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Corticotrophin Releasing Factor

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I. Introduction

MANY years ago Geoffrey W. Harris and his colleagues suggested that the adrenocorticotrophic activity of the anterior pituitary gland is controlled by a chemical transmitter substance, later named the corticotrophin releasing factor, liberated by neurones in the hypothalamus and conveyed to the adenohypophysis via the hypothalamo-hypophysial portal vessels. This proposal naturally stimulated attempts to isolate and identify the active substance. However, while there can be no doubt that the hypothalamus contains a substance, or possibly substances, capable of stimulating corticotrophin (ACTH) secretion, its chemical identity remains a mystery. Nevertheless, something is known about the mechanisms that control its (their) secretion. In the sections that follow, studies on the chemistry, physiology, and pharmacology of the corticotrophin releasing factor(s) are reviewed.

II. Evidence for the Existence of Corticotrophin Releasing Factor

When Harris (105) and Brooks (30) first suggested, perhaps rather tentatively, that the secretory activity of the adenohypophysis may be controlled by humorally transmitted stimuli from the hypothalamus little interest was expressed in their hypothesis. This was probably mainly because it was thought, at that time, that the direction of blood flow in the hypothalamo-hypophysial portal vessels (which form the only anatomical link between the hypothalamus and the anterior pituitary gland) was from the adenohypophysis to the hypothalamus. The direction of flow in these vessels subsequently became a matter of controversy and it was not until 1949, after the development of an elegant technique for the direct observation of blood flow in the portal vessels of rats, that Green and Harris (96) were able to demonstrate conclusively that blood passes from the hypothalamus to the pituitary gland.

It is now firmly established that the synthesis and release of the hormones of the adenohypophysis are controlled by substances released from nerve endings in the hypothalamus and conveyed to the anterior pituitary gland via the portal vessels. The evidence that demonstrated the fundamental importance of the hypothalamus and the hypothalamo-hypophysial portal vessels in the control of corticotrophin secretion came mainly from experiments that involved transection of the pituitary stalk, transplantation of the pituitary gland to a site remote from the sella turcica, or electrical stimulation of the hypothalamus.

Ordinary transection of the pituitary stalk is followed first by adrenal atrophy and subsequently by the recurrence of normal adrenocortical activity. Histological studies demonstrated that, unless a wax paper plate was inserted between the two cut ends, transection of the stalk was followed, almost invariably, by regeneration of the portal vessels, and that the degree of regeneration was correlated with the degree of restoration of pituitary activity (107).

The simple experiment of removing the pituitary gland from the sella turcica and transplanting it to another site in the body also demonstrated the role of the hypothalamus in the control of pituitary adrenocorticotrophic

function. When the transplanted tissue was placed in a site remote from the sella turcica, for example, the anterior chamber of the eye, the kidney capsule, or temporal lobe of the brain, partial or complete atrophy of the adrenal cortices followed. When, however, the transplanted tissue was placed in the vicinity of the hypothalamus and pituitary stalk, regeneration of the hypophysial portal vessels occurred and anterior pituitary function was restored (108).

As early as 1936 it was realised that diffuse electrical stimuli applied to the head or lumbar spinal cord of rabbits (176) or rats (104) enhance adenohypophysial activity. In an attempt to delimit the neural structures involved, localised electrical stimuli were applied directly to regions of the hypothalamus and anterior pituitary gland of anaesthetised animals. Electrical stimulation of the pituitary gland was ineffective but stimulation of discrete areas of the hypothalamus evoked the release of anterior pituitary hormones. Corresponding lesions in the hypothalamus were, however, not always effective in suppressing pituitary activity. Moreover, since the electrical stimulation of the hypothalamus was performed in anaesthetised animals, the possibility existed that the observed endocrine activity was the result of a complication induced by the anaesthesia.

Harris (106) believed it important to study endocrine function in conscious animals, and, accordingly, developed an ingenious method for "remote control stimulation" of the hypothalamus in unanaesthetised rabbits. With the aid of this technique, experiments were performed for relatively long periods without concomitant operative trauma. Furthermore, the experiments were repeated many times in the same animal, thereby reducing the chances that variable factors, such as differences in the nutritional or oestrous state of the animal, influenced the result. The method involved a preliminary operation in which a small flat coil was inserted between the skull and scalp. The inner turn of the coil was connected to an electrode implanted in the hypothalamus and the outer turn of the coil was connected to a second electrode, the indifferent electrode.

Stimulation of the hypothalamus was readily achieved by placing the animal's head in an electromagnetic field and inducing a voltage in the buried coil by remote control. The experiments that followed demonstrated clearly that electrical stimulation of discrete areas of the hypothalamus increased markedly the activity of the adrenal cortex (98). This finding led Harris to postulate that the hypothalamus liberates a chemical transmitter substance into the hypophysial portal vessels that stimulates the adrenocorticotrophic activity of the adenohypophysis. This substance was subsequently named the corticotrophin releasing factor (CRF) by Saffran et al. (224).

Slusher and Roberts (251) were the first to report the extraction of corticotrophin releasing activity from hypothalamic tissue. They isolated a lipoprotein fraction from bovine posterior hypothalami that caused adrenal ascorbic acid depletion (an indicator of increased circulating ACTH) in intact but not in hypophysectomised rats. However, later work indicated that this effect was nonspecific and that the substance was inactive in rats in which the mobilisation of endogenous CRF was prevented either by hypothalamic lesions or by pretreatment with chlorpromazine, morphine, or cortisol (288). Moreover, some additional doubt concerning the ability of hypothalamic tissue to evoke ACTH release arose when Saffran and Schally (223) showed that the amount of ACTH released in vitro by pituitary tissue alone was equal to that released by that tissue when incubated in the presence of hypothalamic, neurohypophysial, or liver tissue. Subsequently, however, the existence of a hypothalamic CRF was convincingly demonstrated by the experiments of Porter et al. (210). They showed that extracts of plasma from the sella turcica of stressed hypophysectomised dogs caused adrenal ascorbic acid depletion in cortisol-treated rats. No similar activity was found in the carotid blood of the same dogs and it was suggested that the corticotrophin releasing activity of the portal blood was due to a substance acquired by the blood on its passage through the primary capillary plexus of the hypophysial portal vessels (208, 209). Later studies confirmed and extended these observations (29, 218). The ability of crude hypothalamic extracts to stimulate the secretion of adrenocorticotrophic hormone (ACTH, corticotrophin) in vivo in rats with hypothalamic lesions (184, 216, 293) or in animals in which the release of CRF was inhibited by corticosteroids (218) was subsequently described. Moreover, in contrast to the earlier findings (223), it was convincingly demonstrated that hypothalamic extracts but not cerebral cortical extracts evoke the release of ACTH from pituitary tissue in vitro (100), thus providing firm evidence for the existence of a hypothalamic CRF.

III. Chemical Nature of Corticotrophin Releasing Factor

The exact location of the CRF-secreting neurons within the hypothalamus is not known but it is clear that the median eminence is rich in CRF activity. Various attempts have been made to isolate CRF from tissue of both hypothalamic and neurohypophysial origin (neurohypophysial extracts possess CRF activity). It appears that the "hormone" is a polypeptide since the CRF activity of hypothalamic extracts is readily destroyed by the proteolytic enzymes, pepsin or trypsin. After preliminary purification procedures, CRF activity is present in a plasma protein fraction of portal blood. In 1958, Schally et al. (236) described the partial purification of a CRF from the convenient if not logical "protopituitrin" (the posterior pituitary starting material for vasopressin isolation). Two years later, two fractions with CRF activity, α-CRF and β-CRF, were isolated from the same source (231). The β-fraction was the more potent and it also

possessed pressor and antidiuretic activity. A preliminary investigation of its structure suggested a resemblance to lysine vasopressin (232). α-CRF, which also possessed ACTH-like activity (231), was described as two peptides, α_1-CRF and α_2-CRF, both similar to but slightly larger than α-MSH. On the basis of these findings, it was postulated that α-CRF is merely a precursor of ACTH (the sequence of the first 13 amino acids is identical with α-MSH) and that β-CRF is the neurohumoral transmitter that stimulates ACTH release (234). In 1962, scientists turned to hypothalamic tissue for their studies (101, 235). This tissue, in my opinion, provides a more rational ("physiological") source of CRF. Hypothalamic tissue yielded two fractions with CRF activity that possessed chromatographic properties similar to those of the α-CRF and β-CRF obtained from the posterior pituitary gland. However, in contrast to the β-CRF of neurohypophysial origin, the corresponding fraction from the hypothalamus had considerable ACTH-like activity and it was suggested that the fraction that resembled α-CRF was probably the physiological corticotrophin releasing hormone.

In an independent study by Dhariwal et al. (64), a substance with CRF activity was isolated from ovine hypothalami by means of gel filtration and ion exchange chromatography. The substance, structure unknown, was believed to be a single chemical entity devoid of ACTH-like or pressor activity. However, the material was not subjected to countercurrent distribution, a process that can separate two CRF activities and thus the possibility of two distinct CRF's could not be excluded. Chan et al. (51) demonstrated the presence of two CRF's in rat median eminence tissue, a large and a small CRF, and suggested that the activity may reside in a peptide containing aromatic amino acids.

For some time it was thought that vasopressin may be chemically identical with CRF (178, 181, 182) and there is circumstantial evidence to support this hypothesis. For example, stress often results in the release of both vasopressin and ACTH (192). McCann and Brobeck (182) showed that rats with lesions in the median eminence develop diabetes insipidus and that ACTH release can be elicited in such animals by injection of vasopressin. Moreover, there was an association between the intensity of the diabetes insipidus and the degree of inhibition of pituitary adrenocorticotrophic activity in animals with hypothalamic lesions (183), and the adrenal ascorbic acid depletion caused by vasopressin in hypothalamic-lesioned rats was proportional to its pressor activity. Furthermore, the immunoreactive vasopressin content of the hypothalamus, like the CRF content, was elevated in adrenalectomised rats but normal in corticosteroid-treated adrenalectomised rats (66, 256). These latter findings should, perhaps, be treated with some caution since the antibody to vasopressin employed may cross-react with other similar peptides that act as CRF.

There is also a considerable amount of evidence that suggests that vasopressin is *not* the major hypothalamic factor that triggers ACTH release. In an early paper, Briggs and Munson (21) demonstrated that pentobarbitone/morphine blocked the effect of vasopressin on ACTH release. Furthermore, according to Saffran and Saffran (222), the doses used by McCann and Fruit (183) to induce ACTH secretion in hypothalamic-lesioned rats were several thousand times greater than the dose needed to inhibit diuresis maximally. This criticism is not necessarily valid since there are no data available to indicate how much systemic vasopressin is necessary in the peripheral circulation to change the effective concentration in the hypophysial portal vessels (94). De Wied et al. (293) showed that there was a great deal of CRF activity but little pressor activity in extracts of hypothalami from hypophysectomised rats, while McDonald et al. (186) found a complete lack of correlation between the release of these two activities in response to nicotine, water loading, and water deprivation in man. Hypothalamo-pituitary-adrenocorticotrophic activity is reduced in rats with inherited diabetes insipidus (Brattleboro rats), but hypothalamic extracts from such rats possess corticotrophin releasing activity (43, 89, 161) and these animals are capable of responding to stress with a rise in corticosterone concentration (7, 294). The ability of vasopressin and several of its analogues to stimulate pituitary adrenocorticotrophic activity has been studied extensively (37, 45, 92, 204). In one study (45), a sensitive and precise in vitro assay technique using pituitary segments (37) and an in vivo/in vitro system (290) using pentobarbitone/chlorpromazine-treated rats were employed in parallel. Arginine vasopressin and lysine vasopressin stimulate ACTH secretion in both systems (45). However, the slopes of the dose-response lines for vasopressin and hypothalamic extracts differed significantly, indicating that the corticotrophin releasing principle in the hypothalamic extract is not chemically identical with vasopressin (45). The desglycinamide derivatives of lysine and arginine-vasopressin exhibited some activity in the in vitro system but were ineffective when injected into pentobarbitone-chlorpromazine-treated rats (45). Pressinoic acid, its amide, oxytocin, alanine-8-oxypressin, and the "tail fragment" (proline-arginine-glycinamide) of arginine vasopressin did not increase corticotrophin production in either test system (45). Oxypressin and arginine vasotocin exhibited very marked corticotrophin releasing activity in both assays (45) and, unlike the vasopressins, the dose-response relationships of arginine vasotocin closely resembled those of hypothalamic extracts (37). Similar findings have been reported by Gillies et al. (92) but in their bioassay system, in which isolated pituitary cells are exposed to the putative hormone, the dose-response lines for arginine vasotocin, like those for lysine vasopressin, differ significantly from those for stalk-median-eminence (SME) extract. These investigators have raised again the possibility that vasopressin within the hypothalamus is of fundamental importance in the physiolog-

ical control of corticotrophin secretion. They showed (91) that the corticotrophin releasing activity of rat SME extract is abolished by incubation with "specific" arginine vasopressin antiserum. Chromatographic separation of SME extracts yielded two peaks of CRF activity. The major peak occurred in the expected position of arginine vasopressin and, in the isolated pituitary cell column bioassay, its dose-response relationships resembled those of vasopressin and not those of the SME extract. On the basis of these findings, Gillies et al. (92) suggested that the corticotrophin releasing activity of the hypothalamus is due to vasopressin (or a closely related molecule) and that it requires a synergistic factor yet to be identified in order to exhibit its full biological activity (91). This hypothesis is in accord with the earlier suggestion of Saffran and colleagues (205) that CRF requires a synergistic cofactor and is further supported by their finding that recombination of the two "peak fractions" results in a complex with strong CRF-like activity and that the dose-response relationships of the complex closely resemble those of the SME extract (89). However, results from experiments done in the Royal Free laboratory (44), in which the corticotrophin releasing activity of hypothalami from rats with inherited diabetes insipidus (Brattleboro rats) has been studied with an in vitro pituitary segment system, are not in accord with this hypothesis. Hypothalamic extracts from Brattleboro rats and from normal controls (Long Evans) cause dose-related increases in ACTH production but both the activity and the slope of the dose response line of the Brattleboro extract are significantly ($P < .001$) less than those of the controls (44). Vasopressin, in concentrations not sufficient to stimulate directly the secretion of ACTH by pituitary segments in vitro, potentiates the corticotrophin releasing activity of hypothalamic extracts from normal and diabetic rats and renders the slopes of the dose-response lines of the Brattleboro extracts parallel with those of the controls (44). Furthermore, in contrast to the findings of Gillies and Lowry (91), treatment with arginine vasopressin antiserum reduces but does not abolish the corticotrophin releasing activity of hypothalamic extracts from controls and does not affect that from Brattleboro rats. Moreover, the antiserum treatment alters the slopes of the dose-response lines of the control extract so that they are identical with those of the Brattleboro extracts (44). These findings suggest that vasopressin is not the corticotrophin releasing factor but that it acts synergistically with the hypothalamic hormone and is essential for the full expression of hypothalamo-pituitary-adrenocorticotrophic activity.

The explanation for the discrepancies between these results (44) and those of Gillies and Lowry may lie in differences between the methods employed to detect corticotrophin releasing activity. It is well known that new biological assay methods should be "validated" by comparison with older, well-established bioassays. Parallel studies have shown that the results obtained with pituitary segments in vitro are in agreement with those obtained with in vivo assay systems employing rats in which endogenous CRF secretion is prevented either pharmacologically (45) or surgically (46, 140). However, recent studies suggest that the results obtained with isolated pituitary cells are not always in accord with those of other systems. According to M. T. Jones and colleagues (personal communication) the "CRF" secreted by isolated hypothalami in response to 5-hydroxytryptamine (5-HT), although it was clearly active when injected into hypothalamic lesioned rats or incubated with rat pituitary segments, was inactive in the pituitary cell column assay described by Gillies and Lowry (90). The reasons for this discrepancy are not clear. The possibility exists that the separation of pituitary cells with enzymes such as trypsin may lead to damage of the cell membranes and thus alteration in the specificity of the CRF receptor but, as yet, direct evidence is not available to support this explanation. Nevertheless, in my opinion, these findings cast considerable doubt upon the validity of the use of isolated cell systems for the detection of corticotrophin releasing hormone and emphasise the importance of validating new methods against older, well-established systems.

The true corticotrophin releasing factors remain to be identified. One of the major problems associated with the isolation of such small, relatively unstable polypeptides from biological tissue is that the peptides may break down or undergo other chemical transformation during the extraction procedures. Jones et al. (140) have developed a new approach to the study of the chemical nature of CRF in the rat that may overcome this problem. These workers showed that acetylcholine stimulates the secretion of "CRF" and vasopressin from isolated rat hypothalami in vitro but that 5-HT is more selective in this respect and evokes the secretion of "CRF" only (140). Attempts are now being made to isolate and identify the CRF released into the incubation medium in response to 5-HT. Preliminary separation by chromatography on Sephadex G-25 demonstrated two peaks of CRF activity, fractions A and B, with molecular weights of about 2500 and 1300, respectively. Both fractions evoked marked, dose-related increases in pituitary ACTH release either when injected into basal hypothalamic-lesioned rats or when incubated with rat adenohypophysial segments in vitro. These effects appear to be specific since neither fraction markedly influences the release of other pituitary hormones in the rat (46, 140) but the ability of the fractions to influence ACTH release in other species has not been tested. The amino acid sequences of these still not purified peptides have yet to be determined and the identity of CRF remains an enigma.

IV. Methods for the Detection and Quantification of Corticotrophin Releasing Activity

Many methods have been developed for the detection of corticotrophin releasing activity. None of these qualify

as a classical biological assay method, since bioassay involves the comparison of the potency of the unknown with that of a suitable standard preparation and, at the present time, there is no satisfactory standard preparation of CRF. (A stable, potent preparation is not generally available.) Nevertheless, the techniques involve the detection and quantification of a biologically active substance and thus, in evaluating their usefulness, their precision, specificity, and sensitivity should all be considered as far as is possible. Without a standard preparation the comparability of methods for the detection of corticotrophin releasing activity cannot be determined but the index of precision can be assessed readily. In my opinion, only those systems in which the index of precision (λ) (78) is less than 0.15 are satifactory. Since the chemical nature of CRF is not known, the true specificity of methods for its detection is difficult to define. A great many substances present in the hypothalamus are known not to affect directly the secretion of corticotrophin (e.g. acetylcholine, noradrenaline, adrenaline, 5-HT, γ-aminobutyric acid, glycine, glutamine, histamine, enkephalin, endorphin, gonadotrophin releasing hormone, thyrotrophin releasing hormone, growth hormone release inhibiting hormone) and therefore should not be active in an acceptably specific assay method. Similarly, without a suitable standard preparation, the sensitivity (the minimum effective dose of CRF required) of the "assay" methods cannot be truly assessed. Several groups have attempted to express the sensitivity of their respective techniques in terms of the minimum concentration of either hypothalamic extract (90, 117, 206, 207, 247, 248) or lysine vasopressin (214, 289, 290) required to evoke a significant response. Neither is valid. The amount of "CRF" present in an extract of one stalk median eminence or hypothalamus depends on the method of extraction (CRF is more readily extracted in media of low pH), on the time that the organ is removed [the CRF content of the hypothalamus varies according to a circadian pattern (62)], the rapidity of the dissection, and on the "stress-state" of the donor at the time of death [the CRF activity of the hypothalamus is affected profoundly by minor stressful stimuli and this is reflected in the potency of extracts (31, 280)]. Moreover, the responsiveness of anterior pituitary tissue to vasopressin cannot be correlated repeatably with that to hypothalamic extracts. Miahle et al. (191) showed that pituitary tissue in vitro responds to hypothalamic extract immediately after it is removed from the donor but that it responds to vasopressin only after a considerable preincubation period. Similar findings have been reported by Sadow and Thomas (221).

The methods for the detection of corticotrophin releasing activity involve the measurement of the resulting adrenocorticotrophic activity of the adenohypophysis either in vivo, in animals in which the endogenous release of CRF has been inhibited, or in vitro.

Until recently the assay systems were hampered by the lack of satisfactory techniques for the determination of corticotrophin. The development of sensitive, specific, and precise bioassay methods for the estimation of corticotrophin (2, 227) has overcome this problem but, nevertheless, some workers still employ relatively insensitive or indirect indices of ACTH secretion (e.g. adrenal ascorbic acid depletion or adrenal corticosteroidogenesis) that I regard as less appropriate.

Rats in which the nonspecific release of corticotrophin is prevented by the placement of extensive lesions in the median eminence have been used for many years for the detection of CRF (289). The effectiveness of the lesion is assessed either histologically or, more simply, by subjecting the animal to a stressful stimulus and subsequent unilateral adrenalectomy to obtain a gland to pretest. The rate of corticosterone production in vitro by the excised gland serves as the index of ACTH release, which is small in an effectively lesioned rat. The test substance is then administered i.v. to the surviving unilaterally adrenalectomised rat and the rate of corticosterone production in vitro by the second adrenal gland is determined. The sensitivity of the pituitary gland to CRF is lower in rats with hypothalamic lesions than in intact rats but, according to de Wied (290), this problem is reduced by performing the assay within 1 to 3 days of placing the lesion. Nevertheless, the method is still relatively insensitive and lacks satisfactory precision (λ = 0.3). Furthermore, the accurate placement of lesions requires great skill and any minute change in the location of the electrode may reduce the effectiveness of the lesion. These technical difficulties prompted a search for other means of inhibiting the stress-induced secretion of ACTH. Sirett and Purves (247) proposed that hypophysectomised rats bearing pituitary transplants in the kidney capsule might be suitable. In appropriately "grafted" rats, the i.v. injection of an acid extract of 1.0 SME caused a significant rise in plasma corticosterone concentration (245–247) but higher doses failed to elicit an enhanced response. This is probably because the adrenal glands of the "grafted" rat are atrophic and are relatively insensitive to ACTH. Their responsiveness is readily restored by "priming" with corticotrophin and suitably "primed grafted" rats respond to SME with dose-related increases in plasma corticosterone concentration over a range of concentrations from 0.2 to 1.0 SME (248, 249). This assay is reliable and relatively precise (λ = 0.13 to 0.17) (248, 249) but it is difficult to perform and requires much experience and expertise. It could be simplified and improved by the incorporation of a suitable direct assay for ACTH.

Many investigators have preferred to employ pharmacological methods to prevent the stress-induced release of ACTH. High doses of glucocorticoids are effective in preventing ACTH discharge and thus corticosteroid-treated rats have been advocated as a convenient and reliable preparation for the detection of CRF (8, 219, 237, 279). However, the system lacks satisfactory preci-

sion (λ = 0.22) and sensitivity (minimum effective dose = 0.2 HE (HE = extract of one hypothalamus)). Most steroids that prevent the discharge of corticotrophin in response to stress also reduce the capacity of the anterior pituitary gland to release ACTH in response to hypothalamic extracts (32, 113, 280) or lysine vasopressin (37) by virtue of direct actions of the steroids on the pituitary gland.

Many psychotropic drugs prevent the release of ACTH when injected into animals pretreated with pentobarbitone. Briggs and Munson (21) showed that treatment with pentobarbitone and morphine inhibits the stress-induced adrenocorticotrophic activity of the adenohypophysis and this drug combination was subsequently adapted for the assay of CRF (99). According to some workers the pentobarbitone-morphine-treated rat is very sensitive to CRF but Briggs and Munson (21), de Wied et al. (292), and Guillemin et al. (99) found the sensitivity of the adenohypophysis to vasopressin to be impaired, which these investigators considered to be an advantage and an indication of specificity. Arimura et al. (8) reported that the effects of this drug combination were unreliable and that the method could be improved considerably by the incorporation of chlorpromazine. Pentobarbitone-morphine-chlorpromazine-treated rats appear suitable for the assay of CRF and respond consistently to concentrations of arginine vasopressin and hypothalamic extract as low as 12.5 mU and 0.3 HE respectively (8). Other workers have found that chlorpromazine alone inhibits the stress-induced release of ACTH in pentobarbitone-treated rats and this drug combination has been strongly favoured for the assay of CRF by one prominent laboratory (164, 165, 290). Pentobarbitone-chlorpromazine-treated rats respond to i.v. injections of either hypothalamic extracts or vasopressin with dose-related increases in the concentration of ACTH in the plasma (133) and in the amount of corticosterone produced by the adrenal gland (164, 165, 290). The drug treatment does reduce the sensitivity of the adenohypophysis to "CRF" (133) but not so markedly as the placement of lesions in the hypothalamus. This is not surprising. The secretory capacity of corticotrophs is reduced by deprivation of trophic stimuli. Hypothalamic-lesioned rats are not used for assay until 1 to 3 days after the operation but the drug-treated animals are employed within 30 minutes of the injection of chlorpromazine (290).

Although it has been suggested that monoamine oxidase inhibitors abolish the pituitary adrenocortical response to stress (269), attempts to employ animals treated with these drugs for the assay of CRF have not been successful. Schally et al. (233) showed that treatment of rats with pentobarbitone and α-ethyltryptamine does not prevent the stress-induced release of ACTH but reduces markedly the responsiveness of the adenohypophysis to putative corticotrophin releasing factors. Similar findings were reported by de Wied (290). The disadvantage of all of the above methods is that the test substances are administered i.v. and thus are diluted in the systemic circulation before they reach the anterior pituitary gland. Nikitovitch-Winer (199) applied a technique of direct intrapituitary injection and demonstrated, with respect to gonadotrophin secretion, a 10- to 48-fold increase in sensitivity to gonadotrophin releasing hormone compared with the parallel i.v. method. Similar results were reported by Campbell et al. (48). A detailed comparison of the effects of i.v., intramedian eminence, and intrapituitary administration of CRF to rats treated with either pentobarbitone/morphine or pentobarbitone/dexamethasone was made. Dhariwal et al. (65) showed that hypothalamic extracts that were ineffective when given i.v. caused dose-related increases in the plasma corticosterone concentration when injected directly into either the median eminence or anterior pituitary gland but that the latter were more effective. The responses after median eminence injections were probably attributable to the spread of material to the adenohypophysis since positive results were obtained only in the limited region in which the portal vessels were most concentrated. These findings suggested that assay methods in which intrapituitary injections are employed are more sensitive than the parallel i.v. techniques. However, the stereotaxic work required for the method limits its use in the screening of large numbers of samples. Furthermore, according to Hiroshige and Itoh (118), the injection technique employed by Dhariwal et al. has serious shortcomings. Firstly, it is difficult to inject into some areas of the pituitary gland, and secondly, the passage of cannulae through the brain stem to the adenohypophysis may stimulate structures that can modulate ACTH secretion or may allow the spread of injected substances up the cannula back to such structures. Hiroshige and colleagues (117–119) have adopted a parapharyngeal approach to the adenohypophysis that enables the gland to be reached without the risk of damaging brain structures or contaminating the tissue with backflow. By using this technique, Hiroshige (117) reported the detection of concentrations of SME extract as low as 0.02. Although it lacks adequate precision (λ = 0.27), the reported specificity and sensitivity of this method recommend its repetition and use by other investigators of CRF.

The loss of pituitary sensitivity and the possibility of incomplete blockade of the hypothalamo-pituitary tract together with the technical difficulties associated with in vivo methods stimulated workers to develop in vitro systems for the assessment of corticotrophin releasing activity. Initially, hemisected pituitary glands, removed from rats often weighing as much as 200 g (52, 100, 204, 223, 224) were employed. The problems of poor diffusion of substances into the gland and the possibility of necrosis at the centre of the tissue coupled with the use of indirect or insensitive methods for the determination of ACTH made the early in vitro methods insensitive and impre-

cise. The dangers of poor diffusion and necrosis have been substantially reduced by the use of quartered (instead of hemisected) pituitary glands removed from small rats (75 g) (37). Various attempts have been made to increase the sensitivity of pituitary segments to "CRF". Seiden and Brodish (238) reported that lowering the pH and increasing the osmolarity of the incubation medium were helpful. Other groups have found that the capacity of adenohypophysial tissue to secrete ACTH in vitro is enhanced either by using glands removed from rats adrenalectomised some days previously (31, 46) or, more simply, by "priming" the segments in vitro with either "CRF" (46) or lysine vasopressin (37). These modifications, coupled with the use of a sensitive bioassay for corticotrophin (2) has resulted in the development of a highly sensitive (minimum effective dose = 0.02 HE) and precise ($\lambda = 0.06$) method for the measurement of CRF (37).

The most "sensitive" assays described so far are those in which suspensions of isolated pituitary cells are challenged with "CRF". The first such method was described by Portanova and Sayers (206, 207). These workers assayed the ACTH secreted by their pituitary cells with the sensitive adrenal cell method and reported that they could detect amounts of hypothalamic extract as small as 0.005 HE. Several modifications of their technique have subsequently been described. Takebe et al. (264) showed that dispersed, pooled, rat adenohypophysial cells cultured for several days could be used for the assay of CRF. They demonstrated a positive relationship between the amount of ACTH released by the cultured cells and the logarithm of the dose of hypothalamic extract over a range of concentrations from 0.0125 to 1.25 HE. This system is reasonably precise ($\lambda = 0.15$) and the same cultured cells can be satisfactorily used in repetitive assays performed on the same or different days, thus providing a relatively economical and simple technique for assessing corticotrophin releasing activity. Gillies and Lowry (90) have developed a system in which columns of isolated rat adenohypophysial cells suspended in Biogel are perfused with test substances. In this system, fresh oxygenated media or samples are continually passed through the cells and thus neither the secretagogue nor metabolic waste products are allowed to accumulate in the media bathing the cells. This method, like that of Takebe et al. (264), has the advantage that many samples may be processed on the same cells with no apparent change in the sensitivity of the cells to trophic stimuli. This is in complete contrast to the findings with pituitary fragments, which respond to repetitive dosing with hypothalamic extracts or vasopressin with significantly elevated increments in ACTH secretion (37, 46). It is surprising, with the availability of sensitive, accurate, specific, and precise bioassays for the determination of ACTH, that Gillies and Lowry (90) chose to use a radioimmunoassay method. Doubtless, the immunoassay is simpler to perform but I do not agree with their statement that the results obtained with the immunoassay are "more reliable" than those achieved with bioassay techniques. It is true that marked discrepancies between the results obtained with immuno- and bioassay methods have been reported, but they are probably associated with the lack of specificity of the immunoassay rather than with unreliability of the bioassay. Moreover, the statement by Gaddum, "when biological methods and chemical methods for the assay of a pharmacologically active substance disagree so widely that the disagreement cannot be due to the error of the tests, the biological method is, by definition, right and the chemical method is wrong" (47) could apply to immunoassays as well as to chemical methods.

It has sometimes been suggested that the pituitary receptors in vitro may lack specificity for CRF and thus that in vitro systems may not be suitable for the detection and quantification of physiological corticotrophin releasing factors. Since there is no standard preparation of corticotrophin releasing factor it is possible at present to express CRF activity only in terms of the amount of ACTH produced by the anterior pituitary gland. It is essential, therefore, that any method for the detection of CRF should be validated as far as is possible by comparing the activity of the same samples on other, preferably in vivo, methods. Comparative studies (45, 46, 140) have shown a good correlation between the results obtained using pituitary fragments in vitro and those achieved with in vivo systems, employing rats in which the endogenous release of CRF was prevented either by lesions in the hypothalamus or treatment with pentobarbitone and chlorpromazine. However, there are doubts (M. T. Jones and colleagues, personal communication) concerning the correlation of the results obtained with isolated pituitary cells (90) and those achieved employing either hypothalamic lesioned rats or pituitary segments in vitro. This problem requires a great deal of further investigation and emphasises the need to evaluate new bioassay methods by comparing them with older, well-established, preferably in vivo systems.

V. Control of the Secretion of Corticotrophin Releasing Factor

Many years ago, Selye (239, 240) discovered that toxic doses of drugs and other noxious treatments (stressful stimuli) produced hypertrophy of the adrenal cortex in intact but not in hypophysectomised rats. This effect obviously was mediated by ACTH. Enormous interest in the mechanisms that control the secretion of ACTH was stimulated. It is now known that the adrenocorticotrophic activity of the pituitary gland is correlated with the corticotrophin releasing activity of the hypothalamus. For example, the peak of the circadian excursion in adrenocortical activity in the rat is accompanied by a rise in the CRF content of the hypothalamus (62, 117, 215, 263, 270). Elevated levels of CRF in the hypothalamus have also been reported in female rats during the morning

of prooestrous (121) before the preovulatory surge of pituitary-adrenocorticotrophic activity (33). Exposure of rats to ether vapour causes a rapid fall and subsequent rise in hypothalamic CRF content together with an increase in the ACTH content of the adenohypophysis and the plasma (31, 120, 280). These changes are followed by a rise in the plasma concentration of corticosterone (fig. 1). Moreover, the stress-induced changes in hypothalamic CRF content, like those in pituitary adrenocorticotrophic activity, are exaggerated by adrenalectomy and reduced by corticosteroid treatment (31, 280).

A great deal of attention has focussed on the mechanisms that control the secretion of CRF. It appears that the production of this hypothalamic hormone involves the integration and differentiation of a torrent of afferent impulses, which may be either excitatory or inhibitory, from several regions of the brain. Studies involving electrical stimulation or lesions of specific areas of the brain suggest that the amygdala (155, 156, 179, 213, 250) and hippocampus (69, 147, 155–157) are of fundamental importance in this respect but other brain areas, notably the thalamus, basal septal area, and rostral midbrain reticular formation, may also be involved. There have been many attempts to characterize the neurones that influence the activity of the hypothalamic corticotrophin releasing hormone cells. Most of these studies have involved conventional in vivo techniques in which drugs that influence the activity of the central nervous system are implanted directly into the brain or, in the case of substances that readily cross the blood-brain barrier, are administered peripherally. The results from such experiments are sometimes hard to interpret. The doses of the implanted drugs are frequently very high and the possibility that the drugs diffuse to other brain areas cannot be disregarded. Moreover, the drugs administered are often rather nonspecific. For example, reserpine, a substance widely used in experimental neuroendocrinology, not only depletes the brain of noradrenaline but also of 5-HT and dopamine. Noradrenaline biosynthesis is effectively inhibited by 6-hydroxydopamine but the animals thus treated show signs of nervousness and aggression that in themselves may influence the secretion of CRF. The development of an in vitro method in which whole rat hypothalami are incubated in the presence of neurotransmitter substances has provided a new approach to the study of the neural mechanisms controlling the secretion of CRF and has overcome many of the problems associated with in vivo work. Of course, the validity of results translated from in vitro experiments to the situation in vivo must be questioned but, nevertheless, this system, in which a whole hypothalamus is challenged with putative neurotransmitter substances, has provided valuable information concerning the receptors *in the hypothalamus* that influence CRF production.

It appears that several neurotransmitter substances are involved in the regulation of CRF secretion. The possibility that a central cholinergic nervous pathway controls the release of CRF was first proposed by En-

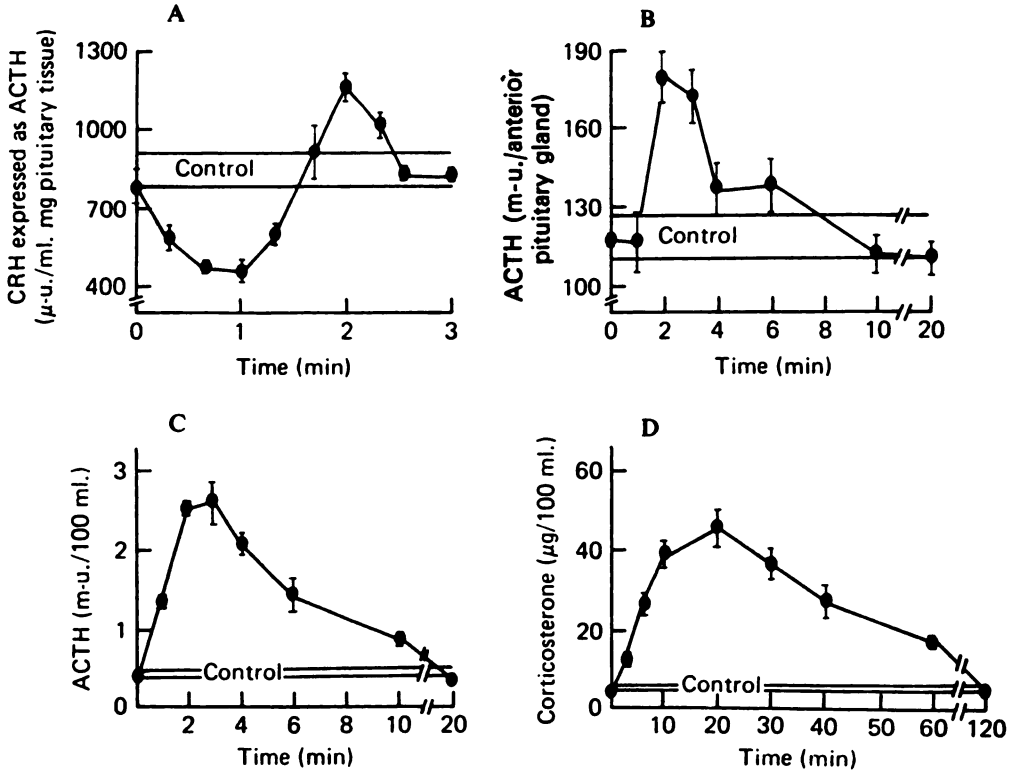

FIG. 1. A, hypothalamic CRF (CRH) content; B, pituitary ACTH content; C, plasma ACTH concentration; and D, plasma corticosterone concentration at various times after stress. Each point is the mean of five determinations and is shown with its standard error. (Reprinted with permission from J. C. Buckingham, *J. Physiol.* (*London*), **286**:331–342, 1979.)

droczi et al. (70), who showed that implantation of carbachol into various regions of the central nervous system stimulates pituitary-adrenocorticotrophic activity. A considerable amount of evidence now supports the hypothesis of Endroczi et al. For example, implantation of atropine into the anterior hypothalamus reduces the adrenocortical response to stress (110, 112, 145) and carbachol increases the plasma corticosterone concentration when infused into the lateral ventricles of the rat (1). Furthermore, picomolar concentrations of acetylcholine evoke both the synthesis and release of CRF by isolated rat hypothalami in vitro (20, 39, 114). It is not clear from the results of experiments in vivo whether the actions of acetylcholine are mediated by stimulation of nicotinic or muscarinic cholinoceptors since pilocarpine (259, 260) and nicotine (260) have been shown to stimulate pituitary-adrenocorticotrophic activity. The actions of acetylcholine on CRF secretion in vitro are mimicked by bethanechol and nicotine but the maximum response to either of these cholinomimetic agents is significantly less than to acetylcholine (fig. 2). Furthermore, these actions of bethanechol and nicotine are inhibited by their respective specific antagonists, atropine and pempidine, while those of acetylcholine are reduced by each of these drugs but completely abolished only when the two drugs are given together (41). These data suggest that the actions of acetylcholine are effected by the stimulation of a mixed population of nicotinic and muscarinic cholinoceptors.

The role of 5-HT in the control of CRF secretion is less well understood. It appears that distinct 5-hydroxytryptaminergic systems within the brain may be involved in the stimulation and inhibition of hypothalamo-pituitary-adrenocorticotrophic activity. Studies in vivo that involve implantation of 5-HT either into the lateral ventricles or into various regions of the hypothalamus suggest that the indoleamine has no effect on the basal secretion of CRF but inhibits its release in response to stressful stimuli (267, 276, 277). Several other findings support this idea (242, 282). The possibility that a 5-hydroxytryptaminergic system may stimulate the production of CRF was first suggested by Krieger and Rizzo (162). They proposed that the circadian excursion of 17-hydroxycorticosteroids is controlled to some extent by 5-HT. More recent studies indicate that the amine may also play a positive role in the regulation of stress-induced adrenocortical function. Results from experiments in vivo and in vitro substantiate these observations. Infusion of 5-hydroxytryptophan, a precursor of 5-HT, into the rhesus monkey (50) or man (136) significantly elevates the plasma cortisol, while cyproheptadine (a rather nonspecific drug that inhibits the actions of 5-HT, acetylcholine, and histamine) reduces the plasma cortisol concentration both in normal subjects and in patients with Cushing's syndrome of "hypothalamic origin" (53, 63, 160). Very small doses of 5-HT stimulate both the synthesis and release of CRF by hypothalamic tissue in vitro (39, 141).

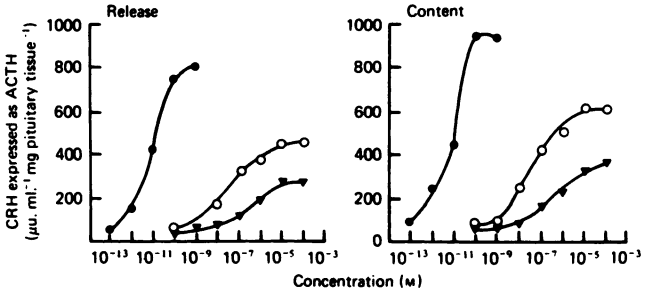

FIG. 2. Effects of acetylcholine (●——●), nicotine (○——○), and bethanechol (▼——▼) on hypothalamic CRF release and content in vitro. Each point is the mean of five determinations. Standard errors are omitted since in every case they were within ± 10% of the mean. (Reprinted with permission from J. C. Buckingham and J. R. Hodges, *J. Physiol.* (*London*), **290**:421–431, 1979.).

Moreover, its effects are completely inhibited by appropriate doses of methysergide or cyproheptadine, which suggests that the indoleamine is acting at specific 5-HT receptors (41). Jones et al. (141) proposed that these actions of 5-HT may be mediated by a cholinergic interneurone since, in their system, its effects were abolished by hexamethonium. These findings have not been confirmed in the Royal Free laboratory. The production of CRF in vitro in response to 5-HT was not influenced by the addition of either pempidine, hexamethonium, or atropine to the incubation medium in concentrations sufficient to inhibit maximally the actions of acetylcholine (41). The reason for this discrepancy between the two laboratories is not clear but it is worth mentioning that the concentrations of hexamethonium employed by Jones et al. (141) were found by the Royal Free group to be sufficiently high to exert variable nonspecific effects (41).

Neurones that secrete γ-aminobutyric acid (GABA) and catecholamines are believed to exert a tonic inhibitory influence over the hypothalamic secretion of CRF. Both the basal and stress-induced activity of the hypothalamo-pituitary-adrenocortical (HPA) complex in the rat were reduced when GABA was implanted into the third ventricle and enhanced by the injection of the GABA-receptor antagonists, picrotoxin and bicuculline (174). Furthermore, very small doses of GABA markedly reduced both acetylcholine- and 5-HT-stimulated CRF production by isolated rat hypothalami in vitro and its effects were inhibited by picrotoxin (141) and bicuculline (41).

The role of the catecholamines in the control of the secretion of CRF has been extensively studied by Ganong and colleagues. The catecholamine precursor, L-dihydroxyphenylalanine (L-DOPA), inhibited the 17-hydroxycorticosteroid response to stress in dogs when administered either i.v. (271) or into the third ventricle (272). Similar inhibitory effects were also evident after intraventricular implantation of L-noradrenaline, dopamine, α-ethyltryptamine, or tyramine (272), while inhibition of catecholamine biosynthesis by administration of α-methylparatyrosine resulted in a rise in the plasma concentra-

tions of corticosterone (230) and ACTH (228). On the basis of these and other (1, 3, 79, 82, 111, 266) findings, it was proposed that both noradrenergic and dopaminergic mechanisms are involved in the secretion of CRF, but more recent evidence indicates that only the former is of any functional significance. Apomorphine, implanted into the third ventricle, did not influence pituitary adrenocortical activity. Inhibition of dopamine-β-hydroxylase with bis(1-methylhexahydro-1,4,-diazepinyl-4-thiocarbonyl) disulphide decreased the hypothalamic noradrenaline content and increased ACTH secretion, while treatment with dihydroxyphenylserine (which causes a selective decrease in hypothalamic dopamine) overcame the rise in ACTH secretion induced by α-methylparatyrosine (81). Moreover, noradrenaline inhibited both basal and acetylcholine- or 5-HT-stimulated CRF secretion from the rat hypothalamus in vitro but dopamine was ineffective in this respect (39, 141). Despite some reports to the contrary, it is now generally agreed that the inhibitory influence of noradrenaline is mediated by stimulation of α-adrenoceptors. Ganong (80) showed that intraventricular injection of phenoxybenzamine (an α-adrenoceptor antagonist) prevented the L-DOPA-induced suppression of the stress response while Eisenberg (68) reported that phentolamine (another α-adrenoceptor antagonist) augmented the pituitary-adrenocortical response to stress in normal rats but that the β-adrenoceptor antagonist, propranolol, was ineffective in this respect. Others have shown that the basal secretion of corticosteroids is also elevated by treatment with α-adrenoceptor antagonists (229). Furthermore, the inhibitory effect of noradrenaline on CRF secretion in vitro was mimicked by adrenaline and the α-adrenoceptor agonists, methoxamine and phenylephrine, but not by the β-adrenoceptor agonist, isoprenaline, and it was antagonised by phentolamine but not by atenolol (a β-adrenoceptor antagonist) (41, 141).

In summary, it appears that central cholinergic nervous pathways stimulate and that GABA-ergic and adrenergic pathways inhibit the production of CRF. Both stimulatory and inhibitory roles have been ascribed to neurones that secrete 5-HT and much further work is necessary to elucidate their true physiological function.

It is generally agreed that both corticotrophin (195, 283) and corticosterone (cortisol in appropriate species) are capable of influencing the functional activity of the HPA system by negative feedback mechanisms. Little is known about the inhibitory actions of ACTH, but those of the corticosteroids have been examined extensively. Nevertheless, the precise site and mode of action of the steroids and their physiological importance as "neuromodulators" have been the subject of controversy. Studies in adrenalectomised, adrenal-enucleated, and intact animals with and without corticosterone treatment under nonstress conditions have demonstrated a distinct inverse relationship between the pituitary and plasma ACTH concentrations and the concentration of corticosterone in the blood (34, 35, 53, 77, 130, 180). Although there are some reports to the contrary (115, 296), the majority of observations indicate that the marked changes in the basal secretion of ACTH that these experimental procedures cause are accompanied by small differences in hypothalamic CRF content (32, 38, 249, 281), which suggests that the blood corticosteroids are important in the control of the activity of the hypothalamo-hypophysial complex under basal nonstress conditions.

The role of the blood corticosteroids in the control of the stress-induced secretion of corticotrophin is less well understood. There can be no doubt that chronic changes in the level of circulating corticosteroids influence markedly the HPA response to stress. The stress-induced adrenocorticotrophic activity of the adenohypophysis is elevated in adrenalectomised rats (13, 35, 123, 130, 132) and is inhibited in intact animals that have received prolonged treatment with corticosteroids (36, 122, 124, 226). Moreover, the rapid fall and subsequent rise in hypothalamic CRF content that occurs in response to stressful stimuli is exaggerated in adrenalectomised rats but normal in adrenalectomised rats maintained on physiological doses of corticosterone (31) (fig. 3). However, the results of experiments that involve the acute administration of steroids suggest that the hypothalamo-pituitary-adrenocorticotrophic response to stress is independent of the blood corticosteroid concentration at the time of stress. Smelik (253) found no direct correlation between the plasma corticosterone concentration and inhibition of stress-induced pituitary-adrenocortical activity in rats given single large doses of the steroid, either i.p. or s.c.; inhibition of ACTH release occurred only some time after the blood corticosterone concentration had returned to the resting level. This observation has been confirmed by using both direct (34) and indirect (127) indices of ACTH secretion and the concept of a "delayed" feedback mechanism that controls the release of corticotrophin in response to stressful stimuli is now widely accepted.

Recently a "rapid" feedback mechanism has also been implicated in the control of stress-induced HPA activity. Dallman and Yates (61) demonstrated that infusion of corticosterone inhibited ACTH secretion in response to stress but that the duration of action of the steroid was very short (less than 5 minutes). This observation was confirmed by Jones et al. (137), who found the system to be sensitive to the rate of increase of the concentration of corticosterone in the plasma and to be saturated at concentrations above the "physiological range". Several authors have disputed the physiological significance of these results. Only indirect indices of ACTH secretion were employed and the rate of infusion of corticosterone was very high. Furthermore, the experiments were performed under pentobarbitone anaesthesia and the possibility that this influenced the activity of the HPA system could not be excluded. In subsequent experiments

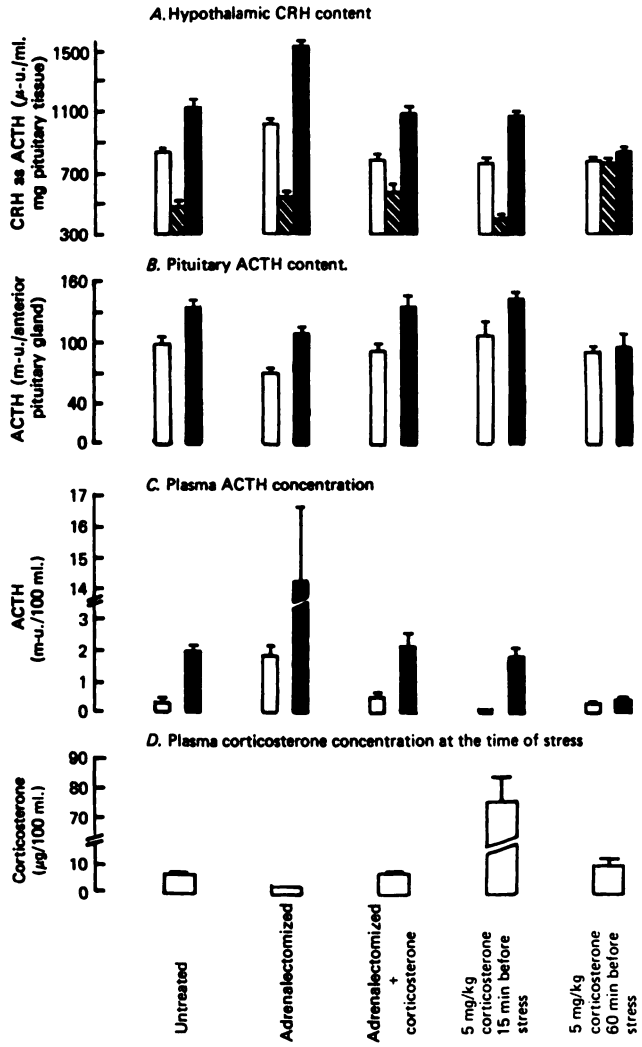

FIG. 3. Hypothalamo-pituitary adrenocorticotrophic activity in untreated, adrenalectomised, and corticosterone-treated rats before and after stress. □, before stress; ▨, 1 minute after stress; ■, 2 minutes after stress. Each column is the mean of five determinations and is shown with its standard error. (Reprinted with permission from J. C. Buckingham, *J. Physiol.* (*London*), **286**: 331–342, 1979.)

performed on conscious rats, Jones and Tiptaft (143) found that the adrenocortical response to stress was impaired 10 minutes after a s.c. injection of corticosterone. In this study no estimations of the plasma concentration of the injected steroid were made; furthermore, the investigators did not discuss their previous suggestion (137) that the rapid feedback mechanism is sensitive to the rate of rise of the plasma concentration of corticosterone. Other papers, based on indirect indices of ACTH secretion, have provided additional evidence that is in accord with the existence of a rapid feedback mechanism (58, 144) but the concept of its being "rate-sensitive" was not discussed further in more recent papers (139, 142, 172).

Corticosteroids are believed to exert their inhibitory effects on HPA activity by acting on specific receptors in the adenohypophysis (6, 37, 75, 76, 142, 172, 220), the hypothalamus (31, 39, 72, 142, 172, 264), and a variety of centres higher in the brain (59, 60, 71, 149, 154, 187, 297). Evidence for inhibition at the pituitary level has been obtained from studies both in vivo (6, 220) and in vitro (37, 38, 75, 76, 196). It appears that corticosteroids act directly on the adenohypophysis to inhibit the release but not the synthesis of ACTH induced by CRF (37, 38). Experiments in vitro indicate that corticosterone may also inhibit CRF-stimulated ACTH biosynthesis but only after prolonged contact of the steroid with the tissue (31). The existence of corticosteroid receptors in the hypothalamus is well documented. Corticosteroids inhibit adrenocortical activity when implanted into the hypothalamus (72, 264). They also inhibit the secretion of CRF by isolated rat hypothalami in vitro in concentrations considerably lower than those needed to influence the adrenocorticotrophic activity of the anterior pituitary gland in vitro (31, 39, 172). Experiments in vivo have yielded similar results (281). Corticosteroid treatment reduces the hypothalamic CRF content (38, 73), depresses hypothalamic unit activity (217), and prevents the release of CRF from already increased stores in the median eminence of adrenalectomised rats (86). These findings may be the result of direct actions of the steroid on the hypothalamus and to actions on higher centres in the brain since in these experiments corticosterone was administered either orally or systemically. A substantial amount of evidence indicates that corticosteroids exert feedback effects at extrahypothalamic sites in the central nervous system (19, 59, 60, 71, 149, 154, 187, 190, 297). For several reasons some of this work is difficult to interpret. Firstly, the concentration of the steroid used in implantation studies may be unphysiologically high. Secondly, the steroid may diffuse away from the site of injection. Thirdly, the selective uptake of corticosteroids by brain structures after peripheral injection does not demonstrate the existence of specific receptors but merely reflects the tissue's capacity to bind the steroid. The presence of receptors can only be postulated when the binding is associated with the specific changes in HPA activity or other specific steroid action. Nevertheless, the importance of the amygdala and the hippocampus (153, 154) as sites of corticosteroid feedback has been well documented and the possibility that other brain areas may also be involved should not be disregarded (19, 154, 190).

It is hard to evaluate the relative importance of each of the "feedback sites" in the control of HPA activity under normal physiological conditions. Experiments in our laboratory (37) demonstrated that stress-induced adrenocorticotrophic activity is always paralleled by concomitant changes in hypothalamic CRF content (fig. 3) and it seems reasonable to infer that the regulatory effects of corticosteroids on the stress-induced secretion of ACTH are exerted predominantly at the hypothalamus or on centres higher in the brain. The pituitary receptors may be involved in the control of ACTH secretion under nonstress conditions. Several groups (31, 38,

115, 249, 296) have shown that the adrenocorticotrophic activity of the adenohypophysis is influenced more readily by changes in the level of circulating corticosteroids than is the CRF content of the hypothalamus. However, the absolute hormone content of a tissue does not necessarily reflect its secretory capacity (123, 132) and the abilities, in vitro, of hypothalami and anterior pituitary segments to secrete CRF (31, 115) and ACTH (31), respectively, are exaggerated by adrenalectomy and reduced by corticosteroid treatment, possibly as a result of direct effects on the tissue itself or to actions on neurones that control its activity. Both in vivo and in vitro studies have suggested that the hypothalamus is more sensitive to the inhibitory effects of corticosterone than is the anterior pituitary gland (31, 172, 281). If this is the case the receptors in the adenohypophysis and perhaps those in centres higher in the brain would be stimulated only when relatively severe suppression of HPA activity is required. Teleologically this hypothesis is appealing since the hypothalamus is the beginning of the final common pathway leading to ACTH secretion. Much further work on this aspect of HPA physiology will be necessary to test this hypothesis.

The possibility that steroid hormones other than the glucocorticoids, corticosterone and cortisol, are capable of influencing the activity of the HPA system has been suggested and the actions of other adrenocortical hormones have been investigated. Progesterone appears to be inactive. However, the delayed inhibitory effects of corticosterone on the stress-induced secretion of ACTH are mimicked by 11-deoxycortisol, 11-deoxycorticosterone, 11β-hydroxyprogesterone, and 11β,17α-dihydroxyprogesterone but not by 11-epicortisol, while 17-hydroxyprogesterone and 18-hydroxy-11-deoxycorticosterone oppose these actions of the glucocorticoids (143). In vitro studies suggest that the actions of these steroids, like those of cortisol and corticosterone, are exerted predominantly on the hypothalamus and to a lesser extent on the adenohypophysis (172). With the exception of the mineralocorticoid, 18-hydroxy-11-deoxycorticosterone, none of these steroids either mimicked or opposed the "rapid" feedback effects of the glucocorticoids (139, 143). 18-Hydroxy-11-deoxycorticosterone antagonised this action of corticosterone (139, 143). On the basis of these findings, it was suggested (139, 143) that only those adrenocortical steroids that possess both the 21-hydroxyl group and 11β-hydroxyl group activate the "rapid" feedback mechanism while the delayed feedback mechanism is stimulated by those steroids that possess either the 21-hydroxyl or the 11β-hydroxyl group. However, the findings with 11-epicortisol and 18-hydroxy-11-deoxycorticosterone (both of which possess 21-hydroxyl groups) are hard to reconcile with this hypothesis. Aldosterone has also been implicated in the control of HPA activity. In concentrations approaching the upper end of the physiological range, aldosterone inhibits the secretion of CRF by rat hypothalami in vitro but does not affect the CRF-stimulated production of ACTH by pituitary segments in vitro (32). Birmingham (18) suggested that the inhibitory effects of this mineralocorticoid on ACTH secretion may be greater than those of corticosterone. This is surprising, since, to my knowledge, there is no evidence of severe impairment of HPA activity in patients with primary hyperaldosteronism.

The possibility that the sex steroids influence the functional activity of the HPA system has been considered. It is unlikely that androgenic steroids are active in this respect. The abilities of hypothalami and adenohypophysial segments to secrete CRF and ACTH respectively in vitro in response to trophic stimuli were unaffected by the presence of testosterone, androsterone, or androstenedione in the incubation medium (32). Moreover, the corticotrophin releasing activity of hypothalami removed from rats was not affected by pretreatment with either testosterone or dehydroepiandrosterone (139). A great deal of circumstantial evidence suggests that oestrogenic steroids affect the functional activity of the hypothalamo-pituitary complex. For example, both the adrenal weight and the plasma concentration of glucocorticoids are higher in the female than in the male rat and are raised still further during the final stages of pregnancy. Furthermore, the concentrations of corticosterone in the plasma (33, 212), ACTH in the plasma (33) and pituitary gland (55), and CRF in the hypothalamus (121) are elevated in the female rat during pro-oestrous as also is the plasma concentration of oestradiol. It has been suggested that oestrogens stimulate the biosynthesis of ACTH and sensitise the pituitary gland to CRF (54, 151). More recent studies with pituitary tissue in vitro have failed to confirm these findings (37). Positive feedback effects of oestrogenic steroids, with respect to adrenocortical activity, appear to be exerted at the hypothalamic level. Although they did not affect the spontaneous secretion of CRF by isolated rat hypothalami in vitro, oestradiol, oestriol, and oestrone potentiated markedly both the increases in the synthesis and the release of this hypothalamic hormone that occur in response to either acetylcholine or 5-HT (32). Thus the possibility exists that not only adrenocortical hormones but also oestrogenic steroids influence the activity of the HPA system. The physiological significance of this is not clear and requires further investigation.

VI. Mechanism of Action of Corticotrophin Releasing Factor

Detailed investigations of the mechanisms by which CRF evokes the secretion of corticotrophin will not be possible until pure preparations of the hormone are available. Nevertheless, some information about its mode of action has been obtained with impure hypothalamic extracts. CRF acts on the corticotrophs in the anterior pituitary gland and causes increases in both the amount of ACTH released and the ACTH content of the cells (37). The effects of inhibitors of protein synthesis on the

rise in pituitary ACTH content have not been thoroughly studied, but the rapidity of this striking response suggests that it cannot be due solely to de novo synthesis of the hormone. ACTH is stored in the corticotrophs in the form of a biologically inactive precursor molecule (162) and it is probable that the rapid CRF-induced increase in ACTH content reflects merely breakdown of the prohormone.

It is widely believed that the action of CRF, like that of other hypophysiotrophic hormones, is mediated via cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (163). A variety of cyclic AMP derivatives, substituted at N^6 and C^8, have been shown to evoke the secretion of ACTH by pituitary tissue in vitro (74). Similarly, theophylline, a drug that inhibits phosphodiesterase and thus the metabolism of cyclic AMP, stimulates ACTH release (74). Adenyl cyclase in the pituitary gland is activated by prostaglandins (298, 299) and thus the possibility has also been raised that prostaglandins are involved in the sequence of events within the corticotroph that lead to ACTH secretion. More recent studies are not in accord with this hypothesis for although prostaglandins do evoke the secretion of corticosteroids when administered to intact control animals (102, 205a), the effect is blocked or inhibited by pentobarbitone/morphine (205a), and they do not affect ACTH secretion either when injected into the pituitary gland in vivo (109) or when added to pituitary tissue in vitro (37).

Until the pure synthetic hormone is available it is unlikely that any competitive antagonists to CRF will be found. The ability of corticosteroids to act as physiological antagonists of CRF has already been discussed. The actions of CRF at the pituitary level are also inhibited by substance P (138). Substance P is present in the hypothalamus in relatively high concentrations and thus it is possible, if it enters the hypophysial portal vessels, that it may act as a physiological corticotrophin release inhibiting factor.

VII. Pharmacology of Corticotrophin Releasing Factor

The secretion of corticotrophin releasing factor is readily evoked by minor stressful stimuli, e.g. inhalation of ether vapour. Many substances are capable of blocking the response to specific stimuli; for example, the release of CRF that occurs in response to insulin or histamine is prevented by pretreatment with glucose or promethazine respectively. However, there are only a few drugs that abolish the secretion of CRF in response to *all* stressful stimuli by inhibiting, either directly or indirectly, its release from the hypothalamus. The drugs that have been most widely studied in this respect are essentially those that have been employed to prevent the secretion of endogenous CRF in animals used for the assay of this hypothalamic hormone, i.e. reserpine, chlorpromazine, morphine, and glucocorticoids. In the sections that follow the effects of these drugs on the production of corticotrophin releasing factor are considered.

A. Reserpine

A single injection of reserpine evokes a prolonged hypersecretion of ACTH (6, 67, 129, 225, 275, 287), the duration of which depends upon both the dose and the route of administration. The mechanism by which reserpine induces this response is not clear. Reserpine causes marked decreases in the concentrations of 5-HT, noradrenaline, and dopamine in the central nervous system (23, 135) by blocking the Mg^{++}-ATP-dependent uptake-storage mechanism in the intraneuronal amine granules (56). Since it is widely believed that central noradrenergic neurones inhibit (41, 228–230, 271, 272) and that 5-hydroxytryptaminergic nervous pathways inhibit (243, 267, 276, 277, 282) and stimulate (41, 141, 160, 162) the secretion of CRF, it is probable that the alterations in availability of these monoamines are associated with the effects of reserpine on pituitary adrenocortical activity. Several groups have shown that CRF release is related indirectly to brain monoamine levels (22, 287), For example, Martel et al. (177) demonstrated that treatment of rats with a monoamine oxidase inhibitor blocked the reserpine-induced depletion of brain monoamines and prevented the hypothalamo-pituitary-adrenocorticotrophic activity and sedative effects evoked by the alkaloid. They suggested that the actions of the drug on the HPA system are an integral part of its pharmacological effects on the central nervous system and not the result of direct actions on the adenohypophysis. It is not yet clear whether the reserpine-induced discharge of CRF is related to changes in brain noradrenaline, 5-HT, or both. It seems unlikely that reserpine exerts its effects by reducing the tonic inhibitory noradrenergic input to the hypothalamus since rats pretreated with α-methyl-paratyrosine (a drug that selectively inhibits tyrosine hydroxylase) respond normally to reserpine. On the basis of these findings Martel et al. (177) suggested that reserpine exerts its effects on CRF secretion by actions on central inhibitory 5-hydroxytryptaminergic pathways but convincing evidence for this hypothesis has not been forthcoming. It was anticipated that direct evidence that the actions of reserpine are related to changes in cerebral 5-HT would be obtained by the use of parachlorophenylalanine (a substance that depletes the brain of the indoleamine without affecting the concentrations of noradrenaline or dopamine). However, this drug is not specific, and simultaneous fluctuations in the concentrations of brain 5-HT, noradrenaline, and dopamine have been described (188, 262, 285). The interpretation of the effects of reserpine on CRF secretion are further complicated by the finding that the alkaloid also influences other neurotransmitter substances implicated in the regulation of its secretion. Reserpine has been reported to increase the content of acetylcholine in the hypothalamus (175) and

to deplete the brain of GABA (11), both of which may lead to enhanced CRF production.

The hypothalamo-pituitary-adrenocorticotrophic response to a single injection of reserpine is reduced and ultimately disappears if the injections are repeated at daily intervals (129, 150, 286). Furthermore, the stress-induced activity of the system is also inhibited once reserpine-adaptation is achieved (129, 152, 173, 286). Several authors have attempted to explain this inhibition but the precise mechanism whereby it is effected is not understood. The observation that rats bearing hypothalamic lesions exhibit a delayed hypothalamo-pituitary-adrenocorticotrophic response to stress (25, 26) prompted the suggestion that the apparent inhibition of CRF secretion in rats that had received prolonged reserpine treatment is due to an alteration in the time-course of the response. This seems unlikely since Vellucci (275) found no evidence of increased ACTH secretion up to 40 minutes after the final injection of reserpine. Kitay et al. (152) reported that repeated injections of reserpine cause a sustained release of ACTH and a fall in pituitary ACTH content. They proposed that the reduction in ACTH content would diminish the ability of the pituitary gland to respond to the CRF secreted in response to an additional stress. However, there are convincing reports that show that a fall in pituitary ACTH content cannot be correlated with an inability to respond to stress (123, 273). Moreover, Hodges and Vellucci (129) found that the inhibition of ACTH release after adaptation to reserpine occurs at a time when the pituitary stores are the same as those in the corresponding vehicle-treated controls, while others (273) showed that the adrenocortical response to aspirin or histamine was normal in rats given a single large dose of reserpine at a time when the pituitary ACTH stores must have been depleted. The possibility has been raised that the reserpine-induced suppression of hypothalamo-pituitary-adrenocorticotrophic activity is due to a "feedback" effect exerted by the elevated levels of corticosteroids previously evoked by the drug (93). However, although very high, nonphysiological doses of corticosteroids inhibit the stress-induced release of CRF and do so more effectively 18 to 20 hours after administration than during the first few hours (12), it is unlikely that any "feedback" effect from endogenous corticosteroids would be so effective or persist for so long. The bulk of the evidence available indicates that, like its stimulatory effects, the inhibitory actions of reserpine are exerted at the level of the hypothalamus or on centres higher in the brain. Reserpine reduces the corticotrophin releasing activity of the hypothalamus (16) probably either by altering the balance of stimulatory and inhibitory nervous inputs to the CRF neurones or by interfering with the CRF storage mechanism. Most groups favour the former hypothesis since, despite one report to the contrary (252), it appears that the reserpine-induced decrease in hypothalamic corticotrophin releasing activity is accompanied by alterations in brain monoamine content. Since noradrenaline inhibits CRF production (41, 68, 80, 81, 229) and dopamine is ineffective in this respect (29, 81, 141), it is unlikely that the reserpine-induced inhibition of CRF secretion is associated with the fall in brain catecholamines. However, the possibility that the stimulatory effects of 5-HT on CRF production are impaired or even abolished as a result of the decrease in the brain content of the indoleamine cannot be disregraded. It appears that reserpine exerts its effects on CRF release predominantly by depleting brain monoamines that are involved in the control of its production. This action may take place at the hypothalamus or at centres higher in the brain. As a result, CRF production is initially stimulated and subsequently inhibited as monoamine stores become progressively depleted. However, it is only when the turnover rates of the various central neurotransmitter substances and the functional activity of the hypothalamus are assessed simultaneously that it will be possible to ascribe the response to specific effects on brain monoamine storage mechanisms.

B. Chlorpromazine

Although there are some reports to the contrary (148, 171), it is generally agreed that a single injection of chlorpromazine causes a marked rise in the plasma concentrations of ACTH and corticosterone and concomitant fall in the content of CRF and ACTH in the hypothalamus and anterior pituitary gland respectively (16). Early studies, in which indirect indices of ACTH secretion were used, suggested that the hypothalamic response to chlorpromazine, like that to reserpine, is prolonged, and one group (255) proposed that it persisted for as long as the sedative effects of the drug. However, experiments in which corticotrophin was measured directly show clearly that this is not the case and that the plasma concentration of ACTH returns to normal some time before the sedative effects disappear. The actions of chlorpromazine on CRF secretion are probably caused by changes in neurotransmitter activity in the brain. The drug interferes with catecholamine neurotransmission by causing postsynaptic blockade of the receptors for dopamine and noradrenaline (4, 49). Since it is believed that noradrenergic pathways inhibit the secretion of CRF, it is tempting to postulate that the stimulatory effects of chlorpromazine on ACTH secretion are due simply to its actions on adrenoceptors (16, 291). However, chlorpromazine also increases the turnover (15) and synthesis (14) of 5-HT, antagonises the actions of various cholinomimetic agents (257), increases the biosynthesis of acetylcholine (268), and raises the concentration of GABA in the cerebellum and the cortex (116). Thus the effects of chlorpromazine on CRF secretion are probably the result of alterations in the balance of stimulatory and inhibitory signals from cholinergic and 5-hydroxytryptaminergic neurones and noradrenergic and GABA-ergic neurones respectively.

Several groups have suggested that a single injection of chlorpromazine causes partial (103) or total (10, 171) inhibition of the hypothalamo-pituitary-adrenocorticotrophic response to stress. These findings have not been confirmed. Hodges and Witek (133) showed that chlorpromazine-treated rats respond to stress with a normal rise in plasma ACTH concentration, thus confirming the earlier findings of Holzbauer and Vogt (134) and Olling and de Wied (201). However, there can be no doubt that chlorpromazine prevents the stress-induced release of CRF when given to pentobarbitone-treated rats (201). This interesting drug interaction is discussed in the following section.

C. Pentobarbitone/Chlorpromazine

Olling and de Wied (201) were the first to show that chlorpromazine abolishes the hypothalamo-pituitary-adrenocorticotrophic response to stress in rats anaesthetised with sodium pentobarbitone. This observation was subsequently confirmed by using both direct and indirect indices of ACTH secretion (133, 241, 290, 291). The mechanisms whereby this drug combination inhibits the stress response are not understood. Several groups have suggested that pentobarbitone anaesthesia prevents the stress-induced release of CRF but experiments in which ACTH was determined by bioassay (133) and radioimmunoassay (97) techniques respectively demonstrate that pentobarbitone-treated rats respond to stress with a normal rise in plasma ACTH concentration. Olling and de Wied (201) and Sevy et al. (241) proposed that pentobarbitone prevents the chlorpromazine-induced hypersecretion of CRF but the results of Hodges and Witek (133) did not support this hypothesis. Since corticotrophin release is readily evoked in pentobarbitone/chlorpromazine-treated rats by injection of either hypothalamic extracts or lysine vasopressin (165, 241) it is probable that the drugs exert their inhibitory effects predominantly on the hypothalamus or on centres higher in the brain. According to de Wied (290) pituitary function is normal in pentobarbitone/chlorpromazine-treated rats but other workers have shown a reduction in the ability of pituitary glands from rats treated with this drug combination to secrete ACTH in response to CRF in vivo (M. T. Jones, personal communication) and in vitro (133). This loss of sensitivity is probably due both to direct inhibitory actions of chlorpromazine on the pituitary gland (295) and to the removal of the trophic drive from the hypothalamus. Both the hypothalamic CRF content and the capacity of the organ to secrete CRF in vitro in response to trophic stimuli are reduced by pentobarbitone/chlorpromazine treatment (133). These effects are probably the result of actions of the drug on centres higher in the brain rather than direct actions on the hypothalamus since the addition of chlorpromazine to the incubation medium does not affect the secretory capacity of hypothalami removed from normal or pentobarbitone-treated rats (295). As discussed in the previous section, chlorpromazine influences markedly the activity of cholinergic and monoaminergic neurones in the central nervous system. Since such neurones influence the secretion of CRF, it is probable that the effects of the drug are related to these actions. The role of pentobarbitone remains to be explained. Chlorpromazine potentiates the actions of central depressant drugs (24) and, accordingly, it has been proposed that it potentiates the "inhibitory" effect of pentobarbitone on CRF secretion (255, 291). Certainly the two drugs need to be given together to block CRF secretion effectively. They probably act synergistically and reduce the ratio of stimulatory and inhibitory impulses controlling the secretion of CRF.

D. Opioids

It has been known for many years that hypothalamo-pituitary-adrenocorticotrophic activity is influenced profoundly by opiate substances and hence the recent isolation and identification of the endogenous opioid peptides, enkephalins and endorphins, have raised the possibility that opioid receptors are involved in the control of CRF secretion. The actions of the opiate drugs have been the subject of controversy, probably for two reasons. Firstly, morphine, the drug most widely employed in these studies, is only a partial agonist and thus its effects are concentration-dependent (159). Secondly, the recent demonstration of the existence of more than one type of opioid receptor has raised the possibility that different receptors mediate different effects and that the overall response depends on the balance of stimulated receptors (284). However, although only indirect indices of CRF and ACTH secretion have been employed, it is generally agreed that acute administration of morphine to conscious animals stimulates hypothalamo-pituitary-adrenocorticotrophic activity. A single injection of morphine into rats causes a fall in the adrenal ascorbic acid concentration (21, 84, 198) and a rise in the concentration of corticosterone in the plasma (83, 158, 167, 197). Its effects are antagonised by nalorphine (84). Moreover, opioid receptor agonists, normorphine and methionine enkephalin (administered intracerebroventricularly), potentiate the adrenocortical response to ether stress (87, 88). Despite one report to the contrary (203), it seems unlikely that the drugs act directly on either the adrenal cortex or the anterior pituitary gland. Opioids do not influence adrenocortical activity in hypophysectomised (84) or median eminence-lesioned (85) rats. Moreover, the secretion of ACTH by anterior pituitary tissue in vitro is not affected by the addition to the incubation medium of morphine, methionine- or leucine-enkephalin, β-endorphin, or naloxone (42, 90). However, receptors in the hypothalamus may be involved. The secretion of CRF by isolated rat hypothalami in vitro is evoked by low concentrations of morphine, leucine-, and methionine-enkephalin. β-Endorphin alone does not affect CRF secretion but, like naloxone, it antagonises the stimulatory

actions of morphine and enkephalin (42). These findings suggest that endogenous enkephalins are involved in the control of CRF secretion and that their actions are modulated by endorphin. β-Lipotrophin, the parent protein of β-endorphin, is released from the adenohypophysis at the same time as ACTH and thus it seems reasonable to suggest that β-endorphin, like ACTH (195a), exerts a short-loop feedback effect inhibiting the further secretion of CRF. The actions of opioid substances on CRF secretion are probably also affected by actions on centres higher in the central nervous system. Certainly they influence the activity of cholinergic and noradrenergic neurones, both of which are implicated in the control of CRF secretion. Morphine and β-endorphin inhibit the release of noradrenaline from the rat cerebral cortex (5) and reduce the turnover of acetylcholine in certain brain nuclei (193, 300). Thus, the effects of opioid substances on CRF secretion are probably due partially to direct actions on the hypothalamus and partially to alterations in the ratio of stimulatory and inhibitory signals received from higher centres in the brain.

In contrast to the effects of a single injection, Briggs and Munson (21) showed that chronic morphine treatment blocked the adrenocortical response to histamine stress and suggested that tolerance develops to the stimulatory actions of the drug. Another group (158) found that after repeated daily injections of morphine the HPA system was no longer stimulated by the drug but the response to stress (cold or insulin) persisted. The bulk of the evidence available suggests that chronic morphine treatment causes impairment but not total inhibition of HPA activity (202). It seems unlikely that this is due to direct actions of the opiate on the adrenal cortex (83). Two mechanisms of action have been proposed. Firstly, that prolonged morphine treatment inhibits CRF secretion and secondly, that chronic treatment increases CRF secretion to such an extent that the pituitary ACTH stores become depleted. George (83) favoured the latter theory because prolonged treatment with morphine resulted in adrenal hypertrophy and hyperplasia (258), effects that are probably due to excessive ACTH secretion, and reduces the pituitary ACTH content to such an extent that ACTH is released only in response to severe stressful stimuli. However, this argument is not tenable. It is well known that animals in which the pituitary ACTH content is substantially reduced respond to mild stressful stimuli with a marked hypersecretion of ACTH (123, 278). At the present time there is little information available concerning the functional activity of the hypothalamo-hypophysial complex in rats given prolonged treatment with morphine. Preliminary experiments in the Royal Free laboratory indicate that the capacity of hypothalami to secrete CRF in vitro is impaired after prolonged incubation with morphine. Furthermore, reduced plasma corticosterone levels have been described after chronic morphine treatment (243), which suggests that CRF secretion may be inhibited by prolonged treatment with the opiate. In this event it is difficult to explain the adrenal hypertrophy and hyperplasia induced by repeated injections of morphine. However, according to Dallman et al. (57) adrenal hypertrophy is not necessarily due to elevated circulating levels of ACTH but may be mediated by a neural mechanism. Perhaps this mechanism also operates in morphine-treated rats. Clearly much further work in which the functional activity of the hypothalamo-hypophysial complex is studied is necessary to explain the true mode of action of morphine.

E. Pentobarbitone/Morphine

The ability of morphine to inhibit the HPA response to stress in anaesthetized rats was first reported by Briggs and Munson (21). It was shown that a single dose of morphine given to rats anaesthetized with sodium pentobarbitone prevented the adrenal ascorbic acid depletion normally produced by laparotomy, unilateral adrenalectomy, or by low doses of histamine or vasopressin. This inhibitory action of morphine was subsequently confirmed both in the rat (200) and in man (185) and was shown to be competitively antagonised by nalorphine (46a). The site and mode of action of this drug combination are not understood. The finding that rats treated with pentobarbitone/morphine respond to hypothalamic extracts with a rise in ACTH secretion suggest that the drugs exert their effects predominantly on the hypothalamus or on centres higher in the brain. No studies on this aspect of the pharmacology of CRF have been made. It is known that opioid substances influence the activity of cholinergic and noradrenergic neurones in the central nervous system, both of which are implicated in the control of CRF secretion. It seems reasonable to suggest that morphine, like chlorpromazine, acts synergistically with pentobarbitone to modulate the activity of neurones controlling the secretion of CRF.

F. Glucocorticoids

The pharmacological effects of corticosteroids on the secretion of CRF are essentially exaggerations of their physiological actions. Treatment of either rats or man with high, nonphysiological doses of corticosteroids abolishes the stress-induced release of ACTH (36, 95, 124, 126) and the normal circadian excursion in hypothalamo-pituitary-adrenal activity (95, 124). Many of the semisynthetic corticosteroids (e.g. betamethasone and dexamethasone) are considerably more potent in this respect (124, 131, 244) than the naturally occurring corticoids. The corticosteroid-induced suppression of hypothalamo-pituitary-adrenocorticotrophic activity is associated with adrenal atrophy (36, 125), a reduction in the plasma concentrations of corticosterone (cortisol in man) and ACTH (36, 95), and a fall in the contents of ACTH and CRF in the anterior pituitary gland and the hypothalamus respectively (38). The sensitivities of the adrenal cortex (17, 95, 124, 128, 189), the adenohypophysis (38) and the hypothalamus (40) to trophic stimuli are also

impaired. Endocrine tissue, unlike neural tissue, rapidly loses its secretory capacity when deprived of trophic stimuli. Thus the apparent insensitivity of the individual components of the HPA system is probably partially due to the absence of positive stimuli. Direct actions of the steroid on the tissue itself are also important. There are numerous reports in the literature that show that corticosteroids act directly on both the hypothalamus and the adenohypophysis to inhibit the secretion of CRF (31, 39, 142, 172) and ACTH (6, 37, 75, 76, 142, 172, 220), respectively, that occurs in response to trophic stimulation, although it is unlikely that they affect the secretory activity of the adrenal cortex (124). Steroids also act on higher centres in the brain to modulate the activity of neurones that control the secretion of corticotrophin releasing factor (59, 60, 71, 149, 154, 187, 297).

VIII. Concluding Comments

In the 43 years that have elapsed since Harris first proposed that the secretory activity of the adenohypophysis is controlled by chemical transmitter substances produced by the hypothalamus, only three of these regulatory hormones (thyrotrophin releasing hormone, gonadotrophin releasing hormone, and growth hormone release inhibiting hormone) have been successfully isolated and identified. These hypothalmic hormones and their synthetic analogues have been effective in the treatment of certain endocrine disorders as also have a variety of drugs that influence their secretion. Thyrotrophin releasing hormone (TRH) and gonadotrophin releasing hormone (GnRH) are employed to assess the capacity of the adenohypophysis to secrete the appropriate trophic hormones, thus facilitating accurate differentiation between diseases of the pituitary gland and the hypothalamus as causes of thyroid or gonadal dysfunction. Clinical neuroendocrinology with respect to HPA function is rather limited. There are reports of successful attempts to treat patients with Cushing's disease of hypothalamic origin with the 5-HT and acetylcholine antagonist, cyproheptadine (e.g., 160). However, since pure CRF is not available it is not possible at the present time to study directly the capacity of the pituitary gland to secrete ACTH. Vasopressin is sometimes used clinically as a test of pituitary ACTH reserve but since it is not chemically identical with CRF the validity of the test is questionable.

There is no reason to assume that the so-called "hypothalamic hormones" are concerned only with endocrine function. Immunofluorescence techniques have demonstrated the presence of some hypothalamic hormones both in extrahypothalamic sites within the central nervous system (9, 211) and in the gut (169). It has been suggested that these peptides may be involved in the control of physiological functions as diverse as behaviour (TRH, GnRH) (146, 194), glucose metabolism (somatostatin) (265), and memory (vasopressin) (274). As yet no extrapituitary effects of CRF have been described nor has the "hormone" been located outside the hypothalamus. However the possibility has been raised that an extrahypothalamic tissue-corticotrophin releasing factor also exists (170). Tissue CRF has been distinguished from CRF of hypothalamic origin on the basis of physicochemical properties, potency, prolonged action on the pituitary-adrenal system, and existence in the blood long after the hypothalamus has been removed. The factor, which appears to originate from damaged tissue, may play a role in the pituitary-adrenocortical response to stress. It appears that during chronic stress of severe intensity (e.g. extensive surgery) the needs of the organism may not be met by the hypothalamic-CRF and that tissue-CRF may be necessary to sustain prolonged pituitary-adrenal activity (27). This fascinating subject has recently been extensively reviewed (28).

A further understanding of the chemistry, physiology, and pharmacology of CRF should ultimately lead to the development of tests that enable differentiation between dysfunction of the hypothalamus and anterior pituitary gland as causes of adrenal disease, to the synthesis of "super-active" CRF receptor agonists and antagonists that may be of therapeutic value, and possibly to the successful treatment of diseases of hypothalamic origin with drugs that influence the activity of the releasing hormone cells.

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