with previous morphologic observations that lymphocytes occur less frequently in the center of MS lesions than in the adjacent normal white matter (*6*). However, more precise identification of these small mononuclear cells as T cells or T cell subsets has not previously been possible in the CNS in this or any other condition. With the aid of monoclonal antibodies, we have characterized further these infiltrating cells and have demonstrated that T cells, in particular $T11^+$ and $T4^+$ cells, are numerous at the lesion margin and in the normal-appearing white matter surrounding the active chronic MS plaque but occur only in small numbers within the lesion center.

This distribution of T cells and Ia^+ cells in the CNS of patients with MS suggests several possibilities of relevance to the pathogenesis of this disease. In addition to supporting the concept of MS being a T cell–mediated condition, the presence of dense infiltrates of $T4^+$ cells in nearby normal white matter might herald an early pathogenetic event. These cells could function to recruit macrophages or mediate cytotoxicity themselves. In this regard, it has been shown that a subset of $T4^+$ cells is cytotoxic for class II MHC (major histocompatibility complex) molecules (*7*). Also, the presence of Ia^+ macrophages in the normal white matter might be indicative of antigen presentation, supported perhaps by the observed close association between Ia^+ macrophages and lymphocytes. Furthermore, ultrastructural studies are in agreement with the present conclusion that active demyelination in MS depends on the presence of macrophages (*6*). The apparent restriction of $T8^+$ (suppressor-cytotoxic) cells to the edge of the lesion and to perivascular areas might reflect a difference in the mobility of helper and suppressor T cells. Whether these cells trigger suppression of autoreactivity or mediate another function, such as conventional cytotoxicity against class I MHC molecules, is unknown. The absence of background staining of normal CNS elements with anti-T8 is of interest because it has been reported that anti-T8 cross-reacts with sheep oligodendrocytes and the suppressor-cytotoxic subpopulation of human T lymphocytes (*8*).

It thus appears that in MS lesion progression is associated with the presence of large numbers of helper (inducer) T cells in the normal white matter adjacent to an existing lesion, whereas suppressor-cytotoxic T cells are limited to the lesion margin. However, demyelination seems to depend on the presence of Ia^+ macrophages. These observations might prove of relevance to future considerations on the pathogenesis of multiple sclerosis.

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12 October 1982

Evidence for the Neuropeptide Cholecystokinin as an Antagonist of Opiate Analgesia

Abstract. *The endogenous neuropeptide cholecystokinin, when administered systemically or perispinally, potently antagonizes opiate analgesia produced by foot shock and morphine. Nonopiate foot-shock analgesia is not reduced by this neuropeptide. The spinal cord appears to be a critical site of cholecystokinin action. These experiments suggest a physiological role for cholecystokinin as a specific opiate antagonist in analgesia-mediating systems. A similar mode of action may explain other behavioral effects of cholecystokinin, such as suppression of food intake.*

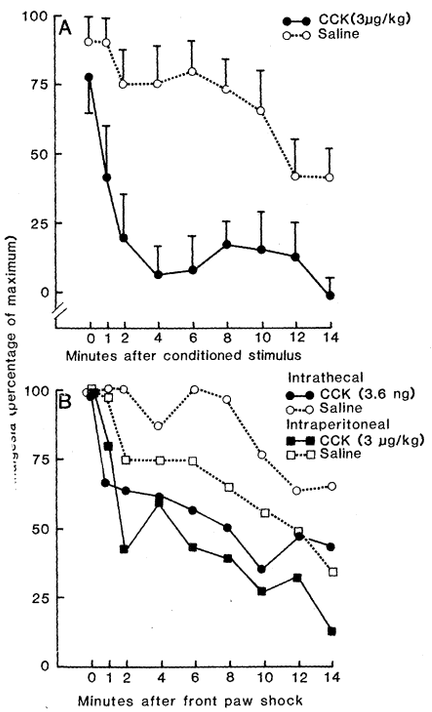
Cholecystokinin (CCK), a polypeptide existing in a variety of amino acid chain lengths, has been isolated from peripheral and central nervous system tissue (*1*). Physiological actions attributed to CCK include regulation of satiation (*2*), modulation of catecholaminergic activity (*3*), and regulation of hypothalamic peptides (*4*). Under certain conditions several of these actions are opposite those of the opiates. Food intake decreases after administration of CCK intraperitoneally or intracerebroventricularly (*2*), whereas food consumption increases after administration of morphine or β-endorphin by the same routes (*5*). Dopamine turnover is decreased by CCK (*3*) and increased by morphine in specific brain regions (*6*). Stress-induced feeding is mediated by endogenous opiates (*7*) and blocked by CCK (*8*). Also, genetically obese mice have elevated levels of β-endorphin in the brain (*9*) but reduced levels of CCK (*10*). We therefore hypothesized that CCK antagonizes endogenous opiates.

Since the endogenous opiates are best known for their analgesic properties, we tested our hypothesis by determining whether the sulfated octapeptide of CCK (CCK-8) blocks opiate-mediated analgesia produced by certain environmental stimuli. Watkins *et al.* (*11*) showed that analgesia can be produced by brief shock of the front or rear paws. Only shock of the front paws was found to produce opiate-mediated analgesia. They also demonstrated that opiate-mediated analgesia can be evoked by a classical conditioning paradigm in which brief foot shock is used as the unconditioned stimulus (*12*). We therefore used these paradigms to determine whether CCK-8 an-

tagonizes opiate-mediated, but not opiate-independent, analgesia (*13*).

In the first experiment we examined the effect of CCK-8 on front paw (opiate) foot shock–induced analgesia (FSIA). Doses of 0.75, 1.5, 3.0, or 6.0 μg per kilogram of body weight or an equal volume of saline vehicle were administered intraperitoneally 30 minutes before the delivery of foot shock. Analgesia was assessed by the tail flick test (*14*). Tail flick latency was measured before exposure to shock and 0, 1, 2, 4, 6, 8, 10, 12, and 14 minutes afterward (*15*). All but the lowest dose of CCK-8 significantly reduced FSIA (Fig. 1B) (*16*).

We then determined whether CCK-8 can attenuate analgesia (opiate) classically conditioned to front paw shock. Rats were exposed to the electrified grid (unconditioned stimulus) for 90 seconds on two consecutive days. On the third day, the animals were injected with CCK-8 (3.0 μg/kg) or saline. Thirty minutes after this injection, the animals were subjected to the same shock procedure as on the two preceding days, except that the current remained off during exposure to the grid (conditioned stimulus). Analgesia was assessed in the same manner as in the previous experiment. As shown in Fig. 1A, classically conditioned analgesia was potently attenuated by CCK-8.



. 1. Attenuation of opiate-mediated FSIA CCK-8. (A) Analgesia classically condi- ed to front paw shock was significantly gonized by CCK-8 (3 μg/kg) administered peritoneally 30 minutes before the condi- ed stimulus [$P < .0001$, analysis of vari- (ANOVA)]. A significant reduction in evel of analgesia (*15*) occurred as early as inute after the conditioned stimulus .005, one-tailed Student's *t*-test) and ted throughout the testing period. (B) eduction of FSIA caused by intraperito- CK-8 (3 μg/kg) ($P < .0001$, ANOVA) omparable in magnitude to that caused ng of CCK-8 administered intrathecally lumbosacral region of the spinal cord 0001, ANOVA).

Since CCK-containing neurons and receptors have been identified in the dorsal horn of the spinal cord (*17*) and since both front paw FSIA and classically conditioned analgesia are antagonized by intrathecally administered naloxone (*18*), it seemed likely that sites mediating the antagonistic effect of CCK-8 on front paw FSIA are present in the spinal cord. We therefore applied CCK-8 directly to the lumbosacral region of the spinal cord (the level mediating the tail flick response) to determine whether this would attenuate analgesia induced by front paw shock. Rats were implanted with permanent intrathecal catheters terminating in the lumbosacral region (*19*). After 2 weeks of recovery, the animals received 3.6 ng of CCK-8 in 5 μl of saline immediately before exposure to front paw shock. Analgesia was tested after the shock. The administration of CCK-8 to the lumbosacral area significantly attenuated the analgesic effect of front paw shock (Fig. 1B).

The fact that this antagonism cannot be accounted for by drug diffusion to the periphery (the dose was below the minimum effective peripheral dose) and the finding that lumbosacral and intraperitoneal administration of CCK-8 result in a comparable degree of antagonism (Fig. 1B) strongly suggest that a CCK-sensitive site mediating the attenuation of front paw FSIA is present in the lumbosacral region of the spinal cord. This antagonism appears to be specific to the biologically active, sulfated variant of CCK, since intrathecal administration of 3.6 ng of desulfated CCK-8, a gastrin-like peptide, did not significantly affect the level of analgesia resulting from front paw shock.

To determine whether the antagonistic effect of CCK-8 is specific to opiate analgesia, we examined the effect of CCK-8 on hind paw (nonopiate) FSIA. CCK-8 (3 μg/kg) was administered intraperitoneally 30 minutes before exposure to hind paw shock. In this instance CCK-8 did not attenuate the resultant analgesia, but slightly potentiated hind paw FSIA. Thus CCK-8 appears to selectively inhibit opiate-mediated analgesia.

If CCK-8 does indeed function as a selective opiate antagonist of environmentally induced analgesia (*20*), then it should also antagonize the analgesic effect of morphine. To examine this possibility, we injected rats intraperitoneally with morphine sulfate (10 mg/kg) 20 minutes after giving them CCK-8 (5 μg/kg) or saline by the same route. Tail flick latencies were measured immediately after administration of the first drug and then at 10-minute intervals for 120 minutes. As illustrated in Fig. 2, morphine-induced analgesia was significantly attenuated by CCK-8. Therefore, CCK-8 is effective in blocking analgesia produced by either exogenous or endogenous opiates.

Cholecystokinin octapeptide, like a variety of other neurally active substances (such as naloxone, opiates, and substance P) (*21*), exerts a dose-dependent, biphasic effect. Whereas low doses of CCK antagonize opiate action, as in the present study, doses ten times higher have been reported to produce analgesia (*22*). This may reflect the dose-dependent activation of multiple biochemical mechanisms.

The biochemical mechanism underlying the antagonism by CCK-8 of opiate-induced analgesia remains to be elucidated. The opiate receptor may be the site of CCK-8 action, although it has been reported that the sulfated heptapeptide of CCK is not a ligand of the opiate receptor (*23*). The binding of CCK-8 to its own receptor may cause conformational changes in the opiate receptor, resulting in decreased affinity for opiate agonists (steric inhibition). An additional possibility is that CCK- and opiate-containing neurons converge on the same neural circuitry, where they exert opposite neuromodulatory effects.

The finding that low doses of CCK-8

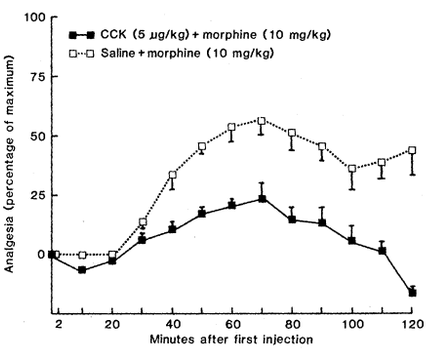


Fig. 2. Antagonism of morphine-induced analgesia by CCK-8. The neuropeptide was administered intraperitoneally at time zero and was followed 20 minutes later by morphine sulfate. Analgesia was significantly lower in the group given CCK and morphine than in the control group as early as 20 minutes after the morphine injection ($P < .05$). Significance levels ranged from $P < .005$ at 60 minutes to $P < .025$ at 80 minutes.

antagonize opiate-mediated analgesia suggests that blocking the endogenous release of CCK may potentiate opiate action. Conversely, elevated levels of CCK may account for the absence of analgesia in response to morphine administration, as in morphine tolerance. Therefore blockade of CCK action may be an effective supplement to morphine administration in the treatment of chronic pain.

It is likely that CCK, in addition to modulating opiate involvement in analgesia, antagonizes other opiate-mediated behaviors, such as feeding. This idea is supported by developmental studies of the functional onset of endogenous satiety mechanisms. For instance, naloxone suppresses milk intake in suckling rat pups on day 14 of age but not earlier (*24*), and CCK first induces satiety around day 15 (*25*). However, empirical data supporting an antiopiate action of CCK for behaviors other than pain responsiveness are yet to be obtained.

Note added in proof: Itoh *et al.* (*26*) recently reported that CCK-8 suppresses β-endorphin–induced analgesia.

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15. If a tail flick did not occur within 8 seconds the radiant heat was terminated to prevent tissue damage. The degree of analgesia was expressed as a percentage of the maximum by applying the following equation: [(EL − BL)/(8 − BL)]100, where EL is experimental tail-flick latency and BL is baseline latency (3.5 to 4.0 seconds).
16. When FSIA values for experimental animals were compared to values for saline-treated controls, the following P values were obtained by analysis of variance: at 0.75 μg of CCK-8 per kilogram, $P > .05$; at 1.5 μg/kg, $P < .0001$; at 3.0 μg/kg, $P < .0001$; and at 6.0 μg/kg, $P < .0001$. Desulfated CCK-8 (1.5 μg/kg, intraperitoneally) failed to attenuate front paw FSIA. Therefore, the antagonism by CCK-8 is a specific effect of the sulfated variant of CCK rather than a general gastrin-mediated effect.
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28 September 1982

Regulation of Queen Number by Workers in Colonies of Social Insects

Abstract. *Experiments with fire ants suggest that queen pheromones act quantitatively in the regulation of queen number in colonies of social insects. Specific mechanisms probably include recognition by workers of unique quantitative blends of pheromones produced by queens, and quantitative effects of pheromones acting at the level of the colony on workers and at the level of the individual on queens. Several aspects of this quantitative hypothesis of pheromone action were tested.*

A major question concerning sociality among the insects is how the single queen status of most colonies is maintained. Wilson (*1*) has argued that the simplest explanation of monogyny is that it evolved through competition between queens—that is, unless queens are closely related, it is ultimately advantageous for them to avoid sharing the same nest. Although his hypothesis is supported by the common occurrence of animosity between queens (*2*), in many social insects the workers participate in the elimination of supernumerary queens, and this seemed to Wilson (*1*) to constitute a difficulty for his hypothesis. He therefore suggested that "the queen-worker complex could evolve so as to remove queens with the least familiar odor, if some of the odor differences were genetic in origin" (*3*). We have developed a hypothesis to explain the maintenance of monogyny by workers using the fire ant, *Solenopsis invicta* Buren.

Because polygynous colonies sometimes occur (*4*) in the North American population of *S. invicta*, we were able t test the responses of workers from mc nogynous and polygynous colonies t queens from both types of colony (*5* Workers that we made queenless usual accepted the unfamiliar queens mo readily than did those that were quee right, and workers from monogyno colonies tended to be more discrimin ing than those from polygynous coloni Queens from polygynous colonies w distinctly less acceptable to work from monogynous colonies, even w the colonies were queenless (Fig. 1a

In another experiment, we introdu multiple (25) foreign queens into qu less colonies; we predicted that wor from monogynous colonies would ki but one queen, whereas workers polygynous colonies would retain r than one. With few exceptions ou perimental results were in agree with the prediction (Fig. 1b) (*6*) weeks after we introduced the qu we subjected the surviving ones oviposition test (*4*). The mean num