as an additional pollutant. At 0.5 ppm of DEHA, there was an initial 1-hour inhibition of smog and then subsequently higher pollution levels than in the control case. At very high DEHA concentrations, inhibition was effective for the entire 6-hour experimental period. In all these experiments DEHA was added initially and allowed to decay away. The kinetic results reported here predict that DEHA maintained at 0.1 to 0.2 ppm for the entire experimental period could produce total inhibition of smog. It would be very interesting to test this hypothesis.

Our preliminary mechanistic information is consistent with OH abstraction of the H of the DEHA hydroxyl group in both the aqueous and gaseous phases, as opposed to the abstraction of the H of the ethyl groups or OH addition to DEHA. The results from studies in aqueous solution indicate a rapid reaction between OH and DEHA but one that is well below the diffusion-controlled rate. This result implies that hydration of the DEHA decreases the probability of a reactive encounter. This would be the expected result if the OH abstracts the H of the DEHA hydroxyl group and the oxygen atom of DEHA is the site of hydrogen bonding to the solvent. The solvent would then act to shield the H of the DEHA hydroxyl group from attack by OH.

We are presently investigating the gas-phase reactivity of HO_2 (*12*) and H with DEHA and determining the absorption spectra of the products of the primary processes. Our aqueous studies have been extended to include other means of measuring OH rates and of determining DEHA reactivity with e^-_{aq}, CO_3^-, and O_x^-. We are making further attempts to identify the intermediates and final products, using the techniques of chemically induced dynamic electron polarization (CIDEP), chemically induced dynamic nuclear polarization (CIDNP), electron spin resonance, and nuclear magnetic resonance coupled with pulse radiolysis (*9*).

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12. Preliminary results on the HO_2-DEHA reaction indicate a rate constant of about 2×10^8 M^{-1} sec^{-1}, which would mean that the DEHA would also be effective at influencing the atmospheric HO_2 concentration.
13. This work was carried out under the auspices of the Division of Physical Research of the U.S. Energy Research and Development Administration.

21 March 1977; revised 16 May 1977

β-Endorphin and Adrenocorticotropin Are Secreted Concomitantly by the Pituitary Gland

Abstract. *The opiate-like peptide β-endorphin and adrenocorticotropin are concomitantly secreted in increased amounts by the adenohypophysis in response to acute stress or long-term adrenalectomy as well as in vitro in response to purified corticotropin releasing factor and other secretagogues. Conversely, administration of the synthetic glucocorticoid dexamethasone inhibits the secretion of both adrenocorticotropin and β-endorphin. Thus, both hormones possess common and identical regulatory mechanisms and there may be a functional role for circulating β-endorphin.*

The two biologically active polypeptides adrenocorticotropin (ACTH) and β-endorphin have been shown by Mains, Eipper, and Ling (*1*) originally to be part of a much larger precursor glycoprotein (31,000 daltons, referred to as 31K-precursor), as synthesized by the cloned pituitary cells of the (mouse) cell line AtT-20/D-16v. The common precursor concept is supported by earlier data on immunocytochemistry of normal pituitary tissue: ACTH [1–39] (residues 1 to 39); β-lipotropin (β-LPH [1–91]) the immediate endorphin-precursor, and the biologically active peptides β-endorphin (that is, β-LPH [61–91]) and α-endorphin (that is, β-LPH [61–76]) are all present in the same cells in the anterior and intermediate lobes of the pituitary gland (*2*). These observations raise the possibility that the biologically active forms of ACTH and β-endorphin might be normally secreted concomitantly. While work over the last 30 years has elucidated the physiological mechanisms involved in the secretion of ACTH, particularly as it relates to the response to stress, the recently discovered endorphins could not be studied in similar circumstances until specific methods to measure their concentration in blood or tissue extracts became available. We have recently devised, described, and validated such methodology (*3*). We now show that, in all conditions studied so far, ACTH and β-endorphin are secreted simultaneously by the pituitary gland.

Thirty-three male rats (Holtzman, 200 ± 15 g of body weight) were kept for 3 weeks, six animals per cage, with lights on at 0700 hours and off at 2000 hours. For studies on acute response to stress, each animal had the right tibia-fibula broken instantaneously, trunk blood being collected by decapitation in tubes containing EDTA (sodium salt) at intervals ranging from 60 seconds to 30 minutes. To study a possible adrenal steroid feedback mechanism on the secretion of endorphin, rats received dexamethasone acetate, a total of 12 mg over 12 days, in two daily subcutaneous injections; other animals were bilaterally adrenalectomized and maintained for 16 weeks on 1 percent NaCl as drinking fluid. Finally three rats from a larger pool of hypophysectomized animals were stressed and processed as above 10 months after total hypophysectomy.

As is shown in Fig. 1 and Table 1, plasma and pituitary concentrations of ACTH and β-endorphin vary concomitantly and in remarkable parallelism in all experimental situations described here, indicating that ACTH and endorphins are secreted simultaneously.

Hypophysectomy abolishes the response to stress, indicating that the peptides (β-endorphin and ACTH) measured in these studies are of hypophysial origin. Addition of purified corticotropin-releasing factor and of other secretagogues to monolayer cultures of adenohypophysial cells also stimulates concomitant secretion of immunoactive ACTH and β-endorphin (*4*). Thus it would appear that the regulatory mechanisms (hypothalamic releasing factor,

feedback by glucocorticoids) involved in the secretion and biosynthesis of both ACTH and β-endorphin are common and identical (*5*).

The antiserum used here (RB100–10/76) to measure plasma and tissue levels of β-endorphin is directed to the region Asn^{20}-His^{27} (Asn, asparagine; His, histidine) of the primary sequence of β-endorphin (*3*). Because of the commonality of that sequence in β-endorphin and its precursor β-LPH, the antiserum recognizes the two peptides as well as 31K-precursor on an equimolar basis (*1*, *3*). Estimation of the molecular size by well-calibrated filtration columns (P-60 Biogel in 4*M* guanidine hydrochloride) has shown that the major component (about 90 percent) in the rat pituitary extracts recognized by the antiserum used here corresponds to β-endorphin (3200 daltons) with a minor (about 10 percent), larger component corresponding to β-LPH (10,000 daltons) and a still smaller amount (< 1 percent) corresponding to 31K-precursor; moreover, gel filtration on Sephadex G75 of the plasma of stressed rats shows also a large peak of immunoreactive endorphin with a retention coefficient identical to that of synthetic β-endorphin.

In other studies, we have observed that the elevated spontaneous secretion of ACTH by fragments incubated in vitro of the hyperplastic pituitary of a patient with the Nelson syndrome was also accompanied by correspondingly elevated secretion of β-endorphin (*6*); also extracts of an ectopic ACTH-secreting adenoma of the pancreas were shown to contain ACTH, β-LPH, and β-endorphin (*7*). In normal rats, electric shocks to the footpad, a type of acute stress other than that described above, also led to elevated levels of plasma β-endorphin.

Thus, the conclusion appears inescapable that, in all conditions studied here in which the pituitary secretes ACTH, it also secretes β-endorphin.

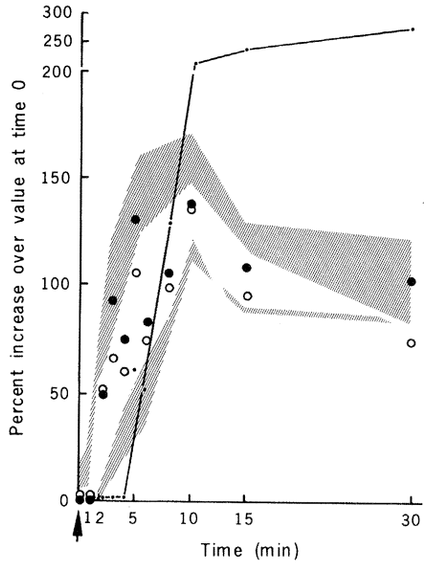

Fig. 1. Plasma levels of ACTH (closed circles) and β-endorphin (open circles) measured by radioimmunoassays (*3*, *15*) in trunk blood obtained from rats killed at times shown on the abscissa; acute stress occurred at time zero. Solid line shows plasma levels of adrenal corticosterone measured by fluorometry (*16*). Shaded areas show confidence limits of the measurements. The correlation coefficient, ρ, between the two populations of ACTH and β-endorphin concentrations is 0.9708 for values of means (d.f. = 20) and 0.7785 for all individual values (d.f. = 64).

Since the early studies of Selye (*8*), ACTH has been recognized as the primary pituitary hormone secreted in response to acute stress in all species studied. The teleological proposal has been to relate the acute secretion of ACTH to the corresponding immediate activation of the adrenal cortex for the secretion of glucocorticoids necessary for immediate increase of neoglucogenesis and the ensuing availability of energy rich carbohydrates. In many, although not all, species, prolactin and growth hormone may also be released in similar conditions (*9*). Growth hormone, ACTH, and prolactin are pituitary responses to stress, all affecting metabolism. Our results show that β-endorphin is also released in response to acute stress.

Evidence has been presented (*10*) that peripheral injection of β-endorphin in mice produces analgesia; this result was obtained with doses of the peptide leading to plasma concentrations four to five orders of magnitude greater than those observed in response to stress. We have failed to observe in rats such central effects of similarly large doses of β-endorphin (up to 20 mg per kilogram of body weight) injected in a peripheral vein. This is in contradistinction to the profound effects (analgesia, catatonia) exerted by small amounts (≥ 0.5 μg) of β-endorphin when injected directly in the brain and unanimously observed (*11*). It may be that our current criteria for assessing possible central effects of the circulating endorphins are naive and of small power. Should such effects of the peripherally released β-endorphin eventually be detectable, our results demonstrate that the system necessary for its immediate secretion and availability in response to stress is highly functional and coupled with activation of the ACTH-adrenal cortex axis.

Thus, a holistic response of the organism to stress would involve the immediate secretion of pituitary hormones, some (such as ACTH and growth hormone) involved in somatotropic (metabolic) adaptive reactions, whereas others (such as β-endorphin, β-melanocyte stimulating hormone, or γ-lipotropin) are endowed with neurotropic (*12*), compartmental, or psychotropic adaptive reactions. Suggestive of a vascular pathway from pituitary to brain, recent observations (*13*) have shown high

Table 1. Plasma and pituitary concentrations of adrenocorticotropin (ACTH) and β-endorphin as modified by adrenalectomy, administration of dexamethasone, and for plasma levels, as modified by hypophysectomy. Plasma and pituitary samples were obtained in nonstress conditions, after decapitation in all groups, except in the case of hypophysectomy. The hypophysectomized animals were first bled through the jugular vein within 2 minutes of initiating ether anesthesia. This provided baseline sample. The right tibia and fibula were then broken as in the stress procedures (Fig. 1); blood was obtained again 10 minutes later by decapitation. In these hypophysectomized animals, either before stress or after stress, neither ACTH nor β-endorphin were measurable. For the radioimmunoassay, plasma samples were processed with no concentration procedure; whole pituitary glands or adenohypophyses were frozen on Dry Ice within 1 minute of the animals' decapitation; later (1 to 2 hours) they were extracted in 1*N* acetic acid at 100°C for 10 minutes.

Treatment	Plasma (ng/ml)		Whole pituitary (μg/gland)		Adenohypophysis (μg/gland)	
	ACTH	β-Endorphin	ACTH	β-Endorphin	ACTH	β-Endorphin
None (controls)	0.8 ± 0.2 (3)*	1.5 ± 0.2 (9)	4.8 ± 0.3 (3)	2.6 ± 0.2 (11)	2.7 ± 0.5 (3)	1.1 ± 0.2 (3)
Adrenalectomy	8.8 ± 1.6 (3)	8.8 ± 1.0 (6)†	9.9 ± 1.1 (3)†	10.8 ± 1.5 (3)†	8.3 ± 1.1 (3)†	5.4 ± 0.7 (3)†
Dexamethasone	< 0.2 (6)‡	< 1 (7)‡	2.1 ± 0.1 (6)§	1.2 ± 0.1 (7)†		
Hypophysectomy	< 0.2 (3)‡	< 1 (3)‡				

*Numbers in parentheses represent the number of replicates for that treatment; a total of 40 rats was used in three separate experiments, the results of which were pooled after demonstration of homogeneity of their variance (χ^2 test); results were studied by analysis of variance in a randomized (no block) design. †$P < .01$. ‡Indicates that the content of each (plasma) sample was at or below the lower limits of sensitivity of the assay. §$P = .05$ between experimental group and control group.

concentrations of pituitary hormones from all three lobes of the pituitary, in the long portal vessels. Functional significance of these observations remains to be proved; there is as yet no evidence that blood flows retrograde from pituitary into brain in a functional mechanism able to deliver pituitary peptides. On the contrary, we have evidence that profound variations in the pituitary secretion of β-endorphin are not reflected in concomitant variations of brain levels of β-endorphin (*14*). Thus, with all this conflicting evidence, we have to search for one or several peripheral targets for the β-endorphin secreted in response to stress in the dynamic fashion and large amounts that we have demonstrated here.

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27 June 1977

Antiallatotropins: Inhibition of Corpus Allatum Development

Abstract. *Treatment of newly eclosed adult milkweed bug* (Oncopeltus fasciatus) *females with precocene 2 prevents secretion of juvenile hormone by inhibition of postimaginal development of the corpus allatum. Ovarian development which is dependent upon juvenile hormone is prevented or reversed, depending upon the timing of precocene treatment. Juvenile hormone secretion is shown to be related to the development of the corpus allatum.*

The juvenile hormones (JH) of insects regulate insect metamorphosis and reproduction (*1*). Treatment of several heteropterous insect species with certain simple chromenes, called precocenes (Fig. 1) induces precocious metamorphosis of the immature stages and prevents ovarian development in the adult stage. Since these effects are fully reversible by treatment with JH, the precocenes are clearly blocking the normal physiology of JH during synthesis, release, transport, or at its site of action. In view of these actions the precocenes have initially been called anti-juvenile hormones (*2*, *3*).

Although the precocenes produce the full range of anti-juvenile and anti-gonadotropic actions against many heteropterous insects, other insect groups, especially Lepidoptera, Coleoptera, and Diptera, may be sterilized in the adult stage but do not undergo precocious metamorphosis. An understanding of the mode of action of the precocenes is crucial to the further development of methods that interfere with normal endocrine function and may thus be used for insect control. Since the actions of the precocenes can be reversed by treatment with JH, it seemed possible that precocenes produce their anti-juvenile hormonal actions through interference with the secretion of JH by the corpora allata.

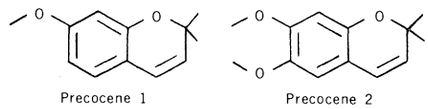

Fig. 1. Anti-juvenile hormones: precocene 1 and 2.

Fluctuations in the volume of the corpora allata have been correlated with cyclic ovarian growth and regression in several insects (*4*). In the milkweed bug, Johansson (*5*) has shown that the corpora allata are very small at eclosion and rapidly enlarge to a maximum volume prior to ovarian development, but the allatal volume does not decrease significantly even during senescence. Johansson has also reported that starved females fail to develop their ovaries, and allatal volume remains subnormal. Since these data taken together suggest that the corpora allata must undergo a postimaginal period of development prior to JH secretion, we investigated the effect of precocene 2 on allatal volume and oocyte development.

We found that precocene treatment of newly eclosed female milkweed bugs prevented allatal development and that the ovaries of treated insects fail to grow, indicating that the undeveloped allata fail to secrete JH. Furthermore, precocene treatment of mature females stops ovarian development and induces regression of the corpora allata.

Newly eclosed milkweed bug (*Oncopeltus fasciatus*) adult females were collected from a stock culture. Groups of ten insects were treated within 24 hours after eclosion or at 120 hours by contact with 8 μg/cm² of precocene 2 in a petri dish for 48 hours as reported previously (*2*), and then transferred to untreated dishes for the remainder of the trials. Insects were maintained on milkweed seeds and water during and after treatment. Juvenile hormone III (10 μg in 1 μl of acetone) was applied topically to the abdomens of some insects. Control insects received acetone only. All experiments were performed in duplicate.

The corpora allata were dissected under Ringer solution and measured with an ocular micrometer, and their volume was calculated as the volume of a sphere. Ovarian development was determined by measurement of the length of the last oocyte of the ovaries with the ocular micrometer.

The volume of the fused corpora allata increased rapidly (Fig. 2A) during postimaginal development, reaching maturity at 6 days. Under our rearing conditions (26°C, 76 percent relative humidity, 16 hours of light, 8 hours of darkness) oviposition occurred on day 6. If insects were treated with precocene on day 1 the corpora allata did not develop (Fig. 2B). When juvenile hormone was applied to precocene-treated insects at 120 hours, there was no effect on the volume of the corpora allata (Fig. 2C). However, as shown in Fig. 2D, when normally devel-