Note added in proof: A fourth chemical company, using *N*-(3,4-dichlorophenyl)-hydroxylamine, has reported an outbreak of chloracne involving more than 40 workers and is attributing the disease to TCAB.

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Endorphins: Profound Behavioral Effects in Rats Suggest New Etiological Factors in Mental Illness

Abstract. *The endogenous morphinomimetic brain peptides Met⁵-enkephalin and α-, β-, and γ-endorphins have been evaluated in rats after intracerebrospinal fluid injection. β-Endorphin produces marked, prolonged muscular rigidity and immobility similar to a catatonic state, counteracted by the opiate antagonist naloxone; this effect occurs at molar doses 1/100 to 1/400 that at which the other peptides or morphine block the response to painful stimuli. All peptides evoked dose-related, naloxone-reversible, wet-dog shakes in rats that had not been exposed to drugs. β-Endorphin produced hypothermia, whereas γ-endorphin produced hyperthermia. Such potent and divergent responses to naturally occurring substances suggest that alterations in their homeostatic regulation could have etiological significance in mental illness.*

Five endogenous peptides with morphine-like biological properties have now been isolated from brain and characterized chemically: Met⁵-enkephalin and Leu⁵-enkephalin by Hughes *et al.* (*1*, *2*) from whole brain extracts; and α-, β-, and γ-endorphins by Guillemin *et al.* (*3–5*) from extracts of hypothalamus-neurohypophysis. Met⁵-enkephalin is structurally identical (*2*) to the fragment of residues 61 to 65 of the pituitary hormone β-lipotropin (β-LPH) (*6*). α-Endorphin (*3*) is structurally identical to β-LPH-(61-76), γ-endorphin (*4*, *5*) is structurally identical to β-LPH-(61-77), and β-endorphin [see (*7*)] to β-LPH-(61-91) (*5*, *6*). The pharmacological properties of endorphins have so far been screened through application of tests in vitro or in vivo previously used to characterize opiate agonists and antagonists (*1–5*, *8–15*). Most (*1–5*, *8–11*, *13*, *15*), but not all (*12*, *14*) of these effects are counteracted by specific opiate antagonists.

Although early reports of the effects of the endorphins suggested their relative equipotency to enkephalin pentapeptides in classical opiate assays (*8*, *9*), much recent work indicates that β-endorphin is from 2 to 40 times more potent than Met⁵-enkephalin in opiate displacement assays (*4*, *15*) and in analgesia assays (*10*), and 4 to 5 times more potent than Met⁵-enkephalin in the guinea pig ileum assay (*5*). We now report that endorphins affect several behavioral and physiological measures in addition to responses to noxious agents and that each of the peptides exhibits different dose-effect profiles on these measures: β-endorphin induces a marked catatonic state lasting for hours (Fig. 1) (*16*) at molar doses 1/100 that at which Met⁵-enkephalin transiently inhibits responses to

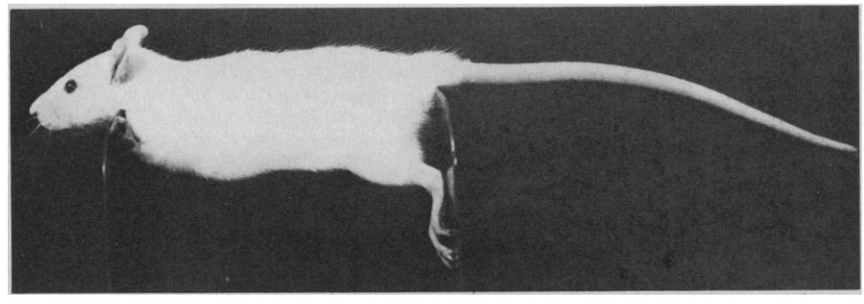

Fig. 1. Thirty minutes after the intracisternal injection of β-endorphin (14.9×10^{-9} mole) this rat exhibited sufficient rigid immobility to remain totally self-supporting when placed across metal bookends which are in contact only at the upper neck and base of the tail. Such postures were maintained for prolonged periods. Note the erect ears and tail, widely opened eyelids and extended lower limbs.

Table 1. Dose-effect profiles of endorphin peptides on rats after injection into a lateral ventricle. For each of the four test criteria (generalized rigidity, loss of corneal reflex, loss of tail-pinch response, and elicitation of wet-dog shakes), the number of animals tested with each substance is indicated, as are the dose ranges (nanomoles injected into the lateral ventricle in a 10-μl volume) over which the effect was obtained. For those cases in which no response was obtained, the highest dose tested is indicated. All positive effects except wet-dog shakes were also seen after intracisternal injections at similar dose ranges.

Substance	Rats (No.)	Rigidity		Loss of corneal reflex		Loss of tail-pinch reflex		Wet-dog shakes	
		Dose (nmole)	Response	Dose (nmole)	Response	Dose (nmole)	Response	Dose (nmole)	Response
Met^5-enkephalin	8	1030	None	340–1030	+	1030	None	340–1030	+
α-Endorphin	24	1210	None	120–1210	+	1210	None	76–1210	+
γ-Endorphin	9	281	None	281	+	281	None	110–281	+
β-Endorphin	9	7.4–14.9	+	3.0–14.9	+	3.0–14.9	+	3.0–14.9	+
Morphine	16	132	None	8–132	+	13.2–132	+	132	*
Saline	8		None		None		None		None

*One of three animals tested with 5.4 nmole of morphine showed episodic shakes, but this effect was not obtained on repeated testing at this dose, or at doses above or below this dose.

noxious agents (*10, 13, 14*). This potent effect of a naturally occurring substance suggests its regulation could have etiological significance in mental illness.

Peptides were injected into the cerebral spinal fluid of rats ($N = 90$) either through the cisterna magna (*17*) or permanently implanted lateral ventricular cannulae (*18*). Other animals were injected similarly with saline vehicle, morphine sulfate, or normorphine chloride. The peptides Met^5-enkephalin and α-, β-, and γ-endorphins were prepared by solid phase synthesis and purified (*19*). Solutions for tests were prepared daily from dry powders and used that day only (*20*).

After injections, rats were observed for gross abnormalities of behavior within open cages; responses to noxious stimuli were tested before injections and at 5-minute intervals after injections by evocation of corneal and eyelid reflexes, and by tail-pinch and pin-prick evoked responses. Rectal temperatures were monitored at 5 to 10 minute intervals for 1 to 4 hours. In general, the overall effects of the individual peptides were similar, regardless of the route of injection into the cerebral spinal fluid, but no gross behavioral effects or responses to noxious stimulation could be seen after intravenous injection in doses up to 1 mg/kg.

In terms of molar dose-effectiveness on the various properties examined, β-endorphin is clearly the most potent substance tested (Table 1). Within 5 to 10 minutes after injection, corneal reflexes disappeared, and general motor activity became depressed; transient episodes of nystagmus could be seen in this period. Within 15 minutes, at doses as low as 3×10^{-10} mole (administered intracisternally) animals showed a total lack of responsiveness to pin-prick or tail-pinch stimuli. After 15 to 30 minutes, animals injected with 7.4×10^{-9} mole of β-endorphin began to exhibit a profound catatonic state (*16*) characterized by extreme generalized muscular rigidity, loss of the righting reflex, and total absence of spontaneous movement. As a result of these effects, animals could be placed in and would retain abnormal body positions (Fig. 1) for indefinite periods. Respiratory movements shifted to the abdomen, and rectal temperature decreased ($-2.3°C \pm 0.1$; $N = 3$; 90 minutes after 7.4×10^{-9} mole). While in this state, the animals' eyes remained widely open (Fig. 1), with no spontaneous blinking, and showed loss of corneal and lid reflexes and often showed exophthalmos. With doses of 7.4×10^{-9} mole, rats remained in this state for approximately 2½ hours. Full spontaneous recovery then occurred rapidly, with no detectable aftereffects. All these actions of β-endorphin were reversed within seconds after intravenous injection of naloxone (1.0 mg per kilogram of body weight); after naloxone-induced recovery, rats frequently showed several episodes of "wet-dog" shakes (*21*) even though they had no prior exposure to endorphins, to exogenous opiates, or to opiate antagonists. Rats given seven daily intracisternal injections of 14.9×10^{-9} mole of β-endorphin ($N = 6$) continued to show the full set of responses and duration of action. However, 8 to 24 hours after as few as five daily injections, such animals also showed spontaneous wet-dog shakes.

The catatonic state induced by β-endorphin could not be simulated with the other endorphin peptides, even at considerably higher doses (Table 1). Doses of morphine or normorphine (8×10^{-9} to 132×10^{-9} mole) which suppressed reflexes to noxious stimulants did produce marked sedation with fixed open eyes and loss of corneal reflexes; but such animals retained considerable spontaneous locomotion (until doses of 1.3×10^{-7} mole), and showed no muscular rigidity, and only a moderate decrease in rectal temperature (0.8 to 1.2°C). At very high doses of α-endorphin, γ-endorphin, or Met^5-enkephalin, transient losses of corneal reflexes were also observed, and α-endorphin seemed more potent in this regard than either γ-endorphin or Met^5-enkephalin. No significant depressions of responsiveness to tail-pinch or pin-prick stimuli were observed with Met^5-enkephalin, α-endorphin, or γ-endorphin, but such effects (*13–15*) could have been missed by the 5-minute interval after injection and before testing began (*17, 18*). In contrast to the syndrome induced by β-endorphin, rats given γ-endorphin showed consistent elevations in rectal temperature (about $2.0° \pm 0.2°C$ at 30 minutes after 281×10^{-9} mole), and sometimes exhibited some degree of hyperresponsivity to sensory testing and handling, although there were individual variations in this response.

Met^5-enkephalin and α-, β-, and γ-endorphins also shared an unexpected, but highly reproducible effect: all produced acute episodes of wet-dog shaking behavior in animals not previously treated with drugs, the response beginning within 90 seconds after lateral ventricular injection (Table 1). These shaking episodes were more prolonged and intense with α- and γ-endorphins (25 or more vigorous shaking episodes 3 to 5 seconds in duration in the first 15 minutes). Rats treated with β-endorphin also exhibited shakes for the first 5 minutes after injection, but this effect was not observed after the onset of the catatonic state. Shaking responses were not observed in saline or morphine injected rats (Table 1) or after intracisternal injection of peptides (*21*). When animals were first treated with naloxone (2 mg/kg subcutaneously, 30 minutes), wet-dog shaking episodes from lateral ventricular peptide injections were almost completely abolished.

Thus, β-endorphin in relatively small amounts induced in rats a naloxone-re-

versible catatonic-like (*16*) state reminiscent of some aspects of schizophrenia. Depending on the dose level, α- and γ-endorphins and Met^5-enkephalin also exhibited subsets of the other behavioral and physiological effects of β-endorphin, in which morphine-like (that is, loss of response to noxious stimuli) actions appeared to be only a portion of a larger neuropsychopharmacological picture. As with the separate nicotinic and muscarinic actions of acetylcholine, all endorphin-mediated actions may not necessarily be explicable in terms of the alkaloid agonist morphine. Extremely puzzling in this regard is the finding that all three endorphin peptides and Met^5-enkephalin can elicit from the lateral ventricle, in drug-naive rats, the wet-dog shaking behaviors ordinarily attributable to opiate withdrawal, and that these responses are counteracted by naloxone (*21*). All of our observations suggest that normal variations—either qualitative or quantitative—in the homeostatic mechanisms regulating the postulated (*4*) conversion of β-LPH as a prohormone to its several endorphin cleavage products could constitute a system fundamentally involved in maintaining behavioral homeostasis.

Furthermore, we propose that subtle derangements in any of the biochemical or physiological mechanisms normally regulating β-lipotropin-endorphins homeostasis could lead to signs and symptoms of mental illness. Such a potential psychophysiological role of endorphins could logically be testable through the therapeutic administration of available opiate antagonists. In fact, at a recent presentation of these results and concepts (*22*), Terenius (*23*) reported Gunne and Lindström's observation that administration of naloxone to two chronic schizophrenics halted their auditory hallucinations within minutes. The ultimate identification of endorphin-sensitive behavioral events and specific treatment of their dysfunctional states may require the development of more specific "anti-endorphins" than those now available, and other naturally occurring brain peptides (*24*) have already been reported to be endorphin antagonists.

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17. Rats anesthetized with ether were fixed into a stereotaxic device, and 50 μl of test material was injected percutaneously into the cerebrospinal fluid through the cisterna magna. Placements were confirmed by withdrawal of spinal fluid before and after injection. Rats commonly remained anesthetized for 5 to 7 minutes.
18. Seven days or more before testing, stainless steel cannulae were stereotaxically implanted over the right lateral ventricle. Awake rats, briefly restrained by wrapping for attachment of injection tubing, were injected with 10 μl of test material administered over 60 seconds. Placements were confirmed by subsequent injection of 10 μl of methylene blue dye at the time animals were killed.
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The C-fragment of β-Lipotropin: An Endogenous Neuroleptic or Antipsychotogen?

Abstract. *Microinjection of the C-fragment (also called β-endorphin), which is amino acid sequence 61-91 of the endogenous pituitary hormone, β-lipotropin (β-LPH), in the periaqueductal gray of the rat resulted in profound sedation and catalepsy, while microinjection of smaller fragments—that is, methionine-enkephalin [sequence β-LPH-(61-65)] and its related pentapeptide, leucine enkephalin, and α-endorphin [sequence β-LPH-(61-76)] resulted in attenuated forms of this behavior. This indicates that the C-fragment is an important neuromodulator of the central nervous system. The similarity of this behavior to that seen after systemic administration to experimental animals of exogenous neuroleptics suggests that a disturbance in the bioavailability of this neuropeptide to receptor sites in brain—perhaps due to lack of enzymatic cleavage from the circulating parent hormone, β-lipotropin—may be an etiological factor in those psychopathological states for which the exogenous neuroleptics exert an ameliorative influence.*

Reports have suggested that various fragments from the pituitary hormone, β-lipotropin (β-LPH), have opioid-like analgesic properties in the central nervous system (CNS). High doses of methionine-enkephalin (which is sequence 61-65 of β-LPH) and its related pentapeptide, leucine-enkephalin, were reported to have transient analgesic effects resulting from intracerebroventricular (*1*) or intracerebral injections into the periaqueductal gray (PAG) (*2*) of rats or mice. Recently, interest has shifted to larger fragments of the hormone, that is, α-endorphin [sequence β-LPH-(61-76)] and the C-fragment (β-LPH-(61-91) (also called β-endorphin); the latter has been reported to exert potent long-lasting analgesia when injected intracerebroventricularly in the cat (*3*) and rat and mouse (*4*). We now report that the C-fragment exerts a profound sedative and cataleptic influence when microinjected in the PAG, a site shown to mediate multiple morphine action, including potent analgesia (*5*), similar to the action of