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Self-Administration of Methionine Enkephalin into the Nucleus Accumbens

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GOEDERS, N. E., J. D. LANE AND J. E. SMITH. *Self-administration of methionine enkephalin into the nucleus accumbens*. PHARMACOL BIOCHEM BEHAV **20**(3) 451–455, 1984.— Microinfusions of the endogenous opiate neurohumor, methionine enkephalin, into the nucleus accumbens initiated a reinforcing stimulus in a dose-related manner. The reinforcing nature of this intracranial self-administration was evaluated with intermittent schedules of reinforcement and a two-lever discrimination procedure. Opiate receptors are likely responsible for the initiation of this reinforcing stimulus since naloxone effectively blocked self-administration. These data suggest the mediation of opiate reinforcement through interactions with opiate receptors in brain regions outside the ventral tegmental area, questioning the current dopamine hypothesis for the initiation of these reinforcement processes.

Methionine enkephalin Intracranial self-administration Opiate receptors Opiate reinforcement
Nucleus accumbens Endogenous opiate peptides

THE abuse liability of opiates depends in part upon their reinforcing properties. Numerous investigations have sought to identify the neuronal circuitry responsible for these effects. Mesolimbic dopaminergic neurons have been proposed to mediate the reinforcing properties of opiates at the level of the ventral tegmental area [29] where the cell bodies sending projections to forebrain structures (including the nucleus accumbens) are localized [4]. This hypothesis has been concluded from data reporting an attenuation of intravenous opiate self-administration and place preference conditioning by systemic pretreatment with neuroleptics [19,20] and from data demonstrating the intracranial self-administration of 150 pmol of morphine into the ventral tegmental area (VTA) but not into VTA projection areas [1]. However, other investigations do not support this hypothesis. Intravenous opiate self-administration is disrupted following the systemic delivery of both cholinergic [5] and noradrenergic receptor antagonists [6]. In addition, neurotransmitter turnover rates measured in the brains of rats intravenously self-administering morphine are not consistent with an exclusive role for dopamine in reinforcement processes [24]. However, studies of the neurobiological substrates of opiate reinforcement that utilize intravenous administration may be equivocal since these drugs have peripheral actions that are reflected centrally [24] making the identification of the neuronal circuitry involved primarily in reinforcement difficult to assess. The effects of systemic drug administration on input-output pathways (i.e., the ability of the animals to respond, stimulus properties, etc.) make specific conclusions difficult. The direct intracranial administration of a drug stimulus onto discrete neuronal systems is more likely to identify pathways specifically initiating and propagating activity indicative of the occurrence of a reinforcing event than is the intravenous route. However, such studies may also result in erroneous conclusions when multiple receptor subpopulations are involved. It is not surprising that concentrations of morphine that maintain self-administration in the VTA are not adequate in other brain regions since several opiate receptors have been demonstrated to be heterogenously distributed in the central nervous system [8]. Rats will self-administer morphine directly into the lateral hypothalamus [14], septum [26], and nucleus accumbens [16], and D-Ala^2-Met-enkephalin (a stable enkephalin analogue) into the lateral hypothalamus [15]. The concentrations producing maximal rates of self-administration generally correspond to the *in vitro* affinity of the agonist for the major receptor subtype in that region. The dopamine hypothesis for the initiation of opiate reinforcement does not consider this differential distribution of receptor subtypes and the affinities of the receptors for various agonists. For example, the VTA contains primarily mu opiate receptors, while delta opiate receptors are predominant in the nucleus accumbens [8]. Morphine has approximately a 10-fold higher affinity for the mu receptor than for

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the delta receptor [21] and is intracranially self-administered (ICSA) into the VTA [1] at concentrations 10-fold lower than required in the nucleus accumbens [16]. This investigation was initiated to determine if methionine enkephalin (met-enkephalin), a putative neurohumor and endogenous ligand for delta opiate receptors, would be self-administered into a region in the central nervous system containing primarily delta receptors at concentrations comparable to the ICSA of morphine into the VTA.

METHOD

Eleven male Fischer strain F-344 (90 to 150 day-old) rats were unilaterally stereotaxically implanted with 22 gauge guide cannulae (Plastic Products Co.) into the nucleus accumbens under pentobarbital anesthesia (50 mg/kg IP). The stereotaxic coordinates were determined using the atlas of König and Klippel [12]. With the incisor bar set at −2.4 mm, the cannulae placements were: 9.5 mm anterior to lamda; 1.2 mm lateral to the midline; and 5.1 mm ventral to the dura. Each cannula was secured to the calvarium using self-tapping 00 stainless steel screws and dental acrylic. After surgery, a stylet was inserted (extending 0.5 mm beyond the end of the guide cannula) and remained in place except during testing. The animals were housed in individual cages on a 12-hr light, 12-hr dark cycle with free access to food and water.

Intracranial microinjections were delivered by an adaptation (Plastic Products Co.) of the electrolytic microinjection transducer system [1,7], which mounted directly onto the guide cannula on the rat's head (Fig. 1). Microinfusions were produced by passing a direct current (200 μA) between a silver anode and a platinum cathode contained in an air-tight drug reservoir, with the resulting evolution of hydrogen gas forcing a reproducible amount of the drug solution out through the 28 gauge injection cannula extending 0.5 mm beyond the tip of the guide cannula. A small quiescent current (6 μA) was used to prevent the redissolution of hydrogen evolved during previous infusions. This system was calibrated with a radioactive procedure to deliver reliable 100±7 nanoliter microinjections. A flexible lead connected the microinjection system to a counter-balanced mercury commutator allowing relatively unrestrained movement of the animal during testing.

The experimental sessions were conducted in a rectangular, Plexiglas operant conditioning chamber (29×27×30 cm) contained in a ventilated, sound attenuated enclosure. A red stimulus light directly above a response lever located on one wall of the experimental chamber indicated the availability of reinforcement. Following the successful completion of the response requirement, the red light was extinguished, a white light was illuminated and 100 nanoliters of the met-enkephalin solution were delivered over 5 seconds. Each infusion was followed by a 30-second time out period with the presentation of a tone and all stimulus lights darkened. The animals were tested during fifteen-hour sessions on alternate days beginning at the start of their active cycles.

Concentrations of zero to 750 pmol of methionine enkephalin dissolved in artificial cerebral spinal fluid (CSF) were tested for self-administration, with fresh drug solutions mixed prior to the start of each experimental session. Initially, the rats were tested on a continuous reinforcement schedule (CRF) with 500 pmol of the drug for three sessions. If this failed to engender the lever-pressing response, the concentration of methionine enkephalin was either decreased or increased by increments of 100 pmol. This procedure was repeated until the animals demonstrated an acquisition of the lever-pressing response or until all concentrations of the drug in the relevant dose range had been examined. When lever pressing was engendered, the animals were tested with various concentrations of methionine enkephalin to determine the relationship between the dose of the drug and the number of microinfusions self-administered. Each dose was evaluated during two experimental sessions, with the concentration of methionine enkephalin producing maximal rates of responding determined for each rat. When stable responding was observed at this optimum concentration (i.e., three consecutive sessions with the number of infusions varying less than 10 percent), the response requirement was gradually increased on fixed-ratio schedules of reinforcement.

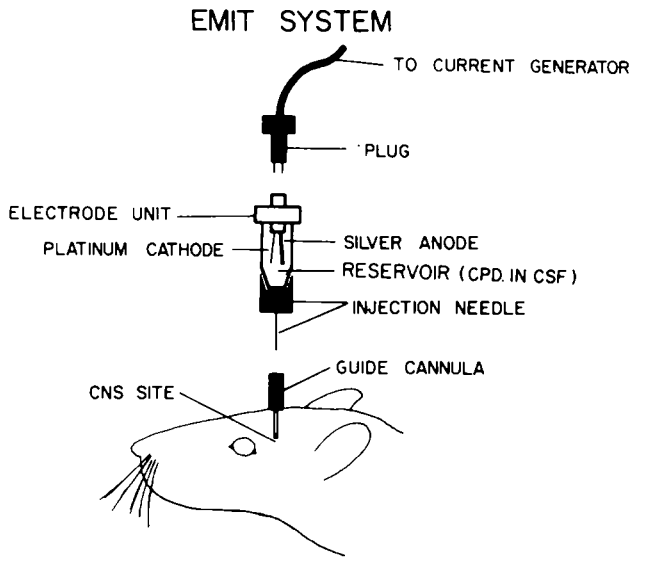

FIG. 1. The Electrolytic Microinjection Transducer System. Microinfusions were produced by passing a 200 μA direct current between the silver anode and platinum cathode, with the resulting evolution of hydrogen gas forcing reproducible 100 nanoliter volumes of drug solution out through the injection cannula.

A two-lever choice procedure was employed to demonstrate that self-administration resulted from the reinforcing properties of methionine enkephalin and not through a generalized increase in activity. In these experiments, a second lever identical to the first was installed on the opposite wall of the experimental chamber. Responses on this lever were counted but had no scheduled consequences. When more than 90 percent of the total number of responses were made on the active lever for three consecutive sessions, the contingencies were reversed between the two levers. An opiate receptor antagonist was used to demonstrate that the reinforcing properties associated with met-enkephalin ICSA were mediated through opiate receptors. For these experiments, naloxone hydrochloride (500 to 1500 pmol) was mixed with the methionine enkephalin solution in the microinjection systems of two of the rats. Each concentration of naloxone was tested during two experimental sessions.

Cannulae placements were verified histologically following the completion of the testing regimen. Each animal was sacrificed by decapitation, and the brains were rapidly re-

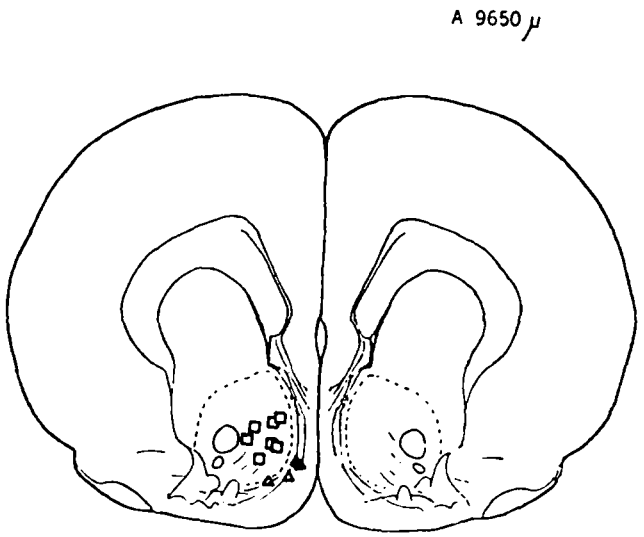

FIG. 2. Histological assessment of guide cannula locations. Squares represent the seven sites that supported the intracranial self-administration of met-enkephalin, and triangles the four sites that did not. Animals with cannulae implanted into the nucleus accumbens exhibited a rapid acquisition of the lever pressing response.

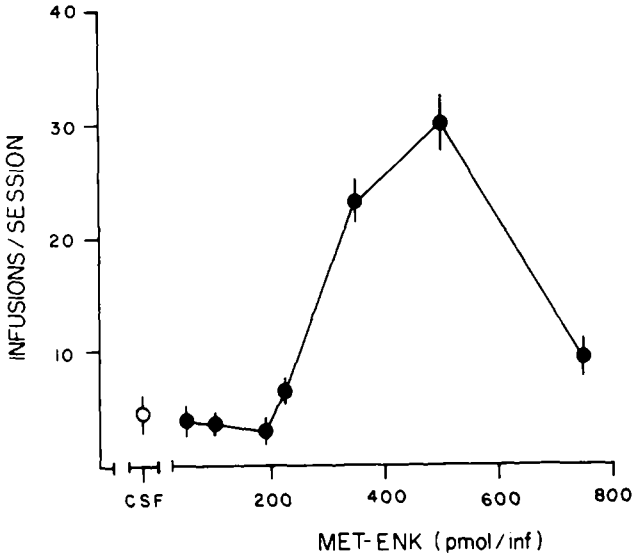

FIG. 3. Dose-response relationship for the intracranial self-administration of vehicle (artificial CSF) and methionine enkephalin into the nucleus accumbens on continuous schedules of reinforcement. Points are means and the error measurements, standard deviations, for double determinations in seven animals.

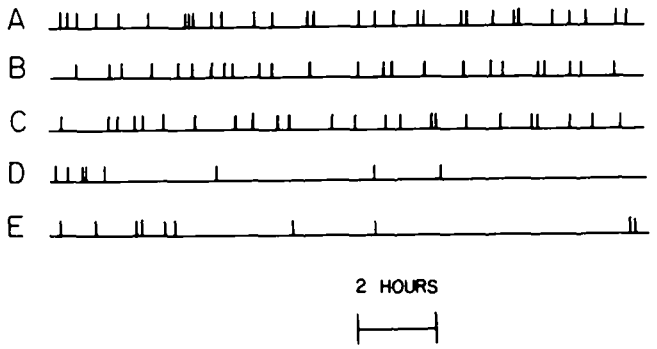

FIG. 4. Patterns of responding maintained by 500 pmol microinjections of methionine enkephalin into the nucleus accumbens from a representative animal during four different experimental conditions: (A) continuous schedule of reinforcement; (B) fixed-ratio 5 schedule of reinforcement; (C) and (D) active and inactive lever presses during a two-lever discrimination experiment; and (E) responding maintained by artificial cerebral spinal fluid alone.

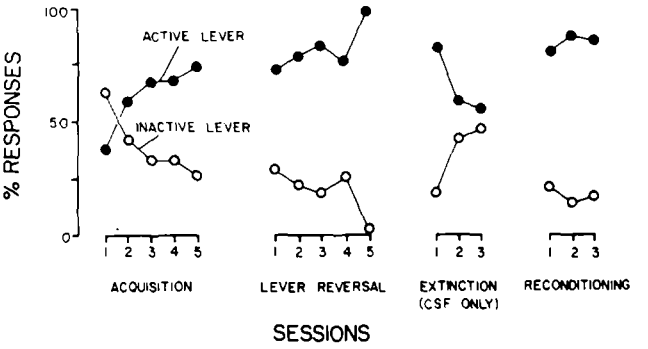

FIG. 5. Percent responding maintained by intracranial microinjections of methionine enkephalin (500 pmol) into the nucleus accumbens in a two-lever choice procedure on a continuous schedule of reinforcement. Responding rapidly increased on the active lever. When the active and inactive levers were reversed, the rat switched responding to the new active lever. Extinction resulted in a loss of this preference and reconditioning in a rapid return to appropriate responding.

moved and frozen over dry ice. Frozen coronal sections (16 micron) were cut with a cryostat microtome (Damon/IEC Division) at −16°C and were mounted onto subbed slides. The slide mounted sections were fixed and stained using the methodologies of Klüver and Barrera [11], and cannula placements were determined by light microscopy.

RESULTS

Seven of the eleven rats self-administered met-enkephalin. Histological analysis demonstrated that microinjections of the drug into the nucleus accumbens resulted in a rapid acquisition of the lever-pressing response (Fig. 2), while infusions near the structure did not maintain responding at any concentration tested in the other four rats. The rate of responding was dose-related (Fig. 3), with maximal rates obtained with 350 to 500 pmol. The response requirement was increased from one to five lever presses per microinfusion (FR1 to FR5) at these concentrations, with the animals increasing their rates of responding to maintain equivalent interinfusion intervals (Fig. 4). The intracranial self-administration of met-enkephalin was maintained in four of these animals for more than fifty consecutive experimental sessions with no evidence of tolerance.

In the lever reversal experiments, the animals discriminated between the two levers, responding significantly more on the active lever (Fig. 5). When the active contingency was reversed several times between the two levers, the animals quickly switched responding to the new active manipulandum. During extinction probes (with artificial CSF substi-

tuted for met-enkephalin) this discrimination was lost and the animals responded on both levers equally at low rates. When the peptide was replaced in the microinjection system, the animals again responded primarily on the active lever.

Including low concentrations of naloxone (500 to 1000 pmol) in the met-enkephalin solution resulted in a transient initial increase in the number of microinfusions delivered per session (Fig. 6). This effect diminished as responding returned to baseline rates with further testing. However, ICSA was effectively blocked with 1500 pmol of naloxone, with the number of microinfusions delivered approaching vehicle (artificial CSF) levels and the animals exhibiting a characteristic extinction pattern of responding. Microinfusions containing only the antagonist did not affect operant levels of responding.

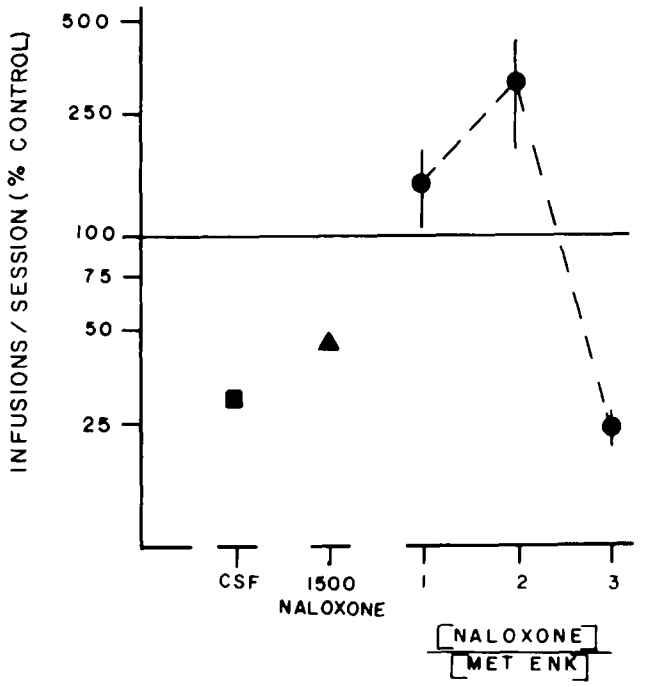

FIG. 6. Effect of naloxone on met-enkephalin ICSA into the nucleus accumbens presented as the percent of responding maintained by 500 pmol of met-enkephalin (100%). Including equimolar concentrations of naloxone resulted in a transient increase in ICSA, while tripling the molar ratio of naloxone to met-enkephalin resulted in extinction. Points are the means and the error measurements, standard deviations, for double determinations in two animals.

DISCUSSION

The intracranial microinjection of methionine enkephalin into the nucleus accumbens initiates a reinforcing stimulus. This is concluded from the rapid acquisition of the lever-pressing response with 350 to 500 pmol of the drug, the regular spacing of interinfusion intervals, the maintenance of responding on intermittent schedules of reinforcement and the ability of the animals to discriminate between active and inactive response levers in a choice procedure. Since the animals responded primarily on the active lever (over 90 percent) during several lever reversals, this self-administration cannot be attributed to a generalized behavioral stimulant action of the drug. The involvement of opiate receptors is implicated since 1500 pmol of naloxone attenuated this self-administration. Greater than equimolar concentrations of naloxone may be required to block ICSA since naloxone has a greater affinity for the mu receptor than for the delta receptor which probably mediates the behavioral effects of met-enkephalin. The increase in ICSA observed with 500 to 1000 pmol of the antagonist indicates that the drug is not altering the ability of the animals to respond (input-output decrements) but rather may be partially antagonizing the reinforcing effects of the peptide. The increased intake suggests that the animals may attempt to maintain an optimum concentration of met-enkephalin at the receptor site. Similar increases in intravenous opiate and stimulant self-administration have been reported following the injection of low doses of neurotransmitter receptor antagonists [13].

Opiate receptors in the nucleus accumbens may be involved in other reinforcement processes. The injection of opiates into this region facilitates intracranial electrical self-stimulation [2]. This structure has also been suggested to be a component of a neuronal circuit mediating morphine reinforcement identified in neurotransmitter turnover rate studies [25]. Furthermore, kainic acid and 6-hydroxydopamine lesions of this region increase intravenous morphine self-administration and shift the dose-effect curve to the right [23], indicating that intrinsic and efferent neurons and dopaminergic innervations (which may all contain opiate receptors) are excitatory to opiate reinforcement processes.

Immunohistochemical investigations have reported the discrete localization of enkephalin immunoreactive sites in rat brain [10, 18, 27, 28]. The widespread distribution of this substance suggests that it may influence physiological functions regulated by these diverse brain regions. For example, enkephalin-containing fiber terminals and cells are concentrated in the periaqueductal grey area [27], with the release of enkephalin possibly modulating pain perception [17]. In addition, the localization of enkephalin in the hypothalamus [28] suggests that it may serve a neuroendocrine regulatory role [3]. Opiate receptors in this structure may also modulate reinforced behaviors since naloxone reduces water intake [22] and β-endorphin increases feeding [9] in rats when applied into the hypothalamus. Morphine [14] and D-Ala^2-Met-Enkephalinamide [15] ICSA into this region provide further support for its role in the mediation of reinforcement. The identification of enkephalinergic cell bodies and nerve endings in the nucleus accumbens [10,28] indicates that it may be an endogenous neurohumor in this brain region. The delivery of methionine enkephalin to delta opiate receptors in the nucleus accumbens results in the initiation of a reinforcing stimulus at concentrations of the same magnitude employed to demonstrate morphine ICSA into the VTA [1]. Presumably, the release of endogenous enkephalin in this brain region should also initiate reinforcing neuronal events. The results of this investigation demonstrate that activation of opiate receptors associated with dopaminergic neuronal cell bodies in the VTA [29] may be sufficient but not necessary for reinforcement. Opiate reinforcement appears to be mediated through the interactions of opiates with receptors localized in other brain regions.

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REFERENCES

1. Bozarth, M. A. and R. A. Wise. Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci* **28:** 551–555, 1981.
2. Broekkamp, C. L. E. and J. M. Van Rossum. The effect of microinjections of morphine and haloperidol into the neostriatum and the nucleus accumbens on self-stimulation behavior. *Arch Int Pharmacodyn Ther* **217:** 110–116, 1975.
3. Bruni, J. F., D. Van Vugt, S. Marshall and J. Meites. Effects of naloxone, morphine and methionine-enkephalin on serum prolactin, leutinizing hormone, follicle stimulating hormone, thyroid stimulating hormone and growth hormone. *Life Sci* **21:** 461–466, 1977.
4. Carter, D. A. and H. C. Fibiger. Ascending projections of presumed dopamine-containing neurons in the ventral tegmentum of the rat as demonstrated by horseradish peroxidase. *Neuroscience* **2:** 569–576, 1977.
5. Davis, W. M. and S. G. Smith. Central cholinergic influence on self-administration of morphine and amphetamine. *Life Sci* **16:** 237–246, 1975.
6. Davis, W. M., S. G. Smith and J. H. Khalsa. Noradrenergic role in the self-administration of morphine or amphetamine. *Pharmacol Biochem Behav* **3:** 447–484, 1975.
7. Goeders, N. E. and J. E. Smith. Cortical dopaminergic involvement in cocaine reinforcement. *Science* **221:** 773–775, 1983.
8. Goodman, R. R., S. H. Snyder, M. J. Kuhar and W. S. Young, III. Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. *Proc Natl Acad Sci USA* **77:** 6239–6243, 1980.
9. Grandison, L. and A. Guidotti. Stimulation of food intake by muscimol and beta-endorphin. *Neuropharmacology* **16:** 533–536, 1977.
10. Khachaturian, H., M. E. Lewis, V. Hollt and S. J. Watson. Telencephalic Enkephalinergic systems in rat brain. *J Neurosci* **3:** 844–855, 1983.
11. Klüver, H. and E. Barrera. A method for the combined staining of cells and fibers in the nervous system. *J Neuropathol Exp Neurol* **12:** 400–403, 1953.
12. König, J. F. R. and R. A. Klippel. *The Rat Brain*. New York: Robert E. Krieger Publishing Co., 1967.
13. Koob, G. F. The dopamine anhedonia hypothesis: a pharmacological phrenology. *Behav Brain Sci* **5:** 63–64, 1982.
14. Olds, M. E. Hypothalamic substrate for the positive reinforcing properties of morphine in the rat. *Brain Res* **168:** 351–360, 1979.
15. Olds, M. E. and K. N. Williams. Self-administration of D-Ala^2-Met-Enkephalinamide at hypothalamic self-stimulation sites. *Brain Res* **194:** 155–170, 1980.
16. Olds, M. E. Reinforcing effects of morphine in the nucleus accumbens. *Brain Res* **237:** 429–440, 1982.
17. Pert, C. B., A. Pert, J. K. Chang and B. T. W. Fong. [D-Ala^2]-Met-enkephalinamide: a potent, long-lasting synthetic pentapeptide analgesic. *Science* **194:** 330–332, 1976.
18. Sar, M., W. E. Stumpf, R. J. Miller, K. Chang and P. Cuatrecasas. Immunohistochemical localization of enkephalin in rat brain and spinal cord. *J Comp Neurol* **182:** 17–38, 1978.
19. Schwartz, A. S. and P. L. Marchok. Depression of morphine-seeking behaviour by dopamine inhibition. *Nature* **248:** 257–258, 1974.
20. Sherman, J. E., C. Pickman, A. Rice, J. C. Libeskind and E. W. Holman. Rewarding and aversive effects of morphine: Temporal and pharmacological properties. *Pharmacol Biochem Behav* **13:** 501–505, 1980.
21. Simantov, R., S. R. Childers and S. H. Snyder. The opiate receptor binding interactions of ^{3}H-methionine enkephalin, an opiate peptide. *Eur J Pharmacol* **47:** 319–331, 1978.
22. Siviy, S. M., F. Bermudez-Rattoni, G. A. Rockwood, C. M. Dargie and L. D. Reid. Intracranial administration of naloxone in water-deprived rats. *Pharmacol Biochem Behav* **15:** 257–262, 1981.
23. Smith, J. E., C. Co, C. M. Crenshaw, T. S. Barr and J. D. Lane. The effects of kainic acid lesions of the nucleus accumbens on intravenous morphine self-administration. *Soc Neurosci Abstr* **7:** 51, 1981.
24. Smith, J. E., C. Co, M. E. Freeman and J. D. Lane. Brain neurotransmitter turnover correlated with morphine-seeking behavior of rats. *Pharmacol Biochem Behav* **16:** 509–519, 1982.
25. Smith, J. E. and J. D. Lane. Brain neurotransmitter turnover correlated with morphine self-administration. In: *Neurobiology of Opiate Reward Processes*, edited by J. E. Smith and J. D. Lane. Amsterdam: Elsevier/North Holland Biomedical Press, 1983, pp. 361–402.
26. Stein, E. A. and J. Olds. Direct, intracerebral self-administration of opiates in the rat. *Soc Neurosci Abstr* **3:** 302, 1977.
27. Uhr, G., R. R. Goodman, M. J. Kuhar, S. R. Childers and S. H. Snyder. Immunohistochemical mapping of enkephalin containing cell bodies, fibers and nerve terminals in the brainstem of the rat. *Brain Res* **166:** 75–94, 1979.
28. Wamsley, J. K., W. S. Young, III and M. J. Kuhar. Immunohistochemical localization of enkephalin in rat forebrain. *Brain Res* **190:** 153–174, 1980.
29. Wise, R. A. and M. A. Bozarth. Brain substrates for reinforcement and drug self-administration. *Prog NeuroPsychopharmacol* **5:** 467–474, 1981.