

BRIEF COMMUNICATION

Effect of bestatin, an aminopeptidase inhibitor, on memory in inhibitory-avoidance and Y-maze discrimination tasks

SHI-YI ZHANG

*Chinese Academy of Medical Sciences
Beijing, People's Republic of China*

and

INES B. INTROINI-COLLISON
and JAMES L. MCGAUGH

University of California, Irvine, California

The findings of recent studies provide extensive evidence that opioid peptides influence learning and memory. Generally, when administered posttraining, retention is enhanced by opiate receptor antagonists (e.g., naloxone and naltrexone) and impaired by agonists (e.g., morphine, β -endorphin, enkephalins) (Castellano, 1975; Gallagher & Kapp, 1978; Introini, McGaugh, & Baratti, 1985; Izquierdo, 1979; Martinez et al., 1981; McGaugh, 1989; McGaugh & Gold, 1989; Zhang, McGaugh, Juler, & Introini-Collison, 1987). The finding that retention is influenced by drugs affecting opiate receptors suggests that endogenous opioid peptide systems are involved in the regulation of memory storage (McGaugh, 1989).

Most previous studies investigating the possible role of the enkephalins in memory storage have examined the effects produced by systemic injections of enkephalins and opiate antagonists. The involvement of enkephalins in memory can also be addressed by using drugs that affect the metabolism of enkephalin (Martinez, Weinberger, & Schulteis, 1988). Two enzymes, aminopeptidase and dipeptidylcarboxypeptidase, seem to play a predominant role in the inactivation of enkephalins (Schwartz, 1983). Enkephalin-hydrolysing aminopeptidase has been found to be sensitive to bestatin, a potent aminopeptidase inhibitor (Schwartz, 1983). Enkephalin hydrolysis is inhibited by bestatin both in vivo and in vitro (Chaillet et al., 1983; Giros, Gros, Solhonne, & Schwartz, 1986). If, as previous findings suggest, enkephalins impair memory, then memory should also be impaired by treatments, such as bestatin, that inhibit the metabolism of enkephalin. To examine this implication, in the present experiments, we investigated the effects of posttraining administration of bestatin on retention in two aversively motivated training tasks.

Male CFW mice, 50–60 days old, were used as subjects. After acclimation to the laboratory for a week, the mice were trained on a one-trial step-through inhibitory-avoidance (IA) apparatus (Zhang et al., 1987) using a 0.6-mA or 0.9-mA, 2-sec, 60-Hz footshock. Immediately after training, the animals received i.p., 0.9% saline or drug injection in a volume of 0.3 ml per mouse. On a test 24 h later the latency to step through was recorded as a measure of retention. The ceiling latency on the retention trial was 300 sec.

After a 2-week interval, the mice were then trained in a Y-maze discrimination (YMD) task (Zhang et al., 1987). The mice were first trained to enter the left, illuminated alley of the Y-maze in order to escape from footshock delivered through stainless steel floor plates. They were trained to a criterion of three successive correct choices of the left alley. The animals then received immediate posttraining drug or physiological saline injections (0.3 ml/mouse). On a 24-h retention test, the discrimination was reversed: each animal received six trials, as on original training, but could escape only by entering the right, darkened alley. The number of choices of the left alley on the six reversal-training trials was used as the measure of retention. The use of this measure is based on the assumption that entries into the left alley, which was the correct alley on the original-training trials, reflect memory of the original training. This assumption is supported by findings of previous studies indicating that errors on the reversal-training trials vary directly with the amount of original training (Introini-Collison & McGaugh, 1986).

The drugs, bestatin hydrochloride (Sigma) and naloxone hydrochloride (Endo), were dissolved into 0.9% saline. All doses are expressed as salt weights. Data were analyzed by two-tailed Mann-Whitney *U* tests; *p* values less than .05 were considered significant.

In the first experiments, we examined dose-response effects of posttraining bestatin on retention. The results obtained in the IA task are shown in Table 1 (left). Retention was impaired by bestatin in doses of 1.0 and 2.0 mg/kg. Lower and higher doses were ineffective. The effects of administering bestatin following training in the YMD task are also shown in Table 1 (right). Significant impairment of retention was obtained with a dose of 1.0 mg/kg. The dose of 2.0 mg/kg was ineffective.

In the second experiment, we examined the effects of bestatin in animals also treated with the opiate receptor blocker naloxone to determine whether the influence of bestatin on memory retention is mediated by opiate receptors. The findings obtained in the IA task are shown in Figure 1A. As can be seen, posttraining bestatin impaired retention. Although there was no significant effect of naloxone administered alone, naloxone attenuated the ef-

This research was supported by a World Health Organization Fellowship (to S.Z.), Research Grant MH12526 from NIMH and NIDA, and Office of Naval Research Contract N00014-87-K-0518 (to J.L.M.). Correspondence may be addressed to James L. McGaugh, Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Table 1
Effect of Bestatin on Retention of Inhibitory-Avoidance and Y-Maze Discrimination Tasks in Mice

| Treatment | Dose (mg/kg) | Inhibitory Avoidance* | | | Y-Maze Discrimination† | | |
|-----------|--------------|-----------------------|---------------------|-------------------|------------------------|------------------|-----------|
| | | No. of Mice | Retention Latencies | | No. of Mice | Retention Scores | |
| | | | <i>Mdn</i> | <i>IQ Range</i> ‡ | | <i>M</i> | <i>SE</i> |
| Saline | | 28 | 235 | 63-300 | 12 | 4.00 | 0.44 |
| Bestatin | 0.5 | 21 | 180 | 20-300 | | | |
| Bestatin | 1.0 | 21 | 145 | 18-232§ | 10 | 2.80 | 0.38§ |
| Bestatin | 2.0 | 22 | 146 | 20-254§ | 12 | 3.33 | 0.38 |
| Bestatin | 4.0 | 21 | 176 | 22-300 | | | |
| Bestatin | 8.0 | 15 | 205 | 24-300 | | | |

Note—All treatments were given (i.p.) immediately after training. *Footshock: 0.9 mA, 60 Hz, 2 sec. †Footshock: 0.4 mA, 60 Hz. ‡Interquartile range. § $p < .05$ as compared with the saline-injected control group.

fects of bestatin: the retention latencies of the naloxone + bestatin group did not differ significantly from those of the controls. Comparable effects were obtained in the YMD task (Figure 1B): the error scores of the bestatin group were significantly lower than those of controls, whereas the retention errors made by the naloxone + bestatin group did not differ significantly from those of the controls.

In a third set of experiments, we examined the effects of bestatin and naloxone in animals trained with lower footshock levels in both training tasks. With the higher

footshock levels used in the experiments reported above (IA = 0.9 mA; YMD = 0.4 mA) the controls were at ceiling on the IA retention test and the controls trained in the YMD task had high retention scores. Under these conditions, naloxone administered alone did not affect retention. The findings obtained with experiments using a lower footshock levels (IA = 0.6 mA; YMD = 0.3 mA) are shown in Table 2. As can be seen, posttraining naloxone enhanced retention in both tasks. Furthermore, bestatin, in a subeffective dose (0.5 mg/kg) which did not cause amnesia when administered alone, (Table 1) attenuated the facilitatory effects of naloxone in both tasks (Table 2).

The findings of these experiments indicate that (1) posttraining parenteral injections of the aminopeptidase inhibitor bestatin produce dose-dependent impairment of retention of IA and YMD training, (2) the effects are attenuated by the opiate receptor antagonist naloxone, and (3) the retention-enhancing effects of posttraining naloxone are attenuated by bestatin.

These experiments were based on previous findings indicating that memory is impaired by posttraining administration of opioid peptides. Our findings, indicating that retention is impaired by posttraining systemic injections of bestatin, are highly comparable to the findings of our previous studies of the effects of posttraining systemic injections of met-enkephalin (Zhang et al., 1987).

The dose-response effects obtained with the two tasks used in this study are similar to those obtained in previous studies of the effects of morphine, β -endorphin, and enkephalins (Introini, 1984; Introini & Baratti, 1984; Introini et al., 1985). In those studies, high doses did not affect memory. Thus, the lack of an effect of high doses of bestatin seems likely to be due to high concentrations of enkephalins. It remains unclear, however, why memory-impairing effects of opiate agonists are generally found only at modest dose levels.

In the previous (Zhang et al., 1987) and the present experiments, the dose-dependent memory-impairing effects of both met-enkephalin and bestatin were attenuated by naloxone. The finding that the retention-enhancing effects of posttraining naloxone are blocked by a low (and subeffective) dose of bestatin provides additional support for the view that the effects are mediated by the activa-

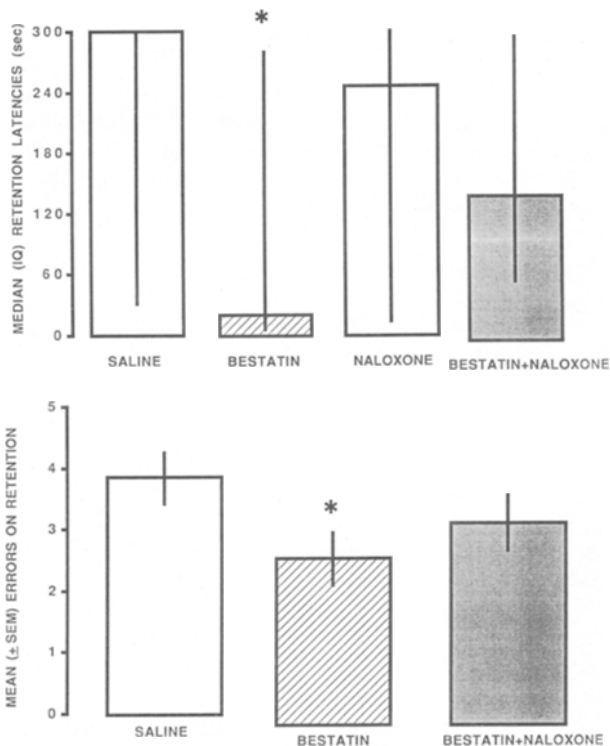


Figure 1. Effect of naloxone on the impairment of retention induced by bestatin in mice trained in (A) inhibitory-avoidance and (B) in Y-maze discrimination tasks. (A) Footshock 0.9 mA, 60 Hz, 2 sec; $N = 15$ mice per group. (B) Footshock 0.4 mA, 60 Hz; $N = 14$ mice per group. * $p < .05$ as compared with the saline group. All treatments were given i.p., immediately posttraining. Bestatin 1.0 mg/kg, naloxone 0.1 mg/kg.

Table 2
Influence of Bestatin (0.5 mg/kg) on the Retention Enhancement of Naloxone (0.3 mg/kg)
in Mice Trained in Inhibitory-Avoidance and Y-Maze Discrimination Tasks

| Treatment | Inhibitory Avoidance* | | | Y-Maze Discrimination† | | |
|---------------------|-----------------------|---------------------|-----------|------------------------|------------------|-------|
| | No. of Mice | Retention Latencies | | No. of Mice | Retention Scores | |
| | | Mdn | IQ Range‡ | | M | SE |
| Saline | 30 | 61 | 11-282 | 18 | 3.00 | 0.35 |
| Naloxone | 24 | 221 | 50-300§ | 18 | 4.06 | 0.34§ |
| Bestatin | 23 | 35 | 9-200 | 18 | 3.17 | 0.29 |
| Naloxone + bestatin | 24 | 134 | 11-239 | 17 | 3.29 | 0.38 |

Note—All treatments were given (i.p.) immediately after training. *Footshock: 0.6 mA, 60 Hz, 2 sec. †Footshock: 0.3 mA, 60 Hz. ‡Interquartile range. § $p < .05$ as compared with the saline-injected control group.

tion of opiate receptors, and is consistent with the view that endogenous enkephalins may play a role in the regulation of memory storage. There is evidence suggesting that aminopeptidase may affect the metabolism of peptides other than enkephalin (Turner, Matsas, & Kenny, 1985). Thus, our finding that naloxone only partially attenuated the effect of bestatin may be due to bestatin influences on nonopioid neuropeptides.

In the present experiments, bestatin was administered intraperitoneally. Thus, we do not know whether the drug effects on memory are due to influences in the brain or in the periphery. In previous studies, we have found that the amnesic effects of posttraining systemic injections of met-enkephalin are blocked by the centrally acting opiate antagonists naloxone and naltrexone, as well as by systemic injections of an opiate antagonist (MR2263) that does not pass the blood-brain barrier (Introini et al., 1985; Zhang et al., 1987). Thus, these findings suggest that the effects of enkephalin on memory may be initiated at peripheral opioid receptors.

REFERENCES

- CASTELLANO, C. (1975). Effects of morphine and heroin on discrimination learning and consolidation in mice. *Psychopharmacologia*, **42**, 235-242.
- CHAILLET, P., MARCAIS-COLLADO, H., COSTENTIN, J., YI, C. C., DELA BAUME, S., & SCHWARTZ, J. C. (1983). Inhibition of enkephalin metabolism by, and antinociceptive activity of, bestatin, an aminopeptidase inhibitor. *European Journal of Pharmacology*, **86**, 329-336.
- GALLAGHER, M., & KAPP, B. S. (1978). Manipulation of opiate activity in the amygdala alters memory processes. *Life Science*, **23**, 1973.
- GIROS, B., GROS, C., SOLHONNE, B., & SCHWARTZ, J. C. (1986). Characterization of aminopeptidases responsible for inactivating endogenous (met⁵)enkephalin in brain slices using peptidase inhibitors and antiaminopeptidase M antibodies. *Molecular Pharmacology*, **29**, 281-287.
- INTROINI, I. B. (1984). *Participacion de peptidos opioides endogenos en el proceso de consolidacion del la memoria: Su posible interaccion con otros sistemas neuronales*. Unpublished doctoral dissertation, Universidad de Buenos Aires.
- INTROINI, I. B., & BARATTI, C. M. (1984). The impairment of retention induced by β -endorphin in mice may be mediated by a reduction of central cholinergic activity. *Behavioral & Neural Biology*, **41**, 152-163.
- INTROINI, I. B., MCGAUGH, J. L., & BARATTI, C. M. (1985). Pharmacological evidence of a central effect of naltrexone, morphine, and β -endorphin and a peripheral effect of met- and leu-enkephalin on retention of an inhibitory response in mice. *Behavioral & Neural Biology*, **44**, 434-446.
- INTROINI-COLLISON, I. B., & MCGAUGH, J. L. (1986). Epinephrine modulates long-term retention of an aversively-motivated discrimination. *Behavioral & Neural Biology*, **45**, 358-365.
- IZQUIERDO, I. (1979). Effect of naloxone and morphine on various forms of memory in the rat: Possible role of endogenous opiate mechanisms in memory consolidation. *Psychopharmacology*, **66**, 199-203.
- MARTINEZ, J. L., JR., RIGTER, H., JENSEN, R. A., MESSING, R. B., VASQUEZ, B. J., & MCGAUGH, J. L. (1981). Endorphin and enkephalin effects on avoidance conditioning: The other side of the pituitary-adrenal axis. In J. L. Martinez, Jr., R. A. Jensen, R. B. Messing, H. Rigger, & J. L. McGaugh (Eds.), *Endogenous peptides and learning and memory processes* (pp. 305-324). New York: Academic Press.
- MARTINEZ, J. L., JR., WEINBERGER, S. B., & SCHULTEIS, G. (1988). Enkephalins and learning and memory: A review of evidence for a site of action outside the blood-brain barrier. *Behavioral & Neural Biology*, **49**, 192-221.
- MCGAUGH, J. L. (1989). Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. *Annual Review of Neuroscience*, **12**, 255-287.
- MCGAUGH, J. L., & GOLD, P. E. (1989). Hormonal modulation of memory. In R. B. Brush & S. Levine (Eds.), *Psychoendocrinology* (pp. 305-339). New York: Academic Press.
- SCHWARTZ, J. C. (1983). Metabolism of enkephalins and the inactivating neuropeptidase concept. *Trends in Neuroscience*, **6**, 45-48.
- TURNER, A. J., MATSAS, R., & KENNY, A. J. (1985). Are there neuropeptide-specific peptidases? *Biochemical Pharmacology*, **34**, 1347-1356.
- ZHANG, S., MCGAUGH, J. L., JULER, R. G., & INTROINI-COLLISON, I. B. (1987). Naloxone and Met-enkephalin effects on retention: Attenuation by adrenal denervation. *European Journal of Pharmacology*, **138**, 37-44.

(Manuscript received February 7, 1989;
 revision accepted for publication May 27, 1989.)