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. I thank H. O. J. Collier for helpful discussions and J. Simoons of Organon Pharmaceutical for ACTH. Supported by NIDA grant 00367.

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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

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steroids are not involved in the deprivation-induced decline in ODC that occurs during maternal deprivation (*3*), evidence from several sources suggests that changes in the serum concentrations of other hormones could mediate the decline. Peripheral administration of growth hormone (GH) and prolactin (protein hormones) to preweanling rat pups causes a rapid increase in brain ODC activity (*4*). The serum concentrations of several polypeptide and protein hormones, including the anterior pituitary hormones GH, prolactin, and thyrotropin (TSH), as well as hormones of peripheral origin such as corticosterone, thyroxine, and renin change in response to many types of stress in both neonatal and adult rats (*5*). Furthermore, impaired secretion of GH in response to insulin and decreased adrenocorticotropin (ACTH) secretion have been observed in human "maternal deprivation-failure-to-thrive" syndrome, whereas other indices of neuroendocrine function such as serum thyroxine concentration appeared to be normal (*6*). These reports suggest that decreased secretion of one or more anterior pituitary hormones might be involved in the biochemical sequelae of maternal deprivation stress. To investigate this possibility, we have measured serum GH, prolactin, TSH, and corticosterone concentrations and brain ODC activity during maternal deprivation in preweanling rat pups.

Gravid Sprague-Dawley rats (Zivic-Miller) were obtained 1 week before delivery, housed individually, and given free access to food and water. When the pups were 10 days old, the mother and pups were transferred from the vivarium to our laboratory. All pups were removed from the mother and either placed in a warm incubator (*7*) or returned to the mother. Previous experiments demonstrated that the biochemical response to maternal deprivation was not influenced by the presence or absence of the home cage environment, including used or clean shavings, or littermates (*3*). In the present experiments, sibling pups were maternally deprived as a group by being placed in a plastic cage containing clean shavings. At the end of an experiment, all pups were killed rapidly by decapitation. Trunk blood was collected in polystyrene tubes and allowed to clot at 4°C. Samples were centrifuged, and serum from two to three pups was pooled and stored at −20°C. Prolactin, TSH, and GH were assayed in each sample by radioimmunoassay with reagents supplied from the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD) rat pituitary program. Assays were performed according to the recommendations supplied with the NIAMDD kit. Results are expressed in terms of NIAMDD rat PRL-RP 1, GH-RP 1, and TSH-RP 1. Serum corticosterone was measured by the competitive protein binding method described by Cameron and Scarisbrick (*8*), with rat serum being used as the souce of binding protein and Sephadex G-25 being used to separate bound and unbound corticosterone as reported by Bassett and Hinks (*9*). The activity of ODC was determined by measuring release of ^{14}CO from DL-[1-^{14}C]ornithine (43 mCi/mole) as described previously (*10*).

Table 1. Concentration of growth hormone in serum and brain ODC activity in 10-day-old rat pups maternally deprived and killed immediately or maternally deprived and returned to the mother for the indicated times before being killed. Results are expressed as percentages of nondeprived controls ± standard error of the mean. Growth hormone concentrations in controls were 29 ± 1 ng/ml.

Experimental conditions	Growth hormone		Brain ODC activity	
	N	Percentage of control	*N*	Percentage of control
Nondeprived	30	100 ± 3	40	100 ± 10
Deprived				
1 hour	10	53 ± 8*	6	58 ± 7*
2 hours	16	60 ± 6*	6	36 ± 7*
6 hours	5	59 ± 12*	6	41 ± 3*
Deprived and returned				
15 minutes	5	155 ± 32		
1 hour	8	99 ± 7	6	252 ± 17*
2 hours	13	94 ± 7	6	268 ± 31*
4 hours	5	100 ± 4	6	150 ± 8*

*Statistically different from control ($P < .05$, Student's *t*-test).

Because decreased GH secretion in response to pharmacologic stimuli had been reported to occur in maternally deprived human infants, we measured the concentration of GH in the serum of deprived pups. Pups were deprived for 1, 2, or 6 hours and then killed, or they were deprived for 2 hours and put back with the mother for 15 minutes, 1, 2, or 4 hours before being killed. Control pups were left with the mother for the entire time period of the experiment. Serum GH concentration in deprived pups was significantly lower than in the control pups throughout the deprivation period but rapidly increased to normal levels when pups were returned to the mother (Table 1). Fifteen minutes after pups were returned to the mother, their GH values appeared elevated relative to nondeprived controls, but this increase was not statistically significant. The activity of ODC in the brains of deprived pups decreased rapidly and remained lower than in controls throughout the deprivation period, but increased rapidly when pups were returned to the mother. The ODC activity in brains of deprived pups actually exceeded that of control pups as early as 1 hour after return and gradually decreased to control levels.

These data indicate that decreases in both brain ODC activity and serum GH occur during maternal deprivation, and that these decreases are reversed when pups are returned to the mother. The increase in serum GH concentration in deprived pups that was observed 15 minutes after they were returned to the mother suggests that a spike in GH secretion might have preceded the overshoot in brain ODC activity. The reason for the overshoot in brain but not in heart ODC activity which occurs when pups are returned to the mother is unclear.

The decrease in GH associated with maternal deprivation could reflect a specific inhibition of GH secretion, a general inhibition of secretion or synthesis of anterior pituitary hormones (panhypopituitarism), or one component of a pattern of response to stress involving several hormones. Measurement of serum concentrations of other anterior pituitary hormones could help distinguish among these possibilities. If secretion of all hormones of the anterior pituitary were suppressed during maternal deprivation, then the serum concentration of prolactin and TSH as well as GH would decrease. If maternal deprivation represented an intense "nonspecific" stress, a more complex pattern similar to that observed after immobilization, electric shock, venipuncture, exercise, or exposure to a novel environment would be expected; that is, a decrease in GH concentration and an increase in ACTH and prolactin concentrations accompanied by an increase or decrease in TSH, depending on the intensity, duration, and type of stimulus (*5*, *11*).

To determine whether the decrease in GH associated with maternal deprivation represented a selective suppression of

Table 2. Serum growth hormone, prolactin, and thyrotropin concentrations in 10-day-old rat pups deprived for 2 hours and killed. Results are expressed as nanograms per milliliter ± standard error of the mean ($N = 16$).

	Nondeprived	Deprived	Percentage of control
Prolactin	1.4 ± 0.1	1.7 ± 0.2	121
Thyrotropin	237 ± 30	216 ± 10	94
Growth hormone	28 ± 4	18 ± 2	64*

*Statistically different from control ($P < .05$, Student's t-test).

Table 3. Effect of cyproheptadine on serum GH and brain ODC activity in 9- to 11-day-old pups. The pups were injected intraperitoneally with cyproheptadine (10 mg/kg) and killed 1¼ hours later. Serum GH and brain ODC activity were then determined. Values are means ± standard error. The ODC activity is expressed in terms of counts per minute per 3 mg of protein per 30 minutes.

Group	N	Serum GH		Brain ODC	
		Concentration (ng/ml)	Percentage of control	Activity	Percentage of control
Control	6	28 ± 8	100	8710 ± 360	100
Cyproheptadine	6	8 ± 3*	34	5270 ± 380*	60

*Significantly different from saline-injected control ($P < .02$).

GH secretion, we measured serum GH, TSH, and prolactin in pups separated from the mother for 2 hours, a time period sufficient for maximum depression of both brain ODC activity and serum GH concentration. After 2 hours of deprivation, GH levels were decreased by 40 percent, whereas serum prolactin and TSH levels were unchanged (Table 2). In a second experiment, serum corticosterone levels, which reflect pituitary ACTH secretion, were measured in deprived and nondeprived pups. Neither 90 nor 120 minutes of deprivation caused a change in serum corticosterone levels relative to nondeprived littermate controls: after 90 minutes of deprivation, the concentration of corticosterone was 2.1 ± 0.2 μg/100 ml, whereas the concentration in controls was 2.0 ± 0.1 μg/100 ml; after 120 minutes, the concentration of corticosterone was 2.0 ± 0.3 μg/100 ml, whereas in the control it was 1.8 ± 0.2 μg/100 ml. These data suggest that the decrease in plasma GH induced by maternal deprivation represents a fairly specific endocrine response and is not simply the reflection of a general suppression of the anterior pituitary or a "nonspecific stress" reaction.

The finding that maternal deprivation evoked a decline in brain ODC activity and serum GH concentration that was unaccompanied by changes in prolactin, TSH, or corticosterone suggested that decreased secretion of GH might be responsible for the decline in brain ODC activity. However, while there is considerable evidence that increased concentration of serum GH results in increased tissue ODC activity, the effects of lowered serum GH on tissue ODC activity are less well known (*2*, *3*). We therefore measured brain ODC activity in rat pups that remained with their mother but were treated with cyproheptadine, a serotonergic blocking agent that decreases serum GH in neonatal rats (*12*). Pups were injected with cyproheptadine (10 mg/kg) or saline and killed 1¼ hours later. Table 3 shows that both the serum GH concentration and the brain ODC activity were significantly lower in cyproheptadine-treated pups than in controls. These findings suggest that suppression of GH secretion results in a decrease in brain ODC activity in neonatal rat pups and support the hypothesis that decreased serum GH concentration during maternal deprivation mediates the decline in brain ODC activity.

Our data indicate that the reduction in serum concentration of GH following maternal deprivation represents a fairly discrete neuroendocrine response rather than a general suppression of the pituitary. That both brain ODC activity and serum GH concentrations decrease during maternal deprivation or after cyproheptadine administration further suggest that the decline in serum GH mediates the maternal deprivation-induced decrease in brain ODC activity. We have shown previously that this decline in brain ODC activity appears to result from removal of a metabolic or hormonal stimulus and that peripheral administration of GH markedly stimulates brain ODC activity (*2*, *3*). These results strongly support the hypothesis that maternal deprivation elicits a neuroendocrine response involving a decline in serum GH levels and that this decrease in GH is responsible for the decrease in brain ODC activity.

These data also are consistent with findings of clinical studies that the decreased GH response to the arginine stimulation test that occurs during human maternal deprivation may be involved in the suppression of growth which occurs in this syndrome. The similarity of the neuroendocrine response to maternal deprivation in preweanling rat pups to the neuroendocrine profile of "failure-to-thrive" children suggests that the removal of rat pups from the mother might provide a useful animal model of the human disease. Further studies are necessary to characterize the endocrine response to maternal deprivation more thoroughly and to determine whether the decline of serum GH represents decreased synthesis or release, or changes in peripheral clearance of the hormone similar to that reported for prolactin (*13*).

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