Conclusion

An array of unneeded chemicals, relatively small in quantity but chemically diverse, is an unavoidable consequence of the various activities conducted in laboratories. Laboratory management has the responsibility to limit the quantities of such chemicals that must be disposed of as waste and to provide financial resources and personnel to ensure safe and legal disposal of unneeded chemicals. The disposal of such chemicals would be facilitated by regulatory recognition of the difference between waste generated in laboratory operations and waste resulting from large-scale industrial operations.

Academic institutions can provide a valuable service by incorporating features of waste handling and disposal into their curricula. New generations of scientists need to be trained in sound practices for the disposal of hazardous chemicals, and from these scientists may come concepts of new ways to dispose of or destroy hazardous chemicals.

References and Notes

1. Committee on Hazardous Substances in the Laboratory, National Research Council Assembly of Mathematical and Physical Sciences, *Prudent Practices for Disposal of Chemicals from Laboratories* (National Academy Press, Washington, D.C., 1983).
2. The committee included Robert A. Alberty, Massachusetts Institute of Technology, chairman; Edwin D. Becker, National Institutes of Health; Larry I. Bone, Dow Chemical U.S.A.; Alain DeCleve, Stanford University; Margaret C. Etter, 3M Company; Irving H. Goldberg, Harvard Medical School; Clayton Hathaway, Monsanto Co.; Blaine C. McKusick, Wilmington, Del.; John F. Meister, Southern Illinois University; William G. Mikell, E. I. Du Pont de Nemours & Co.; Adel F. Sarofim, Massachusetts Institute of Technology; William P. Schaefer, California Institute of Technology; Alfred W. Shaw, Shell Development Co.; Fay M. Thompson, University of Minnesota; P. Christian Vogel, BASF Wyandotte; Kenneth L. Williamson, Mount Holyoke College. William Spindel, National Research Council, was study director. This committee evolved from one that earlier issued a report on handling hazardous substances in laboratories [see B. C. McKusick, *Science* **211**, 777 (1981)].
3. Application for this exemption (DOT-E 8129) can be made to the Associate Director of Hazardous Materials Regulations, Materials Transportation Bureau and Special Programs Administration, U.S. Department of Transportation, Washington, D.C. 20590.
4. For EPA regulations, see 40 CFR, chapter 1; for DOT regulations, see 49 CFR, chapter 1.

Stress Hormones: Their Interaction and Regulation

Julius Axelrod and Terry D. Reisine

The constancy of the "milieu interieur" is the condition of a free and independent existence.

—CLAUDE BERNARD (*1*)

The body responds to increased physical or psychological demands by releasing adrenocorticotropin (ACTH) from the anterior pituitary, glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from sympathetic nerves. These hormones serve to adapt the body to stressors ranging from the mildly psychological to the intensely physical by affecting cardiovascular, energy-producing, and immune systems. It was the 19th-century physiologist Claude Bernard who recognized the importance of adaptive mechanisms with one of the most cogent statements (cited above) framed by a biological scientist (*1*). Walter Cannon referred to the complex biological responses necessary to maintain a steady state in the body as homeostasis (*2*). In a series of landmark experiments during the early part of the 20th century, Cannon recognized the importance of the sympathomedullary system in reacting to stressful events evoked by acute physical or psychobiological stressors (*3*). He observed that the tissues liberate a humoral agent which he termed "sympathin." This was later identified as epinephrine (adrenaline) and norepinephrine (noradrenaline) (*4*).

In 1936 Selye reported that diverse noxious agents cause an enlargement of the adrenal cortex as a consequence of the "stress syndrome" (*5*). During the following three decades many investigators observed that a variety of stressful events cause a release of ACTH from the anterior pituitary (*6*). The secreted ACTH stimulates the synthesis of corticosteroids in the adrenal cortex. The elevated corticosteroid levels in plasma then inhibit the further release of ACTH from the pituitary. In a series of elegant experiments, Harris demonstrated that the release of ACTH from the pituitary is regulated by a corticotropin-releasing factor (CRF) from the hypothalamus (*7*). The CRF synthesized in the hypothalamus reaches the pituitary by a private portal blood supply. It then stimulates the secretion of ACTH from the pituitary. After a long period of intensive investigations CRF was isolated and purified, and its structure was characterized as a 41 amino acid peptide by Vale and co-workers (*8*). CRF was thought to be the major if not the sole means of releasing ACTH from the pituitary. Recent experiments indicate that ACTH can also be released and regulated by catecholamines and other hormones.

Catecholamines, Glucocorticoids, and Sympathoadreno Activity

A variety of stressors cause an increased activity of the sympathetic nervous system and adrenal medulla (*2*). This activity results in a discharge of epinephrine and norepinephrine into the blood stream and changes in the activity of enzymes that synthesize catecholamines and in the concentrations of norepinephrine and epinephrine in the brain. With prolonged stress, marked compensatory changes in the activity of the catecholamine biosynthetic enzymes tyrosine hydroxylase, dopamine β-hydroxylase, and phenylethanolamine *N*-methyltransferase (PNMT) occur. These changes in enzyme activity are regulated to varying degrees by glucocorticoids, ACTH, and neuronal activity.

When mice are subjected to psychosocial stressors through competition for food and living space, they show increases in blood pressure, adrenal weight, and catecholamine concentrations in the adrenal medulla (*9*). The biosynthetic enzymes tyrosine hydroxylase and PNMT are both increased in mice experiencing excessive social stimulation. Forced immobilization of rats

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Summary. Stress stimulates several adaptive hormonal responses. Prominent among these responses are the secretion of catecholamines from the adrenal medulla, corticosteroids from the adrenal cortex, and adrenocorticotropin from the anterior pituitary. A number of complex interactions are involved in the regulation of these hormones. Glucocorticoids regulate catecholamine biosynthesis in the adrenal medulla and catecholamines stimulate adrenocorticotropin release from the anterior pituitary. In addition, other hormones, including corticotropin-releasing factor, vasoactive intestinal peptide, and arginine vasopressin stimulate while the corticosteroids and somatostatin inhibit adrenocorticotropin secretion. Together these agents appear to determine the complex physiologic responses to a variety of stressors.

with the use of a model that combines emotional stress (escape reaction) and physical stress (muscle work) activates both the sympathetic adrenal medullary and adrenocortical systems and the pituitary gland (*10*). The activities of tyrosine hydroxylase, dopamine β-hydroxylase, and PNMT are increased in this type of stress (*11*). Stress such as that associated with swimming, electroshock, and insulin also elevate these biosynthetic enzymes (*11*). Forced immobilization of rats can cause an increase in the plasma epinephrine and norepinephrine concentrations (*12*). Mild stressors such as opening a cage door or handling a rat produces an eightfold increase in plasma epinpehrine concentrations.

The adrenal gland, important in stress reactions, consists of at least two anatomically and chemically distinct structures: an inner medullary area containing catecholamine-producing chromaffin cells and an outer cortical region in which glucocorticoids are synthesized. In most mammals, the adrenal cortex and medulla are contiguous and the main catecholamine produced is epinephrine. In some species, however, the chromaffin tissue is separated from the adrenal cortex and the predominant catecholamine found is norepinephrine (*13*). This suggested that a substance produced in adrenal cortical tissue might gain access to the medulla and regulate the conversion of norepinephrine to epinephrine. Such substances were thought to be glucocorticoids whose synthesis is stimulated by ACTH. Whether or not glucocorticoids and indirectly ACTH modify the formation of epinephrine in the adrenal medulla was determined by subjecting animals to hypophysectomy and, several days later, measuring the activity of PNMT, the epinephrine-forming enzyme in the medulla (*14*). The activity of PNMT showed marked decrease. Furthermore, the repeated administration of ACTH or the potent glucocorticoid dexamethasone to hypophysectomized rats restored PNMT activity to almost normal values. PNMT activity in the adrenal medulla is much lower in hypophysectomized rats subjected to immobilization stress (*15*), but this decrease can be prevented or reversed by treatment with ACTH or glucocorticoids. These experiments indicated the interrelationship of ACTH in the pituitary and glucocorticoids in the adrenal cortex in effecting the synthesis of epinephrine (Fig. 1).

The activity of tyrosine hydroxylase, a rate-limiting enzyme in catecholamine biosynthesis, can increase considerably in response to stressors (*11*). In acute stress the activity of tyrosine hydroxylase is rapidly elevated in the adrenal medulla without any change in the number of enzyme molecules (*16*). In chronic stress, however, the activities of tyrosine hydroxylase (*17*) and dopamine β-hydroxylase are increased (*18*) in sympathetic nerves and the adrenal medulla as a result of an increase in the number of enzyme molecules (*16*). If the preganglionic innervation to sympathetic nerves of the superior cervical ganglia of the rat are cut, the enzyme activity in cell bodies and nerve terminals is not increased, indicating that this mode of compensatory regulation of the enzyme is a transsynaptic event (*19*). The transsynaptic induction of tyrosine hydroxylase appears to be due to stimulation of acetylcholine nicotinic receptors (*20*). Increased stimulation of sympathetic nerves also elevates the activity of dopamine β-hydroxylase (*18*) and to a smaller extent PNMT (*21*). Cutting the presynaptic nerves will block the increase of dopamine β-hydroxylase and PNMT, indicating a transsynaptic induction of these enzymes. As in the case of PNMT, removal of the pituitary gland of the rat results in a gradual reduction of tyrosine hydroxylase and dopamine β-hydroxylase activity in the adrenal medulla (*22*). In hypophysectomized rats subjected to immobilization stress the reduced dopamine β-hydroxylase activity in the adrenal medulla can be restored by ACTH and dexamethasone. From these experiments it appears that the stress-induced increase in tyrosine hydroxylase is due mainly to neuronal activity, whereas dopamine β-hydroxylase is affected by both nerve activity and the pituitary adrenal axis and PNMT is controlled mainly by ACTH and glucocorticoids (Fig. 1). By means of immunocytochemistry and double labeling with radioisotopes, the splanchnic nerve innervating the adrenal medulla has been shown to induce de novo synthesis of dopamine β-hydroxylase and PNMT while glucocorticoids inhibit degradation of these enzymes (*23*).

Rats subjected to a variety of stressors show considerably reduced concentra-

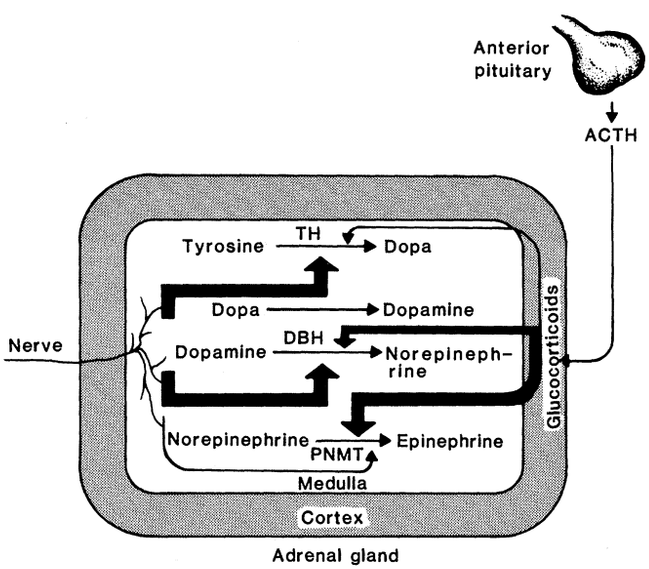

Fig. 1. Regulation of catecholamine biosynthesis in the adrenal medulla. The expression of catecholamines in the adrenal medulla is regulated at a number of different steps in the biosynthetic pathway. Tyrosine hydroxylase (*TH*) activity, the rate limiting step in dopamine synthesis, is affected by nerve activity and to a minor extent by glucocorticoids of the adrenal cortex. Dopamine β-hydroxylase (*DBH*) activity is regulated by nerve activity and glucocorticoids. Phenylethanolamine *N*-methyltransferase (*PNMT*), the enzyme that converts norepinephrine to epinephrine, is predominantly regulated by glucocorticoids and to a small degree by nerve activity. Glucocorticoid synthesis is stimulated by ACTH which is released from the anterior pituitary.

tions of brain norepinephrine (*24*). By means of a technique for the precise dissection of small areas of the brain (*25*) it was observed that the stress-induced depletion of norepinephrine occurs in specific brain nuclei such as the nucleus tractus solitarius, and the arcuate, periventricular, and ventromedial hypothalamic nuclei (*26*). Epinephrine-containing neurons are also present in the brain and localized in the brainstem (*27*). Immobilization stress causes a selective depletion of epinephrine in nucleus tractus solitarius, locus ceruleus, and paraventricular and arcuate nuclei (*28*). Neurons containing CRF have been found in hypothalamic regions such as the paraventricular nucleus (*29*). Recent studies have shown that inhibiting brain PNMT activity enhances CRF immunoreactivity in the paraventricular nucleus, suggesting that epinephrine may regulate the activity of CRF-containing neurons (*30*). Such an interaction between adrenergic and CRF-containing neurons may be important in mediating stress-related responses.

Although a variety of stressors increase the catecholamine concentrations in urine (*31*), which can serve as a measure of stress, catecholamine concentrations in plasma are generally considered to be a more precise measure of the stress-induced activation of the sympathetic medullary system. Until recently it was difficult to obtain a reliable measure of plasma norepinephrine and epinephrine because of their extremely low concentrations. However, with the introduction of specific and highly sensitive radioenzymatic assays for catecholamines it has become possible to determine their concentrations (*32*) during basal conditions and stressful situations in humans. In general, plasma norepinephrine levels reflect the activity of the sympathetic nerves while epinephrine is a measure of secretion from the adrenal medulla. Postural changes can cause two- to threefold elevations in plasma norepinephrine but negligible changes in epinephrine (*33*). Public speaking results in a twofold increase in plasma epinephrine and a 50 percent increase in norepinephrine (*34*). The reverse is true in performing mental arithmetic (*35*). During harassment, type A individuals (coronary prone) have a greater elevation of plasma epinephrine than type B subjects (noncoronary prone) (*36*). Depressed subjects show increased basal levels of plasma norepinephrine and epinephrine which has been related to their degree of anxiety (*37*). Propranolol, a β-adrenoreceptor blocking drug, relieves somatic symptoms (tachycardia, tremors) or acute panic state in anxious persons but appears to be of little use in other forms of anxiety (*38*). Physical, cold, and thermal stress can cause moderate to marked elevation of plasma catecholamine (*39*). Exercise also results in a rise in plasma catecholamines (*40*) that depends on the duration and severity of the exercise. Stress due to surgery, hemorrhage, myocardial infarction, hypoglycemia, and hypoxia causes considerable increases in plasma catecholamines (*41*). Hypoglycemia induced by insulin or 2-deoxyglucose evokes the greatest increase in plasma catecholamines, especially epinephrine. The high levels of catecholamine achieved in hypoglycemia and myocardial infarction may be enough to stimulate β-adrenoreceptors on the anterior pituitary. Studies in our laboratory have revealed that insulin stress in rats stimulates ACTH release from the pituitary (*42*). This effect is blocked by propranolol and since insulin stress raises plasma catecholamine, the rise in ACTH release appears to be due to the direct action of epinephrine on the pituitary. It would be of clinical interest to examine the effect of propranolol on the plasma concentrations of ACTH and glucocorticoids in patients with hypoglycemia.

Table 1. Multireceptor control of cyclic AMP formation and ACTH release in mouse pituitary tumor cells. An ED_{50} value is the concentration of drug at which 50 percent of the maximum stimulation occurs; an IC_{50} value is the concentration of drug at which 50 percent of the maximum inhibition occurs. N.D., not determined.

Additions (references)	ACTH release		Cyclic AMP	
	Maximum response (percent of basal)	ED_{50} (n*M*)	Maximum response (percent of basal)	ED_{50} (n*M*)
Stimulants				
CRF (*90*)	300 to 400	4 to 5	500	2
β-Adrenergic agonists (*48*)				
(−)-Isoproterenol	200 to 400	1	600	50
(+)-Isoproterenol	100	N.D.	100	N.D.
(−)-Epinephrine	200 to 400	50	600	100
(−)-Norepinephrine	200 to 400	200	400	1,000
VIP (*31*)	250	1	250	1
Forskolin (*51, 91*)	500	1,000	4,000	10,000
Cholera toxin (*61*)	500	N.D.	4,000	N.D.
Potassium (*92*)	400	N.D.	100	N.D.
Calcium ionophores				
A23187 (*92*)	400	N.D.	100	N.D.
Ionomycin (*92*)	400	5,000	N.D.	N.D.
	Percentage inhibition of stimulated response	IC_{50} (n*M*)	Percentage inhibition of stimulated response	IC_{50} (n*M*)
Inhibitors				
Dexamethasone*				
CRF (*90*)	100	1	0	N.D.
Isoproterenol (*48*)	100	1	0	N.D.
VIP (*58*)	60	10	0	N.D.
Forskolin (*51*)	90	5	0	N.D.
Somatostatin†				
CRF (*93*)	100	1	80 to 90	0.1
Isoproterenol (*93*)	100	1	80 to 90	0.1
VIP (*93*)	100	1	80 to 90	0.1
Forskolin (*93*)	100	1	80 to 90	0.1
Potassium (*64, 92*)	100	0.4	80 to 90	0.1
Melittin (*61*)	0	N.D.	N.D.	N.D.
A23187 (*61*)	0	N.D.	N.D.	N.D.
Ionomycin (*92*)	50	N.D.	N.D.	N.D.
X537A (*52, 92*)	100	N.D.	N.D.	N.D.
	Dissociation constant (n*M*)	Maximum binding (fmole/mg protein)		
Receptor binding				
Somatostatin (*64*)	1.7	141		
β_2-Adrenergic (*48*)	11	64		

*Cells were treated with $10^{-7}M$ dexamethasone. The cells were washed and then dexamethasone and various stimulants were added. ACTH released into the medium and intracellular cyclic AMP content were measured after such manipulations. †Somatostatin and various stimulants were added simultaneously to the cells.

Multireceptor Release of ACTH from Anterior Pituitary

Adrenocorticotropin is synthesized and released from the anterior pituitary. The availability of synthetic CRF (*8, 43*) and of a mouse anterior pituitary cell line (AtT-20/D16-16) that secretes ACTH made it possible to study the mechanism of release of ACTH and the involvement of catecholamines, glucocorticoids, and other hormones in this secretory process. Recent investigators of ACTH secretion from the pituitary have usually used primary cultures of the rat adenohypophysis (*44*). Although much useful information has been obtained with such preparations, the heterogeneity of the cell types and the low density of the ACTH secreting cells (2 to 3 percent of the total cell population) in the anterior pituitary has limited the characterization of factors directly controlling ACTH release. The AtT-20 cell line was used previously to examine the processing of ACTH from its precursor protein proopiomelanotropin (POMC) as well as the storage and secretion of ACTH and β-endorphin (*45*). This cell line appears to be homogeneous with regard to cell type and, in contrast to primary anterior pituitary cultures, predominantly releases hormones of the POMC family of peptides.

Normal anterior pituitary cells respond to synthetic CRF by releasing ACTH and β-endorphin (*43*). AtT-20 cells also secrete immunoreactive ACTH and β-endorphin in response to CRF (Table 1). Analogs of CRF show the same order of potency in releasing ACTH from AtT-20 cells as observed in normal corticotrophs and, as shown in intact animals and primary cultures of the pituitary, glucocorticoids block the CRF-stimulated release of ACTH in the tumor cells. These findings prompted the use of AtT-20 cells as a model for investigating the cellular and molecular mechanisms that regulate ACTH secretion from the anterior pituitary.

There were previous indications that catecholamines can stimulate ACTH release in vivo and in vitro (*46*). The complexities involved in using the intact animal and primary cultures made it difficult to interpret the precise mechanism whereby catecholamines induced this release. Norepinephrine stimulated ACTH release from AtT-20 cells in a calcium-dependent manner (*47*). Ligand binding studies with tritiated dihydroalprenolol, a β-adrenoreceptor antagonist, indicated the presence of a β-adrenoreceptor on AtT-20 cell membranes which binds catecholaminergic agents with high affinity (*48*). Isoproterenol, a β-adrenoreceptor agonist, as well as epinephrine induced a potent and stereoselective increase of ACTH release from mouse tumor cells which was calcium-dependent and blocked by the β-adrenoreceptor antagonist propranolol (*48*). Two subtypes of β-adrenoreceptors are known, β_1 and β_2 (*49*). β_2-Adrenoreceptors are most sensitive to epinephrine whereas β_1-adrenoreceptors are equally responsive to epinephrine and norepinephrine. Pharmacologic characterization showed that β_2-receptors are present on AtT-20 cells and could mediate the release of ACTH (*48*). The presence of β_2- but not β_1-adrenoreceptors has been reported in the anterior pituitary, but whether they are located on a specific cell type has not been determined (*50*). As in the case of CRF, dexamethasone (Table 1 and Fig. 2) blocks catecholamine-evoked secretion of ACTH from AtT-20 cells (*51*). The longer these cells have been incubated with glucocorticoids the greater the degree of inhibition of ACTH release.

In anterior pituitary membranes CRF also stimulates adenylate cyclase activity (*44*). CRF increases the formation of adenosine 3′,5′-monophosphate (cyclic AMP) in primary cultures and activates cyclic AMP–dependent protein kinase, an intracellular effector of cyclic AMP (*52*). CRF also stimulates adenylate cyclase and cyclic AMP–dependent protein kinase activity in AtT-20 cells (*53*). Support for a critical role of cyclic AMP in regulating ACTH release comes from studies with forskolin. This diterpene,

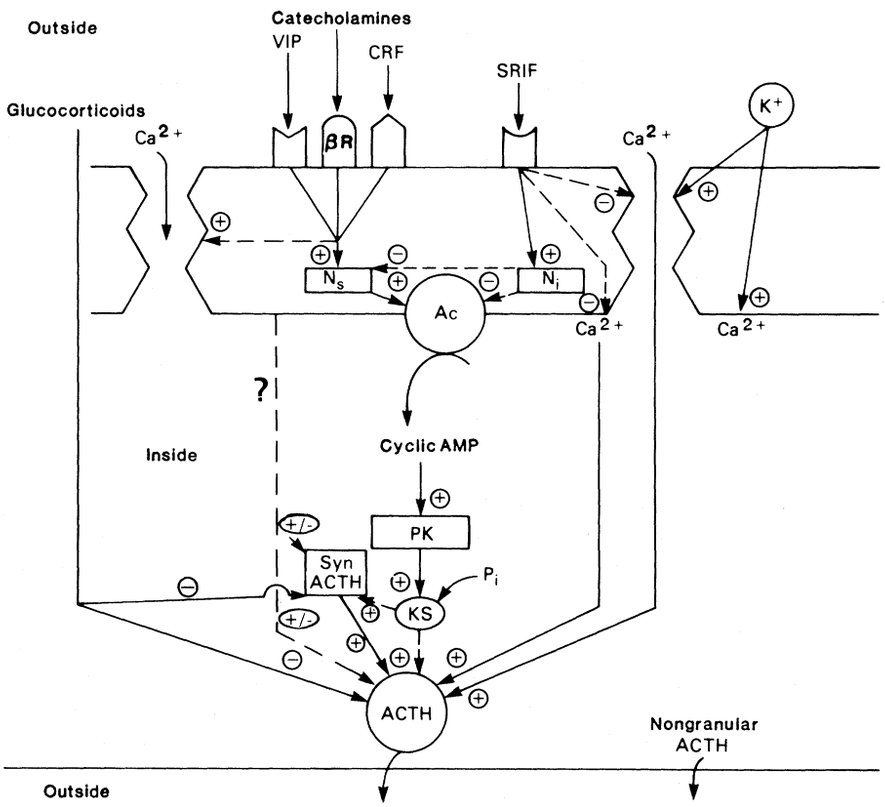

Fig. 2. Molecular mechanisms involved in ACTH release. The release of ACTH can be stimulated (+) by various hormones such as CRF, catecholamines acting on β-adrenoreceptors (*βR*), or α_1-adrenoreceptors (not shown), VIP, or vasopressin (not shown). Each agonist acts on separate and specific receptors. The hormone-induced secretion of ACTH involves a multitude of intracellular second messengers. Secretagogues can activate adenylate cyclase (*Ac*) to form cyclic AMP. A guanine nucleotide stimulatory protein (N_s) is required for hormone activation of Ac. Cyclic AMP activates protein kinase (*PK*) which catalyses the phosphorylation (P_i) of a protein substrate (*KS*). The phosphorylated KS may induce ACTH synthesis (*Syn ACTH*) or the release of granular ACTH (in the circle). The secretion of nongranular ACTH appears to be regulated differently from granular ACTH. Hormones and membrane depolarization, induced by extracellular potassium (K^+) may stimulate calcium (Ca^{2+}) influx or mobilization from intracellular compartments. Other intracellular events (*?*) induced by secretagogues may involve changes in phospholipid methylation, protein carboxymethylation, phosphatidylinositol turnover, C-protein kinase, and glycosyltransferase activity. ACTH release can be inhibited (−) by at least two hormones. Glucocorticoids can inhibit ACTH synthesis or release. Somatostatin (*SRIF*) blocks the activation of Ac or Ca^{2+} mobilization. A guanine nucleotide inhibitory protein (N_i) mediates SRIF's inhibition of Ac. Coupling of N_i with SRIF receptors leads to either a direct blockage of the catalytic subunit of Ac or an inhibition of N_s activity.

which may directly activate adenylate cyclase (*54*), stimulates with equal potency ACTH release (Table 1 and Fig. 2) and cyclic AMP–dependent protein kinase as well as the phosphorylation of several distinct proteins in AtT-20 cells (*53*). It is possible that these endogenous proteins mediate the effect of cyclic AMP on ACTH release. Isoproterenol also stimulates adenylate cyclase activity (Fig. 2) and cyclic AMP–dependent protein kinase in AtT-20 cells, indicating that cyclic AMP may mediate the effect of β-adrenoreceptor agonists on ACTH release (*53*). The increase in ACTH secretion induced by isoproterenol is blocked by calcium antagonists such as verapamil or by the absence of calcium in the extracellular medium (*51*). The activation of adenylate cyclase by isoproterenol is not prevented by calcium ionophore blocking agents nor is the stimulation of cyclic AMP–dependent protein kinase altered by the depletion of extracellular calcium (*53*). This suggests that the actions of Ca^{2+} in the release of ACTH occur at a step distal to kinase activation by cyclic AMP. The secretion of ACTH is stimulated by increasing extracellular calcium concentrations. Furthermore, isoproterenol and catecholamines change the membrane potential of AtT-20 cells and increase the frequency of action potentials on these cells by a calcium-dependent mechanism (*55*). Since catecholamine-evoked ACTH release from AtT-20 cells is also dependent on the presence of calcium, there are probably at least two intracellular mechanisms involving calcium or cyclic AMP that participate in corticotropin secretion.

In addition to the β-adrenoreceptor control of ACTH release, an α_1-adrenergic-like mechanism may also be involved (*56*). In primary cultures of the anterior pituitary, ACTH release stimulated by epinephrine and norepinephrine is blocked by the potent and selective α_1-adrenergic receptor antagonist, prazocin. Dopamine and serotonin antagonists also block epinephrine-induced ACTH secretion. α_1-Receptor agonists such as phenylephrine and methoxyamine are poor stimulators of ACTH release, suggesting the presence of a nonclassical α_1-receptor mediated release of ACTH which does not involve activation of adenylate cyclase.

Besides synthetic CRF and catecholamines, other hormones have been found to stimulate ACTH release. Vasoactive intestinal peptide (VIP), present in the hypothalamus and known to stimulate prolactin release from the anterior pituitary (*57*), also evokes the secretion of ACTH from AtT-20 cells (Table 1) in a dose-dependent manner (*51*). This peptide increases cyclic AMP accumulation in these cells as well as cyclic AMP–dependent protein kinase activity (*53*). VIP also stimulates ACTH release from human anterior pituitary tumor cells (*58*). The effect of VIP on ACTH secretion, like that of other hormones, is blocked by glucocorticoids (*51*). Arginine-vasopressin is also found in hypothalamic neurons and was one of the first hormones proposed to have CRF-like actions. Vasopressin increases the secretion of ACTH from primary cultures of the anterior pituitary (*59*). This releasing action is not mediated by cyclic AMP but may involve some other intracellular effector system (*56*).

Inhibition of ACTH Release

Glucocorticoids consistently block basal and stimulate ACTH release from the anterior pituitary in the intact animal as well as cell preparations in vitro (Table 1). Glucocorticoids may act through several mechanisms to inhibit ACTH secretion (*60*). Long-term treatment of animals or AtT-20 cells with dexamethasone reduces ACTH messenger RNA activity, indicating an inhibition of ACTH synthesis at some pretranslational site. The reduction of ACTH messenger RNA production induced by glucocorticoids correlates with their intracellular glucocorticoid receptor binding activity. Short-term treatment (2 hours) of AtT-20 cells with dexamethasone seems predominantly to effect hormone- and cyclic AMP–stimulated ACTH release rather than ACTH synthesis, since the rise in ACTH release promoted by the calcium ionophore A23187 is not reduced by brief glucocorticoid treatment (*53*). Treatment of AtT-20 cells with dexamethasone for short intervals, while inhibiting isoproterenol-, CRF-, and forskolin-stimulated ACTH release (Table 1 and Fig. 2), does not affect the activation of cyclic AMP–dependent protein kinase by these secretagogues (*53*). The ability of these secretagogues to stimulate cyclic AMP accumulation is not affected by short- or long-term treatment with dexamethasone. The inability of glucocorticoids to block CRF-stimulated cyclic AMP formation was also reported in primary cultures of the anterior pituitary (*44*). Preliminary studies have shown that the enzyme phospholipase A_2 may be involved in releasing ACTH since melittin, an activator of this enzyme, stimulates ACTH secretion (*61*). Glucocorticoids have been observed in some cell systems to induce the synthesis of lipomodulin, a protein that inhibits phospholipase A_2 activity (*62*). If glucocorticoids can rapidly induce either the synthesis or the mobilization of this protein, then the rapid inhibitory effects of dexamethasone on ACTH release may be related to a blockade of phospholipase A_2 activity. Thus, inhibition of phospholipase A_2 activity may serve as another mechanism by which this class of steroids act in the regulation of ACTH secretion.

Somatostatin (SRIF) also inhibits ACTH release in AtT-20 cells (Table 1). This 14 amino acid peptide is of hypothalamic origin and is known to block the secretion of growth hormone, prolactin, and thyroid-stimulating hormone from the anterior pituitary (*63*). AtT-20 cells have SRIF receptors which, when stimulated, cause a reduction in ACTH secretion (*61, 64*) evoked by potassium, CRF, isoproterenol, VIP, cholera toxin, or forskolin. SRIF reduces the ability of these secretagogues to increase cyclic AMP accumulation (*61*) (Table 1). These observations suggest that SRIF can block ACTH release by inhibiting the activation of adenylate cyclase. SRIF also inhibits forskolin-stimulated cyclic AMP formation in *cyc*$^-$ variants of S49 lymphoma cells that are deficient in the guanine nucleotide stimulatory protein (N_s) required for most hormones to activate adenylate cyclase (*65*). From these data it was proposed that SRIF acted through a guanine nucleotide inhibitory protein (N_i) to reduce adenylate cyclase activity (*61, 65*) as well as by a stimulation of guanosine triphosphatase activity (*65*). A useful agent in studying the manner by which hormones inhibit adenylate cyclase is a toxin derived from the bacterium *Bordetella pertussis*. This toxin induces the adenosine diphosphate (ADP)–ribosylation of a 41,000-dalton protein believed to be N_i (*66*). The toxin also blocks the inhibitory effects of hormones and guanine nucleotides on adenylate cyclase in many tissues (*66*). The inhibition of growth hormone release by SRIF from primary cultures of the anterior pituitary is also blocked by the toxin (*67*). In membranes of AtT-20 cells, *Bordetella pertussis* toxin induces the ADP-ribosylation of a 41,000-dalton protein and also prevents the inhibitory effect of SRIF on forskolin-, CRF-, or isoproterenol-stimulated cyclic AMP formation and ACTH release (*68*). These findings suggest that SRIF can act through N_i to inhibit hormone-induced ACTH release.

Interactions of ACTH-Releasing Factors

The ACTH secretagogues can act individually or in concert to regulate the release of ACTH. Hypothalamic extracts are more potent in releasing ACTH than any secretatogue alone. These extracts appear to contain several different CRF-like factors. In primary cultures of the anterior pituitary, vasopressin added together with synthetic CRF induced a greater release of ACTH than CRF alone, indicating that vasopressin can potentiate the action of CRF (*59, 69*). Although vasopressin does not alter cyclic AMP accumulation in the anterior pituitary, it causes a fourfold potentiation in the stimulation of cyclic AMP synthesis by CRF, suggesting that vasopressin improves the efficiency of coupling between CRF receptors and adenylate cyclase. Epinephrine, by activating α_1-adrenoreceptors in the anterior pituitary, also potentiates synthetic CRF's stimulation of ACTH release and, like vasopressin, enhances the cyclic AMP response to CRF (*70*). These findings indicate a synergism between vasopressin, α_1-adrenergic agonists, and synthetic CRF in releasing ACTH. β_2-Adrenoreceptor agonists and CRF also interact to regulate ACTH release. When CRF and isoproterenol are added together, the increase in ACTH secretion is less than additive, suggesting that these secretagogues act through a common mechanism (*51*). This intracellular mechanism is distal to cyclic AMP accumulation since the combined application of CRF and isoproterenol produce additive effects on cyclic AMP formation. VIP, another ACTH secretagogue, appears to release ACTH through a process independent of CRF or β-adrenergic agonists (*50*). VIP, together, with isoproterenol or CRF, causes an additive increase in both ACTH secretion and cyclic AMP production. These findings indicate that CRF, isoproterenol, and VIP may act on separate compartments of adenylate cyclase as well as other second messenger systems (Fig. 2).

Desensitization

Although hormones can induce rapid and pronounced responses from cells, their persistent presence can induce desensitization (*71*). Corticotrophs become refractory to CRF after prolonged exposure to this peptide (*72*). This desensitization is manifest as a reduced maximal ability of CRF to stimulate both cyclic AMP formation and ACTH release. ACTH content is not affected by CRF treatment, indicating that cells are not depleted of the peptide hormone. Forskolin-stimulated cyclic AMP accumulation or ACTH release is not reduced by CRF treatment, suggesting that both adenylate cyclase and the intracellular mechanisms mediating stimulus-secretion coupling are unaffected in the desensitized cells. Thus, either CRF receptors are lowered in density or their coupling to adenylate cyclase is impaired. Studies in which anterior pituitary membranes are exposed to ^{125}I-labeled CRF indicate that desensitization involves the loss of CRF receptors (*73*). Adrenalectomy, a procedure that abolishes the glucocorticoid feedback inhibition of CRF release in the hypothalamus, markedly decreased CRF receptor binding in the pituitary 4 to 6 days after surgery. The density of these sites returns almost to normal after treatment with dexamethasone. Thus, CRF receptors can be down-regulated and this may explain the desensitization observed in primary cultures. Recent studies have also shown that the effect of CRF on ACTH release in vivo is reduced after prior treatment of animals with CRF, suggesting that desensitization can occur under physiologic conditions (*74*).

Vasopressin not only potentiates CRF-stimulated ACTH release but can also increase the ability of CRF to desensitize its own receptor (*75*). Treatment of primary cultures of the anterior pituitary with a fixed concentration of arginine-vasopressin and varying amounts of CRF reduces the amount of CRF needed to desensitize its receptors. Thus, vasopressin and CRF act synergistically to release ACTH and regulate CRF receptors.

β-Adrenoreceptors in many cell types are also readily desensitized (*76*). This was also found to be the case for mouse pituitary tumor cells. Treatment of AtT-20 cells with isoproterenol results in a marked reduction of cyclic AMP formation and release of ACTH after restimulation with the catecholamines (*77*). A decreased binding of [^{3}H]dihydroalprenolol becomes apparent after 20 hours of treatment with isoproterenol. The reduced binding is associated with a decreased density of β-adrenoreceptors but no change in receptor ligand affinity. The desensitization of the cyclic AMP accumulation and ACTH secretion responses were observed before there was a decrease in receptor density. These findings suggest that the desensitization of the β-adrenoreceptor is a two-step process. The first is rapid in onset and shows a reduced capacity of catecholamines to elevate cyclic AMP accumulation and ACTH release. The second step is slower and is associated with a loss of β-adrenoreceptors from cell membranes (down regulation).

The rapid desensitization of the β-adrenoreceptors to stimulation of cyclic AMP synthesis and ACTH release without changes in receptor density could be due to the uncoupling of the receptor from the adenylate cyclase complex, decreased activity of adenylate cyclase, or changes in the ACTH secretory process. These possibilities were examined by first treating AtT-20 cells with isoproterenol to reduce the responsiveness to cyclic AMP elevation and ACTH secretion by about 50 percent (*77*). The cells were then treated with forskolin to directly stimulate adenylate cyclase. The generation of cyclic AMP and release of ACTH in forskolin-treated cells were the same as that of the fully sensitized cells. This experiment indicates that during the early desensitization of the β-adrenoreceptors, the adenylate cyclase and ACTH secretory mechanism is normal and that the desensitization is due to an uncoupling of the receptor from adenylate cyclase.

β-Adrenoreceptor desensitization is homologous (only desensitized to its own receptor) since CRF- and VIP-stimulated cyclic AMP accumulation and ACTH release were unaffected by catecholamine treatment of AtT-20 cells. CRF receptors on normal corticotrophs are also regulated independently of catecholamine receptors (*72, 75*). The independent nature of the desensitization of these receptors indicates that the mechanisms involved are specific for each receptor. Such a property would allow corticotrophs to respond to some stimuli despite the loss of responsiveness to other CRF-like substances.

Somatostatin can also regulate the sensitivity of its own receptor (*78*). Prior exposure of mouse anterior pituitary cells to SRIF lessens SRIF's antagonism of CRF-, VIP-, isoproterenol-, and forskolin-stimulated cyclic AMP accumulation and ACTH release. Treatment with SRIF increases the formation of cyclic AMP in response to forskolin in these cells. This increase is delayed in onset, slow to recover, and is blocked by the protein synthesis inhibitor cyclohexamide. This suggests that prolonged treatment of AtT-20 cells with SRIF desensitizes SRIF receptors and causes a compensatory sensitization of adenylate cyclase through a process requiring protein synthesis. In cultures of brain cells, pro-

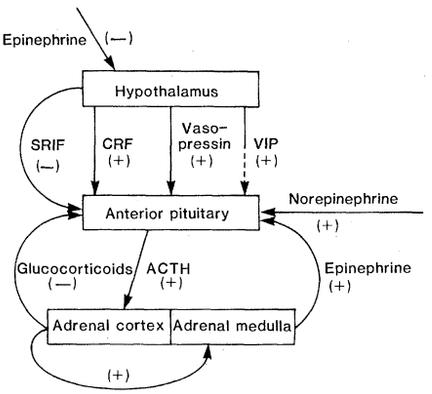

Fig. 3. Multihormonal control of ACTH release. ACTH release from the anterior pituitary is regulated by a number of different hormones. CRF and vasopressin, which are present in hypothalamic neurons, reach the anterior pituitary by a portal system and stimulate (+) ACTH release. The secretion of the releasing factors may be inhibited (−) by an epinephrine input to the hypothalamus. Hypothalamic VIP stimulates ACTH release from both human and mouse tumor cells suggesting a possible role of this peptide as a CRF-like substance. Neuronal norepinephrine or epinephrine released from the adrenal medulla can also evoke ACTH release. ACTH stimulates the synthesis of glucocorticoids in the adrenal cortex. Glucocorticoids can stimulate the synthesis of epinephrine or inhibit ACTH release. Glucocorticoids can either act directly on the anterior pituitary to inhibit the formation of messenger RNA for ACTH or inhibit ACTH release. In addition, these steroids can act in the hypothalamus to effect CRF release (not shown). Somatostatin (*SRIF*) can block the evoked release of ACTH from tumor cells and reduces the plasma concentrations of ACTH in Nelson's syndrome (*94*), suggesting a role for this peptide in ACTH secretion.

longed application of SRIF produced an adaptive response that was manifest as a reduced ability of SRIF to stimulate cell firing activity (*79*). Continued treatment of anterior pituitary cells with SRIF reduces the subsequent ability of the peptide to inhibit the release of growth hormone and thyroid-stimulating hormone (TSH) (*80*). These findings indicate that SRIF receptors on many cell types can be self-regulated. A model detailing the multireceptor regulation in ACTH release in AtT-20 cells is shown in Fig. 2.

The Multireceptor Release of ACTH in vivo

The studies showing that ACTH release in vitro is stimulated by multiple factors raises the question of whether the release of ACTH in vivo is under multihormonal control. The injection of synthetic ovine CRF in rats causes an immediate increase in plasma ACTH concentrations (*81*). This stimulation is dose-dependent and is neutralized by antibodies to CRF. The ACTH release induced by acute ether stress is partially blocked by CRF antibodies, indicating that molecules with immunologic characteristics similar to synthetic CRF are stress mediators (*81*). The lack of total blockage of stress-evoked ACTH release in rats by the CRF antibodies suggests that hormones other than CRF are involved in promoting the release of ACTH in vivo.

Vasopressin also causes the release of ACTH in the intact rat. This stimulation is dose-dependent and prevented by a vasopressin antagonist (*81, 82*). The physiologic condition of the animal has an important role in the stimulation of ACTH secretion by vasopressin. Animals anesthetized with neuroleptics, opiates, and Nembutal (conditions that block CRF release) respond to vasopressin with a smaller elevation in ACTH release than that found in awake, freely moving animals. Immunoneutralization of CRF in nonanesthetized rats also lowers stimulation of ACTH release by vasopressin, suggesting a dependence on CRF for the ACTH releasing action of vasopressin. Vasopressin potentiates CRF-stimulated ACTH release in anesthetized rats, indicating that vasopressin and CRF act in a synergistic manner to regulate ACTH release in vivo as well as in vitro.

Catecholamines also appear to stimulate ACTH release in vivo by a direct action on the anterior pituitary. Peripheral injections of epinephrine increase plasma concentrations of ACTH in intact rats (*46*). The increase in plasma ACTH caused by either epinephrine or (−)-isoproterenol is stereospecifically blocked by propranolol, suggesting that β-adrenoreceptors are linked to the release of ACTH in vivo (*46*). β-Adrenoreceptor agonists have been proposed to stimulate ACTH release in vivo by acting through "central mechanisms," since hypothalamic lesions in female rats prevented the increase in plasma ACTH induced by (−)-isoproterenol (*82, 83*). The process by which catecholamines gain access to the brain to initiate these central effects is not known. Previous work has demonstrated that catecholamines do not cross the blood-brain barrier (*84*). In contrast to these studies, however, Mezey *et al.* (*85*) found that (−)-isoproterenol stimulated ACTH release from male rats in which the hypothalamus was separated from the pituitary by either stalk-transection or lesions of the median eminence. The effect of isoproterenol was blocked by propranolol but not by the selective β_1-adrenoreceptor antagonist practolol. Salmefamol, a β_2-adrenoreceptor agonist, also stimulated ACTH release in stalk-sectioned animals, indicating that β_2-adrenoreceptors can mediate the stimulation of ACTH release by catecholamines in vivo. Isoproterenol-stimulated ACTH release in stalk-transected animals is blocked by dexamethasone, suggesting that the ACTH release induced by β-adrenoreceptor agonists originates from the anterior pituitary. These findings are consistent with the previous studies of Fortier (*86*) and McDermott *et al.* (*87*) who used pituitary transplants to examine the direct action of epinephrine on an ACTH-mediated response. In these studies, the anterior pituitary was placed into the anterior chamber of the eye of hypophysectomized rats. Injection of small quantities of epinephrine into the eye reduced the level of circulating white blood cells (eosinophilia) which is believed to accompany an increase of ACTH release. Similar injections of epinephrine into the other eye did not produce this response. These data as well as the findings in stalk-transected animals indicate that epinephrine can act directly on the anterior pituitary possibly by way of β-adrenoreceptors to stimulate ACTH release.

Conclusion

Studies conducted in vitro and in vivo indicate that the release of the stress hormone ACTH is controlled by complex regulatory mechanisms. Multiple factors such as CRF, vasopressin, catecholamines, and conceivably other hormones stimulate ACTH release by directly acting on the anterior pituitary (Fig. 3). Glucocorticoids and possibly SRIF may inhibit the secretion of ACTH also by a direct action on anterior pituitary. Various hormones can indirectly control ACTH release by acting on central locations to modify the secretion of these releasing or inhibiting factors. In addition, corticotrophs become refractory to hormones that stimulate or inhibit ACTH release. In most cases, the physiologic significance of the desensitization of hormone receptors on corticotrophs is not known. However, down regulation of glucocorticoid receptors in corticotrophs (*88*) may be responsible for the reduced ability of dexamethasone to lower plasma ACTH concentrations in some depressed patients (*89*).

The release of ACTH is the final outcome of the interplay among the hypo-

thalamus, adrenal cortex, adrenal medulla, and possibly other organs. Depending on the type of stress experienced, it is likely that a number of different hormones may singly or in combination affect the amount and duration of ACTH secreted. The interaction of the various stress mediators may act to fine-tune the responsiveness of ACTH-secreting cells.

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