学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏 名　安　燕

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学 位 論 文 題 名
（Title of dissertation）

Biological basis of the anxiolytic-like effect of mirtazapine in the rat conditioned fear stress model
(ラット恐怖条件付けモデルにおける mirtazapine の抗不安効果の生物学的基盤に関する研究)

【Background and Objectives】 Mirtazapine, a NaSSA, which blocks the adrenergic α_2 auto- and hetero-receptors, has been proven to be effective in the treatment of various anxiety disorders in addition to depressive disorders. However, the mechanism whereby mirtazapine exerts its anxiolytic effect has not been fully clarified. Previous studies have shown that the systemic administration of mirtazapine has the anxiolytic-like effect in the contextual fear conditioning model and increases extracellular serotonin levels in the hippocampus. These effects might be related because a number of studies have demonstrated that the facilitation of 5-HT neurotransmission decreases the expression of contextual conditioned freezing. To further explore the anxiolytic mechanism of mirtazapine, in the present study, we investigated the brain area(s) in which mirtazapine exerts its anxiolytic-like effect in the contextual fear conditioning test by using the microinjection of mirtazapine into the median raphe nucleus (MRN) , hippocampus and amygdala. These brain areas are reportedly related to the contextual fear conditioning and the MRN, like the dorsal raphe nucleus, is a main source of serotonergic innervation to the forebrain structures involved in anxiety regulation, such as the hippocampus. Furthermore, I investigated augmentation strategies for the anxiolytic-like effect of mirtazapine. Recently, evidence from animal and clinical studies has shown that lithium has an effect on monoamine systems to potentiate the antidepressant action of various antidepressants. However, only a few studies have investigated the effect of lithium with mirtazapine on anxiety clinically but not preclinically. Previous studies have shown that both lithium and mirtazapine influence serotonergic systems and anxiety. These suggest the possibility that the combination of lithium and mirtazapine may have better efficacies for anxiety disorders through the effect on serotonin.

【Methods】First, I examined the effect of the two intervals between conditioning and exposure to conditioned fear on acute systemic mirtazapine treatment. One day after footshock, the rats received a single injection of mirtazapine 0, 1, 3 and 10 mg/kg at 30 min before testing. One and 7 days after footshock, the rats received a single injection of mirtazapine 10 mg/kg at 30 min before testing. Next, I explored target brain sites of the anxiolytic-like effect of mirtazapine. One day after footshock, mirtazapine (3 μg/site) was directly injected into three brain structures, the MRN, hippocampus and amygdala at 10 min before testing. In chapter 2, I explored augmentation strategies for the anxiolytic-like effect of mirtazapine. In the exepriment of subchronic lithium co-treatment with acute systemic mirtazapine：immediately after footshock, the rats received standard laboratory rat chow containing 0%, 0.05% or 0.2% of Li_2CO_3 for 7 days. On the eighth day, the rats received an intraperitoneal injection of mirtazapine 10 mg/kg at 30 min before testing. In the exepriment of subchronic lithium combined with local mirtazapine treatment：immediately after footshock, rats received standard laboratory rat chow containing 0 or 0.2% of Li_2CO_3 for 7 days. On the eighth day,

mirtazapine was injected into the hippocampus, amygdala and MRN at 10 min before testing.

【**Results**】When the interval between conditioning and testing was 1 day, acute mirtazapine showed a dose-dependent reduction in freezing time. Moreover, intra-MRN injection of mirtazapine reduced freezing behavior significantly, while mirtazapine injections into the hippocampus or amygdala did not. When the interval was 7 days, acute systemic mirtazapine 10 mg/kg treatment did not reduce the expression of conditioned freezing significantly. The combination of subchronic 0.2% Li_2CO_3 but not 0.05% Li_2CO_3 with acute mirtazapine (10 mg/kg) reduced freezing significantly. Subchronic treatment with 0.2% Li_2CO_3 enhanced the effect of mirtazapine (3 μg/site) on freezing behavior significantly when mirtazapine was infused into the MRN but not hippocampus or amygdala.

【Discussion】 Acute systemic administration of mirtazapine dose-dependently reduced freezing one day after fear conditioning, whereas the anxiolytic-like effect of mirtazapine diminished when the interval between fear conditioning and testing was prolonged for seven days. These results are consistent with our previous reports, which showed that systemic mirtazapine reduced anxiety-like behavior, freezing, in the contextual fear conditioning test and that the inhibitory effect of an acute challenge of SSRIs on conditioned freezing diminished by prolonging the interval between conditioning and testing. Memory consolidation processes after the fear acquisition may be involved in this effect. The intra-MRN infusion of mirtazapine showed the anxiolytic effect in the contextual fear conditioning test. The intra-MRN infusion of mirtazapine is supposed to increase extracellular 5-HT levels in the nerve terminal areas such as the hippocampus, because mirtazapine an α_2-adrenergic antagonist but has very weak α_1-adrenergic antagonistic action. Previous reports revealed that the local administration of mirtazapine or the α_2-antatgonist idazoxan into the MRN increased extracellular 5-HT levels in both the MRN and the entorhinal cortex. Their results support my hypothesis that local mirtazapine stimulates MRN activity by blocking α_2-adrenoceptors in the nerve terminals of noradrenergic neurons and increases the extracellular 5-HT levels in the nerve terminal areas of serotonergic neurons, leading to the anxiolytic effect in the contextual fear conditioning test. In the future, the effect of microinjection of a selective α_2-adrenergic antagonist into the MRN on anxiety-like behaviors should be examined. Subchronic 0.2% Li_2CO_3treatment enhanced the anxiolytic-like effects of acute systemic and local mirtazapine administration in the contextual fear conditioning test. Earlier in vivo microdialysis studies have reported that systemic administration of mirtazapine increases extracellular serotonin concentrations in the hippocampus and that subchronic lithium treatment increases extracellular serotonin levels in the medial prefrontal cortex and hippocampus. Taken together, these data suggest that the lithium augmentation of the anxiolytic-like effect of mirtazapine may be mediated by the enhancement of 5-HT neurotransmission in the hippocampus. The mechanism, by which lithium facilitates central 5-HT neurotransmission, is not yet completely understood at present. Previous biochemical studies have shown that lithium is linked to several factors affecting the extracellular 5-HT levels, such as synthesis, storage, release, reuptake and metabolism. The result of lithium-induced enhancement of the anxiolytic-like effect of the intra-MRN mirtazapine administration supports the hypothesis that this enhancement is mediated by the effect on serotonin.

In conclusion, this study showed that the anxiolytic-like effect of mirtazapine was mediated by its action on the MRN, and that subchronic 0.2% Li_2CO_3 treatment enhanced the anxiolytic-like effect of mirtazapine probably through its effect on 5-HT.