spheric oxidation to selenite in control flasks (without soil) amounted to less than 0.03 μg/ml. Enrichment cultures were then plated on medium B (containing 1.5 percent Difco Noble agar) and incubated at 28°C for 4 to 5 days. From these plates individual colonies were isolated, checked for purity, and again tested for their ability to oxidize elemental selenium to selenite as above.

A spore-forming bacterium capable of oxidizing elemental selenium was isolated by this procedure and identified as *Bacillus megaterium* (*11*). It oxidized elemental selenium to selenite (Fig. 1) at a rate that varied linearly with the square root of time to a close approximation [$R^2 = .67$ (multiple coefficient of determination), and the quadratic term was nonsignificant at the 5 percent level]. Conformity of the rate of production of selenite to a relation with the square root of time suggests that the rate-limiting step could be one of diffusion of particles through what would be effectively a semi-infinite medium. An analysis of variance was carried out on the parabolic coefficients of the straight line thus fitted for each of the four replicate flasks (Table 1).

Selenium was oxidized to selenite by atmospheric oxygen at an average rate of 0.011 ± 0.003 μg ml^{-1} $day^{-1/2}$ (95 percent confidence interval) in uninoculated control flasks containing the medium and elemental selenium. The organism increased the rate of oxidation ($P < .001$) (Table 1). The greater increase in rate for red than for gray selenium ($P < .01$, *t*-test) can probably be attributed to the difference in surface area rather than to the allotropic form. After incubation for 40 days, approximately 1.5 percent of the red selenium had been oxidized to selenite.

Trace amounts of selenate were found at the end of the incubation (Table 1) and were significantly ($P < .05$) different among the four treatments. Although this observation indicates that the organism is capable of oxidation to selenate, the amount of selenate produced represents less than 1 percent of the amount of selenite formed.

The production of only trace amounts of selenate and substantial amounts of selenite by this strain of *B. megaterium* is in contrast to the two earlier reports on the oxidation of elemental selenium by microorganisms (*6*, *7*). Both investigators claimed, but without supporting evidence, that selenium was oxidized to selenate and the formation of selenite was not discussed.

To prevent selenium deficiency, grazing ruminants require only 0.02 to 0.03 μg/g in pasture (*3*). Although the levels of oxidation reported here are small (≤ 1.5 percent of the added selenium), they represent a definite link in the selenium cycle. If achieved on natural or added selenium in soils, they could be of importance in providing sufficient concentrations of selenium in herbage to prevent animal deficiency of this element.

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Effects of Vasopressin on Human Memory Functions

Abstract. *Arginine vasopressin and a number of its synthetic analogs augment memory functions in experimental animals. One of these analogs, 1-desamino-8-D-arginine vasopressin (DDAVP), influences human learning and memory. Cognitively unimpaired, as well as cognitively impaired adults, treated with DDAVP for a period of several days, learn information more effectively, as measured by the completeness, organization, and consistency (reliability) of recall. DDAVP also appears to reverse partially the retrograde amnesia that follows electroconvulsive treatment.*

Arginine vasopressin (AVP) is one of the few peptides synthesized entirely within the central nervous system in several nuclei located in the medial hypothalamus. It is transported from magnocellular hypothalamic nuclei to the posterior pituitary, where it is stored and released into the systemic circulation to regulate renal free water clearance. It is also transported from the hypothalamus to the third ventricle. The cerebrospinal fluid is thought to mediate a variety of AVP's putative central nervous effects, including those related to information processing (*1*, *2*). The mechanisms by which AVP affects learning and memory have not been elucidated, but evidence suggests that it acts as a neuromodulator and that it can influence not only the functional activity of biogenic amines thought to be involved in memory processes but also the release of other peptide modulators, including the endorphins (*3*–*5*).

In a number of experiments, different small groups of informed and consenting subjects volunteered to be treated with 1-desamino-8-D-arginine vasopressin (DDAVP) (in doses of from 30 to 60 μg administered intranasally by a calibrated catheter three times a day), and placebo similarly administered. Placebo and DDAVP treatments were repeated each day over a period of from 2 to 3 weeks. Prior to placebo and drug treatment, all subjects had become familiar through practice with all cognitive procedures used in the study. During both the placebo and the active drug periods, subjects had their weight and serum electrolytes recorded regularly, and their fluid intake was regulated daily to give an average of 1400 ml in 24 hours. The manner in which treatments were administered was designed to ensure the double-blind nature of the study and protect subjects against DDAVP-induced water retention.

The cognitive response (learning and memory) after DDAVP treatment was contrasted with placebo effects (i) in six young unimpaired college students (four males and two females); (ii) in a similar group of young unimpaired subjects treated only with placebo for a period of 8 weeks; (iii) in four patients with histories of endogenous mood disturbance with accompanying cognitive impairment (all females between the ages of 36 and 47 years); and (iv) in two female mood disorder patients, who had been unresponsive to antidepressant medication, tested after electroconvulsive therapy (ECT), which would be expected to

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induce retrograde amnesia for events processed just prior to ECT. All studies were completed on an inpatient psychiatric research unit of the National Institutes of Health.

The learning-memory tasks used to access the cognitive response after DDAVP treatment had each been previously tested and validated in clinical and pharmacological studies of memory and learning (*6–10*). Equivalent forms of these procedures were used and designed to provide measures of serial learning and memory (*6*); recall consistency or reliability (*11*); and organizational determinants that affect encoding of information and, thereby, learning and memory (*9*). In testing the potential effects of DDAVP in attenuating the retrograde amnesia produced by ECT, a previously untested cognitive procedure was designed, tested, and then used to specifically explore disruptions of memory consolidation produced by ECT and its potential attenuation following DDAVP treatment.

Six young unimpaired subjects demonstrated statistically significant increases in learning and memory when treated with DDAVP, in contrast to placebo treatment (Fig. 1). Increases were seen in (i) serial learning [treatments versus subjects by replication, $F(1, 25) = 5.8$, $P < .05$]; (ii) prompted free recall, most marked at the end of a period of 2 weeks of DDAVP treatment [treatments versus subjects by replications, $F(1, 25)=4.62$, $P < .05$]; and (iii) recall of semantically related words [$F(1, 25) = 6.2$, $P < .05$]. There was no DDAVP-related change, however, in the consistency of recall of previously remembered words (information) stored in long-term memory, probably because even under placebo conditions subjects reliably recalled previously remembered words (consistency of recall, 91 ± 4 percent). Similar unimpaired college student volunteers, treated three times a day, for a period of months with the vehicle nasal spray placebo alone and tested twice weekly, failed to demonstrate similar changes in any of these measures of learning and memory.

Four depressed patients, diagnosed on the basis of research diagnostic procedures (*12*) and demonstrating impaired cognition related to their disordered moods, were treated with placebo and DDAVP for at least 2 weeks after completing a period of baseline assessment of learning, memory, and mood. Three of the four patients demonstrated significant cognitive enhancement beginning 2 days after DDAVP treatment (*13*) and continuing for 2 weeks. This cognitive improvement was independent of changes in mood. The statistically reliable increase in learning and memory was evident on measures that included (i) increased recall of information on the prompted recall task, an average increase of 23 ± 7 percent (tested by a repeated measure analysis of variance using the ten learning recall test trials for each patient for each biweekly testing) [F (1, 16) = 9.1, $P < .01$]; (ii) a related marked increase in the consistency (or reliability) of recall of previously remembered words from a mean of .46 to .63 ± .06 ($P < .01$); and (iii) an increase in free recall of semantically related words, along with an increase in the organization of remembered words, as measured by the tendency to cluster or remember together related words on recall (index of clustering increased from .42, poor clustering, to .23, more clustering or organization) (standard error = ± 0.08, $P < .01$). Serial learning, a particularly difficult task for these patients (few reached criterion) was not similarly altered after DDAVP treatment. Only 4 weeks after active drug treatment had ceased did patients return to their baseline learning and memory performance. These changes in learning and memory were not significantly correlated with changes in the severity of clinical depression, although some patients did spontaneously report feeling more activated while being treated with DDAVP. A second group of cognitively less-impaired mood-disorder patients treated with DDAVP for a much shorter period of time demonstrated a less robust and more variable learning-memory response to DDAVP treatment.

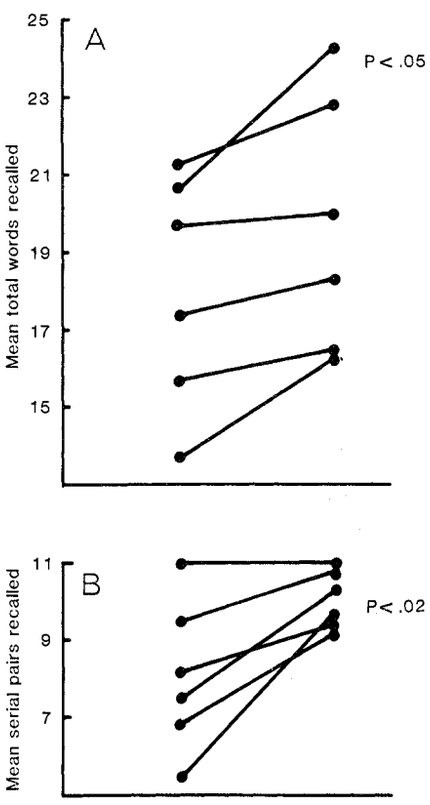

Fig. 1. Cognitive effects of DDAVP in unimpaired subjects. Each data point represents the mean value of at least five tests per subject of (A) memory for related words and (B) number of correct responses for the first four serial-learning trials (a point at which no subject had reached criterion of two perfect serial list repetitions).

To test for a possible attenuation of a retrograde amnesia after ECT treatment, two depressed patients were studied after familiarization and practice with the procedure and at a point midway in their course of ECT treatment, just after a lifting of mood had already been observed (weeks 3 and 4 of a 6-week series of standardized biweekly unilateral ECT treatments). During week 3, subjects were treated with placebo, and during week 4, they received DDAVP.

Subjects practiced a task in which they listened to eight equivalent lists of eight related words each, controlled for their frequency in the language, the degree of semantic relatedness, and the frequency of occurrence within a category (*14*). The first list was presented 45 minutes before ECT, the second 5 minutes later, and so on, with the last list presented 10 minutes before ECT treatment. Recall of these words was attempted 5 hours later when patients were alert. On recall, subjects were first given the name of a category from which items had been drawn and were asked to try to remember the words. This procedure was repeated three times with equivalent materials. The first time, patients did not receive ECT. After 1 week, they were treated with placebo and listened to eight lists of words; they then received ECT, and, after 5 hours, attempted recall. In the third condition, the same procedure was followed, but subjects were treated with DDAVP (instead of placebo) for 3 days at doses of 40 to 60 μg. After ECT, with saline preliminary treatment, both patients demonstrated retrograde amnesia by a systematic decrease in recall as a function of time of presentation prior to ECT treatment. Recall of words presented at least 25 minutes before ECT was not disrupted, but recall of the last four lists, presented between 25 and 10 minutes before ECT, was reduced to 1/3 that of baseline values (Fischer exact probability test, $P < .01$). This retrograde am-

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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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.

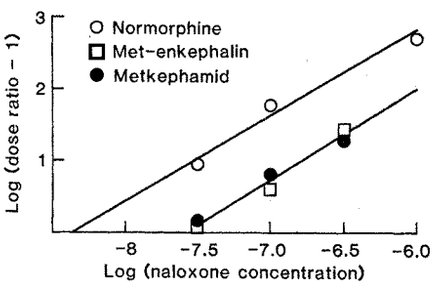

Fig. 1. Schild plots (*14*) for naloxone versus (○) normorphine, (□) methionine enkephalin, and (●) metkephamid. The intercepts of the lines with the abscissa give the pA_2 values for naloxone versus these opioid agonists. The pA_2 value with 95 percent confidence limits (*20*) for normorphine is 8.32 (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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