Hypothalamic Control of the Anterior Hypophysis and its Metabolic Implications

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The importance of the anterior lobe of the pituitary gland in the establishment and course of diabetes mellitus suspected by clinicians for many years (see the review in 1938 by Atkinson on 817 cases of acromegaly) has been demonstrated by the now classical experiments of Houssay, Long, Lukens and Young. That this Society should be concerned with the relationships between "Brain and Diabetes" is most appropriate. There is now ample evidence that the functions of the anterior lobe of the pituitary gland are to a considerable extent dependent upon the integrity of its anatomical relationships with certain formations of the hypothalamus of the diencephalon.

Even though the hypothalamus is morphologically an ill-defined structure, anatomists recognize various areas* in the hypothalamus-pituitary complex particularly at the junction of hypophysial and hypothalamic tissues. These are the median eminence, the infundibular stem, and the infundibular process, all constituting the neurohypophysis or posterior pituitary. The pars tuberalis, the pars intermedia, and pars distalis all form the adenohypophysis or anterior hypophysis. The main body of the pituitary gland is connected to the hypothalamic structures in the floor of the third ventricle by the pituitary stalk.

Just as they have two different embryological origins, the two lobes of the pituitary also have two different types of connection with the hypothalamus.

The posterior pituitary gland is connected to the nuclei of the anterior hypothalamus (n. supra opticus, n. para ventricularis) and the nuclei of the tuber by a well-known tract of nerve fibers first described by Cajal and usually known as the hypothalamo-hypophysial tract. The two nuclei, supra opticus and para ventricularis, the hypothalamo-hypophysial tract and the posterior lobe of the pituitary are presently known to be concerned with the secretion of vasopressin and oxytocin.

The anterior pituitary is connected to the hypothalamus through an entirely different system. It is generally agreed that no secretory nerve fibers of hypothalamic origin similar to those of the hypothalamo-hypophysial tract are to be found in the adenohypophysis. An extremely well-developed and peculiar vascular system, however, connects the median eminence and anterior lobe of the pituitary gland. The original description by Popa and Fielding[2] of this portal system was later confirmed by Wislocki and King,[1] who furthermore concluded that blood flows in these vessels from their primary plexus toward the pituitary gland and that their secondary plexus irrigates the adenohypophysial parenchyma only. It was almost ten years later that Hinsey, and Green and Harris[3] suggested that this peculiar vascular formation might serve as a connecting link between the hypothalamus and the anterior lobe of the pituitary. The hypothalamic control of the functions of the anterior hypophysis would thus be of a humoral nature, the hypothalamo-hypophysial portal vessels serving as a carrier of the hypothetical mediators involved and liberated at the nerve endings which in the median eminence are in close relation to the capillary loops of the primary plexus.

The extent and the mechanism of the hypothalamic control of the anterior pituitary are still incompletely understood. That the anterior lobe of the hypophysis requires for a normal function the integrity of its connections with the hypothalamus has, however, been demonstrated in a variety of experimental circumstances. This short review will be mostly concerned with the secretion of ACTH, TSH and growth hormone, three hypophysial factors with important metabolic activities which are well known to the members of this audience.

*These various structures were defined and shown graphically by means of projection slides during presentation of the paper at the meeting of the Society. Since excellent reviews dealing with the anatomy of this region have recently appeared, the interested reader is referred to these.[1,2]

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Interruption of the stalk of the pituitary gland prevents the release of ACTH which follows exposure to certain stress situations. If a simple transection of the stalk is performed, the inhibition of the stress response first observed will, however, disappear and the normal pattern of ACTH release will be re-established. It is possible to correlate the return of normal functions with the regrowth of the portal vessels through the cut ends of the stalk. If the regeneration of the portal vessels is prevented, as by placement of a polyethylene sheath between the cut ends of the stalk, the blockage of the ACTH discharge will persist. It is interesting to note here that this blockage of ACTH release is not absolute and that some stimuli (laparotomy, traumatism, scalding, etc.) may still induce an apparently normal adrenocorticotrophic discharge. Also, after stalk section the adrenal cortex allegedly does not become as atrophic as it does following hypophysectomy. It would thus appear that a certain basal secretion of ACTH is still possible, at least for some time, in the denervated hypophysis.

Results very similar to these have been obtained after transplantation of the anterior lobe of the pituitary in areas distant from the hypothalamus. The growth of these animals with pituitary stalk section or peripheral pituitary grafts does not appear to be completely arrested; various authors have reported resumption of growth of the hypophysectomized animal after subcutaneous implantation of pituitary anterior lobes. Thus, the secretion of growth hormone may not require intact hypothalamo-pituitary connections. This question deserves further investigation in view of several reports somewhat at variance with these results.

The placement of minute lesions in various regions of the hypothalamus with the aid of a stereotaxic apparatus, a technic widely used recently in several laboratories, has also permitted localization of areas within the hypothalamus related to particular secretions of the anterior pituitary. Lesions in the anterior hypothalamus are usually followed by depression of thyroid function with maintenance of normal adrenocorticotrophic secretion. More posterior lesions or specific destruction of the median eminence have been reported to inhibit ACTH release in response to stress. According to McCann[5] the hypothalamic lesion which inhibits ACTH release always damages the hypothalamo-hypophysial tract, thus producing diabetes insipidus along with the ACTH-block. These questions should not be considered as satisfactorily settled. It is to be regretted that in most instances adequate histological control of the exact extent and localization of the lesions produced were not reported or performed; also, the recent introduction of more sensitive and more direct criteria of ACTH release (as compared to those used by these investigators) may make necessary a reappraisal of some aspects of these questions. For instance, in a recent series of experiments we have observed that minute lesions in the posterior hypothalamus may inhibit ACTH release without producing diabetes insipidus, and conversely that diabetes insipidus of considerable degree and several weeks' duration can be produced without interference with the release of ACTH.

Instead of localized stereotaxic lesions, functional inhibition of the normal activated state of the hypothalamus, as after administration of various pharmacological drugs (barbiturates, phenothiazine or reserpine derivatives), can inhibit the release of several pituitary hormones. Recent studies on the mechanism of these drugs have shown, furthermore, that their primary locus of action in inhibiting pituitary function may be at levels other than the hypothalamus (midbrain) even though their pituitary inhibitory actions are transhypothalamic. The integrity of various ponto-mesencephalic formations, parts of the reticular activating system, appears to be necessary for a normal hypothalamic control of pituitary release of ACTH and gonadotrophins. This concept has been strengthened by the results of electrical stimulations and of recordings of the electrical activity of various hypothalamic areas performed concurrently with endocrine studies.

The survival of fragments of pituitary tissue in vitro, with tissue culture or organ culture methods, has shown that the isolated hypophysial tissue ceases to secrete ACTH in the culture fluid within a few hours after explantation, although it will survive for months and differentiate its outgrowth into the classical type of pituitary cells. If, several weeks after placement of the pituitary tissue in culture, small fragments of ventral hypothalamus are introduced into the tube, ACTH activity reappears in the fluid. This was considered as suggestive evidence in favor of a hypothalamic control of the corticotrophic function of the pituitary.[3,4] It is interesting that injection of the fluids from these cultures to hypophysectomized animals did stimulate growth, disregarding age of the culture or absence of hypothalamic fragments. Here again, as mentioned earlier in the animal with pituitary stalk section or pituitary transplant, a fair degree of autonomy from the hypothalamus seems to characterize the secretion of growth hormone.

It would thus appear that the hypothalamic nuclei exert an important control over the function of the anterior lobe of the pituitary. The exact modalities and

the extent of this control are not definitely known. If intact hypothalamo-pituitary connections are necessary for adequate response of the pituitary to acute stimuli, some degree of pituitary autonomy is possible regarding ACTH and TSH secretion and to an even larger extent regarding growth hormone.

It was mentioned earlier, especially in view of the vascular connections existing between median eminence and anterior hypophysis, that a hypothalamic control of the pituitary should be neurohumoral in its ultimate effector. The results from the tissue culture experiments and the recent demonstration of an ACTH-releasing activity in the portal blood draining from the upper end of the cut pituitary stalk, have considerably strengthened this hypothesis. The only attempts made so far to identify the hypothetical mediators involved in the control of the secretion of the various pituitary hormones have been related to ACTH release. Using various in vitro technics as simple bio-assays, a corticotrophin releasing factor (CRF) of hypothalamic origin has been concentrated and partially purified.[1-6] It is none of the previously known neurohormones; it appears to be closely related by its physico-chemical characteristics to the small peptides of the posterior lobe of the pituitary. Recent studies have confirmed with in vivo technics the results previously obtained in vitro. The physiological significance of the factor isolated leaves little doubt, since it is highly active in stimulating release of ACTH in animals with a hypothalamic lesion which inhibits the ACTH secretion normally induced by exposure to stress.

The same material when administered intravenously to humans stimulates the release of ACTH as measured by levels of peripheral plasma 17-OH corticosteroids. Twenty-five to forty-five micrograms of the purified material given in one single intravenous injection elevate the plasma corticoids within a few minutes (figures 1 and 2).

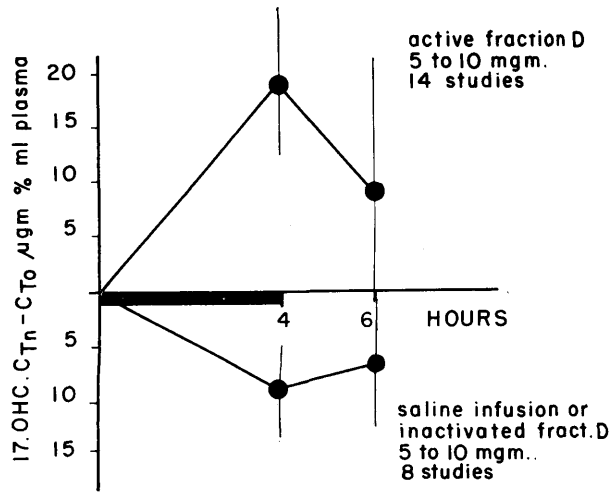

FIG. 1. Variations of the plasma 17 OH-corticosteroid concentration in normal individuals after a perfusion of the corticotrophin releasing factor (fraction D) purified on the basis of its activity in vitro. Bottom of diagram, control studies.

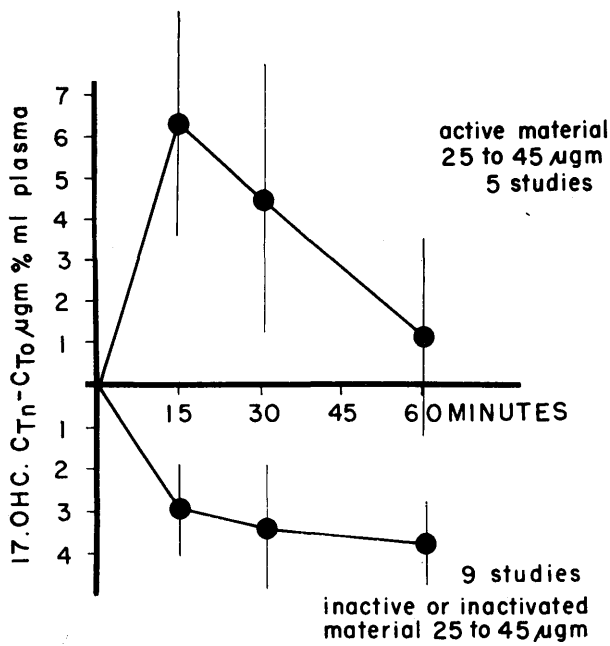

FIG. 2. Variations of the plasma 17 OH-corticosteroid concentration in normal individuals receiving a single intravenous injection of purified corticotrophin releasing factor (electrophoretic fraction DΔ). Bottom of diagram, control studies.

SUMMARY

The existence of a hypothalamic control of the anterior pituitary is thus well established. At a time when peripheral metabolic effects of purified pituitary hormones are being discovered, such as with corticotrophin and thyrotrophin, the metabolic implications of this diencephalic control are numerous. They may well encompass more than a simple transfer to the hypothalamus, of the hypophysial control of its various target glands.

Extensive investigations are still necessary to have a clear understanding of this problem. Of considerable interest also is the fact that this hypothalamic control of the anterior pituitary is humoral in nature. We have seen earlier that microgram quantities of a partially purified corticotrophin releasing factor injected into the human may influence considerably levels of pituitary adrenal function. We have preliminary evi-

dence that these metabolic factors of hypothalamic origin may be recovered in urine. Thus, in some not too distant future we may be able to study not only the pituitary but also possible diencephalic dysfunctions. It is even possible that we may be in a position to correct these dysfunctions by some replacement therapy, when we know more about the chemical structures of the neurohumors involved.

SUMMARIO IN INTERLINGUA

Le Governantia Hypothalamic del Hypophyse Anterior e le Inferentias Metabolic de Su Existentia

Le existentia de un governantia hypothalamic del hypophyse anterior es documentate e establite como facto foras de dubita.

A un tempore quando peripheric effectos metabolic de purificate hormones pituitari es discoperite progressivemente—per exemplo in le caso de corticotrophina e de thyrotrophina—le inferentias metabolic de un tal governantia diencephalic es numerose. Il es ben possibile que ille inferentias excede in lor signification un simple transferimento al hypothalamo del functiones governatori que le hypophyse possede pro varie glandulas dependente de illo.

Extense investigationes additional va esser necessari pro clarificar nostre comprension de iste problema. Un facto de interesse considerabile es que iste governantia hypothalamic del hypophyse anterior es de natura humoral. Nos ha vidite que microgrammas de un partialmente purificate factor liberatori de corticotrophina, quando injicite in subjectos human, pote exercer un influentia considerabile super le nivellos del function pituitari-suprarenal. Nos ha provas preliminari que iste factores metabolic de origine hypothalamic pote esser traciate in le urina. Assi il pare possibile que in un futuro non troppo distante nos va poter studiar non solmente dysfunction pituitari sed etiam le possibile dysfunction diencephalic. Il es mesmo possibile que nos va trovar medios pro corriger ille typo de dysfunction per le un o le altere therapia de reimplaciamento basate super nove cognoscentias del structuras chimic del neurohumores in question.

ACKNOWLEDGMENT

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REFERENCES

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DISCUSSION

LOUIS J. SOFFER, M.D.: I have had occasion to follow the work of Dr. Guillemin for a good many years, and in a much more modest way to attempt some studies in this area. I have been impressed with the results that he has obtained and the ingenuity of his experiments.

The problem is very interesting. In essence it revolves around whether the anterior hypophysis is stimulated to secrete its hormones essentially by fractions or agents originating somewhere in the brain or whether it is capable of being stimulated by some peripheral mechanism. The problem which has interested me primarily is the ability of the pituitary to elaborate corticotropin, and I shall confine my remarks to this aspect of the problem.

The first thing to bear in mind is that the adenohypophysis is a unique structure devoid of any neurosecretory fibers. We must assume therefore that in whatever way the anterior lobe of the pituitary is stimulated, it is via a humoral agent. This is the first concept that we have to accept.

In attempting to establish the nature and origin of this humoral agent, we are provided with some insight if we examine the anatomical nature of the blood supply to the adenohypophysis, and Dr. Guillemin has done this in detail. I would add one point and that is that in addition to the portal-hypophyseal system originating from the internal carotid artery and breaking up into capillaries in the region of the median eminence and then again collected in the form of a rather large blood vessel which goes to the adenohypophysis, there are branches which go directly from the internal carotid artery to the adenohypophysis, and not through the stalk or the hypophyseal system.

The implications of this observation are significant. They mean that the adenohypophysis is available for stimulation both by agents which find their way through vascular channels present in the hypothalamic area and by agents which reach the pituitary directly from the systemic circulation. In short, it seems to me that nature has been generous, and a variety of contingencies has been provided for.

The question then arises, are there any systemic agents and are there any agents originating within the hypothalamus which are capable of exercising an effect on the function of the adenohypophysis? There is the well-established concept that the secretion of the adenohypophysis, as far as ACTH is concerned, is influenced by the level of the circulating adrenocortical steroids. The mechanism of this effect is perhaps more debatable. The fact remains that as there occurs an increase in the circulating level of the glucogenic corticoids, there is a decrease in the secretion of corticotropin, and the antithesis is similarly true.

In the experimental animal, at least, the administration of epinephrine has resulted in a discharge of corticotropin from the adenohypophysis. This has been subject to some discussion, because although it could be demonstrated in the dog that following the administration of epinephrine, there is an increase in the plasma levels of the 17-hydroxycorticoids, similar observations have not been established in man.

It was suggested by Sayers that epinephrine acts by causing an increase in the utilization of the adrenocortical steroids and, therefore, an increase in the elaboration of ACTH.

In any event, the question arises whether the adenohypophysis is capable of reacting to epinephrine directly or whether epinephrine, if it exercises an effect on the adenohypophysis, does so essentially through the elaboration of the neurohormonal agent in the hypothalamus. The fact remains that in the experimental animal in which viable adenohypophysis is transferred to the anterior chamber of the eye, the local administration of small amounts of epinephrine resulted in an eosinopenia and lymphopenia. There is therefore some reasonably valid evidence to indicate that the adenohypophysis is capable of responding to an agent, systemically or locally introduced, as the case may be, without the intercession of the hypothalamus.

The next question that arises: Does the hypothalamus elaborate an adenohypophyseal stimulating agent? Dr. Guillemin has discussed this in detail.

I should like to describe again the nature of the experiments dealing with this problem, since they are interesting and the conclusions are based somewhat on what we conceive to be the merits or faults of this experimental approach. We have already discussed the portal hypophyseal system, and therefore it would seem very natural to introduce a barrier between the pituitary and the rest of the brain in an effort to determine whether the secretion of, say, corticotropin is thus interfered with.

When the stalk is sectioned, there occurs a decrease in the secretion of corticotropin, but it should be pointed out that the secretion of corticotropin does not cease and that indeed the experimental animal in which the stalk is sectioned is capable of responding to stress.

There is a question that arises in relation to this experiment. When the stalk is sectioned, the circulation of the adenohypophysis is interfered with. Therefore, is the decrease in adenohypophyseal secretion the result of the unavailability of a neurohumoral agent or is it due to necrosis of the pituitary resulting from the interruption of its blood supply?

I think that the stalk experiments raise as many questions as they answer. The stereotaxic experiments, perhaps, reveal some additional information. When minute areas of medial and posterior hypothalamus and the median eminence are destroyed, there is some evidence to indicate that there does occur an interference with the adenohypophyseal secretion of corticotropin. And, on the contrary, when these are stimulated, there is an increase in the secretion of this hormone. These results, too, although suggestive, are by no means entirely conclusive.

Perhaps the most rewarding experimental studies today are those of Dr. Guillemin and his group and Dr. Saffran and his co-workers. These have to do with efforts to isolate a fraction from the hypothalamus, which, in tissue culture of viable adenohypophyseal slices is capable of causing an increase in the secretion of ACTH.

In some earlier experiments by other investigators, explants of the posterior hypothalamus of the bull were capable of causing some increase in the secretion of ACTH, in contrast to those of the anterior hypothalamus, spleen and liver. These experiments, however, were crude and not entirely convincing.

Dr. Guillemin modestly passed over the nature of his experiments rather hurriedly, but I think it is worth while to examine them more closely. When one takes small areas of tissue or extracts of the medial or posterior hypothalamus, or of the median eminence, which are added to a culture medium containing vi-

able adenohypophyseal tissue, the resultant fluid when added to adrenal cortical slices causes an increase in the concentration of those steroid fractions which have a double bond between C_4 and C_5 and a ketone on C_3. We can conclude from the results of this experiment that there has occurred an increase in secretion of corticotropin by the adenohypophyseal tissue thus treated. Saffran and his group have similarly demonstrated that posterior pituitary extract and protopituitrin when added to adenohypophyseal tissue yield similar results.

These studies would therefore indicate that an agent or agents obtained from the medial and posterior hypothalamus, the median eminence and posterior pituitary extract are all capable of causing an increase in the secretion of ACTH by the adenohypophysis. When these various extracts were chromatographed, Dr. Guillemin found that one spot with identical R_f was common to all and which Dr. Guillemin referred to as compound D. When compound D, which proved to be a small polypeptid, was eluted it was found capable of causing secretion of corticotropin by adenohypophyseal tissue slices. This substance was effective in minute amounts varying from one to three gamma. This problem was further complicated by the fact that compound D was also present in a substance obtained from horse gut by Von Euler which he referred to as substance P. In any event an ACTH releasing agent is apparently elaborated by certain areas of the hypothalamus and perhaps even of the posterior pituitary lobe although the latter may simply serve only as a way station for the storage or transmission of this agent. In addition an apparently similar substance is secreted by horse intestine.

What are the conclusions that can be arrived at? It would appear to me that the available evidence suggests that the adenohypophysis can be stimulated to secrete corticotropin both by a neurohumoral agent having its origin probably in the posterior hypothalamus and surrounding area and by agents reaching it from the general circulation. Since the integrity of the adrenal cortex is essential for life such a dual control system would seem not unreasonable since a considerable degree of protection is thus provided.

I will conclude my remarks by expressing once more my appreciation to Dr. Guillemin and his co-workers for their most interesting and provocative studies.

DR. GUILLEMIN: I wish to thank Dr. Soffer for an interesting discussion of the experimental evidence on which are based the concepts which I presented earlier. The question of the possible ubiquity of the hypophysiotropic mediator, I did not intend to discuss here. I am glad, however, that Dr. Soffer introduced it in his discussion. Our studies indicated, indeed, that extrahypothalamic origins of the corticotropin releasing factor were perhaps to be considered. Teleologically it would make sense. The question is still with us and it is too early to answer it. The results obtained with a fraction of substance P mentioned by Dr. Soffer have to be confirmed in vivo. When composition and structure of the hypothalamic factor have been established, it should be easier to compare with it substances with hypophysiotropic activity and of extrahypothalamic origin.

In the Western world we are fortunate in being free from the worst enemy of expanding knowledge, authoritarianism, with which seekers after new knowledge have often had to fight. Nevertheless, it is in human nature to be allergic to new ideas, and discoveries are rarely received with undiluted enthusiasm. This is particularly true when the discovery impinges upon some vested interest or conflicts with the views of a scientific hierarchy. Many years ago T. H. Huxley pointed out that it is the common fate of knowledge to start as heresy and end as a superstition, and recognition that the end of this cycle has been reached is usually belated. Countless examples of opposition to new knowledge could be cited. This year has seen the centenary of the birth of Ronald Ross, whose discovery of the malaria parasite in the mosquito has had a decisive influence on human history. Yet, in the words of a recent appreciation, "at the time of his original discovery he received every possible discouragement from officialdom. He persisted in his work because he was a dedicated scientist." More appropriately today, we may recall that Addison's description of the disease which now bears his name was received with skepticism in many quarters and that reports of two or three cases presented to the London Medical and Chirurgical Society were refused publication in the *Transactions.*

A. S. Parkes, in "The Art of Scientific Discovery" from *Perspectives in Biology and Medicine* 1:377, Summer, 1958. Copyright 1958 by The University of Chicago.