Pharmacology Biochemistry & Behavior, Vol. 19, pp. 561–565, 1983. © Ankho International Inc. Printed in the U.S.A.

Arginine Vasopressin Enhances Retention of Morphine Tolerance[1]

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Received 22 March 1983

MOORE J. E. *Arginine vasopressin enhances retention of morphine tolerance.* PHARMACOL BIOCHEM BEHAV 19(4) 561–565, 1983.—The hot plate method was used to assess tolerance in rats following daily injections of morphine. Following analgesia assessment, or a time equated rest period, rats were injected with either saline or a pituitary peptide. Arginine vasopressin, but not ACTH 4–10, prolonged the retention of morphine tolerance when assessed five weeks after the last injection. Neither the rate nor the degree of tolerance development were influenced by either peptide. These hormones had no effect on retention of tolerance in rats not assessed for analgesia during the period of tolerance development. The effects of pituitary peptides on morphine tolerance are analogous to the effects they have on learning and memory processes, suggesting that similar adaptational processes are occurring in both phenomena.

Morphine tolerance Arginine vasopressin ACTH 4–10 Hot-plate test

VASOPRESSIN, adrenocorticotropin (ACTH), and related pituitary peptides have been implicated in learning and memory processes [4, 12, 16, 35, 45]. Vasopressin facilitates both retention and retrieval of avoidance and appetitive responses [2,13], and is believed to act on the central nervous system to aid consolidation of memory [35,45]. Administered following training, it retards extinction of learned behaviors [3, 6, 11]; given prior to a retention test, it facilitates retrieval [6]. Vasopressin also reverses impaired avoidance behavior produced by hypophysectomy [5], corrects impaired memory in rats with hereditary diabetes insipidus [14], and reverses experimentally produced amnesia [31, 36, 37]. Conversely, vasopressin antiserum hampers the retention of avoidance behavior [46].

ACTH and behaviorally active ACTH analogs facilitate acquisition and delay extinction of both avoidance and appetitive responses if administered prior to training trials, and enhance retrieval if administered prior to tests of retention [4, 10, 16, 19, 24, 25, 34, 35]. These effects vary depending on dose employed, training parameters, and time of administration [35], and appear to be mediated by sensory, attentional, motivational, or other performance enhancing effects of the peptides [4,35]. If administered post-training, however, only ACTH enhances retention [21–23]; shorter analogs are generally without effect ([34,44] cf., [18,41]). The effects of ACTH analogs, therefore, are possibly restricted to enhancement of acquisition and retrieval, and possibly do not include enhancement of consolidation or retention [16,35].

The development of morphine tolerance has many similarities to the acquisition of learned responses. Inhibitors of protein synthesis interfere with the acquisition of learned behaviors, and also prevent the development of morphine tolerance [9,33]. Electroconvulsive shock and cortical stimulation similarly interfere with both learned responses and morphine tolerance [29]. Additionally the development of morphine tolerance is topographically similar to learning [32,40].

Since morphine tolerance and learning appear similar in many ways, and since pituitary peptides influence learned behavior, it is quite possible that these hormones will also influence tolerance to morphine. Rats unable to synthesize vasopressin are both slower to develop tolerance to morphine and slower to acquire a learned response [14,15], and normal rats given vasopressin antiserum demonstrate impaired tolerance development [47].

The effect of vasopressin on morphine tolerance development has not been clearly determined [43]. Using extremely large doses of desglycinamide^9-lysine vasopressin (DG-LVP), investigators found that this peptide facilitated the development of morphine tolerance [30]. Similarly, van Ree and de Wied [42] reported that physical dependence on morphine developed more rapidly in rats treated with desglycinamide^9-arginine vasopressin (DG-AVP) rather than a placebo. Others, however, were unable to find this facilitory effect of vasopressin on tolerance development and concluded that the role of vasopressin as an endogenous modulator of opiate effects is minimal or nonexistent [38].

Holaday, Dallman, and Loh reported that ACTH had little effect on the magnitude of tolerance and physical dependence following morphine pellet implantation [27]. This study, however, did not evaluate the rate of development or the maintenance of morphine tolerance as a consequence of ACTH administration. In addition, the morphine pellet

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technique may not be as effective as repeated injections of morphine in maximizing the learning components of morphine tolerance.

Thus far, the data suggesting an effect of vasopressin on morphine tolerance are promising, but unclear, and no effect of ACTH-like peptides has yet been reported. The present study evaluated the effects of both arginine vasopressin (AVP) and ACTH 4–10 on the development and maintenance of tolerance to morphine. Procedural improvements permit clarification of previous contradictory reports.

METHOD

Subjects

The subjects were 48 naive, female, Sprague-Dawley derived rats, 150–180 days old, and weighing between 300 and 400 grams. They were housed individually in a normal 12-hour light-dark cycle (light from 0700–1900) with Purina Laboratory Chow and water continuously available. Rats were allowed to acclimate to the colony room maintained at 70°F, and to the light-dark cycle for two weeks prior to the beginning of the study.

Apparatus

Analgesia was assessed using a modified hot plate technique [17]. Animals were placed onto a metal plate, heated to 55°C (±0.5°C), and the latency with which a rat either licked one of its paws or attempted to jump out of the restraining Plexiglas cylinder was recorded. Following 60 seconds elapsed time, the animals were removed from the hot plate apparatus whether or not they had already responded.

Drugs

The compounds employed were: morphine sulphate (S. B. Penick, Lyndhurst, NJ), synthetic arginine vasopressin, 100 IU/mg (U. S. Biochemical Corporation, Cleveland, OH), and ACTH 4–10 (Peninsula Laboratories, Inc., San Carlos, CA). All drugs were dissolved in 0.9% NaCl and injected subcutaneously (SC). A volume of 1 ml/kg was used for all injections.

Procedure

Rats were divided into 4 groups of 12 rats each. On Days 1–7, all rats received two daily injections 30 minutes apart. Three of these groups were administered morphine (30 mg/kg) on the first daily injection and either ACTH 4–10 (80 μg/kg), AVP (100 μg/kg), or saline on the second daily injection. The fourth group received saline injections on each daily drug administration. Half the rats in each group were tested for analgesia on the hot plate immediately prior to the second injection on each of the seven treatment days. ACTH 4–10 was used, rather than the whole peptide, because it has no adrenal effects but does have complete behavioral potency [12]. Rats were injected at the same time each day, between 1600 and 1700 hours.

Following the seven treatment days all animals were left undisturbed in their home cages for the next 35 days. On Day 42, all rats were injected with morphine (30 mg/kg) and assessed for analgesia on the hot plate 30 minutes following the injection.

RESULTS

Logarithmic transformations of hot plate response latencies were computed to normalize skewed distributions. A two-way analysis of variance of the logarithmic transformations of hot plate response latencies on Days 1–7 was performed. The factors were drug combination (morphine/saline (MS)—morphine/ACTH (MA)—morphine/AVP (MV)—saline/saline (SS)) and days (1 through 7) with subjects nested within drug combination. A significant main effect was found for days, $F(6,120)=12.82$, $p<0.0001$, with tolerance increasing from Day 1 to Day 7. The main effect for drug combination was not significant, but a significant interaction was found for drug combination × days, $F(18,120)=2.92$, $p<0.0003$. This effect was due to shorter latencies on subsequent days for groups hot plate tested following morphine but not for the group tested following saline injections (Fig. 1). There were no differences in either the rate or the degree of tolerance development among the three groups that received seven daily injections of morphine followed by hot plate assessments, indicating that neither AVP nor ACTH 4–10 had any effect on tolerance development.

A two-way analysis of variance of the logarithmic transformations of hot plate response latencies on Day 42 was also performed. The factors were hot plate (yes—no) and drug combination (MA—MS—MV—SS). When all animals were hot plate tested on Day 42, those that had been assessed for analgesia on the hot plate on each of the treatment days demonstrated more tolerance than animals that were not assessed for analgesia on each treatment day, $F(1,22)=13.26$, $p<0.0008$. The main effect for drug combination was not significant. However, there was a significant interaction effect for hot plate × drug combination, $F(3,5)=3.37$, $p<0.05$. Animals from groups MA and MV that were hot plate tested on Days 1–7 demonstrated response latencies on Day 42 that were significantly shorter than those demonstrated by MA and MV animals that were not tested on Days 1–7, $t(15)=2.28$, $p<0.05$ and $t(15)=3.46$, $p<0.01$, respectively. There were no differences between animals tested and not tested on the hot plate on Days 1–7 for drug combinations MS and SS on Day 42 (Fig. 2).

To directly determine the effect of AVP and ACTH 4–10 on tolerance retention in hot plate tested animals, the mean response latency change from Day 7 to Day 42 was compared across groups MS, MA, and MV. On Day 42, MV animals had retained significantly more tolerance than both MS and MA animals, $t(15)=3.11$, $p<0.005$ and $t(15)=1.79$, $p<0.05$, respectively (one tailed tests). MS and MA animals did not differ significantly on tolerance retained. Thus, AVP prolonged the retention of morphine tolerance.

DISCUSSION

The previously reported facilitory effect of vasopressin on the development of tolerance to morphine was not found in the current study [30,42]. Krivoy *et al.* [30], however, employed extremely large doses of DG-LVP and van Ree and de Wied [42] measured tolerance indirectly, by evaluating the antagonistic effects of naloxone. That is, they observed a naloxone-precipitated weight loss and the blockage of hyperthermia after fewer daily trials in morphine-treated animals if they were also injected with DG-AVP rather than saline. The present study employed doses of the peptides closer to those used in the majority of previous behavioral research and evaluated tolerance in a more direct manner. The lack of an effect of AVP and ACTH 4–10 on tolerance development found in the current study is consistent with the results of Schmidt *et al.* [38] who recently found no facilitory

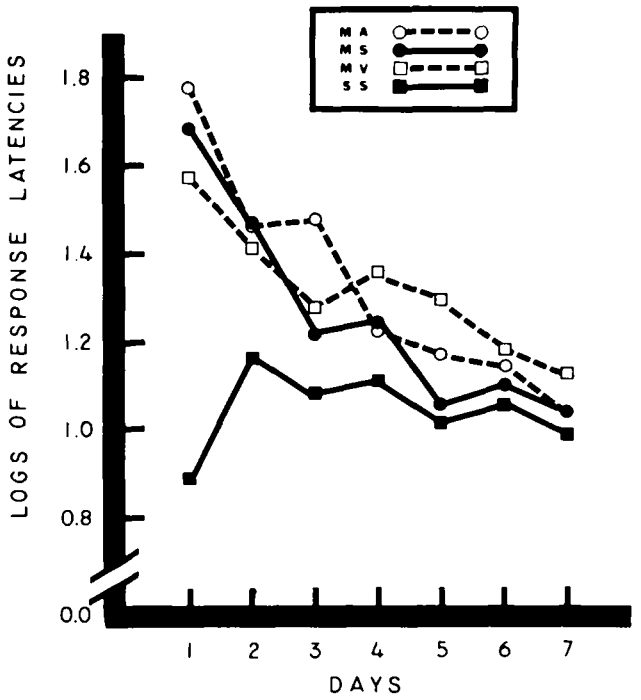

FIG. 1. Means of logarithmic transformations of hot plate response latencies for groups hot plate tested on Days 1–7. Groups received drug combinations of morphine/ACTH (MA), morphine/saline (MS), morphine/AVP (MV), or saline/saline (SS). There were no differences in the rate or degree of tolerance development among MA, MS, and MV groups.

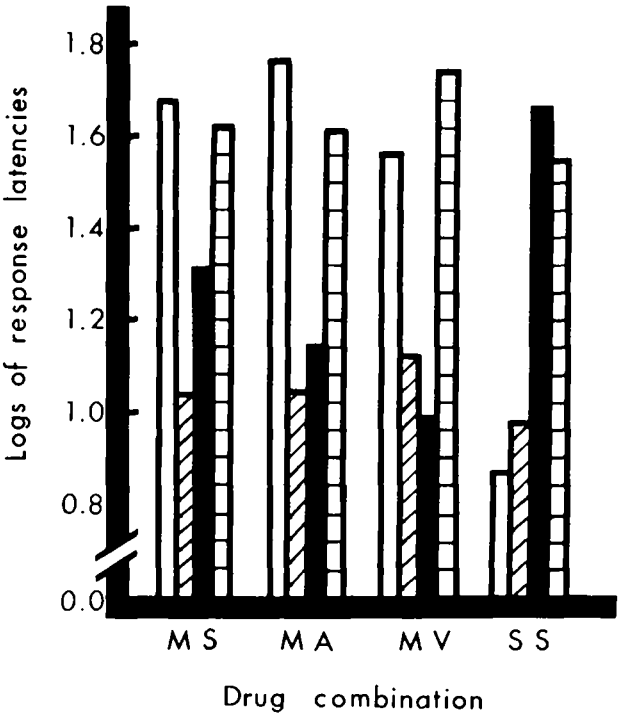

FIG. 2. Mean logarithmic transformations of hot plate response latencies on Days 1, 7, and 42 for groups treated with drug combinations of morphine/saline (MS), morphine/ACTH (MA), morphine/AVP (MV), and saline/saline (SS), and either tested or not tested on the hot plate on Days 1–7. □=Hot plate tested animals on Day 1. ▨=Hot plate tested animals on Day 7. ■=Hot plate tested animals on Day 42. ▤=Non-hot plate tested animals on Day 42. On Day 42, for rats previously hot plate tested, animals that received AVP (MV) demonstrated significantly shorter latencies than all other groups, $ps < 0.05$.

effect of either vasopressin or oxytocin on the development of tolerance to morphine and with Holaday *et al.* [27] who failed to find evidence of a direct role of pituitary peptides in chronic opiate effects.

The ability of AVP, but not ACTH 4–10, to prolong the retention of morphine tolerance are findings largely consistent with the reported effects of these peptides on acquired behaviors. Vasopressin has consistently been shown to enhance retention, whereas ACTH 4–10 more likely affects only retrieval and other performance measures. Since peptides were administered only after daily trials, and since a relatively long retention period was employed in this study, it is unlikely that processes other than retention were involved. In a related study, Hoffman *et al.* [26] found a similar AVP-enhanced retention of tolerance in ethanol tolerant mice.

That tolerance development was not enhanced is also consistent with behavioral research. The most frequent finding involving pituitary peptides is their ability to retard extinction of active and passive avoidance responses. Their influence on the acquisition of avoidance responses in not nearly as well documented. The variability across studies regarding response acquisition may be due to differences in dosage, target responses, degree of stressfulness of training, timing of hormone administration, or other procedural variations.

In the present study, peptides were administered following each treatment session, thus allowing only for a test of retention to be made. It remains to be determined whether peptides administered prior to training or retention trials will facilitate tolerance development or retrieval, respectively, perhaps via enhanced motivational or attentional mechanisms. These findings would not be unexpected given previous behavioral research with these peptides.

That tolerance was not demonstrated on Day 42 in any of the groups that were not assessed on the hot plate apparatus prior to this time can be explained several ways. Siegel has reported [39] that tolerance to morphine, as measured by shorter latencies on the hot plate, does not develop in the absence of hot plate assessments repeatedly paired with the systemic effects of morphine. Other investigators have demonstrated that tolerance to morphine will develop in the absence of hot plate assessments, but to a lesser degree or less rapidly [1,28]. This may be due to the fact that the hot plate apparatus functions as part of a complex conditioned stimulus in the Pavlovian conditioning of morphine tolerance [39].

A second possibility is that morphine tolerance, at least in part, involves instrumental conditioning processes. It has been suggested that the performance of an adaptive response while under the influence of a drug is the mediating factor in tolerance development to that drug [8,32]. It is conceivable that paw-licking on a hot plate is an adaptive response, and therefore facilitative of tolerance development.

As a third alternative, Carder [7] has presented a stress model of tolerance. According to this model, hot plate testing would facilitate tolerance development simply because it

is a stressful experience, and not because it involves Pavlovian or operant conditioning phenomena. He suggested that stress induced changes in biochemistry mediate tolerance development.

Hot plate testing, being a stressful experience, should also cause the release of pituitary peptide hormones. If this experience produces the release of additional hormones, beyond those released in response to morphine administration, then it might facilitate the development of morphine tolerance. It was recently reported [20] that stress can augment the release of additional pituitary peptides in morphine-treated mice. These data point to the possibility that morphine tolerance is facilitated by analgesia assessment on the hot plate because hot plate testing produces the release of additional peptide hormones and these hormones facilitate adaptations to morphine and/or to analgesia assessment. However, the present results indicate that additional hormones are without effect in non-hot plate tested animals, indicating that the experience itself is an integral part of the facilitory mechanism.

The present findings, that tolerance development is dependent upon contextual and /or behavioral experiences, and that AVP enhances tolerance retention, provide further support for the hypothesis that morphine tolerance is mediated, at least in part, by central nervous system adaptations similar to those involved in learning and memory.

ACKNOWLEDGEMENTS

The author wishes to thank R. Adron Harris, Bruce Horwitz, Bernadette Craven, Dennis Wright, William Waters, and Lawrence Siegel for their technical assistance and valuable comments.

REFERENCES

1. Adams, W. J., S. Y. Yeh, L. A. Woods and C. L. Mitchell. Drug-test interaction as a factor in the development of tolerance to the analgesic effect of morphine. *J Pharmacol Exp Ther* **168:** 251–257, 1969.
2. Ader, R. and D. de Wied. Effects of lysine vasopressin on passive avoidance learning. *Psychon Sci* **29:** 46–48, 1972.
3. Bohus, B. Effect of desglycinamide-lysine vasopressin (DG-LVP) on sexually motivated T-maze behavior of the male rat. *Horm Behav* **8:** 52–61, 1977.
4. Bohus, B. and D. de Wied. Actions of ACTH- and MSH-like peptides on learning, performance and retention. In: *Endogenous Peptides and Learning and Memory Processes*, edited by J. L. Martinez, Jr., R. A. Jensen, R. B. Messing, H. Rigter, and J. L. McGaugh. New York: Academic Press, 1981, pp. 59–77.
5. Bohus, B., W. H. Gispen and D. de Wied. Effect of lysine vasopressin and ACTH 4–10 on conditioned avoidance behavior of hypophysectomized rats. *Neuroendocrinology* **11:** 137–143, 1973.
6. Bohus, B., G. L. Kovács and D. de Wied. Oxytocin, vasopressin and memory: Opposite effects on consolidation and retrieval processes. *Brain Res* **157:** 414–417, 1978.
7. Carder, B. Environmental influence on marihuana tolerance. In: *Behavioral Tolerance: Research and Treatment Implications*, edited by N. A. Krasnegor. Washington, DC: U. S. Government Printing Office, 1978, pp. 90–102.
8. Chen, C. S. A study of the alcohol-tolerance effect and an introduction of a new behavioural technique. *Psychopharmacologia* **12:** 433–440, 1968.
9. Cox, B. M. and O. H. Osman. Inhibition of the development of tolerance to morphine in rats by drugs which inhibit ribonucleic acid or protein synthesis. *Br J Pharmacol* **38:** 157–170, 1970.
10. de Wied, D. Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. *Proc Soc Exp Biol Med* **122:** 28–32, 1966.
11. de Wied, D. Long-term effect of vasopressin on the maintenance of a conditioned avoidance response in rats. *Nature* **232:** 58–60, 1971.
12. de Wied, D. Pituitary-adrenal system hormones and behavior. In: *The Neurosciences: Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge, MA: MIT Press, 1974, pp. 653–666.
13. de Wied, D. and B. Bohus. Long-term and short-term effects on retention of a conditioned avoidance response in rats by treatment with long-acting pitressin and α-MSH. *Nature* **212:** 1481–1486, 1966.
14. de Wied, D., B. Bohus and T. B. van Wimersma Greidanus. Memory deficit in rats with hereitary diabetes insipidus. *Brain Res* **85:** 152–156, 1975.
15. de Wied, D. and W. H. Gispen. Impaired development of tolerance to morphine analgesia in rats with hereditary diabetes insipidus. *Psychopharmacologia* **46:** 27–29, 1976.
16. de Wied, D. and J. Jolles. Neuropeptides derived from pro-opiocortin: Behavioral, physiological, and neurochemical effects. *Physiol Rev* **62:** 976–1059, 1982.
17. Eddy, N. B. and D. Leimbach. Synthetic analgesics: II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exp Ther* **107:** 385–393, 1953.
18. Flood, J. F., M. E. Jarvik, E. L. Bennett and A. E. Orme. Effects of ACTH peptide fragments on memory formation. *Pharmacol Biochem Behav* **5:** Suppl 1, 41–51, 1976.
19. Garrud, P., J. A. Gray and D. de Wied. Pituitary-adrenal hormones and extinction of rewarded behavior in the rat. *Physiol Behav* **12:** 109–119, 1974.
20. Gibson, A., M. Ginsburg, M. Hall and S. L. Hart. The effects of opiate receptor agonists and antagonists on the stress-induced secretion of corticosterone in mice. *Br J Pharmacol* **65:** 139–146, 1979.
21. Gold, P. E., R. P. Rose, C. W. Spanis and L. L. Hankins. Retention deficit for avoidance training in hypophysectomized rats: Time-dependent enhancement of retention performance with post-training ACTH injections. *Horm Behav* **8:** 363–371, 1977.
22. Gold, P. E. and R. van Buskirk. Effects of posttrial hormone injections on memory processes. *Horm Behav* **7:** 509–517, 1976.
23. Gold, P. E. and R. van Buskirk. Enhancement and impairment of memory processes with posttrial injections of adrenocorticotrophic hormone. *Behav Biol* **16:** 387–400, 1976.
24. Gray, J. A. Effect of ACTH on extinction of rewarded behavior is blocked by previous administration of ACTH. *Nature* **229:** 52–54, 1971.
25. Guth, S., S. Levine and J. P. Seward. Appetitive acquisition and extinction effects with exogenous ACTH. *Physiol Behav* **7:** 195–200, 1971.
26. Hoffman, P. L., R. F. Ritzmann, R. Walter and B. Tabakoff. Arginine vasopressin maintains ethanol tolerance. *Nature* **276:** 614–616, 1978.
27. Holaday, J. W., M. F. Dallman and H. H. Loh. Effects of hypophysectomy and ACTH on opiate tolerance and physical dependence. *Life Sci* **24:** 771–782, 1979.
28. Kayan, S., R. K. Ferguson and C. L. Mitchell. An investigation of pharmacologic and behavioral tolerance to morphine in rats. *J Pharmacol Exp Ther* **185:** 300–306, 1973.
29. Kesner, R. P., D. J. Priano and J. R. DeWitt. Time dependent disruption of morphine tolerance by electroconvulsive shock and cortical stimulation. *Science* **194:** 1079–1081, 1976.

30. Krivoy, W. A., E. Zimmerman and S. Lande. Facilitation of development of resistance to morphine analgesia by desglycinamide^{9}-lysine vasopressin. *Proc Natl Acad Sci USA* **71:** 1852–1856, 1974.
31. Lande, S., J. B. Flexner and L. B. Flexner. Effect of corticotropin and desglycinamide^{9}-lysine vasopressin on suppression of memory by puromycin. *Proc Natl Acad Sci USA* **69:** 558–560, 1972.
32. LeBlanc, A. E. and H. Cappell. Tolerance as adaptation: Interactions with behavior and parallels to other adaptive processes. In: *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*, edited by K. Blum. New York: Academic Press, 1977, pp. 65–77.
33. Loh, H. H., F. H. Shen and E. L. Way. Inhibition of morphine tolerance and physical dependence development and brain serotonin synthesis by cycloheximide. *Biochem Pharmacol* **18:** 2711–2721, 1969.
34. Martinez, J. L., Jr., B. J. Vasquez, R. A. Jensen, B. Soumireu-Mourat and J. L. McGaugh. ACTH 4–9 analog (ORG 2766) facilitates acquisition of an inhibitory avoidance response in rats. *Pharmacol Biochem Behav* **10:** 146–147, 1979.
35. McGaugh, J. L. Hormonal influences on memory. *Annu Rev Psychol* **34:** 297–323, 1983.
36. Pfeifer, W. D. and H. B. Bookin. Vasopressin antagonizes retrograde amnesia in rats following electroconvulsive shock. *Pharmacol Biochem Behav* **9:** 261–263, 1978.
37. Rigter, H., H. van Riezen and D. deWied. The effects of ACTH- and vasopressin-analogues on CO_2-induced retrograde amnesia in rats. *Physiol Behav* **13:** 381–388, 1974.
38. Schmidt, W. K., J. W. Holaday, H. H. Loh and E. L. Way. Failure of vasopressin and oxytocin to antagonize acute morphine antinociception or facilitate narcotic tolerance development. *Life Sci* **23:** 151–158, 1978.
39. Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J Comp Physiol Psychol* **89:** 498–506, 1975.
40. Siegel, S. A Pavlovian conditioning analysis of morphine tolerance. In: *Behavioral Tolerance: Research and Treatment Implication*, edited by N. A. Krasnegor. Washington, DC: U. S. Government Printing Office, 1978, pp. 27–53.
41. Soumireu-Mourat, B., J. Micheau and C. Franc. ACTH 4–9 analog (ORG 2766) and memory processes in mice. In: *Endogenous Peptides and Learning and Memory Processes*, edited by J. L. Martinez, Jr., R. A. Jensen, R. B. Messing, H. Rigter and J. L. McGaugh. New York: Academic Press, 1981, pp. 143–158.
42. van Ree, J. M. and D. de Wied. Prolyl-leucyl-glycinamide (PLG) facilitates morphine dependence. *Life Sci* **19:** 1331–1340, 1976.
43. van Ree, J. M. and D. de Wied. Vasopressin, oxytocin, and dependence on opiates. In: *Endogenous Peptides and Learning and Memory Processes*, edited by J. L. Martinez, Jr., R. A. Jensen, R. B. Messing, H. Rigter and J. L. McGaugh. New York: Academic Press, 1981, pp. 397–411.
44. van Wimersma Greidanus, T. B. Effects of MSH and related peptides on avoidance behavior in rats. *Front Horm Res* **4:** 129–139, 1977.
45. van Wimersma Greidanus, T. B., B. Bohus and D. de Wied. Vasopressin and oxytocin in learning and memory. In: *Endogenous Peptides and Learning and Memory Processes*, edited by J. L. Martinez, Jr., R. A. Jensen, R. B. Messing, H. Rigter, and J. L. McGaugh. New York: Academic Press, 1981, pp. 413–427.
46. van Wimersma Greidanus, T. B. and D. deWied. Modulation of passive avoidance behavior of rats by intracerebroventricular administration of antivasopressin serum. *Behav Biol* **18:** 325–333, 1976.
47. van Wimersma Greidanus, T. B., H. Tjon Kon Fot-Bronstein and J. M. van Ree. Antisera to pituitary hormones modulate development of tolerance to morphine. In: *Characteristics and Function of Opioids*, edited by J. M. van Ree and L. Terenius. Amsterdam: Elsevier North Holland, 1978, pp. 73–74.