foragers to ignore a resource where they had previously obtained a reward. To explain the results, I postulate that stingless bees abandoned the study plots. During the 75 hours in which Africanized honey bees were observed, I saw no aggression by the bees. Africanized honey bees also fail to exhibit interspecific aggression at artificial nectar feeders (*10*). If Africanized honey bees interfered with stingless bees, this was incidental to their foraging or involved a subtle chemical interaction.

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15 March 1978; revised 30 May 1978

Opiate Effects After Adrenocorticotropin or β-Endorphin Injection in the Periaqueductal Gray Matter of Rats

Abstract. *Injections of adrenocorticotropic hormone (ACTH) into the periaqueductal gray matter of drug-naive rats resulted in a dose-dependent opiate abstinence syndrome characterized by fearful hyperreactivity and explosive motor behavior. Injecting shorter chains of ACTH caused attenuated forms of this behavior. Injections of β-endorphin at this same site caused opposite behavior: sedative, analgesic, and catatonic. If the effects of morphine are mediated by two classes of receptor) and the other which is not stereospecific and naloxone-insensitive—the endogtor)—and the other which is not stereospecific and naloxone-insensitive the endogenous ligand of the second receptor may be ACTH. The neuropeptides ACTH and endorphin may be part of an integrated neuromodulatory system, and the opiate abstinence syndrome may be the result of an altered interaction between the two receptor systems.*

We have previously reported (*1*) that morphine effects in the central nervous system (CNS) appeared to be mediated by two classes of receptors: an endorphin receptor that was naloxone-sensitive, showed stereospecific affinity for opiates, and mediated the analgesic, catatonic effects of morphine; and a second receptor that was naloxone-insensitive, showed nonstereospecific affinity for opiates, and mediated the hyperexcitability [explosive motor behavior (EMB)] induced by morphine. We hypothesized that animals given morphine systemically did not normally manifest EMB because of a masking inhibitory action exerted by the endorphin receptor, which was simultaneously activated by morphine. Furthermore, since the morphine abstinence syndrome is similar or identical to the behavioral syndrome of EMB, we suggested that opiate abstinence symptoms might be due to morphine exciting the second receptor when naloxone blockade of the endorphin receptor ended the inhibitory action. However, the endogenous ligand for the second receptor was not known.

We now report that injections of the endogenously occurring peptide adrenocorticotropic hormone (ACTH) in the periaqueductal gray matter (PAG) of opiate-naive rats result in behavior similar to the opiate abstinence syndrome. We suggest that the mechanism underlying opiate abstinence behavior may consist of the stimulation by morphine of an ACTH receptor after removal (by naloxone blockade) or weakening (by tolerance development) of the inhibitory action of the endorphin receptor. This implies that the multiple pharmacological effects of opiates are dissociable and mediated by separate receptors; that is, narcotic analgesia is mediated by the endorphin receptor, and narcotic dependence by the ACTH receptor.

Adult male Wistar rats, weighing approximately 300 to 350 g, were each surgically implanted with bilateral intracerebral cannulas aimed at PAG sites (*2*). The guide cannulas were made of 30-gauge stainless steel tubing (outer diameter = 0.30 mm); the tips were aimed at sites just lateral to the aqueduct: 1 mm anterior to lambda, 0.75 mm lateral to the midline, and 4 mm below the level of the skull surface (we used horizontal head position, with bregma and lambda at the same horizontal plane). Injection needles were prepared from 35-gauge stainless steel tubing (outer diameter = 0.13 mm) and calibrated to extend precisely 2 mm beyond the tip of the guide cannula. From 5 to 7 days elapsed before any testing was conducted. Placement of cannulas was subsequently histologically verified in all animals.

Injection of ACTH(1–24) in the PAG resulted in all nine animals becoming hyperreactive to previously neutral auditory and visual stimuli and making 60-cm-high leaps in repeated attempts to escape from the plastic bin. (Normal rats are incapable of such high leaps.) Other signs of excitation such as tachycardia, tachypnea, and hyperthermia were also observed. Onset of jumping occurred approximately 10 minutes after the injection and lasted approximately 30 minutes; after this time other signs were observed, such as wet-dog shakes, teeth chatter, ear blanching, abnormal posture, squeal on touch, scratching, grooming, and exploration (rearing on hind legs) (*3*). These behaviors have been termed recessive signs and are suppressed during the period of occurrence of dominant signs such as jumping (*4*). Injections into the PAG of lower doses of ACTH(1–24) or of shorter chains of the peptide, such as melanocyte-stimulating hormone (α-MSH), which coincides with ACTH(1–13), and ACTH/MSH(4–10) resulted in an immediate onset of recessive signs lasting approximately 20 minutes, but never in the dominant sign of jumping. These results (Table 1) suggest that the longer chain ACTH(1–24) resulted in fuller expression of the abstinence syn-

0036-8075/78/0915-1032$00.50/0

drome by providing a better fit for the receptor. The relatively high doses of ACTH employed in our study indicate that ACTH may undergo rapid enzymatic degradation after injection into brain, with only small amounts ever reaching receptor sites (*5*).

We and others (*6*) have reported that the midbrain PAG is a unique site of morphine action. Morphine injections in the PAG resulted in two seemingly paradoxical behavioral syndromes (Table 2): (i) analgesia, accompanied by a late-occurring catatonia (1 hour until onset) and (ii) an early-occurring (within 10 minutes) fearful hyperreactivity characterized by EMB. Systemic injections of naloxone (1 mg per kilogram of body weight), an opiate antagonist, blocked or reversed the analgesia and catatonia but not the EMB. Injections of β-endorphin in the PAG at a dose of 4 μg resulted in analgesia and catatonia but never in EMB. These effects were fully reversible by naloxone. High doses of (+)-morphine, the inactive stereoisomer of natural (−)-morphine, injected in the PAG, resulted in EMB but not in analgesia and catatonia. Systemically administered naloxone (1 mg per kilogram of body weight) did not block or reverse the EMB. Among various nonopiate compounds injected into the PAG (including atropine, substance P, adenosine, somatostatin, FK 33–824, the synthetic analog of enkephalin, methionine-enkephalin, and leucine-enkephalin), none resulted in EMB. The EMB syndrome is similar to that seen after injections of either (+)- or (−)-morphine in the PAG, suggesting that a common mechanism may underlie both. We previously characterized the receptor that mediates EMB as having nonstereospecific recognition of morphine and insensitivity to naloxone and being activated only by high local concentrations of morphine (*1*); we now report that it is also activated by ACTH, a result that suggests that ACTH may be the endogenous ligand for this receptor. The receptor that mediates analgesia and catatonia was previously characterized as having stereospecific recognition of morphine and sensitivity to naloxone; we suggested that its endogenous ligand was β-endorphin (*7*).

Recent evidence suggests that ACTH and β-lipotropin (β-LPH) (the putative precursor of β-endorphin) share a common precursor with a molecular weight of either 31,000 (*8*) or 28,500 (*9*). Both β-LPH and ACTH have been reported in the same pituitary cell; after stress, ACTH and β-endorphin are simultaneously released, causing a fall in levels of both peptides in the pituitary (*10*). Whether brain is a target organ for this release is unknown, since it is uncertain how these pituitary polypeptides could reach the brain (*11*), despite reports of a reverse portal system from pituitary to hypothalamus (*12*). We have observed diametrically opposed behavioral patterns after injection of either ACTH or β-endorphin (*13*) in the PAG, although both behaviors are seen in the same animal after morphine injection at this same site. These observations suggest that administration of an exogenous opiate such as morphine results in the stimulation of both classes of receptors, perhaps by fortuitous receptor occupancy, which are normally stimulated only by their endogenous ligands.

Our results suggest a modulating interaction between these two classes of receptors (Table 2). When rats were first treated with morphine given intraperitoneally, presumably resulting in activating the inhibitory influence of the endorphin receptor, and were then injected with morphine in the PAG, they became analgesic and catatonic but did not manifest EMB. Treatment of animals with naloxone given intraperitoneally, presumably resulting in blockade of the endorphin receptor and removal of its inhibitory influence, followed by morphine injection in the PAG caused EMB but not analgesia and catatonia (*14*). Treatment with morphine intraperitoneally before ACTH injection in the PAG blocked the occurrence of EMB, while treatment with naloxone intraperitoneally before ACTH injection in the PAG did not block EMB. When morphine is given systematically, we suggest that the opiate activates multiple neuronal circuits in the CNS. Some of these may have endorphin receptors which exert an inhibitory influence on the ACTH receptor. Thus the excitatory effects of morphine at the ACTH receptor would be masked by its inhibitory effects at the

Table 1. Percentage of animals displaying abstinence signs after injection of peptides into the periaqueductal gray of drug-naive rats. The numbers in parentheses indicate the number of animals in each group. Abbreviations: ACTH, adrenocorticotropic hormone; α-MSH, melanocyte-stimulating hormone.

Abstinence signs	Percentages			
	ACTH (1–24)		α-MSH 10 to 34 μg (6)	ACTH/MSH (4–10) 10 to 28 μg (5)
	50 μg (9)	25 μg (4)		
Dominant sign				
Jumping (> 25 times)	100	0	0	0
Recessive signs				
Teeth chatter	56	25	33	20
Wet-dog shakes	56	100	83	20
Squeal on touch	100	25	83	40
Rear on hind legs	44	50	67	40
Abnormal posture	44	25	17	0
Hyperthermia	89	50	100	60
Mean body temperature (°C)	38.3	37.6	38.1	37.5

Table 2. Effect of intraperitoneal naloxone or morphine on the presence of analgesia or explosive motor behavior (EMB) after bilateral intracerebral injection in the periaqueductal gray matter (PAG) of drug-naive rats of opiate or peptide. Doses for intraperitoneal injections (number in parentheses) are expressed as milligrams per kilogram of body weight.

Intraperitoneal injection		PAG injection		Analgesia	EMB
Drug	Amount (mg/kg)	Drug	Amount (μg)		
Previous results					
None		Morphine	10	+	+
Naloxone	1	Morphine	10	−	+
None		β-Endorphin	4	+	−
Naloxone	1	β-Endorphin	4	−	−
None		(+)-Morphine	80	−	+
Naloxone	1	(+)-Morphine	80	−	+
New results					
Morphine	30	Morphine	10	+	−
Morphine	30	ACTH	50	+	−
Naloxone	1	ACTH	50	−	+
Morphine + naloxone	30 + 1	Morphine	10	−	+
Morphine + naloxone	30 + 1	ACTH	50	−	+

endorphin receptor located at other CNS sites. We further suggest that it is the residual presence and not the absence of morphine that is correlated with opiate abstinence behavior (*15*). This view is compatible with the finding (*16*) that opiate abstinence behavior can be precipitated in vivo by naloxone as early as 10 minutes after a high dose of morphine to drug-naive animals. It is further supported by an inverse correlation between the degree of opiate dependence and the dose of naloxone required to precipitate abstinence symptoms (*17*); this can be viewed as being due to the selective development and increase of tolerance to morphine by the endorphin receptor but not by the ACTH receptor. This view would also account for the observation (*18*) that opiate abstinence can be precipitated by naloxone in mice that have been addicted to opiates as long as 43 days after drug cessation only if they are first given morphine, presumably needed to activate the ACTH receptor: a time-dependent attenuation of opiate tolerance in the endorphin receptor is also suggested by the higher doses of naloxone needed to initiate jumping.

Although experiments with isolated guinea pig ileum (*19*) and neuroblastoma X glioma hybrid cells (*20*) support the proposal that opiate dependence consists of the development of a latent hyperexcitability to compensate for the inhibitory action of opiates (*21*), our results suggest that in the brain another mechanism of opiate action exists, mediated by two classes of receptors—the endorphin receptor and the ACTH receptor (*22*)—and that the opiate abstinence syndrome is caused by an altered equilibrium in this system.

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23 March 1978; revised 20 June 1978

Selective Depression of Serum Growth Hormone During Maternal Deprivation in Rat Pups

Abstract. *Maternal deprivation was associated with a decline in immunoreactive growth hormone in the serum of rat pups. Pups that were returned to the mother showed a rapid reversal in this deprivation-induced decrease. The change in growth hormone concentration was not accompanied by changes in the concentrations of prolactin, thyrotropin, or corticosterone in the serum, but were correlated with alteration in the activity of ornithine decarboxylase in the brain. Treatment of neonatal rat pups with cyproheptadine, a serotonin antagonist that suppresses growth hormone secretion, resulted in a significant decline in both serum growth hormone concentration and brain ornithine decarboxylase activity. These findings suggest that maternal deprivation elicits a specific suppression of growth hormone release which mediates the decrease in ornithine decarboxylase activity. The study is consistent with clinical findings of impaired growth hormone "responsivity" in human maternal deprivation syndrome.*

Interruption of mother-infant interactions is a "stressful" experience that has adverse biochemical, physiological, and behavioral consequences for the offspring (*1*). Previously, we reported that ornithine decarboxylase (E.C. 4.1.1.17; ODC) activity, a sensitive index of organ growth and differentiation, decreases in the brain and heart of rat pups after just 1 hour of maternal deprivation and increases rapidly when the pups are returned to the mother (*2*). The changes in ODC which result from maternal deprivation are directly associated with the removal of active mothering behavior and not secondary to malnutrition or perturbations in body temperature (*3*). Furthermore, the decline appears to be mediated by a metabolic or endocrine signal rather than from a direct neural stimulus (*3*).

Although we have shown that adrenal

0036-8075/78/0915-1034$00.50/0