Modulation of Memory Processing by Cholecystokinin: Dependence on the Vagus Nerve

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Allowing mice access to food immediately after an aversive training session enhances memory retention. Cholecystokinin-octapeptide (CCK-8), which is a gastrointestinal hormone released during feeding, also enhances memory retention when administered intraperitoneally. This memory-enhancing effect of CCK-8 is blocked when the vagus nerve is cut, indicating that CCK-8 may produce its effect on memory retention by activating ascending fibers in the vagus nerve. Thus, CCK-8, a peripherally acting peptide, may mediate the memory-enhancing effects of feeding.

ONE OF THE BASIC NEEDS OF EVEN the most primitive organism is to be able to find food. The advantage to the animal of maintaining a vivid memory of a successful hunt is obvious. We thus hypothesized that feeding an animal after an aversive training session would enhance memory retention.

To test this hypothesis, two groups of male mice [TAC(SW)], each consisting of 20 mice that were 6 weeks old, were deprived of food for 18 hours, while a third group of 20 mice was allowed free access to food for the same period of time. Mice were given four training trials in which to learn to avoid receiving footshock in a T-maze runway (*1*). The ad libitum group and one of the food-deprived groups were given food immediately after training. The ad libitum group ate very little, whereas the group deprived of food ate voraciously. The other food-deprived group received food 3 hours after training. Thereafter, all groups had free access to food until retention testing was performed 1 week later. On the retention test, mice were trained until they avoided receiving footshock on five out of six consecutive trials. A one-way analysis of variance (ANOVA) indicated that access to food immediately after training had a significant effect on mean trials to reach the criterion [$F(2,57) = 6.38, P < 0.001$] (Fig. 1A, expressed as recall score). Further analysis of the data (Dunnett's t test) showed that the group receiving food immediately after training required significantly fewer mean trials to reach a criterion of five avoidances in six consecutive trials than the ad libitum control group ($P < 0.05$). Between the groups of food-deprived mice, the group receiving food immediately after training required a significantly lower mean number of trials to reach criterion than mice receiving food 3 hours after training ($P < 0.01$, Tukey's t test).

It is well recognized that a number of gastrointestinal peptides are released during feeding that signal the central nervous system to terminate feeding (*2*). The best studied of these signals is the gastrointestinal hormone cholecystokinin (CCK) (*3*). In view of the enhanced memory seen after feeding, we tested whether CCK would enhance memory. We administered sulfated CCK-octapeptide (CCK-8S) (from Peninsula Laboratories, Belmont, California) to mice that had not been deprived of food, since food deprivation would impair memory processing. There were 15 mice in each group, and CCK-8S was given intraperitoneally in one of eight doses (0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, or 10.0 μg per kilogram of body weight). After the last of four training trials, CCK-8S was administered within 30 seconds. Retention was tested 1 week after training. Low doses of CCK-8S had little effect on retention, intermediate doses facilitated retention, and high doses had no apparent effect on retention (Fig. 1B). A one-way ANOVA on mean trials to first avoidance (*4*) indicated that CCK-8S had an effect on retention [$F(7,105) = 2.95, P < 0.01$]. A subsequent comparison of mean trials to first avoidance for the saline control and for each dose of CCK-8S indicated that doses from 0.05 to 1.0 μg/kg significantly reduced the mean trials to first avoidance response ($P < 0.05$, Dunnett's t test), with the greatest reduction in mean trials to first avoidance response occurring at 0.5 μg/kg. This result confirmed the previous findings that CCK-8S enhances memory after parenteral administration (*5*). To test whether CCK-8S enhanced retention by acting within the abdomen or at some other site, we compared the effect of subcutaneously administered CCK-8S to intraperitoneally administered CCK-8S, with 15 mice in each group. When CCK-8S was administered subcutaneously, it was less potent than when administered intraperitoneally; four times as much CCK-8S was required to improve retention when given subcutaneously compared to intraperitoneally (Fig. 1C). Nonsulfated CCK-8 (CCK-8NS) binds less potently to CCK receptors than CCK-8S (*3*). To test the specificity of the CCK effect, we examined the effect of CCK-8NS on retention with 15 mice in each of seven groups (Fig. 1D). CCK-8NS yielded an inverted U-shaped dose-response modulation of retention similar to the CCK-8S dose-response curve. A one-way ANOVA indicated that the effect of CCK-8NS on retention was significant [$F(6,98) = 4.63, P < 0.01$]. Dunnett's t tests run on mean trials to first avoidance response for saline versus responses for each dose indicated that 0.5 to 5.0 μg/kg of CCK-8NS decreased mean trials to first avoidance response significantly ($P < 0.05$), with the greatest decrease in mean trials to first avoidance occurring at 5.0 μg/kg. This finding was similar to the effective range for CCK-8S (0.05 to 1.0 μg/kg), and it indicated that CCK-8NS is only about 1/10 as effective as CCK-8S, on a microgram per kilogram basis, at improving retention.

A number of studies have shown that intraperitoneal administration of CCK-8S inhibits feeding in rats by a mechanism that depends on an intact vagus nerve (*6*). The studies of Smith *et al.* (*7*) showed that this process involved the afferent vagal nerve fibers. Crawley and colleagues (*8*) showed that lesioning of the paraventricular nucleus and the nucleus tractus solitarius, and the connections between them, inhibited the satiety effect of CCK-8S. We hypothesized that the memory-enhancing effects, like the effects on feeding, would be abolished by vagotomy and therefore tested whether the vagus mediated CCK-8S memory-enhancing effects. Nonoperated, sham-operated, and vagotomized TAC(SW) male mice were allowed free access to food (*9*). Half of the mice in each of the three groups received saline immediately after T-maze footshock avoidance training. The other half received CCK-8S intraperitoneally (0.5 μg/kg) immediately after training. There were 15 mice per group, and retention was tested 1 week after training. Retention was poor among the saline-injected mice, with recall scores ranging from 7 to 27% (*10*). The nonoperated and sham-operated mice that were administered CCK-8S after training had recall scores of 73 and 80%, respectively, while the vagotomized mice had a recall score of only 13%. A two-way ANOVA (drug times operations) on either mean trials to first avoidance or to criterion yielded significant

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main effects for saline versus CCK-8S and for type of operation; the two-way interaction was also significant (Fig. 2A, expressed as recall score).

A partitioning of the sum of squares indicated that among those mice with a cut vagus nerve, the effect of CCK on retention was highly significant [$F(1,84) = 23.74$, $P < 0.001$], but the contribution of saline was not significant. Nonoperated and sham-operated mice contributed significantly to the main effect of operation on retention, but the effect of vagotomy was not significant ($F < 1$). The significant interaction of drug and operation was due primarily to the poor retention in vagotomized mice given saline or CCK-8S. A comparison of each CCK-8S–treated group with its saline control indicated that the number of trials either to first avoidance or to criterion were significantly lower for nonoperated mice ($t = 3.23$, $t = 5.23$, both $P < 0.002$) and for sham-operated mice ($t = 3.02$, $P < 0.002$; $t = 2.67$, $P < 0.02$). The vagotomized mice given saline or treated with CCK-8S did not differ significantly from each other by either measure of retention ($t < 1$).

The failure of CCK-8S to improve retention in vagotomized mice could have been due to impaired acquisition. To determine if this was true, three additional sets of mice were trained as above in a single session until they reached the criterion of five avoidances in six consecutive trials. The results of a one-way ANOVA indicate that no significant difference in acquisition among nonoperated, sham-operated, and vagotomized mice occurred ($F < 1$). The mean trials to criterion (± SEM) for these naïve mice were as follows: nonoperated, 5.93 ± 0.26; sham-operated, 6.00 ± 0.20; vagotomized, 6.00 ± 0.23. Thus a lack of differ-

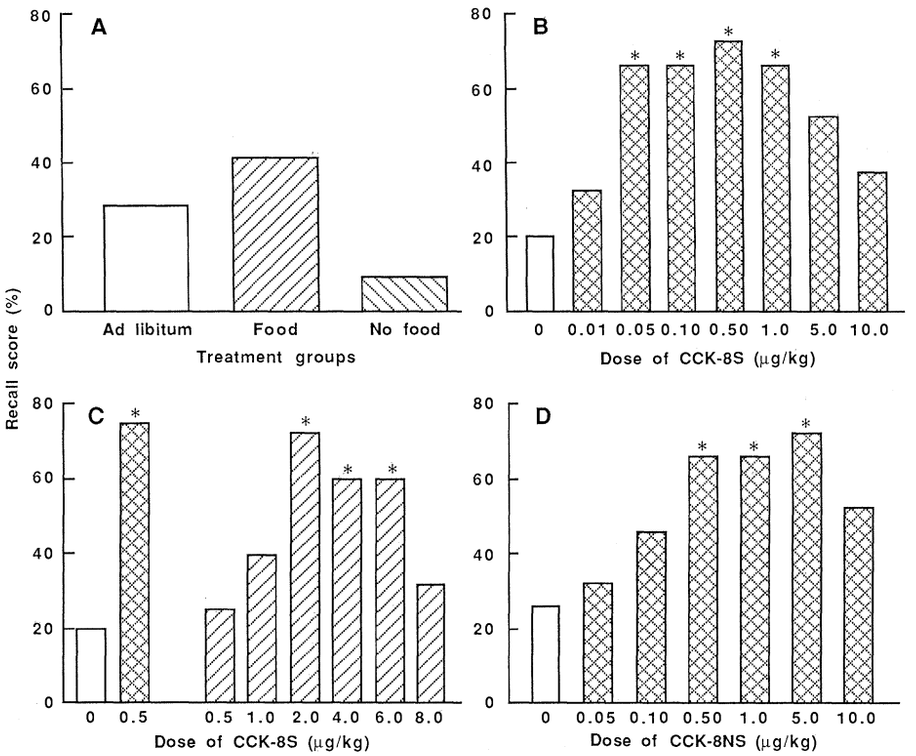

Fig. 1. (**A**) One-week memory retention by mice deprived of food for 18 hours after footshock avoidance training and feeding. Retention in mice deprived of food before training and then given food after training differed from that in mice allowed access to food ad libitum ($P < 0.05$) or that in mice receiving food 3 hours after training ($P < 0.01$). The mean trials to criterion (± SEM) that correspond to the recall scores were: ad libitum, 8.85 ± 0.41; food, 7.8 ± 0.38; no food, 9.55 ± 0.27. Twenty mice were in each group. (**B**) A dose-response relation between the amount of CCK-8S administered intraperitoneally and retention test performance. The inverted-U shape is typical of memory-enhancing compounds. The mean trials (± SEM) to first avoidance that correspond to the recall scores were: 0 μg/kg, 4.53 ± 0.43; 0.01 μg/kg, 4.13 ± 0.40; 0.05 μg/kg, 2.87 ± 0.43; 0.1 μg/kg, 2.93 ± 0.41; 0.5 μg/kg, 2.80 ± 0.32; 1.0 μg/kg, 2.87 ± 0.38; 5.0 μg/kg, 3.40 ± 0.48; and 10.0 μg/kg, 4.25 ± 0.72. Fifteen mice were in each group. (**C**) The optimal dose of CCK-8S administered intraperitoneally (0.5 μg/kg) compared to the dose-response effect of subcutaneous administration. The enhanced potency of the intraperitoneal administration, which was four times that of the subcutaneous method, indicates that the effect of CCK-8S on retention is mediated by effects on abdominal receptors. The mean trials (± SEM) to first avoidance response that correspond to the recall scores were: saline given intraperitoneally or subcutaneously (open bar), 4.33 ± 0.37; CCK-8S given intraperitoneally (cross-hatched bar) at 0.5 μg/kg was 2.6 ± 0.30; and for CCK-8S given subcutaneously (diagonal-line bars) at 0.5 μg/kg, 4.2 ± 0.32; 1.0 μg/kg, 3.60 ± 0.32; 2.0 μg/kg, 2.87 ± 0.28; 4.0 μg/kg, 3.27 ± 0.36; 6.0 μg/kg, 3.07 ± 0.29; and 8.0 μg/kg, 4.07 ± 0.37. There were 15 mice in each group. (**D**) The dose-response effect of the weaker binding CCK-8NS (administered intraperitoneally) on retention for T-maze footshock avoidance training. Comparing the minimal effective dose in (B) (0.05) and (D) (0.5) indicates that CCK-8S is more potent at improving memory retention than CCK-8NS. The mean trials (± SEM) to first avoidance that correspond to the recall scores were: 0 μg/kg, 4.40 ± 0.36; 0.05 μg/kg, 4.5 ± 0.39; 0.1 μg/kg, 4.07 ± 0.37; 0.5 μg/kg, 2.73 ± 0.33; 1.0 μg/kg, 3.07 ± 0.38; 5.0 μg/kg, 2.75 ± 0.34; and 10.0 μg/kg, 3.47 ± 0.40. Fifteen mice were used in each group. An asterisk in (B) through (D) indicates this group differs from the control (0 μg/kg) at $P < 0.05$ by Dunnett's t test. For visual clarity all results are presented as recall scores. Statistical analysis was based on two measures of retention, trials to first avoidance response or trials to criterion as explained in the text. Recall scores and trials to first avoidance response yield a correlation of +0.90 or higher.

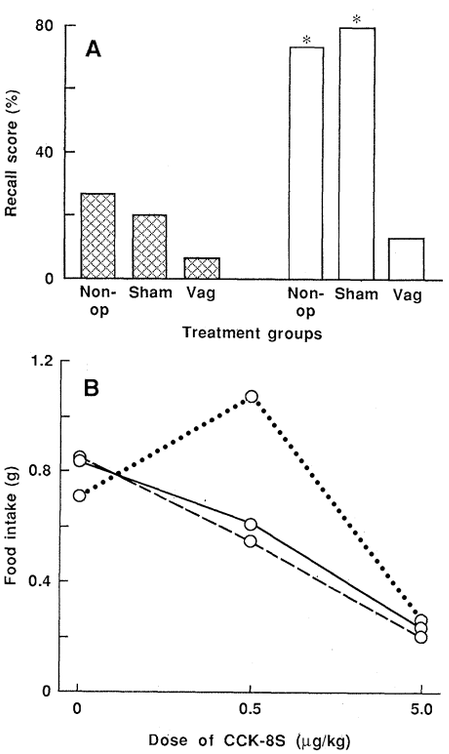

Fig. 2. (**A**) Effect of vagotomy on the memory-enhancing effect of CCK-8S on retention for footshock avoidance training. Vagotomy blocked the memory-enhancing effect of CCK-8S. The means (± SEM, $n = 15$) corresponding to the recall scores for saline-treated groups (cross-hatched bars) were: nonoperated (nonop), 4.47 ± 0.40; sham-operated (sham), 4.87 ± 0.49; vagotomized (vag), 5.33 ± 0.29. The means (± SEM) for CCK-8S–treated (open bars) (0.5 μg/kg, administered intraperitoneally) groups were: non, 2.8 ± 0.30; sham, 3.33 ± 0.29; vag, 5.27 ± 0.36. An asterisk indicates $P < 0.01$ difference for 0 and 0.5 μg/kg dose in each type of operation (Dunnett's t test). (**B**) Effect of CCK-8S administered intraperitoneally on food intake in nonoperated (——), sham-operated (– – –), and vagotomized (· · ·) mice. Vagotomy prevented the suppression of food intake by CCK-8S (0.5 μg/kg) that was seen in nonoperated and sham-operated mice.

ence in ability of vagotomized versus nonoperated and sham-operated mice to learn did not account for the difference in CCK-8S to enhance retention.

At the conclusion of the memory experiments, the same group of mice was used to examine the effect of vagotomy on the inhibitory effect of CCK-8S on feeding (*11*). Vagotomy abolished the CCK-8S effect on feeding at low doses, but not at high doses (Fig. 2B). A similar shift in the dose-response curve for the CCK-8S effect on feeding has been shown in vagotomized dogs (*12*).

At the conclusion of the above experiments, on a separate day, the mice were deprived of food for 18 hours and then allowed to feed for 2 hours, after which stomach weights were obtained from 102 mice in the three groups. The mean ratios of stomach weight to body weight were as follows: nonoperated, 1.0 ± 0.04; sham-operated, 1.0 ± 0.04; vagotomized, 2.8 ± 0.5. A one-way ANOVA indicated that operation had a significant effect on the ratios of stomach weight to body weight [$F(2,100) = 10.15$, $P < 0.001$]. Thus, we were able to confirm the effectiveness of the vagotomies.

Our data show that both feeding and peripherally administered CCK-8S enhance memory in mice. This gastrointestinal hormone seems to produce its effect on memory by activating ascending vagal fibers. Further studies are necessary to determine if CCK-8S is responsible for the entire effect of feeding on memory, or, as appears to be the case in the regulation of feeding, if a combination of gastrointestinal hormones act synergistically to produce this effect (*13*). The concentrations of CCK-8S achieved after administration of the optimum memory enhancing dose would be well within the physiological range seen after feeding in rodents (*14*). A link may have evolved between the release of gastric peptides and memory processing in the central nervous system because of the survival advantages for an animal to remember the details of a successful food-foraging expedition.

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10. Recall score is defined as the percentage of mice in a group making an avoidance on retention test trials 1 to 3 [see (*1*) for details].
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Identification of a T3-Associated γδ T Cell Receptor on Thy-1$^+$ Dendritic Epidermal Cell Lines

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The murine epidermis contains a subpopulation of bone marrow–derived lymphocytes that have a dendritic morphology and that express Thy-1 and T3 cell-surface antigens but not other markers (L3T4 or Lyt-2) characteristic of mature peripheral T lymphocytes. An alternative type of T cell receptor was earlier identified on a subpopulation of murine thymocytes with a similar phenotype (T3$^+$, L3T4$^-$, Lyt-2$^-$), but not on peripheral murine T lymphocytes. Two independently derived Thy-1$^+$, L3T4$^-$, and Lyt-2$^-$ dendritic cell lines of epidermal origin that express a T3-associated disulfide-linked heterodimer composed of a 34-kilodalton γ-chain and 46-kilodalton partner (the δ chain) have now been identified. Analysis of *N*-linked glycosylation revealed that this receptor is similar to that detected on thymocytes. These results demonstrate that Thy-1$^+$ dendritic epidermal cell lines can express γδ T cell receptors in vitro and suggest that Thy-1$^+$ dendritic epidermal cells express such receptors in vivo. The localization of these γδ T cell receptor–expressing cells in the epidermis may be of importance for understanding the function of these receptors.

THE MURINE EPIDERMIS CONTAINS A minor subpopulation of dendritic bone marrow–derived leukocytes that express high levels of the Thy-1 cell-surface antigen but do not express other markers (L3T4 or Lyt-2) characteristic of mature peripheral T lymphocytes (*1*). We recently showed that all Thy-1$^+$ dendritic epidermal cells (DECs) express T3 in situ and thus likely belong to the T lymphocyte lineage (*2*). Analysis of the components of T3-associated T cell receptor (TCR) molecules on Thy-1$^+$ DECs has been facilitated by the availability of several long-term lymphokine-dependent cell lines derived from Thy-1$^+$ DECs that maintain their phenotype in vitro (*1, 2*). We detected a T3-associated 34-kD TCR γ chain on the cell surface of two Thy-1$^+$ DEC cell lines that was apparently disulfide-linked to a 34-kD molecule; this finding suggested that the γ chain might be expressed as a homodimer on these cell lines (*2*). We have now analyzed T3-associated TCR molecules on three additional Thy-1$^+$ DEC cell lines and demonstrate that two lines express a T3-associated disulfide-linked heterodimer composed of 34-kD γ chain and a 46-kD partner (termed the δ chain). These results indicate that Thy-1$^+$ DEC cell lines can express the TCR γ chain as a disulfide-linked component of a heterodimeric TCR and suggest that Thy-1$^+$ DECs express such receptors in vivo and thereby represent one of the major extrathymic T cell populations with this phenotype.

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