nesia was substantially reversed after DDAVP treatment, as indicated by a threefold increase in recall of items susceptible to disruption—those presented closest in time to ECT administration (Fischer exact probability test, $P < .01$).

These findings demonstrate a DDAVP-related enhancement of learning and memory. The determinants of this enhancement include effects on consolidation processes, organization of memories, and "strengthened" trace events in memory (as measured by increased consistency in recall). We have begun to examine whether this synthetic vasopressin might attenuate the cognitive impairments evident in progressive idiopathic dementia (Alzheimer's disease, senile dementia). Some of the six patients we have tested so far have demonstrated reliable increases in learning and recall following DDAVP treatment (*15*). These cognitive changes appear to be determined by increased accessibility to knowledge structures necessary for effective and complete encoding of recallable information. The psychobiological mechanisms that determine the role of vasopressin in influencing memory and learning remain undefined but a continuing challenge to neuroscientists attempting to explore cognition.

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References and Notes

1. D. de Wied, *Life Sci.* **19**, 685 (1976).
2. _____ and W. H. Gispin, in *Peptides in Neurobiology*, H. Gainer, Ed. (Plenum, New York, 1977).
3. S. F. Zornetzer, in *Psychopharmacology*, M. A. Lipton, A. DiMascio, K. F. Killan, Eds. (Raven, New York, 1978).
4. Tj. B. van Wimersma, B. Bohus, D. de Wied, in *Anatomical Neuroendocrinology*, W. F. Stumpf and L. D. Grant, Eds. (Karger, Basel, 1975).
5. M. Tanaka, E. R. de Kloet, D. de Wied, D. H. G. Versteeg, *Life Sci.* **20**, 1799 (1977).
6. G. M. Henry, H. Weingartner, D. L. Murphy, *Psychopharmacology* **27**, 319 (1973).
7. N. Sitaram, H. Weingartner, J. C. Gillin, *Science* **201**, 274 (1978).
8. N. Sitaram, H. Weingartner, E. D. Caine, J. C. Gillin, *Life Sci.* **22**, 1555 (1978).
9. H. Weingartner, R. Cohen, D. L. Murphy, *Arch. Gen. Psychiatry*, in press.
10. E. D. Caine, M. H. Ebert, H. Weingartner, *Neurology* **27**, 2087 (1977).
11. H. Buschke, *J. Verg. Learn. Verb. Behav.* **12**, 543 (1973).
12. R. L. Spitzer, J. Endicott, E. Robins, *Am. J. Psychiatry* **32**, 1187 (1975).
13. P. W. Gold, H. Weingartner, J. C. Ballenger, F. K. Goodwin, R. M. Post, *Lancet* **1979-II**, 992 (1979).
14. W. F. Battig and S. Montague, *J. Exp. Psychol.* **80**, 1 (1969).
15. H. Weingartner, P. Gold, W. Kaye, M. H. Ebert, J. C. Gillin, N. Sitaram, R. C. Petersen, in preparation.

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Metkephamid, a Systemically Active Analog of Methionine Enkephalin with Potent Opioid δ-Receptor Activity

Abstract. *Metkephamid is an analog of methionine enkephalin that retains high affinity for the δ receptor and is a systemically active analgesic. Since it is at least 100 times more potent than morphine as an analgesic when placed directly into the lateral ventricles, and is 30 to 100 times more potent on the δ receptor and yet is roughly equipotent on the μ receptor in vitro, it is concluded that it probably produces analgesia by an action on δ receptors as well as, or rather than, on μ receptors. It has less tendency to produce respiratory depression, tolerance, and physical dependence than standard analgesics, and it is presently undergoing clinical trial.*

There is evidence that methionine enkephalin (*1*) is an inhibitory neurotransmitter in the brain (*2*) and could therefore provide the basis for development of specialized therapeutic entities. The potential utility of such compounds is manyfold. In addition to the role in modulation of nociception, opioid peptides may also have a role in the hypothalamic control of pituitary function (*3, 4*) and may play a part in sexual maturation and function (*5-7*). Furthermore, dysfunction in these systems may be related to various forms of psychiatric illness (*8, 9*). Two different opioid receptor types, the μ receptor and the δ receptor, have been demonstrated (*10*) and there may be more. The natural enkephalins prefer the δ receptor, whereas morphine preferentially utilizes the μ receptor, and it appears that the potent methionine enkephalin analog FK33824 does also (*10-12*). Questions arise as to whether one or both receptors mediate analgesia, whether one or both mediate tolerance and physical dependence, and whether one or both, or some other receptor, mediates some of the other activities listed above. The natural peptide is enzymatically too labile to allow an evaluation of its potential therapeutic utility in the whole animal. If it does have a physiological role in the brain, however, then analogs modified to reach receptors in the brain should have the appropriate pharmacology. A research challenge is to provide structural modifications of methionine enkephalin that confer enzymatic protection without destroying affinity and efficacy at the desired receptors.

We now report the synthesis and pharmacological testing of a minimally modified analog of methionine enkephalin that is a systemically active analgesic and that has high affinity for the δ receptor. We first determined the relation between the structure of methionine enkephalin and its activity. This was conducted in vitro to eliminate pharmacokinetic problems. Having deduced the essential structural requirements for receptor activation (*2, 12, 13*), we modified methionine enkephalin in order to achieve enzymatic protection and increase blood-brain barrier permeation. The affinity of an antagonist for a receptor utilized by a given agonist can be estimated by calculation of its pA_2 value (*14*); that is, the negative logarithm of the concentration of the antagonist that necessitates a doubling of the agonist concentration to achieve a given effect. The pA_2 values for naloxone versus some enkephalin analogs were used to determine whether the analogs were interacting with the same receptors (δ receptors) as the natural peptide. Metkephamid (L-tyrosyl-D-alanylglycyl-L-phenylalanyl-N_2-methyl-L-methioninamide monoacetate) was developed in this manner and proved to have analgesic potency in rodents similar to the potencies of analgesic standards such as morphine and meperidine, but less tendency to produce respiratory depression, tolerance, and physical dependence. It is now undergoing initial clinical trials in human subjects.

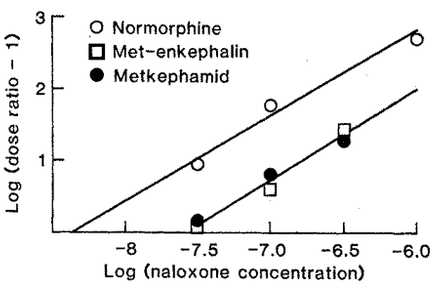

Fig. 1. Schild plots (*14*) for naloxone versus (○) normorphine, (□) methionine enkephalin, and (●) metkephamid. The intercepts of the lines with the abscissa give the pA_2 values for naloxone versus these opioid agonists. The pA_2 value with 95 percent confidence limits (*20*) for normorphine is 8.32 (8.06 to 8.69); for methionine enkephalin, 7.54 (7.38 to 7.74); and for metkephamid, 7.60 (7.43 to 7.80). The pA_2 values for metkephamid and methionine enkephalin are not different and presumably reflect action on the δ receptor. These values are significantly different from the pA_2 value for normorphine, however, which presumably reflects action on the μ receptor. See (*20*) for definition of dose ratio.

Peptides were prepared by solution

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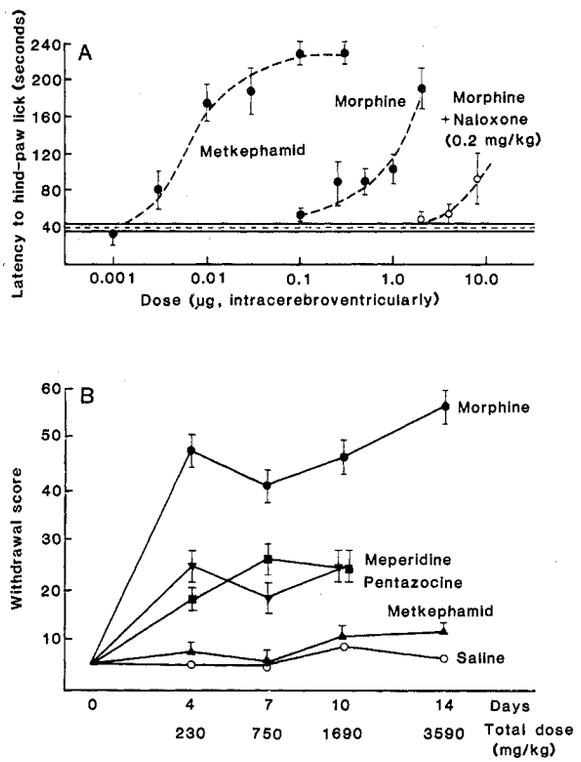

Fig. 2. (A) Comparative dose-response curves of morphine and metkephamid in the hot plate test in mice. The drugs were administered into the lateral ventricles. The ordinate is the latency in seconds of the hind paw lick. The horizontal lines give the mean control (saline-treated mice) latency ± standard error. Latencies were determined 30 minutes after injection of morphine and 15 minutes after metkephamid. Naloxone (0.2 mg/kg, subcutaneously) was given 15 minutes before testing. As indicated on abscissa, 1.0 μg is equivalent to 3.0 nanomoles of morphine free base or 1.5 nanomoles of metkephamid free base. (B) Naloxone-precipitated withdrawal scores after chronic treatment of rats with morphine, meperidine, pentazocine, metkephamid, or saline. Withdrawal was precipitated at days 4, 7, 10, and 14 by the injection of naloxone (10 mg/kg, subcutaneously). Withdrawal signs were scored for 15 minutes after injection of naloxone. The numbers below the abscissa give the total dose of compound in milligrams per kilogram administered by each time of testing. There are no data for meperidine or pentazocine after day 10 because the rats died after further treatment with these drugs.

methodology. The general approach required the dicyclohexylcarbodiimide hydroxybenzotriazole-mediated coupling of an N^a-Boc-protected amino terminal tripeptide with the desired carboxyl terminal dipeptide amide or ester (*3, 13, 15*). The peptides were tested in vitro (*3, 12, 15*) on single vas deferens from mature mice (Cox, 30 to 40 g). The hot plate test for analgesia was conducted by means of an apparatus with an electrically heated, thermostatically controlled (52°C) metal plate (*3, 12, 15*). The time in seconds from contact with the plate until a hind paw was licked was recorded as a response latency. The latency until an escape jump occurred was also recorded. A tail heat test in rats and a writhing test in mice were also used to assess analgesia (*16, 17*). Drugs were administered subcutaneously, intravenously, or intracerebroventricularly. For intracerebroventricular administration, Hamilton microsyringes bearing 27-gauge needles with stops at 2.5 mm from the needle tip were used (*12*).

Male Sprague-Dawley rats (90 to 100 g at start of experiment, Harlan Industries) were used to assess primary dependence liability of the opioids. The rats were given subcutaneous injections of saline or an opioid drug (morphine, meperidine, pentazocine, or metkephamid) four times daily for 14 days, with doses increasing gradually from 10 to 160 mg/kg per injection. Development of dependence was assessed at days 4, 7, 10, and 14 by challenge with naloxone (10 mg/kg, subcutaneously) and scoring of the resulting withdrawal signs (*16, 18, 19*).

Metkephamid produced a potent naloxone-reversible depression of the electrically induced twitch of the mouse vas deferens. The median effective doses (ED_{50}'s) on the vas deferens for metkephamid, methionine enkephalin, and normorphine were 1.2×10^{-8}, 2.0×10^{-8}, and $3.9 \times 10^{-7} M$, respectively. Clearly, the structural modifications of methionine enkephalin that led to metkephamid did not interfere with binding to the opioid receptor. Metkephamid and methionine enkephalin were at least 20 times more potent than normorphine on this preparation. Analysis of pA_2 values (*20*), furthermore, suggested that metkephamid and methionine enkephalin both utilized the δ receptor, whereas normorphine utilized the μ receptor (Fig. 1).

To assess activity of metkephamid on receptors in brain, which mediate analgesia, we first tested it after administration directly into the lateral ventricles to bypass pharmacokinetic barriers. When the analgesic effects of administration by this route were compared by the hot plate test in mice, metkephamid proved to be at least 100 times more potent on a molar basis than morphine and at least 30,000 times more potent than methionine enkephalin (Fig. 2).

Metkephamid was also an effective analgesic after subcutaneous or intravenous administration, as shown by the hot plate and writhing tests in mice and the tail heat test in rats. The comparative intravenous ED_{50}'s with 95 percent confidence limits of metkephamid and meperidine on the jump response were 0.21 (0.03 to 0.57) and 3.1 (1.4 to 5.7) μmole/kg, respectively, and on the hind paw lick response were 0.36 (0.08 to 0.85) and 1.7 (0.89 to 2.7) μmole/kg, respectively. The ED_{50} of metkephamid was thus about 1/15 that of meperidine on the jump response. The dose-response curve for metkephamid on the jump response, however, was shallower than that of meperidine; thus the difference in potency between metkephamid and meperidine was even greater than 15:1 at doses lower than the ED_{50}, and both compounds produced maximum analgesia at about the same dose (~3 mg/kg, intravenously). In tests by the subcutaneous route of administration, metkephamid was more potent on a molar basis than morphine, meperidine, pentazocine, and codeine in the writhing and hot plate jump tests for analgesia in mice; it was about one-half as potent as meperidine and one-third as potent as morphine on a molar basis in the hot plate hind paw lick test in mice. In the tail heat test in rats, it was about one-third as potent on a molar basis as morphine. Time courses of morphine, meperidine, and metkephamid were compared by using doses of each compound that had approximately equal analgesic effects. The duration of activity of metkephamid was intermediate between meperidine and morphine in the writhing test in mice and similar to morphine in the tail heat test in rats.

Physical dependence after chronic treatment was tested in rats showed that morphine produced a high level of dependence, meperidine and pentazocine produced an intermediate level of dependence, and metkephamid produced only slightly more dependence than saline (Fig. 2B). Similar results were obtained in mice in a withdrawal jumping test. Metkephamid also proved to have a much smaller depressant effect on respiration than morphine, as determined from blood gas measurements (partial pressures of O_2 and CO_2, and pH) in rats; morphine depressed respiratory function at doses as low as 1 mg/kg, whereas metkephamid had no significant effect on respiratory measurements at doses below 64 mg/kg.

The pentapeptide enkephalins have significant advantages over the larger

endorphins from the point of view of drug development since pharmacological activity resides in the smaller fragment which is easier and more economical to synthesize. We have described the preparation and pharmacological assessment of metkephamid, an analog derived from methionine enkephalin by slight structural modification that provides enzymatic protection and bioavailability without loss of desired receptor activity. Metkephamid is more active on the mouse vas deferens preparation than methionine enkephalin, which in turn is 20 to 30 times more potent than normorphine. The observation that naloxone had the same pA_2 value versus metkephamid as it had versus methionine enkephalin is evidence that they bind to the same receptor, the δ receptor, rather than the μ receptor preferred by normorphine. Studies with guinea pig ileum and inhibition of binding in brain tissue, however, suggest that metkephamid may be more β-endorphin-like than methionine enkephalin-like (*11*); that is, it is quite active on both the μ and the δ receptors. Studies in vivo indicate that metkephamid acts on receptors in brain, which mediate analgesia, since it is more than 100 times as potent an analgesic as morphine after direct injection into the lateral ventricles. Since metkephamid is only one to three times as potent as morphine at inhibiting normorphine binding in brain (*11*) and yet is more than 100 times as potent at producing analgesia, it is likely that metkephamid utilizes a different receptor, such as the δ receptor, to mediate this activity. Indeed, metkephamid is more active than morphine on the δ receptor as indicated by the data from mouse vas deferens presented above and also the inhibition of [D-Ala^2, D-Leu^5]enkephalin (Ala, alanine; Leu, leucine) binding to brain membranes (*11*). In both δ-receptor systems, metkephamid is at least 30 times more potent than morphine.

Metkephamid is a more potent analgesic than meperidine when given intravenously, as measured by the hot plate test in mice. By the subcutaneous route, the analgesic potency of metkephamid is of the same order of magnitude as that of the standard drugs morphine, meperidine, and pentazocine, and the duration of its analgesic activity is similar to that of meperidine or pentazocine, but metkephamid produces less primary dependence than morphine, meperidine, or pentazocine. Since meperidine and pentazocine have similar analgesic activity and duration of action, the lesser dependence produced by metkephamid reflects more than mere pharmacokinetic differences. Metkephamid is also less potent than the standard drugs in suppressing withdrawal phenomena in morphine-dependent mice, rats, and monkeys and produces less respiratory depression than morphine.

The preclinical pharmacological profile of metkephamid suggests that it should be a parenteral analgesic in humans, with potential advantages over existing drugs, and it has therefore been submitted to clinical testing (*15*). The only other enkephalin analog studied in man (*21*) is FK33824, which appears to have a greater preference for the μ receptor. Preliminary data indicate that metkephamid does have analgesic activity, but it is too early to judge its efficacy and acceptability relative to other analgesics at this time.

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References and Notes

1. J. Hughes, *Brain Res.* **88**, 295 (1975).
2. R. C. A. Frederickson, *Life Sci.* **21**, 23 (1977).
3. _____ and E. L. Smithwick, in *Endorphins in Mental Health Research*, E. Usdin *et al.*, Eds. (Macmillan, London, 1979), pp. 352-365.
4. C. J. Shaar, R. C. A. Frederickson, N. B. Dininger, L. Jackson, *Life Sci.* **21**, 853 (1977).
5. M. S. Blank, A. E. Panerai, H. G. Friesen, *Science* **203**, 1129 (1979).
6. B. J. Meyerson and L. Terenius, *Eur. J. Pharmacol.* **42**, 191 (1977).
7. B. Pellegrini-Quarantotti *et al.*, *Life Sci.* **23**, 673 (1978).
8. L. Terenius, in *Characteristics and Functions of Opioids*, J. Van Ree and L. Terenius, Eds. (Elsevier, Amsterdam, 1978), pp. 143-158.
9. S. J. Watson, H. Akil, P. A. Berger, J. D. Barchas, *Arch. Gen. Psychiatry* **36**, 35 (1979).
10. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **207**, 495 (1977).
11. H. W. Kosterlitz, personal communication.
12. R. C. A. Frederickson, R. Nickander, E. L. Smithwick, R. Shuman, F. H. Norris, in *Opiates and Endogenous Opioid Peptides*, H. W. Kosterlitz, Ed. (Elsevier, Amsterdam, 1976), pp. 239-246.
13. A more detailed explanation is forthcoming (R. C. A. Frederickson, E. L. Smithwick, R. Shuman, in preparation).
14. H. O. Schild, *Br. J. Pharmacol. Chemother.* **2**, 189 (1947).
15. R. C. A. Frederickson, E. L. Smithwick, D. P. Henry, in *Neuropeptides and Neural Transmission*, C. Ajmone Marsan and W. Traczyk, Eds. (Raven, New York, 1980).
16. R. C. A. Frederickson and S. E. Smits, *Res. Commun. Chem. Pathol. Pharmacol.* **5**, 867 (1973).
17. S. E. Smits and M. B. Myers, *ibid.* **7**, 651 (1974).
18. R. C. A. Frederickson, *Nature (London)* **257**, 131 (1975).
19. _____, C. R. Hewes, J. W. Aiken, *J. Pharmacol. Exp. Ther.* **199**, 375 (1976).
20. The dose ratio (the ratio of ED_{50} in the presence of an antagonist to ED_{50} in the absence of antagonist) for each level of antagonist and each compound was fitted to an overall general linear model with a different slope and intercept for each compound. A test for differences among slopes was insignificant ($P = .522$), and consequently a simpler model was fitted, with only a single slope for all three compounds. This slope was not significantly different from unity. On the basis of this reduced model, the intercept with the abscissa (pA_2 value) was computed for each compound, and Fieller's theorem (*22*, *23*) was used to compute the exact 95 percent confidence limits.
21. B. von Graffenried, E. del Pozo, J. Roubicek, E. Krebs, W. Pöldinger, P. Burmeister, L. Kerp, *Nature (London)* **272**, 729 (1978); G. Stacher, P. Bauer, H. Steinringer, E. Schriber, G. Schmierer, *Pain* **7**, 159 (1979).
22. E. C. Fieller, *Q. J. Pharm. Pharmacol.* **17**, 117 (1944).
23. G. O. Zerbe, *Am. Stat.* **32**, 103 (1978).
24. We thank C. Harrell, V. Burgis, and J. D. Edwards for technical assistance, A. Scott for typing the manuscript, B. Sparks for preparing the figures, and Endo Laboratories for naloxone.

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Endogenous Late Positive Component of the Evoked Potential in Cats Corresponding to P300 in Humans

Abstract. *A long-latency component of the averaged evoked potential recorded from cats was present only when the evoking stimulus was relevant to the task. The amplitude of this component varied inversely with stimulus probability and was independent of stimulus modality.*

The late positive component of the human averaged evoked potential (AEP), P300, has been studied extensively since its discovery by Sutton (*1*). It is considered an endogenous component of the AEP as its appearance and amplitude depend upon the circumstances under which stimuli are presented rather than upon their physical parameters. Several investigators have shown P300 to be related to human information processing (*2*). Essentially nothing, however, is known regarding the neural processes giving rise to P300. The requisite intracranial recording and lesion experiments are very difficult to achieve using human clinical material. We report here on a long-latency evoked potential component in cats that meets experimental criteria for classification as an endogenous component analogous to P300. Possible endogenous components have been reported previously in cats and monkeys (*3*); however, these evoked potential components were not comparable to the human P300.

In humans, the P300 component (i) can be elicited by stimuli of different modalities, (ii) occurs only if the stimulus is task-relevant, and (iii) varies inversely in amplitude with stimulus probability (*4*). In addition, it is generally maintained

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