HYPOTHALAMUS AND ADENOHYPOPHYSIS: WITH SPECIAL REFERENCE TO CORTICOTROPHIN RELEASE

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The hypothalamus would appear to be the focal point of convergence of the neural pathways mediating corticotrophin (ACTH) release. The variety of neural pathways entering this brain center in all likelihood match the variety of stimuli which are capable of increasing rate of release of ACTH from the adenohypophysis. The delineation of the pathways, synapses, centers of integration involved represents a formidable task for the future. The other task, more limited in scope but complex enough, is the definition of the link by means of which the hypothalamus modulates adenohypophyseal activities.

Hypothalamic Regulation of Adenohypophyseal Function. The unique vascular connection (portal venous system) and the lack of neural connections between hypothalamus and adenohypophysis suggest that chemical mediators pass from median eminence via a vascular path to release various adenohypophyseal ‘trophic’ hormones (Harris 1955). The following observations support this thesis. Simple stalk section has no appreciable influence on adenohypophyseal function if the portal venous system regenerates. Transplants of adenohypophysis, located at sites other than the median eminence area of the hypothalamus, are relatively inadequate in the maintenance of the functional activities of the adenohypophyseal target organs (Harris & Jacobsohn 1952; Fortier & Selye 1949; Cheng et al. 1949). A striking demonstration of the dependence of adenohypophysis upon hypothalamus is the recent experiment of Nikitovich-Winer & Everett (1958). Transplants of the adenohypophysis, after being located under the capsule of the kidney for a number of weeks, were relocated under the median eminence. The cells of the transplant, uniformly chromophobic at the renal site, regained tinctorial differentiation when relocated to the median eminence and the target organs, ovary, thyroid
and adrenal cortex, although not restored to normal, were significantly improved from a morphological and a functional point of view.

Lesions in the hypothalamus may result in inertia of ACTH release in response to otherwise effective stimuli. McCann (1953) concluded that an effective lesion must involve a major fraction of the median eminence. Lesions anterior or posterior to the median eminence were ineffective. We have been able to confirm these observations of McCann. However, it would be unwise at this time to make a definite statement about the exact location of the area of the hypothalamus most intimately or directly associated with the regulation of ACTH release. In our own laboratory it has not been possible to attain a satisfactory degree of uniformity of production of effective lesions and we are inclined to be conservative about making firm conclusions. Infarction of the adenohypophysis is one consequence of lesioning which results in failure. In a number of instances, lesions would appear to result in the loss of an inhibitory influence upon the adenohypophysis as judged by enlargement of the adrenals and low initial adrenal ascorbic acid concentrations. Animals with such lesions exhibit no or an attenuated response to corticotrophin releasing factor. The phenomenon may be due to failure of an already highly active adenohypophysis to further increase its activity in response to otherwise effective stimuli. That the hypothalamus may be less dominant than indicated above is suggested by the recent report of Egdahl et al. (1959) to the effect that ACTH is released at an accelerated rate in response to burn trauma in dogs in whom the hypothalamus has been completely (or almost completely) destroyed.

Pathways to the Hypothalamus Mediating ACTH Release: Impulses from Below. A neural pathway through spinal cord appears essential for ACTH release in response to cutaneous electrical stimulation. Spinal cord transection in the rat at the level of the upper thoracic vertebrae prevents adrenal ascorbic acid depletion following electric shock to the lower extremities. Stimulation of the forelegs of the cord-sectioned rat elicits ACTH release. Analysis of blood ACTH in adrenalectomized rats with cord section confirmed these observations and in addition indicated that descending paths in the cord to the adrenal medulla are not essential for activation of the adenohypophysis (Redgate, unpublished observations.)

The relative importance of locally produced toxins and of neural connections in the mediation of ACTH release in response to burns and to operative trauma has been evaluated by Egdahl (1959). He concludes that there is no evidence for the production in the wounded area of a substance which induces ACTH release from the adenohypophysis. The peripheral nerves appear to play the major role.

ACTH release elicited during the induction of ether anaesthesia is mediated by a neural pathway which passes through the brain stem. Brain stem transec-
tion (decerebration) at the upper level of pons prevents the sudden rise in blood ACTH titer normally observed in the adrenalectomized rat during the first few minutes of exposure to ether. ACTH release following administration of epinephrine persists in the decerebrate rat but is inhibited by destruction of the median eminence of the hypothalamus. Thus, epinephrine appears to elicit ACTH release by an action on neural elements rostral to the upper level of the pons. The brain stem reticular formation may be an important component in transmission between the spinal cord and hypothalamus. 

Indirect evidence for this arises from observations that anaesthetic agents which depress conduction through this multisynaptic area also inhibit ACTH release (Royce & Sayers 1958a).

Pathways to the Hypothalamus Mediating ACTH Release: Impulses from Above. The hypothalamus has extensive neural connections with the cerebral cortex, basal ganglia, rhinencephalon and thalamus. Experiments designed to evaluate the influence of these higher centers upon the release of ACTH by means of newly developed analytical techniques have been initiated and it is anticipated that they will lead to a more complete understanding of the means by which emotional states, e.g. fear and anger, act upon the endocrine system. For example, Mason (1958) has demonstrated that stimulation of the amygdaloid nucleus results in a sharp rise in plasma 17-hydroxycorticosteroids in the monkey. On the other hand, entorhinalhippocampal stimulation results in a decrease in plasma 17-hydroxycorticosteroids in this species (Mason et al. 1957).

Hypothalamic Corticotrophin Releasing Factor(s) (CRF). The nature of the chemical mediator, which enters the capillary loops in the median eminence, travels via the portal venous system to the adenohypophysis to increase the rate of release of ACTH, has been the subject of a number of investigations. Progress is limited in large measure by the lack of simple and specific methods of bioassay of the chemical mediator (corticotrophin releasing factor, CRF). Of the various assays employed, which include the release of ACTH from isolated incubated pituitaries (Saffran & Schally 1955), the corticosteroid blocked rat (Porter & Jones 1956; Porter & Rumsfeld 1956), the drug blocked rat (Briggs & Munson 1955) and the hypothalamic lesion rat, in our opinion the last mentioned is the most appropriate (see Sayers, Redgate & Royce (1958) for an evaluation of these assay methods). The hypothalamic lesion rat does not respond to epinephrine, ether, unilateral adrenalectomy, glucagon, oxytocin, polymyxin B, Serotonin, acetylsalicylic acid or histamine. This test animal does exhibit adrenal ascorbic acid depletion when callenged with ACTH, with vasopressin or with a corticotrophin releasing factor of hypothalamic origin.

Vasopressin induces adrenal ascorbic acid depletion in the rat with a lesion in the hypothalamus. McCann & Fruit (1957) have demonstrated that synthetic
lysine vasopressin as well as material from natural sources is effective. These observations together with the fact that an effective lesion is associated with diabetes insipidus as well as with inertia of ACTH release has naturally prompted McCann to suggest that vasopressin is the physiological CRF. Large doses of vasopressin (large in comparison to an antidiuretic dose) are required to induce ACTH release. McCann points out that the dose must be large when vasopressin is introduced into the systemic circulation in order to simulate the high concentrations which would normally be confined to the portal venous system.

Royce & Sayers (1958 b) have demonstrated that the action of vasopressin in stimulating the adrenal cortex is not confined to release of ACTH from the adenohypophysis. Vasopressin elevates the level of ACTH in the blood of the decapitated rat (maintained with artificial respiration). The actions of vasopressin may be classified as follows:

1) **Vasopressin acts directly on the adrenal cortex.** Vasopressin increases the concentration of 17-hydroxycorticosteroids in the adrenal vein blood of hypophysectomized dogs (Hume & Nelson 1957) and depletes adrenal ascorbic acid in the hypophysectomized rat (Royce & Sayers 1958 b). Hilton et al. (1959) have demonstrated a steroidogenic action of vasopressin in the isolated perfused adrenal of the dog. These observations are of considerable interest since as far as we know vasopressin is the only substance other than ACTH which depletes adrenal ascorbic acid in the hypophysectomized rat. The action of vasopressin on the adrenal cortex may represent another interesting example of overlapping of biological actions of certain polypeptide hormones (e.g., melanocyte stimulating activity of ACTH, oxytocic action of vasopressin).

2) **Vasopressin inhibits degradation of ACTH when added to anterior pituitary homogenates** (Barrett & Sayers 1958). One explanation is that vasopressin and ACTH complete for binding sites on proteolytic enzymes. This would account in part at least for the potentiating action of vasopressin on ACTH in the hypophysectomized rat (Royce & Sayers 1958). Tyberghein et al. (1956) had earlier interpreted the action of ACTH and of insulin in enhancing the glycolytic activity of glucagon on liver slices as a protection of the glucagon against degradation by proteolytic enzymes.

3) **Vasopressin releases ACTH from extrapituitary binding sites.** A relatively large fraction of injected or endogenously released ACTH is taken up by the kidney. Vasopressin may induce release of ACTH from kidney by competing for binding sites in this organ (Royce & Sayers 1958 b). In this connection, Higginbotham & Dougherty (1957) have attributed the action of ACTH and of other polypeptides in enhancing the toxicity of Polymyxin B to competition between polypeptides for tissue binding sites.

4) **Vasopressin releases ACTH from the adenohypophysis.** The degree of adrenal ascorbic acid depletion induced by vasopressin in the decapitated-hypothala-
mic lesioned rat is less than that induced in the hypothalamic lesioned rat. The difference very likely represents the direct action of vasopressin on the adenohypophysis. Perhaps vasopressin competes with ACTH for binding sites in the cells of the adenohypophysis and this may be the mechanism by means of which ACTH is released by the physiological CRFs. These actions of vasopressin just described may be of pharmacological interest only. They do point up possible complexities which may arise in connection with the assay of substances for CRF activity. From a more general point of view the observations suggest that the binding sites for which polypeptide hormones compete may include target organs, proteolytic enzymes and storage sites. The influence of one polypeptide hormone upon the biological activity of another would obviously be a complex function of their relative affinities for each of these binding sites.

Additional CRFs of Neurohypophysial Origin. Chemical fractionation of neurohypophyseal tissue has yielded polypeptide fractions, apparently distinct from vasopressin, which possess corticotrophin releasing activity. Privat de Garilhe et al. (1958) have fractionated neurohypophyseal extracts on IRC-50 ion exchange resin. A number of fractions when added to incubated pituitary glands increased the amount of ACTH in the incubation medium and when injected into steroid blocked rats induced adrenal ascorbic acid depletion. The active fractions were distinct from vasopressin.

Schally, Saffran & Zimmermann (1958) have reported purification of a neurohypophyseal polypeptide (not vasopressin) by paper chromatography which is exceedingly potent (active at $5 \times 10^{-10} \mu g$) in the in vitro pituitary assay.

Schally & Guillemin (1959) report that chromatography of neurohypophyseal extracts on carboxymethylcellulose yields a protein fraction which induces an increase in plasma corticosteroids in the morphine-pentobarbital blocked rat. CRF activity was closely associated with vasopressin on the chromatograms. However, Schally & Guillemin consider the CRF activity to reside in a polypeptide distinct from vasopressin.

CRF Activity in Hypothalamic Extracts. We have employed the hypothalamic lesioned rat (as described by McCann) for the assay of CRF in extracts of calf hypothalamus. The tissue used consists of the median eminence and immediately adjacent structures. Crude acetic acid extracts of slaughterhouse material exhibit pressor (USP pressor assay in rat), ACTH (hypophysectomized rat), and CRF activities. The ACTH activity is due to contamination with pituitary tissue. Carefully dissected hypothalamic tissue exhibits no significant quantity of ACTH. The depletion of adrenal ascorbic acid induced by crude acetic acid extracts in the lesioned rat is greater than can be accounted for by pressor or ACTH activities. Furthermore, pepsin destroys CRF activity without influencing pressor or ACTH activities.

The crude extracts have been fractionated by discontinuous elution from
carboxymethylcellulose. The 0.1 M ammonium acetate (pH 5.8) eluate, injected intravenously in a dose equivalent to about one third of a median eminence stalk complex and containing about 4 μg of protein nitrogen induces a pronounced depletion of adrenal ascorbic acid in the lesioned rat. At this same dose the fraction exhibits no pressor or ACTH activity. The 0.2 M ammonium acetate eluate contains most of the pressor activity of the extract and the 0.4 M ammonium acetate eluate, the ACTH activity. The 0.1 M fraction which contains the CRF activity is not toxic when injected intravenously. Whereas the CRF activity of the crude extract was stable, the activity of the purified material is labile and we have as yet no explanation for this instability. These observations indicate that a CRF, distinct from vasopressin, is present in hypothalamic extracts. The hypothalamic CRF, unlike vasopressin, exhibits no appreciable adrenal ascorbic acid depletion in the decapitated rat. Additional studies are needed to yield more definitive characterization of its chemical nature and its relation to other CRFs. The experimental data are suggestive, but by no means conclusive that the pepsin labile factor from hypothalamic tissue is the chemical mediator elaborated by the brain which courses down the portal venous system to increase rate of release of ACTH from the adenohypophysis.

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REFERENCES