesion Generator, Radionics Inc., Burlington, U.S.A). The coordinates of the electrode placement were; 3.3 mm anterior to 0 point, ±2.9 mm lateral to the sagital suture, and 0.7 mm dorsal to horizontal plane with the incisor bar placed 2.2 mm ventral to the horizontal plane.11) A stainless-steel electrode (0.25 mm diameter) was inserted into the amygdaloid body and its tip was heated at 70°C for 60 s. In the sham-operated mice, the inserted electrode was not heated. Control mice were only anesthetized. The number of mice in each group was 14–15.

Administration of DX-9386 DX-9386 is composed of ginseng, polygala, acorus and hoelen in the ratio of 1:1:25:50 (dry weight). Aqueous extracts of ginseng, polygala and hoelen and ethanolic extract of acorus were individually prepared and supplied as a mixture of extracts by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). The prescription was dissolved in saline and administrated orally, once a day, every day at doses of 250 and 500 mg/10 ml/kg/d from the day of the amygdala lesioning until the day when the animals were sacrificed, one month after lesioning. The preparation was given to mice 30 min before the step-down test during the behavior testing period. Saline was administered to the intact and sham-operated mice instead of the prescription. DX-9386, at these doses, did not induce any changes in pain sensitivity and general behavior, and had no anti-convulsive effect. All other drugs used were of the highest purity obtainable from commercial sources.

Learning Performance Test After the lesioned mice recovered from the effects of surgery, that is 15 d after the operation, as shown by their change in body weight, they were submitted to a step-down type passive avoidance task. The step-down test was performed according to the
method of Nishiyama et al.\(^{12}\) following motor activity measurement. The apparatus was a rectangular box with a grid floor designed to give a 50 V AC electric shock. A rubber platform was placed at one corner. In the learning trial (day 0), a mouse was placed gently on the platform. When the mouse stepped down and touched the grid floor, it was given a punishing electric shock and jumped back onto the platform. In the testing trials (days 1—9), the same test procedures were performed for 3 min at the same time of day. An electric current was always applied to a mouse when it descended during the period of the testing trials. The parameters recorded in each testing trials were the number of stepping down events (number of errors) and the number of animals which did not step down. The percentage of mice which did not step down to the grid floor during each testing trial was recorded as the memory retention ratio. The accumulated numbers of stepping down events during the whole period of the testing trials were also calculated as the total number of errors.

**Motor Activity Test** The motor activity of the mice was measured with a tilting-type round activity cage, 18 cm in diameter and 18 cm in height (MA001, O’hara & Co., Ltd., Tokyo, Japan). Motor activity was measured for 30 min every day immediately after oral administration of DX-9386.

**Choline Acetyltransferase (ChAT) Activity** After completion of the behavioral tests, that is 31 d after the amygdala lesioning, the mice (\(n=9--10\)) were decapitated after cervical dislocation and their brains immediately removed. The cerebral cortex, hippocampus and hypothalamus were dissected out and their ChAT activities were measured using the method of Fonnun.\(^{13}\) The protein content was determined using the dye method.\(^{14}\)

**Histological Observation** Four to five mice in each group were perfused transcardially with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer, pH 7.4. The removed brains were trimmed and 8 \(\mu\)m paraffin sections were serially cut and stained with cresylviolet. The location and histological alterations at the lesioned site were assessed by microscopic observation.

**Statistics** The numbers of errors in the step-down tests, as well as the motor and ChAT activities were analyzed using the Mann–Whitney’s U-test. The numbers of animals which did not step down in the first retention test and the ratio of the memory retention were examined by using the chi-square test.

**RESULTS**

Lesioning the bilateral amygdala produced a fall in the body weight of the operated mice for 2 to 3 d. However, most mice recovered this weight loss in two weeks. Daily oral administration of DX-9386 did not affect these changes in body weight (data not shown).

In the first testing trial of the step-down test, the number of errors in amygdala lesioned-mice was significantly greater than in either the control or sham-operated group. Administration of DX-9386, at a dose of 250 mg/kg to the amygdala lesioned group reduced the number of errors to the level exhibited by the sham-operated mice (Fig. 1). A higher dose of the preparation (500 mg/kg) tended to reduce the number of errors, but this did not reach statistical significance. The ratio of memory retention in the amygdala-lesioned group during the testing session was significantly reduced compared with that of the control or sham-operated group. Repetitive administration of DX-9386 did not improve the memory retention deficit of the amygdala-lesioned group (data not shown). The total number of errors during the whole period of the testing trials was increased by destruction of the amygdala, while chronic administration of DX-9386 did not affect this parameter (data not shown).

There was no difference in motor activity among control, sham-operated and amygdala-lesioned mice. Daily administration of DX-9386 did not affect the motor activity over all sessions (data not shown).

The ChAT activity in the hippocampus or hypothalamus was not affected by lesioning the amygdala. The lesion, however, caused a 19% decrease in ChAT activity in the cerebral cortex suggesting a functional neural connection between these two structures (Table I). Repeated oral administration of DX-9386 did not alter the enzyme activity in the cerebral cortex or other brain areas (Table I).

Extensive gliosis was observed in the damaged area in the amygdala-lesioned mice. Numerous cells with small nuclei could be observed in and around the lesioned area and a scar had formed. Several blood vessels were observed.

![Fig. 1. Effect of DX-9386 on Step-Down Test Performance in Amygdala-Lesioned Mice](image)

A mouse was placed on a safe platform in a corner of the step-down apparatus and the number of stepping down events in the first testing trial was counted. The bars indicate S.E.M. Abbreviations: Cont, naive mice received only anesthetic; Sham, sham-operated mice; DX 0, amygdala-lesioned and saline treated mice; DX 250 and DX 500, amygdala-lesioned and 250 or 500 mg/kg/d DX-9386-treated mice. a) *p<0.05* vs. Sham, b) *p<0.05* vs. DX 0 (Mann–Whitney’s U-test).

**TABLE I. Effect of DX-9386 on ChAT Activity in Amygdala-Lesioned Mice**

<table>
<thead>
<tr>
<th></th>
<th>Cont</th>
<th>Sham</th>
<th>Amygdala-lesioned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DX 0</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>517±33</td>
<td>547±61</td>
<td>443±29*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>850±96</td>
<td>611±76</td>
<td>632±85</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>415±30</td>
<td>379±20</td>
<td>356±18</td>
</tr>
</tbody>
</table>

Results are given as means±S.E.M. (pmol/mg protein/min, \(n=9--10\)). *\(p<0.05\) vs. Sham (Mann–Whitney’s U-test). See the Fig. 1, caption for abbreviations.
in the injured area suggesting a rich blood supply. No distinct pathological differences were seen when comparing among lesioned-control and DX-9386-treated animals.

DISCUSSION

Lesioning the bilateral amygdala significantly impairs learning performances in mice. This memory deficit was already apparent in the first testing trial and lasted throughout the subsequent trials, suggesting that a long-term memory process was disturbed by amygdala lesioning. A number of studies have shown that a passive avoidance task, such as the step-down test, is dependent upon intact amygdala neurons.\textsuperscript{15–18} The present data supports this. The amygdala consists of basolateral, lateral, basomedial, central, and intercalated nuclei.\textsuperscript{19,20} Although we could not disrupt each subnucleus separately in mice because of their small size, our studies did reconfirm the importance of an intact amygdaloid complex in the memory processing of mice.

Daily administration of DX-9386 ameliorated the memory acquisition impairment, but not the memory retention deficit induced by the amygdala lesions. These results were consistent with our previous findings that the prescription improved a thymectomy-induced learning acquisition deficit without ameliorating the memory retention ability.\textsuperscript{3} This data supports the hypothesis that memory consists of several distinct processes\textsuperscript{21,22} and indicates that our prescription might favorably improve the memory acquisition process. Ginseng, one of the component of the preparation, is well known to affect the central nervous system and improve memory ability.\textsuperscript{23–26} Because no morphological alterations were observed following DX-9386 treatment, the learning improving effect of the prescription in amygdala-lesioned mice may be due to an activation of an other part of the brain to take over the deficit of the amygdala. The effects of DX-9386 in our experiments did not exhibit a clear dose-dependence. The precise mechanism of DX-9386 on memory processes requires further investigations.

Passive avoidance tasks depend on intact cholinergic transmission in the limbic system.\textsuperscript{10} Recently, we found that intra-amygdala injection of scopolamine in mice caused learning deficits which were evaluated by step-through and step-down tests.\textsuperscript{27} Moreover, Segawa observed that a single acute injection of carbachol into a lesioned amygdala transiently ameliorated the learning impairment in the step-through test.\textsuperscript{28} These findings suggest that inhibition of cholinergic activity is involved in the learning impairment in amygdala-lesioned mice. A decrease in the ChAT activity in the cerebral cortex of amygdala-lesioned mice suggests that these two structures have a direct or indirect cholinergic connection. In contrast, the ChAT activities in the hippocampus and hypothalamus were unaffected by the amygdala lesions. However, this does not preclude the possibility that other neurotransmitters are involved in amygdala-hippocampal or amygdala-hypothalamic pathways. Our results suggest that the beneficial effect of DX-9386 on learning was not accomplished by restoring decreased cholinergic transmission in the cerebral cortex.

In conclusion, the present work demonstrates that the amygdala is involved in memory processes, probably by modulating cholinergic transmission in the cerebral cortex. Repeated administration of DX-9386 ameliorated the memory deficits by mechanisms other than those affecting the cholinergic system in the cerebral cortex.

REFERENCES

28) M. Segawa, Doctoral Thesis, the University of Tokyo, 1990.