行政院國家科學委員會專題研究計畫成果報告

恐懼反應習得與長期儲存之神經機制（III）

The Neural mechanisms underlying acquisition and long-term storage of conditioned fear/anxiety (III)

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主持人：梁庚辰 國立臺灣大學心理學系

一、 中文摘要

本研究探討在腦中注入抗焦慮劑buspirone對於提取恐懼記憶之影響。在大白鼠接受抑制性躲避學習作業。在學習後一天或不同的保存時距內接受記憶測試。測試前接受周邊或腦內之buspirone以及相關節抗劑之注射。研究結果發現在回憶測試前於周邊注入buspirone有損於一天或五天的記憶提取，但對於二十一天的記憶提取僅有些微的效果。最有效的時間是記憶測試前三十分鐘施予注射。此一效果可以被血清素1A受體拮抗劑所阻斷，但不受D2或α2受體拮抗劑之影響。如果將buspirone注射於腦中部同的區域，則只有注射在海馬會損及一天的記憶提取而且此效果也與有關。這些研究的發現顯示，Buspirone的抗焦慮效果與其在海馬回的血清素1A受體作用有關。

關鍵詞：抗焦慮劑、杏仁核、海馬、藍班核、縫合核、前額皮質、終紋床

二、 英文摘要

The present study was aimed to investigate the effect of pretest injections of buspirone on memory retrieval. Male Wistar rats were trained on a one-trial step-through inhibitory avoidance task and given subcutaneous (s.c.) or intra-cranial injections of buspirone at various areas shortly before the 1-day or 21-day test. Pretest s.c. injections of buspirone caused a dose-dependent impairment of retention. The effect was more prominent when busprirone was injected 30 min before the retention test than injected 150 or 5 min before. Buspirone given before the 1-day or 5-day test caused a robust effect, but only a marginally effect before the 21-day test. The impairing effect on the 1-day test was attenuated by pretreatment of a 5-HT1A antagonist WAY 100635, but not by a D2 agonist apomorphine or an α2 agonist clonidine. Pretest infusion of the drug into the median raphe, locus coeruleus, amygdala, bed nucleus of the stria terminalis and medial prefrontal cortex caused no significant effect. Pretest infusion of buspirone into the ventral hippocampus impaired performance in the 1-

day test, and the effect was attenuated by the 5-HT1A antagonist WAY100635. The findings, considered with our previous ones, suggest that buspirone may act on 5-HT1A receptors of the hippocampus to suppress expression of a recently acquired affective memory.

Key words: anxiolytics, conditioned fear, amygdala, median raphe, locus coeruleus, medial prefrontal cortex, rats

三、目的與緣起

Buspirone is an atypical anxiolytic dissimilar to benzodiazepines. Evidence accumulated recently showed that this drug could have an effect on learning and memory: Pre- or posttraining injection of buspirone, either systemically or directly into the brain, impaired retention in the inhibitory avoidance or swimming tasks. Additionally, pretest administration of this drug impaired performance in retention tests of the inhibitory, active avoidance or Morris water maze tasks (Liang et al., 1998). In view of these data, it would of interests to know how and where in the brain buspirone might act to influence acquisition and/or memory retrieval of various learning tasks. Findings from the previous year (Liang, 1999) showed that buspirone exxerting its effect on memory formation on 5-HT1A receptors in the amygdala. On the other hand the retrieval affecting effect of buspirone appeared to be related to the hippocampus. However, it was unknown whether this effect was also involved the 5-HT1A receptor. In the present report, we showed that the effect of buspirone on 1-day memory retrieval was related to its action on 5-HT1A receptors of the ventral hippocampus.

四、結果

I. <u>Dose-response curve for pretest systemic injections of buspirone</u>

Rats were trained on the inhibitory avoidance task as described previously. Thirty minutes before the 1-day retention test, various groups of rats received s.c. injections of saline, 1.0 mg/kg, 2.5 mg/kg or 5.0 mg/kg buspirone. Results indicated that pretest s.c. injections of buspirone induced a dose-dependent deficit in the retention test. A Kruskal-Wallis one-way ANOVA revealed a signicant difference among the four groups ($H'(3) = 28.7$, $p < 0.0001$). Further paired comparisons by Mann-Whitney two-tailed U-test indicated that the group receiving 2.5 mg/kg or 5.0 mg/kg buspirone had significantly lower retention scores than the controls ($U = 22$ & 0, $p < 0.01$ & 0.001; respectively). The group receiving 1.0 mg/kg buspirone also appeared to have lower retention scores than the controls, but the difference between the two was not statistically significant. Yet retention scores of the 1.0 mg/kg group were significantly better than those of the 2.5 or 5.0 mg/kg group ($U = 23$ & 4; $p < 0.05$ & 0.001).

To evaluate whether buspirone would have altered the activity level, some rats were subjected to the open field test five minutes after receiving buspirone. The number of line crossing (mean + S.E) for the vehicle, 2.5 mg/kg, or 5.0 mg/kg buspirone treated groups was 32.4 + 4.6, 36.6 + 3.0, 14 + 2.6; respectively. A one-way ANOVA revealed a significant drug main effect ($F(2,30) = 11.9$, $p < 0.0002$). Post-hoc comparisons indicated that the 5.0 mg/kg group showed locomotor activity significantly lower than the controls (Dunnett $t(30) = 4.6$, $p < 0.05$), while the 2.5 mg/kg group did not.

II. <u>Effect of buspirone injected at various times before testing on retention</u>.

To evaluate the best time for injection of buspirone before testing, four

groups of rats were trained and tested 1 day later. One group received vehicle 30 min prior to the testing, while the remaining three groups received 5.0 mg/kg buspirone 5 min, 30 min, and 150 min before testing. Buspirone given 30 min before testing produced the greatest impairment, while that given 150 min before or 5 min before testing produced less of an effect. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the four groups ($H'(3) = 18.9$, $p < 0.001$). Further comparisons indicated that the control group had better retention scores than all buspirone-treated groups ($U = 10, 5 \& 8$; $p < 0.01, 0.001 \&$ 0.01 for 5, 30 & 150 min groups). The 30 min group also had poorer retention performance than the 5 or 150 min group ($U = 12.5 \& 15.5$, $p < 0.01$).

III. Effects of buspirone on retention in tests with various retention intervals.

Six groups of rats were trained as described and were tested for retention at 1, 5, or 21 days after training. For the pair of groups tested at each retention interval, one group received vehicle and the other received 5.0 mg/kg buspirone, injected subcutaneously 30 min before testing. Buspirone injected before each retention test impaired retention performance, but buspirone injected before a 21-day retention test appeared to have less of a deleterious effect. In the 1-day and 5-day tests, the buspirone-treated groups had significantly poorer retention performance than the control ($U = 10 \& 27$, $p < 0.001$ & 0.01, respectively). In the 21-day test, while the buspirone group appeared to be lower than the control group in retention performance, the difference between the two only approached statistical significance ($U = 41.5$, $0.05 < p < 0.10$). Retention scores of the vehicle groups and the buspirone groups were analyzed individually by two Kruskal-Wallis one-way ANOVAs. No significant difference was found among the vehicle groups suggesting little memory decay over the 20-day retention period. On the other hand, there was a significant difference among the buspirone-treated groups ($H'(2) = 7.1$, $p < 0.05$). Further paired comparisons indicated that rats given buspirone before the 21-day test showed better retention performance than rats given buspirone before the 1-day or 5-day test ($U = 30.5 \&$ 34.5, $p < 0.05$ for both tests).

IV. The effect of buspirone was attenuated by 5-HT$_{1A}$ antagonist

Various groups of rats were used to evaluate the involvement of 5-HT1A, D2 or $\alpha 2$ receptors in the effect of buspirone. They were trained as described. They received one of the following treatments: vehicle, buspirone, WAY 100635, buspirone plus the 5-HT1A antagonist WAY 100635, buspirone plus various doses of the D2 agonist apomorphine or buspirone plus various doses of the $\alpha 2$ agonist clonidine. The dose of buspirone was 3.0 mg/kg that was given 30 min. before testing, the dose of WAY 100635 was 0.3 mg/kg. The doses of apomorphine were 0.1, 0.3, 1.0 or 3.0 mg/kg, while those of the clonidine were 0.01, 0.03, 0.1 or 0.3 mg/kg. Injection of buspirone was given subcutaneously 30 min before testing, while injection of the other drug was given intra-peritoneally 10 min before the buspirone injection. Three groups of vehicle- or buspirone-injected rats tested along with groups of rats pretreated with different drugs showed comparable retention scores which did not differ statistically, so they were collapsed into a combined vehicle group or a combined buspirone group. Prestest injections of

buspirone again induced impaired retention as in the previous experiments, pretest injections of WAY 100635 had no effect on retention, but effectively blocked the impairing effect of buspirone. On the other hand, pretest injections of neither apomorphine nor clonidine were able to atteunuate the effect of buspirone. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups given vehicle, buspirone, WAY 100635 or buspirone plus WAY 100635 ($H'(3) = 28.9$, $p < 0.0001$). Further paired comparisons by Mann-Whitney two-tailed U-tests indicated that the rats treated with 3.0 mg/kg buspirone had significantly lower retention scores than the vehicle group ($U = 153$, $p < 0.0001$). The group receiving buspirone alone did not had retention scores different from the controls. On the other hand, the group receiving WAY 100635 plus buspirone showed retention scores significantly better than the buspirone group ($U = 39$, $p < 0.0001$). These findings suggested that the buspirone effect was blocked by the 5-HT1A antagonist. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups treated with vehicle, buspirone, or buspirone plus various doses of apomorphine ($H'(5) = 29.9$, $p < 0.0001$). Paired comparisons by Mann-Whitney two-tailed U-tests indicated all groups treated with buspirone plus apomorphine had retention scores lower than the vehicle group ($U = 50$, 55, 27 & 60 for the groups having 0.1, 0.3, 1.0 or 3.0 mg/kg apomorphine; $p < 0.001$, respectively). No group receiving apomorphine had retention scores better than the group receiving buspirone alone. These findings suggest that D2 receptors appeared not to be involved in the effect of buspirone on retention performance.

A Kruskal-Wallis one-way ANOVA revealed a significant difference among the groups treated with vehicle, buspirone, or buspirone plus various doses of clonidine ($H'(5) = 29.9$, $p < 0.0001$). Paired comparisons by Mann-Whitney two-tailed U-tests indicated all groups treated with buspirone plus clonidine had retention scores lower than the vehicle group ($U = 51$, 15, 44 & 44 for the groups having 0 01, 0.03, 0.1, or 0.3 mg/kg apomorphine; $p < 0.005$). No group receiving clonidine had retention scores better than the group receiving buspirone alone. These findings suggest that $\alpha 2$ receptors appeared not to be involved in the effect of buspirone on retention performance.

V. Sites of action for buspirone to affect retention performance in 1- and 21-day tests

To investigate where in the brain the effect of buspirone on retrieval may be meidated, groups of rats with cannulae implanted in various sites of the brain were trained as described in the Method section and were tested either 1 or 21 days after training. They received infusion of vehicle or 5.0 µg buspirone into the target structure 5 min before the retention test. In the 1-day test, pretest infusion of the drug impaired retention performance only when given into the ventral hippocampus, the buspirone-treated animals had significantly lower retention scores than the controls ($U = 44$, $p < 0.005$). The group given pretest intra-amygdala infusion of buspirone appeared to have lower retention scores than the correspondent controls, but the difference was not statistically significant. Buspirone infused into the locus coeruleus, median raphe nucleus, BNST or medial prefrontal cortex did not significantly

affect performance in the retention test. In the 21-day test, buspirone failed to cause a significant effect when infused into any of the brain regions before testing. The groups receiving infusion of buspirone into the medial prefrontal cortex appeared to have lower retention scores than the correspondent control group, but the difference only approached statitical significance ($U = 72.5$, $0.05 < p < 0.10$). No difference in any other pair of comparisons was close to statistical significance, even though rats receiving buspirone in the locus coeruleus or ventral hippocampus appeared to have lower retention scores.

VI. The effect of intra-hippocampal infusion of buspirone in the 1-day retention test was attenuated by 5-HT1A antagonists.

In an attempt to replicate the above finding and verify that the effect involved indeed the 5-HT1A receptors, six groups of rats with cannulae implanted into the hippocampus were trained as described. Three of them were tested 1 day after training, while the other three were tested 21 days after training. In each test, different groups of rats received 5 min before testing vehicle, buspirone or buspirone plus a specific 5-HT1A antagonistWAY 100635. Results indicated that in replicating findings of the previous experipment, pretest intra-hippocampal infusion of buspirone impaired 1-day retention performance but had no significant effect on the 21-day retention, and further that the effect of buspirone on 1-day retention could be attenuated by the specific 5-HT antagonist infused simultaneously into the hippocampus. A Kruskal-Wallis one-way ANOVA revealed a significant difference among the three groups tested 1 day after training ($H'(2) = 11.3$, $p < 0.05$). Further comparisons by Mann-Whitney two-tailed U-tests indicated that the buspirone-treated rats had significantly lower retention scores than those treated with vehicle ($U = 26$, $p < 0.01$) or buspirone plus UH-301 ($U = 16.6$, $p < 0.05$), while the latter two groups did not differ from each other. In the 21-day test, while the control appeared to have higher retention performance, but the differences among the three groups were not different ($H'(2) = 0.3$, ns).

五、討論

Results of the present study can be recapitulated as follows: 1. In replication of previous findings, pretest injections of buspirone caused a deficit in retention performance and the effect was most robust when buspirone was injected 30 min. prior to the retention test. Further, the retention-deficit induced by pretest infusion of bupirone appeared to reduce as the retention period lengthened. 2. The retention-impairing effect of buspirone could be attenuated by a 5-HT1A antagonist WAY 100635. 3. Pretest infusion of buspirone into the ventral hippocampus also caused a retention deficit in the 1-day test, which could be attenuated by the 5-HT1A antagonist. Yet the same treatment had no effect on retention in the 21-day test. These findings suggest that the hippocampus was involved in retrieving or storage by inference of affective memory in the inhibitory avoidance task shortly after the training experience but became disengaged as the retention intervals extended. and 5-HT1A receptors played an important role in the effect of buspirone suppressing retrieval of an emotional experience.