Induction of Copulatory Behavior in Sexually Inactive Rats by Naloxone

Abstract. *The intraventricular injection of* D-*alanine-methionine-enkephalinamide (*D-*Ala*^{2}*-Met-enkephalinamide), a synthetic analog of Met-enkephalin that is resistant to enzymatic degradation, inhibits copulatory behavior in sexually vigorous male rats in doses which do not influence motor activity or feeding behavior. This effect is prevented by naloxone, a specific inhibitor of opioid receptors. In addition, injections of naloxone induce copulatory behavior in sexually inactive male rats. These results suggest that endorphins play an important role in the regulation of sexual behavior.*

Recent evidence suggests that endorphins, a generic name for endogenous peptides with morphine-like activity comprising enkephalins and endorphins sensu stricto, may play several physiological roles besides that of controlling pain perception. In fact, these peptides, as well as their specific receptors, are unevenly distributed in the brain; they can be found not only in areas such as the periaqueductal gray matter and thalamus, which are involved in the mechanisms of pain perception, but also in striatum, hypothalamus, and limbic system (*1*, *2*). Moreover, the administration of different endorphins into the cerebrospinal fluid of rats, mice, and cats causes not only analgesia but also different behavioral changes, such as hyperactivity, rigid immobility, wet-dog shakes, and grooming, which are prevented by specific narcotic antagonists (*3*).

Meyerson and Terenius (*4*) reported that the percentage of male rats mounting receptive females decreased when the males received an intraventricular injection of 1 μg of β-endorphin, although exploratory activity was not affected; the decrease in mounting activity was prevented by naltrexone, an opiate antagonist. This finding could be of clinical importance, since long-term users of narcotic analgesics often complain of frigidity and impotence (*5*). In investigating the possible role of endorphins in the regulation of sexual behavior, we studied th effect of D-Ala^2-Met-enkephalinamide (DALA) on the copulatory behavior of sexually active rats and that of naloxone on the copulatory behavior of sexually inactive ones.

The compound DALA is a chemical derivative of Met-enkephalin. It has almost the same affinity for opiate receptors as that of the endogenous peptide but is resistant to enzymatic degradation (*1*).

From 230 male Sprague-Dawley rats (Charles River, Como, Italy), we selected 40 that were sexually inactive (IN) and 60 that were sexually active (SA). The rats were 80 to 100 days old and were selected after five screening tests at 3-day intervals with highly receptive females. The SA rats ejaculated at least twice in each of the last three screening tests; the IN rats failed to ejaculate in each of the five screening tests. From 2 weeks before the screening tests until the end of the study the rats were housed individually at 24°C in 20 by 40 cm cages, under a reversed light-dark cycle (with light from 11:00 p.m. to 11:00 a.m.). Food and water were constantly available. Before we began the experiments we anesthetized the SA rats with Equithesin and implanted a permanent cannula into the right lateral ventricle of the brain [coordinates: A + 1.0; L 1.0; DV − 4.0; see Pellegrino and Cushman (*6*)]. The IN rats received no surgery.

One week after surgery, when they were retested for sexual activity, the SA rats were as vigorous as before surgery. Mating tests, in which a highly receptive female was introduced into the male's cage, took place in the home cages under dim red light. These tests lasted 30 minutes, beginning approximately in the middle of the animals' dark cycle. The percentage of male rats copulating and the following measures were registered on an event recorder: (i) mount and intromission latencies, from the introduction of the female into the male's cage until the first mount and intromission, respectively; (ii) ejaculation latency from the first intromission to the first ejaculation; (iii) interval between intromissions or the mean interval separating the intromissions of a series; (iv) post-ejaculatory interval, or the time from an ejaculation to the next intromission; (v) mount frequency, that is, the number of mounts in a series; (vi) intromission frequency, that is, the number of intromissions in a series; and (vii) ejaculation frequency, that is, the number of ejaculations in 30 minutes.

The female lures were ovariectomized Sprague-Dawley rats brought into estrus with estrogen-progesterone treatment. Motor activity was measured in male rats kept under the same conditions as the rats used in the mating tests. These rats, in which a cannula was implanted in the right lateral ventricle 1 week before the experiment, were placed singly in activity cages (Motron, Stockholm) and

Table 1. Effect of DALA on copulatory pattern in sexually experienced male rats. The DALA or saline was given intracerebroventricularly (i.c.) 15 minutes before the test; naloxone (4 mg/kg) or saline was given intraperitoneally (i.p.) 30 minutes before the test. The dose of 6 μg of DALA suppressed copulatory behavior in all animals; naloxone completely prevented such inhibition. The dose of 3 μg of DALA did not influence the percentage of rats achieving ejaculation. Values are means ± standard error obtained from ten animals (*10*). Measures of time are expressed in seconds. Except for ejaculation frequency, copulatory measures refer to the first ejaculatory series. Sixty rats were divided into six equal groups, each of which (ten rats) received one of the reported treatments.

Copulatory measure	Saline (i.p.) plus saline (i.c.)	Saline (i.p.) plus DALA (3 μg i.c.)	Naloxone (i.p.) plus saline (i.c.)	Naloxone (i.p.) plus DALA (3 μg i.c.)	Naloxone (i.p.) plus DALA (6 μg i.c.)
Latencies					
Mount	87.2 ± 16.4	697.6 ± 157.8*	90.3 ± 19.2	137.1 ± 40.9	115.8 ± 21.9
Intromission	137.7 ± 25.6	840.2 ± 190.3*	125.2 ± 30.1	147.8 ± 47.4	134.0 ± 29.1
Ejaculation	543.3 ± 92.9	616.4 ± 122.1	606.7 ± 112.3	605.8 ± 137.1	664.9 ± 157.2
Intervals					
Post-ejaculation	318.7 ± 19.1	265.2 ± 25.8	270.1 ± 16.8	318.1 ± 46.5	296.0 ± 23.5
Interintromission	45.0 ± 9.2	32.5 ± 6.3	30.8 ± 6.1	35.2 ± 5.5	46.5 ± 10.8
Frequencies					
Mount	4.2 ± 0.8	3.2 ± 0.8	4.8 ± 0.8	5.0 ± 1.4	5.1 ± 1.3
Intromission	14.4 ± 2.1	16.0 ± 1.7	15.6 ± 2.1	15.2 ± 3.3	17.8 ± 3.8
Ejaculation	2.3 ± 0.3	2.0 ± 0.3	2.2 ± 0.4	2.2 ± 0.4	2.3 ± 0.4

*$P < .01$ in respect to the value for saline plus saline (Student's t-test).

0036-8075/79/0413-0203$00.50/0

Table 2. Induction of copulatory behavior in sexually inactive (IN) rats by naloxone. Each value is the mean of two or three experiments on 40 animals. Forty IN rats were injected intraperitoneally twice with saline and two and three times with naloxone (doses of 2 and 4 mg/kg, respectively) according to a Latin-square design. Tests were carried out at weekly intervals.

Treatment (30 minutes before test)	Percentage of animals exhibiting at least one*		
	Mounting	Intromission	Ejaculation
Saline	18.0	17.5	0
Naloxone (2 mg/kg)	41.5†	32.5	5.8
Naloxone (4 mg/kg)	90.0†	90.0†	74.0†

*Within a 30-minute observation period. †$P < .01$, calculated by the sign test (*10*).

their activity was measured for 30 minutes.

D-Ala^2-Met-enkephalinamide (Tecnofarmaci, Pomezia, Italy) was dissolved in saline just before use and administered into the lateral ventricle in 6-μl volumes. Naloxone hydrochloride was dissolved in saline at *p*H 7.2 and given intraperitoneally in a volume of 0.1 ml per 100 g of body weight. Control rats received an equal volume of saline intraperitoneally or intraventricularly, or both.

We tried to establish the specific effects of DALA on copulatory behavior, that is, effects that were distinct from impairments of motor performance. We injected different doses of DALA intraventricularly in order to find the maximum dose that did not decrease motor activity. This dose was found to be 6 μg; it failed to produce any noticeable change in other behavioral parameters such as reactivity or motor coordination. However, male rats treated with this dose exhibited a total loss both of copulatory behavior and the ability to ejaculate. The dose of 3 μg of DALA did not influence the percentage of rats achieving ejaculation but influenced their copulatory pattern.

As shown in Table 1, rats treated with 3 μg of DALA initiated copulation (mounts and intromission) significantly later than rats treated with saline. However, this dose failed to influence measures (ii) through (vii) of copulatory performance.

During the interval preceding copulation, the initial response of males to the female was indistinguishable from the behavior of control males; pursuit of the female and sniffing and licking of her anogenital region were observed during the minutes preceding copulation. These behavioral responses were also present in animals treated with the higher dose of DALA, who failed to copulate.

Prior treatment of the rats with naloxone (4 mg/kg), which per se did not interfere with copulatory behavior, completely prevented the inhibitory effect of 3 and 6 μg of DALA.

To further assess the effect of DALA on sexual behavior, we studied the effect of the peptide on feeding. We used 20 male rats, which were trained for 1 month to eat their daily food within 2 hours according to a procedure previously described (*7*). They were implanted with a permanent cannula into the lateral ventricle as were the animals used in the mating tests.

Starting 1 week after surgery, we established a baseline behavior during a period of 8 days in which the animals ate a daily average of 20.3 ± 1.2 g of food (Purina chow pellets) and the latency between food presentation and feeding onset was 5.3 ± 0.3 seconds. On the day of the experiment, 30 minutes before food presentation, half the animals were injected with saline (6 μl) and the other half with 3 μg of DALA. Neither treatment influenced either the amount of food eaten (19.1 ± 1.6 g after DALA) or the interval between food presentation and the feeding onset (4.8 ± 0.5 seconds after DALA). These results support the assumption that the inhibitory effect of DALA on sexual behavior is somewhat specific and is not secondary to a sensory alteration. Moreover these findings suggest that stimulation of opioid receptors results in a loss of the male's ability to initiate copulation.

We then investigated the effect of naloxone on 40 IN rats. Each rat was tested twice after an injection of saline and two and three times after injections (2 and 4 mg/kg, respectively) of naloxone. Treatments were given intramuscularly 30 minutes before each test according to a Latin-square design.

Naloxone at the 4 mg/kg dose markedly increased the number of animals displaying mounting intromission and achieving ejaculation (see Table 2). The dose of 2 mg/kg had a significant effect only on the mounting but not on the other elements of the copulatory behavior. Animals that were improved by naloxone returned to their low basal level of sexual activity when tested 1 week later after saline treatment. Males that achieved ejaculation after an injection of naloxone exhibited a copulatory pattern not significantly different from that of vigorous SA rats (results not shown).

These results indicate that DALA inhibits copulatory behavior in male rats in doses which do not influence motor activity or feeding behavior and do not produce other overt behavioral changes. Naloxone completely reversed this effect, strongly suggesting that this response is mediated by stimulation of opioid receptors in brain. DALA seems to inhibit the animal's willingness or ability to initiate copulation or both. Our results are in agreement with those obtained with β-endorphin by Meyerson and Terenius (*4*). β-Endorphin seems to be more potent than DALA in inhibiting copulatory behavior; the difference in potency roughly parallels the ratio in the affinity of the two peptides for opiate receptors (*8*). The finding that naloxone induces copulatory behavior in IN rats is of great theoretical and practical interest. In fact, since endorphins inhibit copulation by an action which can be antagonized by naloxone, the stimulant effect of the latter might be explained by an antagonism of an endogenous endorphin in the brain.

The fact that naloxone did not further enhance the sexual behavior in vigorous SA rats suggests that during sexual arousal there is no tonic stimulation of opioid receptors involved in the control of sexual behavior. Furthermore, our results may indicate that sexual inadequacy in rats may be due, among other causes (*9*), to endorphins.

It will be interesting to determine whether the results obtained in rats apply to other animal species. If so, opioid antagonists might become potentially useful therapeutic agents for sexual disturbances in man.

G. L. GESSA
E. PAGLIETTI
Institute of Pharmacology, University of Cagliari, Cagliari, Italy
B. PELLEGRINI QUARANTOTTI
Tecnofarmaci S.p.A., Via Ardeatina, Pomezia, Rome

References and Notes

1. C. B. Pert, A. Pert, J.-K. Chang, B. T. W. Fong, *Science* **194**, 330 (1976).
2. G. Urca, H. Frenk, J. C. Liebeskind, A. N. Taylor, *ibid.* **197**, 83 (1977); R. Simantov, M. J. Kuhar, G. W. Pasternak, S. H. Snyder, *Brain Res.* **106**, 189 (1976); H. Y. Yang, J. S. Hong, E. Costa, *Neuropharmacology* **16**, 303 (1977); N. Ling, R. Burgus, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3942 (1976); R. C. A. Frederickson, *Life Sci.* **21**, 23 (1977).
3. E. T. Wei, L. F. Tseng, H. H. Loh, C. H. Li, *Life Sci.* **21**, 321 (1977); J. M. Walker, G. G. Berntson, C. A. Sandman, D. H. Coy, A. V. Schally, A. J. Kastin, *Science* **196**, 85 (1977); M. Meglio, Y. Hosobuchi, H. H. Loh, J. E. Adams, C. H. Li, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 774 (1977).
4. B. J. Meyerson and L. Terenius, *Eur. J. Pharmacol.* **42**, 191 (1977).

5. L. E. Hollister, in *Sexual Behavior: Pharmacology and Biochemistry*, M. Sandler and G. L. Gessa, Eds. (Raven, New York, 1975), p. 85.
6. L. J. Pellegrino and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain* (Meredith, New York, 1971).
7. G. Biggio, M. L. Porceddu, W. Fratta, G. L. Gessa, *Adv. Biochem. Psychopharmacol.* **16**, (1977).
8. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **267**, 495 (1977).
9. G. L. Gessa and A. Tagliamonte, in *Sexual Behavior: Pharmacology and Biochemistry*, M. Sandler and G. L. Gessa, Eds. (Raven, New York, 1975), p. 117.
10. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956).
11. This study was sponsored by a grant from Tecnofarmaci S.p.A., Pomezia, Rome, Italy. Naloxone was supplied by Salars, Como, Italy.

26 September 1978

Thalamic and Cortical Afferents Differentiate Anterior from Posterior Cingulate Cortex in the Monkey

Abstract. *The anterior cingulate cortex receives thalamic afferents mainly from the midline and intralaminar nuclei rather than the anterior thalamic nuclei. In contrast, the posterior cingulate cortex receives afferents primarily from the anterior thalamic nuclei and from extensive cortical areas in the frontal, parietal, and temporal lobes. These contrasting afferents may provide a structural basis for pain-related functions of the anterior cingulate cortex.*

A wide range of clinical and experimental data indicate that anterior and posterior cingulate cortex are functionally distinct regions of the limbic system. These studies consistently demonstrate the involvement of the anterior cingulate cortex in many complex somatic and visceral motor functions as well as in responses to pain, whereas the posterior cingulate cortex has little to do with such functions. Thus, electrical stimulation of the anterior cingulate cortex (area 24) in the cat and the monkey produces cardiac slowing, piloerection, pupillary dilation, and changes in the rate of respiration and muscular tone (*1*). Stimulation of area 24 in humans also produces integrated motor responses (such as stretching and sucking) (*2*). Furthermore, stimulation of the anterior cingulate cortex in the cat inhibits attack behavior evoked by hypothalamic stimulation, whereas stimulation of the posterior cortex does not (*3*). In addition, ablation studies have shown that anterior cingulate lesions produce deficits in alternation learning, but posterior cingulate lesions do not (*4*). Of particular significance is that in humans neurosurgical procedures that interrupt axons coursing beneath the anterior cingulate gyrus alleviate the noxious effects of chronic pain although perception of pain per se is not abolished (*5*). This is interesting since the anterior cingulate gyrus has a much higher affinity for binding opiates than the posterior cingulate gyrus (*6*).

In light of these data, it is surprising that anatomical studies have not yet resolved the issue of precisely which thalamic or cortical areas send afferents to the cingulate gyrus. As a consequence, the available anatomical data do not provide a good structural basis for the extensive functional differences between the anterior and posterior areas of the cingulate cortex nor for the "pain-related" functions of the anterior cingulate gyrus. It has long been thought that the anterior, mediodorsal, and laterodorsal nuclei of the thalamus are the source of thalamic afferents to the cingulate gyrus, with separate divisions of the anterior thalamic nuclei sending afferents to both the anterior and posterior cingulate cortex, while the mediodorsal nucleus sends afferents to the anterior cingulate cortex and the laterodorsal nucleus to the posterior cingulate cortex (*7*). The ablation-degeneration techniques used in such studies are difficult to interpret, however, because the lesions usually damage adjacent fibers of passage (*8*).

Using anatomical techniques that avoid these problems, we have found that the anterior cingulate cortex receives afferents primarily from the midline and intralaminar nuclei rather than from the anterior thalamic nuclei. In contrast, the anterior and laterodorsal thalamic nuclei project to the posterior cingulate cortex. Furthermore, the anterior cingulate cortex receives few cortical afferents, while the posterior area receives extensive afferents from the frontal, parietal, and temporal lobes, maintaining the dichotomy of the thalamic afferents.

In eight rhesus monkeys (*Macaca mulatta*), a 20 percent solution of horseradish peroxidase (HRP) was injected into the cingulate gyrus. The results are based on four cases with injections of 0.14 μl each. These cases were processed according to the perfusion-fixation procedure described by Rosene and Mesulam (*9*) and were reacted with the tetramethyl benzidine reaction procedure of Mesulam (*10*). We tried to keep injections, postoperative survival time, and processing procedures identical to facilitate comparisons. Furthermore, none of the injections appeared to penetrate underlying white matter. Cytoarchitectural division of the cingulate gyrus into area 24 (anterior cingulate cortex) and areas 23 and 29 (posterior cingulate cortex) is based on Brodmann's cytoarchitectonic map (*11*) while parcellation of the thalamic nuclei is according to Olszewski (*12*).

After HRP was injected into the anterior cingulate gyrus (Fig. 1), only small numbers of HRP-labeled neurons were present in a few cortical areas, whereas posterior injections produced extensive labeling of neurons in a larger number of cortical regions. After injections into the anterior cingulate cortex, only a few labeled neurons were found in the dorsolateral prefrontal cortex, in the lateral orbitofrontal cortex, in the insular cortex, and in the caudal parahippocampal gyrus [areas TF and TH (*13*)] (Fig. 1). After posterior injections, however, large numbers of labeled neurons were found in the dorsolateral bank and depths of the principal sulcus, the medial orbitofrontal cortex, areas TF and TH of the parahippocampal gyrus, and in the posterior parietal cortex. The anterior cingulate cortex also contained labeled neurons, as did the posterior cingulate cortex after anterior injections, indicating a reciprocity of connections between the anterior and posterior cortices. For all these cortical areas, anterograde transport of ^{3}H-labeled amino acids confirms a projection to cingulate cortex areas 24, 23, or 29 distinct from adjacent frontal or parietal cortex into which HRP could spread. Afferents from both the amygdala and the hippocampus were also selective in their distribution to the anterior and posterior cingulate gyrus. Thus, the lateral basal nucleus of the amygdala sends afferents to area 24 anteriorly, while the subicular division of the hippocampus sends afferents to the posterior cingulate cortex (*14*).

Even more striking than the differences in cortical afferents to the anterior and posterior cingulate cortices is the contrast in thalamic afferents. Injections of HRP into the anterior cingulate cortex (Fig. 1) produced extensive labeling of neurons throughout the midline and intralaminar thalamic nuclei (Fig. 2, A to C). Surprisingly, these anterior injections failed to demonstrate HRP labeling of neurons in the anterior thalamic nuclei as expected. Instead, the most numerous and heavily labeled neurons were located in the midline and intralaminar nuclei including the paraven-

0036-8075/79/0413-0205$00.50/0