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17. We are indebted to the NIAMDD rat pituitary hormone distribution program for supplying the radioimmunoassay for rat LH and FSH. The excellent technical assistance of H. Laurent, N. Keating, J. Boone, K. von Dessonneck, J. Porter, and R. Kaiser is gratefully acknowledged. Supported by NIH grants HD09690, RFP CD-78-5, the Rockefeller Foundation, the Clayton Foundation for Research, California Division, and the Texas Salk Institute Foundation.

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Excitatory and Inhibitory Effects of Opiates in the Rat Vas Deferens: A Dual Mechanism of Opiate Action

Abstract. *Both natural (−)-morphine and its unnatural enantiomer (+)-morphine exert an excitatory action on electrically stimulated contractions of rat vas deferens. Preexposure to (−)-morphine results in cross-tolerance to the inhibitory action of β-endorphin. (−)-Naloxone and its stereoisomer (+)-naloxone also exert an excitatory action, but only (−)-naloxone blocks the inhibitory action of β-endorphin. Thus morphine exerts a dual action on a peripheral organ: one an inhibitory action mediated by the stereospecific endorphin receptor that is blocked stereospecifically by naloxone, the other an excitatory action mediated by a nonstereospecific receptor that is not blocked by naloxone. The opiate abstinence syndrome is seen as due to the unmasking of the excitatory action of opiates when its concomitant inhibitory influence is removed by selective blockade by naloxone or weakened by selective tolerance. The view that the rat vas deferens is devoid of morphine receptors is now seen as arising from a reverse example of morphine's dual action: the masking of the inhibitory action of morphine by its concomitant and more potent excitatory action.*

We previously proposed a dual mechanism model of morphine action in the central nervous system (CNS) and presented evidence that morphine actions in the CNS are mediated by two classes of receptors (*1*). One, stereospecific for opiates and antagonized by naloxone, mediates the inhibitory actions of opiates (analgesia, sedation, and immobility); the other, not stereospecific for opiates and not antagonized by naloxone, mediates the excitatory actions of opiates (such as hyperreactivity) (*2*). This dual action mechanism implies that the opiate abstinence syndrome is due to the unmasking of the excitatory action of the opiate when its simultaneous inhibitory influence is removed by selective blockade by naloxone or weakened by the development of selective tolerance. We now present evidence for a dual mechanism of opiate action in a peripheral organ, the rat vas deferens.

The rat vas deferens has been reported to be devoid of morphine receptors (*3*), yet β-endorphin, the opiate peptide that is the putative endogenous ligand for the classical morphine receptor, was reported to exert a potent inhibitory action on this tissue (*4*). Morphine was found to exert a stimulatory action (*5*), whereas the potent opiate etorphine was found to exert an inhibitory action (*6*). This seeming contradiction led us to examine the effects of morphine on this tissue. We now report that morphine exerts a dual action—both excitatory and inhibitory—on this preparation, with the excitatory effect masking the inhibitory one.

To demonstrate the inhibitory action of morphine (which is masked by its concomitant and more potent excitatory action), we investigated cross-tolerance to the inhibitory action of β-endorphin after exposure to morphine. This effect was observed to be stereospecific, occurring only after exposure to (−)-morphine, not its unnatural enantiomer (+)-morphine (*7*). The excitatory action was nonstereospecific, occurring after both (−)- and (+)-morphine, and was not blocked by the opiate antagonist (−)-naloxone. While the excitatory action also occurred after exposure to (−)-naloxone or its stereoisomer (+)-naloxone (*7*), only (−)-naloxone blocked the inhibitory action of β-endorphin (Fig. 1) (*8*). These results extend to a peripheral organ our previous findings for the CNS that morphine exerts dual actions that are mediated by stereospecific inhibitory receptors and nonstereospecific excitatory receptors, with the former being blocked stereospecifically by (−)-naloxone.

Vasa deferentia were dissected from freshly killed Sprague-Dawley rats (250 to 300 g), carefully stripped of surrounding tissue, and pressed to remove their seminal contents. Each tissue sample was suspended in a 5-ml bath containing Krebs-Ringer solution (*9*) maintained at 37°C and *p*H 7.4 and gassed with a mixture of 95 percent O_2 and 5 percent CO_2. The resting tension was approximately 1 g; the contractions induced by electrical stimulation (30 V, 0.1 Hz, 0.5 msec) (*6*) were measured isometrically. All strips were equilibrated for 1 hour, with washes every 15 minutes. This was followed by a pretreatment period of 30 minutes, during which the tissue was continuously exposed to one of four drugs or no drug while the electrical stimulation continued. Immediately afterward, the strips

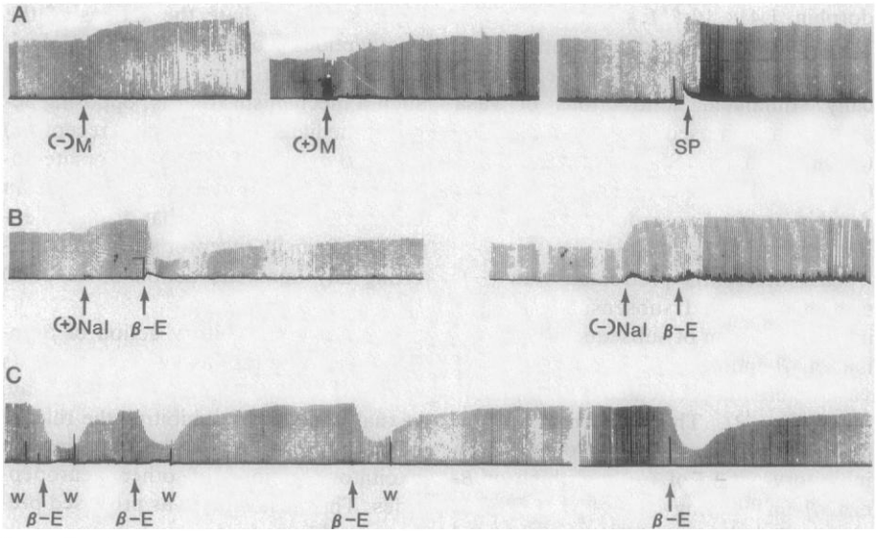

Fig. 1. Tracings showing excitatory and inhibitory actions of opiates and peptides on the electrically stimulated contractions of rat vas deferens. (A) Left, excitation after addition of (−)-morphine (*M*) (final dilution, $1 \times 10^{-4}M$); center, after (+)-morphine ($1 \times 10^{-4}M$); right, after substance P (*SP*) ($2.5 \times 10^{-6}M$). (B) Left, excitation after addition of (+)-naloxone (*Nal*) ($1 \times 10^{-4}M$) followed by inhibition (no blockade) when β-endorphin (*β-E*) ($1.4 \times 10^{-6}M$) was added in its presence; right, excitation after addition of (−)-naloxone ($1 \times 10^{-4}M$); no inhibition (blockade) followed when β-endorphin ($1.4 \times 10^{-6}M$) was added in its presence. (C) Inhibition in a vas deferens preexposed to (+)-morphine ($1 \times 10^{-4}M$); β-endorphin ($1.4 \times 10^{-7}M$) was added 1, 10, 30, and 60 minutes later. Three minutes after the first three additions, the strips were washed (*w*).

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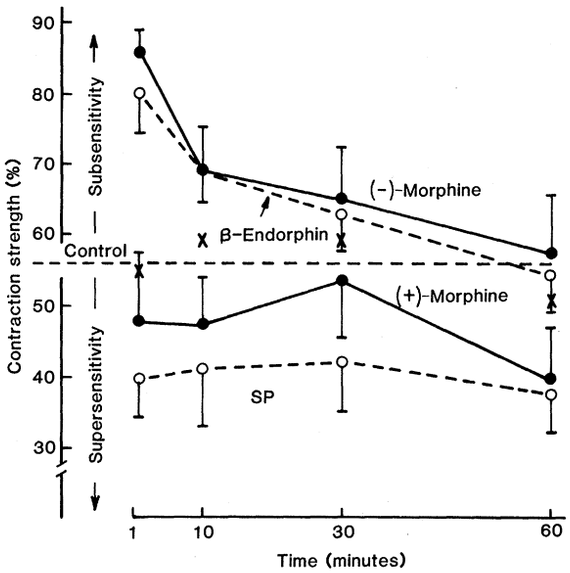

Fig 2. Mean contraction strength (± standard error) of vasa deferentia (six per group) after β-endorphin ($1.4 \times 10^{-7}M$) was added 1, 10, 30, and 60 minutes after a 30-minute exposure to either (−)-morphine ($1 \times 10^{-4}M$), (+)-morphine ($1 \times 10^{-4}M$), β-endorphin ($1.4 \times 10^{-6}M$), substance P (*SP*) ($2.5 \times 10^{-6}M$), or no drug (X). The smallest contraction during the 3-minute exposure to β-endorphin was measured and expressed as a percentage of the contraction height before β-endorphin was added.

were rinsed three times; then 5 μl of β-endorphin (final concentration, $1.4 \times 10^{-7}M$) was added to the bath for 3-minute periods (followed by three more rinses) 1, 10, 30, and 60 minutes after the end of the pretreatment phase (Fig. 1). The smallest contraction during the periods in which β-endorphin was present in the bath was measured and expressed as a percentage of the contraction height before β-endorphin was added. The results were evaluated by a repeated-measures analysis of variance. Five groups of six vasa deferentia each were tested under these conditions. The five pretreatments were (−)-morphine, $1 \times 10^{-4}M$; (+)-morphine, $1 \times 10^{-4}M$; β-endorphin, $1.4 \times 10^{-6}M$; substance P (SP), $2.5 \times 10^{-6}M$; and no drug (control) (*10*). During the pretreatment phase, electrically stimulated contractions of vasa deferentia exposed to (−)-morphine, (+)-morphine, or SP showed excitation (Fig. 1), whereas contractions of β-endorphin-exposed vasa deferentia showed inhibition (Fig. 1).

Vasa deferentia pretreated with (−)-morphine showed subsensitivity to the inhibitory action of subsequently administered β-endorphin similar to that of vasa deferentia pretreated with β-endorphin (Fig. 2). This subsensitivity indicates that tolerance developed to the inhibitory action of (−)-morphine or β-endorphin during the pretreatment phase. This cross-tolerance decreased over time (in the absence of drug), reaching the control level by 60 minutes. The slopes for the five groups differ significantly (*11*). These results indicate that during the pretreatment phase, (−)-morphine exerted an inhibitory action that was masked by its concomitant excitatory action but then revealed by a state of tolerance similar to that shown by the β-endorphin-pretreated group.

This subsensitivity is in contrast with the supersensitivity to β-endorphin shown by vasa deferentia pretreated with SP or with (+)-morphine. The supersensitivity may compensate for the prolonged and exclusively excitatory action of SP or (+)-morphine. Substance P in particular exerted a potent excitatory action during the pretreatment phase. In a separate experiment, SP and β-endorphin exerted a mutually antagonistic action on the rat vas deferens. Substance P reversed the inhibitory action of β-endorphin, and β-endorphin reversed the excitatory action of SP. Although the receptors that mediate the actions of the two peptides probably differ, physiological antagonism in vivo may consist of such a mechanism (that is, opposing actions mediated by separate receptors) (*12*). To date, no endogenous opiate antagonist similar to naloxone (that is, an endogenous substance that displaces endorphin from its receptor site without exerting any agonistic action of its own) has been identified.

The potent inhibitory action of β-endorphin on the rat vas deferens suggests that this opiate peptide may modulate sexual function by inhibiting the release of neurotransmitters or by opposing the excitatory actions of other neuropeptides. This possibility was proposed previously (*13*).

In summary, the apparent lack of morphine action on the rat vas deferens (*3*) can now be seen as due to morphine's concomitant excitatory action masking the inhibitory one. At low to moderate concentrations of morphine (and of other opiates such as levorphanol), the two actions counterbalance each other, with a net result of no overt effect. At higher concentrations (for example, $10^{-5}M$), the balance is tilted in favor of the excitatory action, which then becomes manifest. It is unlikely that the rat vas deferens contains a unique species of opiate receptor that is exclusively responsive to the inhibitory actions of β-endorphin and etorphine and not to morphine or levorphanol (*6*). A more parsimonious view is that a common endorphin receptor mediates the inhibitory action of opiates and opioid peptides, whose action, in the case of morphine and levorphanol, is masked by the concomitant (and more potent) excitatory action of the opiates at the excitatory receptor (*14*). [Etorphine, in contrast to morphine and levorphanol but like β-endorphin, apparently exerts an exclusively inhibitory—or minimally excitatory—action (*15*).]

Finally, it is likely that the well-known withdrawal (contracture) phenomenon of the dependent, isolated guinea pig ileum is another example of the dual action of opiates, the contracture being the unopposed excitatory action of the opiate following naloxone blockade of the opiate's opposing inhibitory action at the endorphin receptor.

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8. This excitatory action of naloxone suggests that naloxone may exert part of its antagonistic action by an excitatory influence at the nonstereospecific receptor in addition to blockade of the stereospecific inhibitory receptor. This was previously suggested by D. R. Stevens and W. R. Klemm [*Science* **205**, 1379 (1979)], who observed withdrawal jumping in mice given a high dose of naloxone followed 1 minute later by morphine (50 mg/kg), or given morphine and then the high dose of naloxone 15 minutes later. This phenomenon supports our view that precipitated abstinence is due to selective naloxone blockade of the stereospecific inhibitory receptor; this results in unmasking of the excitatory presence of morphine at the nonstereospecific receptor. Stevens and Klemm hypothesized that an additional action may arise from an agonistic action of naloxone on nonstereospecific receptors.
9. The Krebs-Ringer contained the following (in grams per 10 liters): NaCl, 68.96; KCl, 3.54; $NaHCO_3$, 21.00; KH_2PO_4, 1.27; glucose, 19.82; and CaCl, 3.68.
10. All drugs and peptides were dissolved in sterile H_2O.
11. There was a significant effect of groups, F (4, 25) = 4.83, $P < .005$, and a significant effect of trials, F (3, 75) = 13.25, $P < .001$; the interaction of these factors was also significant, F (12, 75) = 2.83, $P < .003$. The groups differed

significantly at the first minute. F (4, 100) = 9.04, $P < .001$, and at the tenth minute, F (4,100) = 4.89, $P < .001$, but not at 30 and 60 minutes. Individual group comparisons with t-tests indicate that the groups pretreated with β-endorphin and (−)-morphine responded in a manner significantly different from that of the groups given (+)-morphine and SP at these times and from that of the control group at the first minute.

12. For example, it has been shown that the inhibitory action of clonidine in mouse vas deferens is due to its action at presynaptic α adrenoceptors, whereas the inhibitory action of opiates is mediated by presynaptic opiate receptors [M. G. C. Gillan, H. W. Kosterlitz, L. E. Robson, A. A. Waterfield, *Br. J. Pharmacol.* **66**, 601 (1979)]. The former is selectively blocked by phentolamine, the latter by naloxone. In rat vas deferens, both phentolamine and naloxone exert stimulatory actions that may be due in part to a blockade of the α-adrenoceptor or opiate receptor, respectively.
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14. These two may correspond to the high- and low-affinity opiate receptor-binding sites reported by G. W. Pasternak and S. H. Snyder [*Nature* **253**, 563 (1975)]. Whether the excitatory or inhibitory action is expressed probably depends on the ratio of excitatory:inhibitory receptors activated by the opiate.
15. The lack of excitatory action of etorphine in this assay in vitro parallels previous findings in vivo. A high dose of etorphine administered in the periaqueductal gray region (a site previously determined as mediating morphine analgesia) (*2*) in rats resulted in analgesia but not hyperreactivity, whereas morphine administered in this same brain region resulted in analgesia and hyperreactivity (*2*) [B. E. Thorn and R. A. Levitt, *Neuropharmacology* **19**, 203 (1980)].
16. I thank K. C. Rice for the gifts of (+)-morphine and (+)-naloxone. β-Endorphin (camel) and substance P were obtained from Peninsula Laboratories, Inc. This work was supported by grant DA 00367 from the National Institute of Drug Abuse.

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Elevated Cerebrospinal Fluid Norepinephrine in Schizophrenics: Confounding Effects of Treatment Drugs

The report by Lake *et al.* (*1*) presents evidence that the mean concentration of norepinephrine in the cerebrospinal fluid of a group of schizophrenics was elevated compared to that in healthy controls. A difficulty in studies of this type is the possible confounding effects of drugs used in the treatment of schizophrenia. Lake *et al.* report that the subjects received no medication for at least 2 weeks prior to the study. However, neuroleptic agents persist in the body for much longer periods of time. For example, chlorpromazine may be detected in the urine months after its administration has been discontinued (*2*). Furthermore, it is possible that drugs have persisting effects even after their disappearance from the body. Therefore, the effects of drugs used in the treatment of schizophrenia should be considered in the interpretation of the results reported by Lake *et al.*

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The comment by Lipsky is appropriate and warranted. Most of our 35 schizophrenic patients had been medication-free for more than 2 weeks before being studied, and the mean time since receiving the last dose of psychotic medication was about 4 ± 0.6 weeks, excluding several patients who had never been given antipsychotic drugs. [The concentrations of norepinephrine (NE) in the cerebrospinal fluid (CSF) of the patients who had never received antipsychotic drugs were similar to those measured in the patients who had received such drugs.] These periods are inadequate to ensure complete elimination of neuroleptic drugs, but to withhold therapeutic medication for 2 weeks is difficult; to withhold it for longer periods may be unethical.

There were elevated levels of NE in the CSF of the schizophrenic patients (*1*). The question by Lipsky seems to be whether some persisting antipsychotic drug effect could have been responsible for the elevation. The primary catabolite of brain NE metabolism is 3-methoxy-4-hydroxyphenylglycol (MHPG), and chlorpromazine lowers CSF levels of MHPG in schizophrenic subjects (*2*). In animals a variety of neuroleptic drugs significantly reduce brain NE levels (*3*). Eleven of the patients in our study were subsequently given pimozide, an antipsychotic drug, and restudied after at least 4 weeks of daily drug administration. Norepinephrine levels at the time of the second lumbar CSF tap were significantly reduced ($t = 3.04$, $P < .01$; Student's paired t-test) (*4*, *5*).

It appears that at least some antipsychotic drugs are associated with a decrease rather than an increase in NE levels. Thus, our finding of elevated levels of NE in CSF of schizophrenics is not explained by the effects of residual antipsychotic drugs.

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