PERFUSION OF THE RAT ANTERIOR PITUITARY VIA A CANNULATED PORTAL VESSEL

By

John C. Porter, Renon S. Mical, Jerome G. Ondo and Ibrahim A. Kamberi

ABSTRACT

A procedure is described for exposing parapharyngeally the hypothalamic-hypophysial complex, enabling one to cannulate a pituitary stalk portal vessel, and thereby perfuse the anterior pituitary. When a crude hypothalamic extract is infused into the anterior pituitary via a cannulated portal vessel, LH and FSH release are markedly stimulated, whereas prolactin release is inhibited. The infusion of a cerebrocortical tissue extract has no effect on the release of LH, FSH, or prolactin, showing that the infusion per se does not affect the release of these hormones. The responses of the perfused anterior pituitary to hypothalamic extract are related to the concentration of the extract in the infusate.

In 1936, Wislocki & King discovered that blood in the hypothalamic-hypophysial vasculature flowed from the hypothalamus to the anterior pituitary. Their key observation led promptly to the formulation of the hypothesis that the hypothalamus by way of this vasculature controlled the release of the gonadotrophic hormones through a neurohumoral mechanism. (For the belated

1. This work has been supported by grants from The Population Council, New York, N. Y., and the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases (Grant No. AM 01237).
2. Postdoctoral Training Fellow of the United States National Institutes of Health, Grant HD 00256.
publication of the first presentation of this hypothesis, see Friedgood 1970.) Yet, despite these early advances, our knowledge of the means of regulation of the anterior pituitary is still fragmentary. The slow progress here may be due partly to the fact that in the living mammal it is difficult to expose the ventromedial hypothalamus and pituitary sufficiently to enable one to perform critical experiments upon the individual units of the hypothalamic-hypophysial complex. As it is well known, the pituitary lies deep within the cranium and is protected, as it were, by the brain from above and guarded from below by bones of the skull and jaw as well as by other structures, e.g., the pharynx, muscles associated with eating and head movement, etc.

To circumvent these anatomical impediments, a number of indirect procedures have been used; but, for the most part, these procedures have lacked the precision needed to resolve many critical questions that have arisen about anterior pituitary control. One method, often used in attempts to test the efficacy of various substances to stimulate or to inhibit the release of hormones from the anterior pituitary, involves the injection of test solutions into the peripheral circulation via easily accessible large blood vessels, e.g., jugular vein, femoral vein, etc. Once in the systemic blood, the test substance is then presumed to be transported to the anterior pituitary. The justification for use of such a procedure lies principally in its ease of execution. However, there are at least two major objections to this approach. First, a test substance when injected into the general circulation is diluted to some unknown extent by the blood before reaching the anterior pituitary. Such uncontrolled dilutions make it difficult to obtain results which can be interpreted quantitatively.

The second objection is even more serious, especially in experiments involving substances believed to have releasing or inhibiting activity. It is known that the hypothalamic-hypophysial vasculature is a portal system (Popa & Fielding 1930, 1933) within which blood flows from the hypothalamus to the anterior pituitary (Wislocki & King 1936). The primary capillary plexus of this vasculature lies primarily in the ventromedial hypothalamus. These particular capillaries drain into the stalk portal vessels which in turn empty into a secondary capillary plexus in the anterior pituitary. Thus, any substance injected into the systemic circulation must necessarily pass through the primary plexus before reaching the anterior pituitary. Because of this peculiar vascular arrangement, we are confronted with a dilemma when interpreting the results of an experiment in which the release of a hormone from the anterior pituitary is altered by a test substance which has been injected into the general circulation. We must ask, did the test substance act directly on cells in the anterior pituitary stimulating them to release a trophic hormone? or did the test substance cause secretory elements in the hypothalamus to discharge hypophysiotrophic substances which in turn stimulate or suppress the release of a trophic hormone?
Because these questions are difficult to answer, some investigators have employed methods which enable them to inject test substances directly into the body of the pituitary. This mode of administration has some advantages. First, the hypothalamus is circumvented and its influence minimized. Second, the cannula, through which the test solution is administered, can be held permanently in the pituitary through an attachment to the skull and be used at will for long periods. Third, substances can be infused during the conscious state. Utilizing this approach for the injection of hypothalamic extracts into the anterior pituitary, Nikitovitch-Winer (1962) and Averill & Kennedy (1967) succeeded in causing ovulation and thyrotrophin release, respectively, in rats.

However, objections can be raised against the injection of solutions directly into the body of the anterior pituitary. The normal pressure gradients within the gland are probably distorted greatly by the injected solution. In addition, little is known about the distribution of the injected solution within the gland. We have observed by direct visualization that when a solution is injected rapidly into the body of the anterior pituitary, it tends to accumulate in one site before following a narrow route of egress from the gland. Such an accumulation of fluid could cause a site of hypoxia and distort the response of the tissue. On the other hand, when a solution is injected slowly, it seems to spread minimally; and little tissue is exposed to the test solution.

Some of these objections could be overcome if the test solution were infused directly into one or more portal veins which are, of course, distal to the primary capillaries of the hypophysial portal vasculature. Such a procedure would not only circumvent the hypothalamus but also allow the infusate to the anterior pituitary to follow vascular tributaries which ordinarily receive blood from the portal vessel or vessels. The major difficulty with this approach can be attributed to the fact that in mammals the hypothalamic-hypophysial vasculature is located in a region of the cranium that is difficult to expose in the living animal.

Over the past few years, we have developed a procedure for laying bare this region sufficiently in the anaesthetized rat to enable one to perform certain manipulations under direct visualization upon individual components of the hypothalamic-hypophysial complex. These manipulations include (a) the collection of blood quantitatively from the pituitary stalk (Porter & Smith 1967), (b) the injection of solutions into the third ventricle via a microcannula inserted through the stria terminalis of the hypothalamus (Kamberi et al. 1970), (c) the perfusion of the anterior pituitary via a cannula located in a stalk portal vessel (Porter et al. 1970), and (d) the perfusion of the median eminence and/or stalk via a microcannula placed within an infundibular or peduncular artery (Kamberi et al. 1971a).
MATERIALS AND METHODS

1. Exposure of the ventromedial hypothalamus and anterior pituitary

a. Exposure of the base of the skull. – In general, exposure is effected through a parapharyngeal approach which, in some respects, is similar to that described for hypophysectomy by Smith (1930). In order to keep the head of the animal immobile, the anaesthetized rat is placed on its back in a holder (Fig. 1) which fixes the head firmly by means of ear bars. Then, with the head pointed away from the operator, a midline incision is made in the skin of the neck; and the animal is connected by means of an endotracheal tube (Fig. 2) to a rodent respirator (Harvard Apparatus Co.) which provides him with oxygen during the remainder of the experiment. Subsequent operations are performed with the aid of an operational binocular microscope having a working distance of 20 cm and a range of magnification varying from 5 to 50 X.

After the skin incision in the neck is extended from the sternum to the interramal vibrissae (Fig. 2), the sternohyoid and omohyoid muscles are separated by blunt dissection; and the thyreohyoid muscle on the animal’s left side (operator’s right) is transected slightly below the thyroid. The sternohyoid and

---

![Fig. 1.](image_url)

Rat head holder. The animal is held on its back on base plate (A) by means of ear bars (C) which are inserted in the ear canals. The ear bars are held in vertical supports (b) by set-screws (D). (From Porter et al. 1970).

252
omohyoid muscles on the operator's left side as well as the trachea are retracted to the left while the contralateral muscles are retracted to the right. Now, by means of curved (approximately 30 degrees) blunt forceps, the hyoid bone is lifted and pulled forward to expose the first vertebra and the muscles attaching to the base of the skull (Fig. 3). The muscles adhering to the occipital bone are freed from their attachments by scraping with a blunt instrument. The first vertebra, which lies directly below the thyroid, and the occipital ridge, which lies in the midline of the occipital bone, are useful landmarks for orientation. The occipital ridge can be visually followed anteriorly to the suture which joins the occipital and basisphenoid bones. The suture line of these two bones lies perpendicular to the occipital ridge and delineates approximately the posterior margin of the anterior pituitary.

The tissue overlying the occipital bone is dissected laterally until the internal carotid arteries and much of the tympanic bullae can be seen. The hyoid bone is now pulled anteriorly as far as possible by means of a retractor. The hyoid is usually fractured during this retraction, but this is of no consequence. The tissue adhering to the basisphenoid is dissected anteriorly until the presphenoid
The trachea, sternohyoid and omohyoid muscles are retracted laterally and the hyoid bone is pulled anteriorly to expose the muscles covering the first vertebra and base of the skull.

becomes visible. At this time, additional retractors are placed on either side of the retractor holding the hyoid. These retractors keep the skin, muscles, and the soft pharynx out of the operator’s field of view. The tissue over the basisphenoid is dissected laterally until the pterygoid processes, the middle lacerated foramina, and the medial halves of the tympanic bullae are exposed. Muscles overlying the tympanic bullae are held out of the operator’s view by means of retractors placed near the anterior margins of the tympanic bullae. When this portion of the operation is performed by a skilled individual, only minor blood vessels are broken, and, consequently, little blood is lost. (Caution: If retractors are placed tightly near the posterior margin of the tympanic bullae, the flow of blood through the internal carotid arteries and/or ptergopalatine arteries can be obstructed; and, if so, the rat dies within a short time.)

b. Removal of bone covering the ventromedial hypothalamus and anterior pituitary. – A drill consisting of an electric motor, a 7D handpiece (Foredom
Electric Co., N.Y.), and a dental bit with a No. 8 spherical burr is used to remove most of the basisphenoid bone. The drill is operated at high speeds with minimal pressure exerted on the bit. Some bleeding occurs when the sinus in the basisphenoid is broken, but the haemorrhage is easily controlled with bone wax. The final thin layer of the basisphenoid is removed with the aid of forceps. The opening in the bone in the anteroposterior direction should extend from a point that is approximately 1 mm posterior to the occipital-basisphenoid suture to a point that is approximately 1 mm anterior to the basisphenoid-presphenoid suture. Bone is removed in the lateral direction until the internal carotid arteries can be seen. Thus, it is necessary to remove bone until the anterior lacerated foramina are reached and to remove the antero-medial corner of each tympanic bulla. Satisfactory exposure is obtained when most of the anterior pituitary, pituitary stalk, and optic chiasma can be seen through the meninges. The region of the skull that is removed is illustrated in Fig. 4.

2. Preparation of the microcannula

By means of a micropipette puller (Industrial Science Associates, Ridgewood, N.Y.), borosilicate glass tubing (0.9 to 1.1 mm outside diameter; 100 mm long) is drawn out to form a fine tip. The platinum heating element of the puller is brought to the near maximal temperature, but when the glass shows first signs of softening, as indicated by a slight lengthening of the tube, the platinum element is cooled momentarily by blowing one’s breath briefly on the heating element. Consequently, when the element warms again, the glass tube is drawn out to a short, abruptly-tapered capillary tip. The capillary is next bent approximately 75 degrees. A typical cannula is illustrated in Fig. 5. Because we have found that the diameters of the portal vessels vary slightly from rat to rat, the capillary tip is not shortened to its final length until the portal vessels are exposed and a vessel is selected for cannulation. Then, the tip of the capillary is shortened by cutting with small scissors at a point where the outside diameter of the capillary tip is slightly smaller than the diameter of the vessel to be cannulated. The final outside diameters of most capillary tips range from 20 to 40 μm.

3. Preparation of the infusate

One of the more vexing problems which we encountered during the early stages of the development of the infusion procedure was the result of an accumulation in the cannula tip of debris of microscopic dimensions which obstructed the flow of the infusate through the cannula. This problem was solved by subjecting all solutions that were to be infused to preliminary serial
filtrations through Millipore filters of progressively decreasing pore diameters of 8, 3, 0.8, and 0.3 µm. As a final precautionary procedure, a filter having a nominal pore diameter of 3 µm was placed in the cannula holder to filter the infusate immediately before the fluid entered the cannula. A small amount of lissamine green is usually added to the infusate. The green colour enables us to follow the course of the infusate through the portal vessels and anterior pituitary.

4. Cannula holder

The small size and extreme fragility of the cannula makes it necessary to have a special means of manipulating it during the experiment. To attain this
end, a holder was designed to serve three purposes: First, the holder provides a means of attaching the cannula to the micromanipulator. Second, the cannula holder serves as a conduit through which the cannula and infusion pump are connected. Third, the holder provides a means of placing an inline Millipore filter before the cannula. A design which we have found satisfactory is illustrated in Fig. 6. One end of the cannula holder is made to provide a liquid-tight seal between the holder and a polyethylene tube (Fig. 6a, b, c). The opposite end of the polyethylene tubing is connected to a 2 ml syringe. An infusion pump (Harvard Apparatus Co.) is used to regulate flow through the cannula.

5. Cannulation of a portal vessel

Immediately before cannulating a portal vessel, the dura and arachnoid membranes overlying the ventromedial hypothalamus and pituitary stalk are reflected, bringing into view the stalk portal vessels as illustrated in Fig. 7. (In this photomicrograph, the larger vessels have been darkened for emphasis.)
Details of the cannula holder: 

- a = polyethylene tubing (0.96 mm o. d.) with a flanged tip;
- b = female coupling (11 mm long, 8 mm in diameter) used to hold the polyethylene tube tightly to the male conduit c (75 mm long, 4.8 mm in diameter);
- d and j = male and female parts of a Swinny syringe filter holder (Millipore Corp., Bedford, Mass.);
- c, f, g, h and i = Teflon gasket, steel ring, filter, steel grid and Teflon gasket, respectively, for the Swinny holder;
- m = female coupling (7 mm long, 6 mm in diameter) used to attach the cannula n (Fig. 5) to the male conduit k (15 mm long, 4.8 mm in diameter);
- l = silicon rubber gasket (7 mm long, 3 mm in diameter).

(From Porter et al. 1970).

Photomicrograph of the median eminence and pituitary stalk showing the hypophysial portal vessels in a representative animal. (The major vessels have been retouched for emphasis). (From Porter et al. 1970).
The number of major portal vessels have been found to vary among rats. Although 5 to 8 vessels are present in most animals, as few as 1 or 2 have sometimes been observed. After being exposed, the vessels should be kept covered with an isotonic salt solution as much as possible to prevent them from drying. Should a vessel become dry, one usually encounters difficulty in its cannulation.

After the micromanipulator is placed beside the animal’s head, the cannula and the holder are attached to the manipulator. (We use a Leitz Wetzlar micromanipulator.) Then, using a magnification of 25 to 50 × – depending on personal preference, the operator brings the cannula tip into juxtaposition with a portal vessel. As fluid is pumped slowly through the cannula, the cannula tip is forced into a portal vessel using the universal control of the manipulator. It may be necessary occasionally to adjust the tension exerted on the vessel by the cannula with the vertical control mechanism. Since the inside diameter of the vessel is greater than the outside diameter of the cannula tip, one usually has little difficulty in inserting the cannula as illustrated in Fig. 8. Once the cannula is in the vessel, the green perfusate can be seen to pass quickly down the portal vessel and to become dispersed among the sinusoids of the pars distalis.

The concentrations in plasma of LH, prolactin, and FSH were determined by radioimmunoassay according to Niswender et al. (1968, 1969) and Parlow et al. (1969), respectively.

Fig. 8.
Drawing illustrating the approximate position of the cannula in a portal vessel relative to the pituitary. (From Porter et al. 1970.)

259
1. Infusion and distribution of the infusate

The blood flow of the pars distalis of anaesthetized male rats ranges from 0.6 to 1 $\mu\text{l} \times \text{min}^{-1} \times \text{mg}^{-1}$ of tissue (Porter et al. 1967). Since the pars distalis of adult rats weighs 7 to 10 mg, the total blood flow of such glands can be computed to be between 4 and 10 $\mu\text{l} \times \text{min}^{-1}$. If this volume of blood were delivered to the gland by five portal vessels, for example, the average flow through each vessel would become 0.8 and 2 $\mu\text{l} \times \text{min}^{-1}$. To simulate such flow, we routinely infuse solutions into a portal vessel at the rate of 2 $\mu\text{l} \times \text{min}^{-1}$.

The distribution of the infusate from a given portal vessel within the pars distalis is variable. Occasionally one sees a cannulated portal vessel which is located on one side of the pituitary stalk deliver its effluent to capillaries which are located on the contralateral side of the pars distalis. Although one cannot predict with high precision which portion of the pars distalis will receive blood from a given portal vessel, it does seem that once a pattern is established, there is little tendency for the distribution to change during the infusion period in a given animal. Some representative distribution patterns in glands infused via a cannulated portal vessel which was located centrally on the stalk or laterally on the stalk are illustrated in Figs. 9 and 10, respectively.

Fig. 9.
Gross distribution in the pars distalis of a perfusate administered via a cannula in a portal vessel located ventromedially on the pituitary stalk.
(From Porter et al. 1970).
2. Release of hormones by the perfused pars distalis

The release of trophic hormones is altered quickly when hypophysiotrophic substances are infused into a portal vessel (Fig. 11). When an acidic extract of rat hypothalamic tissue, neutralized to pH 7.2, was infused at the rate of 2 µl × min⁻¹ into a portal vessel of male rats, the mean concentration of LH in the plasma of arterial blood of the recipient animals increased within 10 minutes to a concentration that was 240 per cent of the pre-infusion level (Fig. 11). At the same time in the same set of animals, the plasma FSH level rose to 170 per cent while that of prolactin fell to 63 per cent of the pre-infusion concentration. After 20 minutes of infusion, the plasma LH level was 450 per cent, FSH was 230 per cent, but prolactin was only 50 per cent of the initial level. After 30 minutes of infusion, the plasma levels of LH, FSH, and prolactin were 580 per cent, 300 per cent, and 37 per cent, respectively, of the pre-infusion values. When the infusion was stopped after 30 minutes, the release of LH and FSH slowed markedly as indicated by the fall in the plasma concentrations of these hormones, whereas prolactin release increased (Kamberi et al. 1971b).

The rate of release of LH and FSH during the 30-minute period of infusion varied directly with the concentration of the hypothalamic extract in the in-

---

Fig. 10.
Gross distribution in the pars distalis of a perfusate administered via a cannula in a portal vessel located laterally on the pituitary stalk. (From Porter et al. 1970.)
The simultaneous release of LH, FSH, and prolactin during a 30-minute period of infusion of a hypothalamic extract into a pituitary stalk portal vessel. All values are expressed as a mean percentage of the pre-infusion concentrations of each hormone. The use of a logarithmic representation of the concentrations was arbitrary. (Computed from data reported by Kamberi et al. 1971b).

The infusion of extracts equivalent to 22.5, 45, and 135 µg of hypothalamic tissue (wet weight) per minute caused progressively increasing concentrations of LH (Fig. 12) and of FSH (Fig. 13) in arterial plasma during the 30-minute infusion period. Yet, the infusion of an extract of cerebrocortical tissue equivalent to 135 µg of tissue per minute had no effect on the release of LH (Fig. 12) or of FSH (Fig. 13), showing that the release of these two hormones was not a consequence of the infusion per se. In contrast to LH and FSH, the infusion of an extract equivalent to 22.5, 45, and 135 µg of hypothalamic tissue suppressed the release of prolactin, and the extent of the inhibition varied with the concentration of the extract in the infusate (Fig. 14). The infusion of an extract of cerebrocortical tissue had no effect on prolactin release. Other substances such as dopamine, epinephrine, norepinephrine, serotonin, or melatonin when infused into the pars distalis via a cannulated portal vessel have no effect on the release of LH, FSH, or prolactin (Kamberi et al. 1970, 1971a, 1971c, 1971d).
The release of LH during a 30-minutes period of infusion of different concentrations of an extract of hypothalamic tissue into a pituitary stalk portal vessel. The control consisted of an extract equivalent to 135 µg/minute of cerebrocortical tissue. All values are expressed as a percentage of the pre-infusion concentrations. (Computed from data reported by Kamberi et al. 1971b).

**DISCUSSION**

The procedure for perfusing the anterior pituitary via a microcannula inserted into a portal vessel would appear to offer several advantages when evaluating the efficacy of test solutions to stimulate or inhibit the release of trophic hormones. The investigator can control the composition of the perfusing fluid to a degree that is unrealizable with other in vivo procedures. In addition, such small quantities of materials can be administered that any excess which enters the general circulation is diluted so greatly that re-circulation of an infused substance is insignificant.

When an extract of hypothalamic tissue is infused into a stalk portal vessel, there is a prompt increase in the release of LH and FSH and an inhibition of prolactin release as judged by the changes in the concentration of these hormones in systemic plasma. Unfortunately, it is not possible at present to collect the venous effluent of the anterior pituitary and thereby measure directly the secretory rates of trophic hormones from the anterior pituitary. If it were
The release of FSH during a 30-minute period of infusion of different concentrations of an extract of hypothalamic tissue into a pituitary stalk portal vessel. The control consisted of an extract equivalent to 135 μg/minute of cerebrocortical tissue. All values are expressed as a percentage of the pre-infusion concentrations. (Computed from data reported by Kamberi et al. 1971b).

possible to do so, one might expect trophic hormone release to be affected very quickly after commencing the infusion.

A possible shortcoming of this mode of perfusing the anterior pituitary can be attributed to the fact that several portal vessels usually provide blood to the anterior pituitary, yet only one is cannulated and not all of the gland is perfused. This apparent drawback can possibly be overcome by infusing fluid at a faster rate, but the advantages or disadvantages that might be derived from such an approach may not be realized until better methods are developed for the assessment of secretory rates. The use of changes in hormone concentrations in systemic blood to evaluate secretory rates is far from optimal, and a change in the concentration of a hormone in the general circulation is undoubtedly a highly damped function of an altered secretory rate. Thus, the true dynamics of the response of the anterior pituitary to a perfused substance may differ considerably from the slower changes observed in the concentration of a secretory product in the blood of the general circulation.
The release of prolactin during a 30-minute period of infusion of different concentrations of an extract of hypothalamic tissue into a pituitary stalk portal vessel. The control consisted of an extract equivalent to 135 μg/minute of cerebrocortical tissue. All values are expressed as a percentage of the pre-infusion concentrations. (Computed from data reported by Kamberi et al. 1971b).

ACKNOWLEDGMENTS

The authors express gratitude to Mrs. Jane C. Gottwald for excellent assistance during the preparation of this manuscript. We are indebted to Drs. G. D. Niswender and A. R. Midgley, Jr., for antiserum to LH; L. E. Reichert, Jr., for ovine LH; J. Meites for antiserum to prolactin; S. Ellis for rat prolactin; and the National Institute of Arthritis and Metabolic Diseases Rat Pituitary Hormone Program for the reagents used in the radioimmunoassay of rat FSH. The authors also wish to thank Dr. Paul C. MacDonald for his generous assistance during this research.

REFERENCES


DISCUSSION

Goding: I have two questions, Dr. Porter. Have you put oestradiol into one of the pituitary portal vessels or into the hypothalamus in the region where you put the dopamine? And what about Schally’s marvellous releasing compounds?

Porter: We are conducting experiments in which steroids are being used in an attempt to alter the responsiveness of either the hypothalamus or the pituitary, but the results are still preliminary. The only synthetic releasing factor we have used has been TRF (Porter et al. 1971). The release of TSH is stimulated when TRF is infused into a hypophysial portal vessel. We have also infused purified hypothalamic ovine LRF which was obtained from Drs. Guillemin and Burgus. It stimulated LH release. We have not used synthetic LRF but hope to do so in the near future.

Goding: Did it release FSH as well as LH?

Porter: We did not check this point but will do so at the earliest opportunity.

Serra: In a study done in Dr. Midgley’s laboratory (Serra & Midgley 1970) it was possible to demonstrate that isolated pituitary glands from adult rats, and more recently from human foetuses, in an in vitro system, can respond to separate administrations of hypothalamic extracts with a brief, immediate release of LH, largely limited to the time of exposure to rat or bovine hypothalamic extracts and followed by a drop to a relatively constant basal release rate. In the superfusion technique, the continuous renewal of incubation medium avoids accumulation of endogenous hormone and permits one to study, for short periods of time, the releasing mechanism of pituitary hormones in the absence of short or long feedback, or other complicating mechanisms.

In your experiment, the portal vessel infusion permits a direct stimulation of the anterior pituitary, but since the peripheral blood collection is done in ten minutes’ in-
tervals, it cannot be excluded that endogenous hormones once released into the general circulation can act through a long feedback in both the hypothalamus and the anterior pituitary. If this is the case, could the releasing mechanism be in some way masked?

**Porter:** I am not sure that the long or short feedback systems operate in short-term experiments. Trophic hormones can be infused directly into the anterior pituitary without affecting the release of hormones from the anterior pituitary.

**Martini:** I am pleased to see that Dr. Porter has confirmed that the anterior pituitary is not directly sensitive to pituitary hormone; we have also shown this with less sophisticated techniques.

**Yoshinaga:** Neill (1970) and Wakabayashi et al. (1971) reported that stress or anaesthesia causes an increase in prolactin levels in the rat. In your preparation, the prolactin concentration in the control group did not change for 30 minutes. I would like to know the reason for the difference between your results and the observations of others. Do you think that the irrigation of the pituitary with hypothalamic extract at the rate of 2 μg per minute could eliminate the influence of the hypothalamus in situ? Is it necessary to ligate portal vessels other than the one you cannulate for the elimination of hypothalamic influences?

**Porter:** Actually, I would interpret our results to be in agreement with those of other workers. The values for prolactin were rather high, and these animals certainly would qualify as stressed by most standards that one would care to use to define this operational state. Still, we can only say that it looks like these animals are rather stable in our hands, because the prolactin can remain steady for as long as two hours. One could attribute this finding to the fact that the animals are anaesthetized and that they are subjected to continuous surgical trauma. But, none of this really concerns me. What we desire is a stable preparation.

**Jaffe:** What happens to prolactin when you infuse TRF into the portal circulation?

**Porter:** Unfortunately, I do not know. Prolactin levels in plasma were not determined in those experiments.

**Ahrén:** Is it possible with your technique to infuse the substances for a longer time? If so, then it might be possible to investigate whether the «releasing substances» influence not only release but also synthesis of the anterior pituitary hormones. In other words, have you analysed also the content of pituitary hormones after the release of the hormones?

**Porter:** Thus far, we have not conducted any experiments dealing with synthesis. But this is an interesting possibility.

**Urquhart:** Three short questions: One relates to the question of how distribution of infused dye in the gland depends on the perfusion rate. Second, have you had the opportunity to measure the resistive pressure drop across your cannula system that could allow you to make a statement about the perfusion pressures you have off the cannula tip when you are perfusing at the 2μl/min rate? Third, what is your view about prospects for being able to collect pituitary venous blood directly?

**Porter:** Certainly the extent of spread of the perfusate in the pituitary is flow related. There is no question about that. No attempt has been made to perfuse all of the gland, and the fact that only part of it is perfused may be a limitation of this procedure. We
have not measured the pressure at the tip of the pipette. In regard to your question on collecting pituitary venous blood, I can say that this is a problem that has bothered me a long time. Clearly, this is a very desirable thing to do and, if done, would answer many questions. But it is a formidable task. The pituitary drains into a meshwork of sinuses which would lie on the side of the gland and empty into the cavernous sinuses. Thus far, I have been unable to see a way which would enable one to isolate these structures; but I keep hoping that one day I shall see the light and be able to do it.

Lunenfeld: In your paper you gave the FSH and LH blood concentration increases as percentages of pre-infusion levels. I understand from the slide you presented that the pre-infusion levels were about 5 nanograms LH per ml and one microgram FSH per ml, respectively. Could you tell us to which LH and FSH preparations you refer, what standards and what antisera were used in the assays. Furthermore, I would be interested in the reliability criteria (e.g. sensitivity, precision, specificity) of the assays employed.

Porter: The results were presented as percentages of control values to circumvent the problem of reference standards. The reference standard for FSH was the NIAMD-Rat FSH-RP-1. The values for LH were expressed in terms of the NIH-LH-S1. So, the values for LH and FSH on a weight basis are really not comparable. This is why we presented the results as percentages, so that one could compare changes in the plasma levels of one hormone with those of another. The lowest limit of sensitivity for LH is about 0.1 nanogram. I don't remember the lower limit of sensitivity for FSH, but it is somewhere between 1 and 10 nanograms.

Lunenfeld: How long can you keep the pituitary secreting the amounts of FSH and LH which you reported?

Porter: I don't know. We have not infused longer than 30 minutes.

Diczfalusy: I agree very much with what Dr. Porter said about the complications in bioassays introduced by different standards. Indeed, one of the major complications is that the half life time of circulating gonadotrophins will largely influence the potency estimates. Recently, Dr. Dufau and her husband, Kelvin Catt from the NIH (Dufau et al. 1971) described a four hour in vitro bioassay for LH or HCG, using the decapsulated rat testicle and the secretion of testosterone by this preparation as an endpoint. Their system is so sensitive that it enables one to measure 5–10 milliU. Furthermore, they have shown that desialated HCG which has been deprived of sialic acid and has no biological activity in conventional bioassays is active in this preparation, suggesting that what really happens following desialization is a change in circulating half life time. Dr. Porter, I wonder whether a combination of this assay with a radioimmunoassay wouldn't enable you to get additional information on the nature of the gonadotrophin secreted by the pituitary and whether or not subunits are secreted at the same time.

Porter: Certainly, the possibility is real that a variety of active components are secreted by the pituitary or may be produced from a precursor molecule in the peripheral blood. It would be worthwhile to use an alternate assay, particularly a sensitive, reliable bioassay.

Goding: At what time of the cycle in the rat are these experiments done?
Porter: These were male rats and, in these preliminary experiments, this sex was selected in order to obviate the complexity that one encounters in the female rat.

Goding: It would be quite interesting to me to know if you could for instance, block the response to dopamine infused into the hypothalamus with the simultaneous infusion of progesterone into the pituitary portal vessels. It would be interesting to know whether progesterone could block the LH release without affecting the FSH release. Is this the sort of thing you have in mind?

Porter: Yes, I suspect that some steroids will alter the responsiveness of the anterior pituitary to the releasing factors. We are conducting a set of experiments to test this hypothesis now.

References:


