Kappa Opioid Receptor Agonist Administration in Olfactory Bulbectomized Mice Restores Cognitive Impairment through Cholinergic Neuron Activation

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Received February 13, 2018; accepted March 29, 2018

Olfactory bulbectomized (OBX) mice are characterized by impaired performance in the passive avoidance test and decreased number of cholinergic neurons in the hippocampus. Several studies have reported that k-opioid receptor agonists improve cognitive function in mice. However, their influence on OBX-induced cognitive dysfunction remains unclear. To address this question, we evaluated the effects of the endogenous κ-opioid receptor agonist dynorphin A (Dyn A) and the selective agonist trans-(-)-U-50488 on the behavior of OBX mice in the passive avoidance test. The cognitive dysfunction of OBX mice was significantly recovered by the intracerebroventricular administration of Dyn A or trans-(-)-U-50488. The effects of these two agonists were counteracted by the selective κ-opioid receptor antagonist nor-binaltorphimine or the inhibitor of acetylcholine release β-bungarotoxin. These findings suggest that κ-opioid receptor agonists produce anti-dementia effects through activation of cholinergic neurons in OBX mice.

Key words olfactory bulbectomy; memory; κ-opioid; cholinergic neuron

The heptadecapeptide dynorphin A (Dyn A), an endogenous κ-opioid receptor agonist is thought to play a role in mood and cognitive function.1,2 Several studies have reported that κ-opioid receptor agonists, such as U-50488H, improve cognitive function induced by cholinergic dysfunction in mice.3–6 Especially U-50488H ameliorates scopolamine-induced cognitive dysfunction, an effect that is reversed by nor-binaltorphimine (nor-BNI), a selective κ-opioid receptor antagonist; thus, suggesting that κ-opioid receptor agonists influence memory through the cholinergic system.5,7 Likewise, hippocampal microinjection of Dyn A (2-13) improves mecamylamine-induced amnesia.7 However, nor-BNI does not completely reverse the effects of Dyn A (2-13),7 suggesting that it might additionally act through non-opioid receptors. Moreover, a recent study suggested that Dyn A (1-13) alleviates stress-induced depressive-like behavior in mice.9

Olfactory bulbectomized (OBX) rodents are a useful experimental animal model for depressive disorder, as reported by us and several other researchers.9–12 OBX in rodents leads to various abnormal behaviors, such as impairment of memory related behavior10,13 and hyperemotional behavior.15 Furthermore, OBX animals show neurochemical and physiological changes similar to those present in depression patients, including the reduction of hippocampal neurogenesis, and of choline acetyltransferase (ChAT) activity.13,16 The abnormal behaviors of OBX animals have been shown to be ameliorated by the administration of anti-depressant drugs, such as imipramine and escitalopram.17 It has been reported that δ-opioid receptor agonists, such as SNC80 and KNT-127, improve OBX-induced abnormal behavior such as depressive and hyperemotional behaviors.18,19 However, the influence of Dyn A or other κ-opioid receptor agonists on OBX-induced cognitive dysfunction remains unclear.

In the present study, we examined the effects and underlying mechanisms of Dyn A or the selective κ-opioid receptor agonist trans-(-)-U-50488 on the impairment of memory-related behavior in OBX mice.

MATERIALS AND METHODS

All experiments were performed following the approval of the Ethics Committee of Animal Experiments in Tohoku Medical and Pharmaceutical University (approval number: 17015-cn) and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Efforts were made to minimize suffering and to reduce the number of the animals used.

Animals Male ddY mice (weighing 28–32 g; Japan SLC, Shizuoka, Japan) were used for all experiments (total, n=140). Mice were housed in cages with free access to food and water, under conditions of constant temperature (22±2°C) and humidity (55±5%), on a 12-h light-dark cycle (lights on: 07:00 to 19:00).

Olfactory Bulbectomy Mice anesthetized with pentobarbital Na (50 mg/kg, intraperitoneally; Dainippon Sumitomo Pharma, Osaka, Japan) were placed in a stereotaxic frame. The scalp was incised; two holes were drilled to expose the olfactory bulb (OB) (2 mm prior to the bregma 1 mm lateral to the midbrain), which was then bilaterally aspirated using a suction pump. All mice were sacrificed at the end of the experiment and visually examined to confirm that two thirds of the OB were lesioned (taking care not to cause any damage to the frontal cortex). Data obtained from mice in which the lesion was not extensive enough or extended to the cortex were excluded. Sham operations were performed with the same procedure but without the removal of the OB.

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Drugs The full length of Dyn A (Bachem, Bubendorf, Switzerland, H260) and trans-(−)-U-50488 (Sigma-Aldrich, Inc., St. Louis, MO, U.S.A., U111) were dissolved in Ringer’s solution. Nor-BNI (Sigma-Aldrich, Inc., N1771) and β-bungarotoxin (β-BuTX; Sigma-Aldrich, Inc., T5644) were dissolved in saline. The used doses of nor-BNI and β-BuTX were based on previous studies. 20,21) Dyn A (0.5, 0.7, or 1 nmol) and trans-(−)-U-50488 (10, 20, or 40 nmol) were administered by an intracerebroventricular (ICV) injection in unanesthetized mice, in a volume of 5 µL per mouse, 10 min before the behavioral test. The technique employed herein for ICV injections into mice was the same as that described by Brittain and Handley. 22) Control OBX mice were injected ICV with vehicle (Ringer’s solution). Nor-BNI and β-BuTX were administered at a dose of 0.1 mL/10 g of body weight: nor-BNI intraperitoneally, 24 h before Dyn A or trans-(−)-U-50488 ICV injection and β-BuTX subcutaneously, 30 min before ICV injection.

Step-Through Passive Avoidance Task The passive avoidance test was performed as previously described. 13,23) In the training trial, before the OBX surgery, each mouse was placed in the illuminated compartment and the latency to enter the dark compartment was recorded. As soon as the mouse had completely moved from the lighted compartment into the dark compartment, an electric foot-shock (1 mA for 500 ms) was delivered through the floor grids. In the retention trial, performed 14 d after OBX, each mouse was placed in the illuminated compartment and the latency to enter the dark compartment was once again recorded, allowing a maximum cut-off time of 300 s; no foot-shock was delivered in this case. All measurements were performed between 11:00 and 17:00.

Statistical Analysis Results are expressed as mean±standard error of the mean (S.E.M.). The significance of differences was determined by the student’s t-test for two-group comparisons and by a one-way ANOVA followed by Fisher’s Protected Least Significant Difference test for multi-group comparisons. We considered $p<0.05$ to represent significant differences.

RESULTS

Effects of Dyn A and trans-(−)-U-50488 on Memory Retention of OBX Mice in the Step-Through Passive Avoidance Task To examine whether and how Dyn A and trans-(−)-U-50488 affected memory-related behavior impairment in OBX mice, we employed the step-through passive avoidance task to measure changes in latency after administration of Dyn A (0.5, 0.7, and 1 nmol/mouse; Fig. 1A) or trans-(−)-U-50488 (10, 20, and 40 nmol/mouse; Fig. 1B). The latency to enter the dark compartment in the retention trial was

Fig. 1. Effects of Dynorphin A (Dyn A) and trans-(−)-U-50488 on Memory Impairment Induced by Olfactory Bullectomy (OBX)

Quantification of the latency to enter the dark compartment in the retention trial of the passive avoidance test. Mice were sham- (white bars) or OBX-operated (black and dashed bars) and then treated with Dyn A (A) or trans-(−)-U-50488 (B) at the indicated concentrations. Control OBX mice were treated with vehicle (black bars in A and B). Bars represent mean±S.E.M. One-way ANOVA \([F(4, 45)]=17.46, p<0.01, (A); F(4, 45)=11.71, p<0.01, (B)]\). *$p<0.05$ and **$p<0.01$ versus vehicle-treated sham group; #$p<0.05$ and ##$p<0.01$ versus vehicle-treated OBX group. $n=10$ per group.

Fig. 2. Effects of Nor-Binaltorphimine (Nor-BNI) on Memory-Related Behavior

Quantification of the latency to enter the dark compartment in the retention trial of the passive avoidance test. Mice were sham- (white bars) or OBX-operated (black, dashed, and dotted bars) and then treated with nor-BNI; 24 h before the Dyn A (A) or trans-(−)-U-50488 (B) injection, at the indicated concentrations. Control OBX mice were treated with vehicle (black bars in A and B). Bars represent mean±S.E.M. One-way ANOVA \([F(3, 36)=33.82, p<0.01, (A); F(4, 45)=23.53, p<0.01, (B)]\). *$p<0.05$ and **$p<0.01$ versus vehicle-treated sham group; $^\# p<0.05$ and $^\#\# p<0.01$ versus vehicle-treated OBX group; $^\$ p<0.01$ versus Dyn A or trans-(−)-U-50488-treated OBX group. $n=10$ per group.
assessed 14d after the training trial. The OBX group showed a significantly shorter latency than the sham group, indicating that OBX induced memory deficits. The administration of Dyn A or trans-\(-\)U-50488 to OBX mice 10 min before the retention trial significantly increased latency compared to vehicle injection, suggesting that these drugs improved memory deficits in OBX mice.

\textbf{\textit{k-}
\textit{Opioid Receptor Involvement in Dyn A- and trans-\(-\)U-50488-Mediated Memory Improvement}} In order to determine whether the \textit{k-}
\textit{opioid receptor was involved in the observed memory improvement in OBX mice, mediated by Dyn A- and trans-\(-\)U-50488, we injected the selective \textit{k-}
\textit{opioid antagonist nor-BNI (10 or 20mg/kg). The effects of Dyn A and trans-\(-\)U-50488 were completely inhibited by nor-BNI (Fig. 2).}

\textbf{\textit{Involvement of Cholinergic Neurons in Dyn A- and trans-\(-\)U-50488-Induced Memory Improvement in OBX Mice}} In order to examine whether cholinergic neurons were involved in the observed memory improvement in OBX mice, mediated by Dyn A and trans-\(-\)U-50488, we injected the acetylcholine (ACh) release inhibitor, \textit{\beta-}
\textit{BuTX. Our results showed that \textit{\beta-}
\textit{BuTX significantly inhibited the effects of the \textit{k-}
\textit{opioid receptor antagonists, Dyn A and trans-\(-\)U-50488, in OBX mice (Fig. 3).}

\textbf{DISCUSSION}

Consistent with our previous results, the present study confirmed that OBX mice exhibit long-term memory-related behavioral impairments in the passive avoidance test, 14d after the surgery. We demonstrated that the administration of Dyn A or trans-\(-\)U-50488 improved the cognitive deficits of OBX mice, and this effect was significantly inhibited by pre-administration of the \textit{k-}
\textit{opioid receptor antagonist nor-BNI. These results are consistent with the findings of a previous study that used a rat model of memory deficits, induced by the muscarinic acetylcholine receptor antagonist scopolamine. In contrast, Hiramatsu and Watanabe reported that Dyn A (2-13) improves memory deficits induced by the nicotinic acetylcholine receptor antagonist mecamylamine, but this effect was not reversed by nor-BNI. Our previous study suggested that OBX-induced memory deficits were associated with dysfunction of the cholinergic system in the hippocampus. Thus, we conclude that \textit{\kappa-}
\textit{opioid agonists, such as dynorphins, compensate for hippocampal cholinergic system dysfunctions and that the \textit{k-}
\textit{opioidergic system plays an important role in the modulation of learning and memory but does so via the muscarinic and not the nicotinic acetylcholine receptors.}

The degeneration of cholinergic activity in the brain is associated with mood disorder. OBX induces retrograde degeneration of cholinergic neurons from the medial septum, leading to decreased cholinergic innervation of the hippocampus. Indeed, the levels of ChAT, a presynaptic marker of cholinergic neurons, in the hippocampus were significantly decreased by OBX. Consistent with these findings, the activation of cholinergic neurons improved the OBX-induced memory-related behavioral impairments, suggesting that they are tightly associated with dysfunction of cholinergic activity in the hippocampus. These data are in line with our results on the effects of \textit{\beta-}
\textit{BuTX, an ACh release inhibitor, on Dyn A- or trans-\(-\)U-50488-induced improvement of memory-related behavior in OBX mice. We showed that \textit{\beta-}
\textit{BuTX attenuates the effect of Dyn A or trans-\(-\)U-50488.

Previous studies have suggested that \textit{\kappa-}
\textit{opioid receptor agonists improve muscarinic receptor antagonist-induced cognitive dysfunction in animal models via regulation of hippocampal ACh release. Hippocampal \textit{\kappa-}
\textit{opioid receptors in \gamma-
\textit{aminobutyrate (GABA)ergic neurons regulate the function of the cholinergic system in the hippocampus. These results indicate that Dyn A- or trans-\(-\)U-50488-induced improvement of memory impairments in OBX mice may be associated with the activation of cholinergic neurons in the hippocampus.}

In conclusion, our results indicate that the stimulation of \textit{\kappa-}
\textit{opioid receptors in the hippocampus may ameliorate cognitive dysfunction through the activation of the cholinergic system. These findings may offer a therapeutic alternative approach in the treatment of depression patients’ symptoms.

\textbf{Acknowledgments} This study was supported by Matching Fund Subsidy for Private Universities from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

\textbf{Conflict of Interest} The authors declare no conflict of
interest.

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