Ultrastructure of Hypothalamic Neurons and of the Median Eminence

R. E. CLATTENBURG

SUMMARY: *Our light, and electron microscopic (EM) findings within the hypothalamic supraoptic (SO) and paraventricular (PV) nuclei of the normal female rabbit are in agreement with those reported earlier by other investigators for the same nuclei of the dog and rat. The neurons of these nuclei are the hypothalamic synthesis sites of the neurohypophyseal hormones.*

With the exception of the arcuate nucleus, none of the hypothalamic nuclei associated with the control of adenohypohpyseal function have been studied extensively with the electron microscope. On the basis of our EM findings within the female rabbit hypothalamus, all neurons observed within the preoptic (PO) and suprachiasmatic (SCH) nuclei of the non-mated control animal were morphologically identical to

(Turn to page 54)

RÉSUMÉ: *Nos observations avec microscope-lumière et aussi microscope-électrone, aux noyaux hypothalamiques. SO et PV chez la lapine normale, sont d'accord avec celles antécédentes d'autres investigateurs des mêmes noyaux chez le chien et le rat. Que les neurones de ces noyaux soient les sites de synthèse hypothalamique des hormones neurohypophysaires, est bien établi à ce temps.*

Avec la seule exception du noyau arcué, tous les autres noyaux hypothalamiques, reliés au contrôle de fonction adénohypophysaire n'ont pas été étudiés extensivement avec microscope-électrone. D'après non propres trouvailles utilisant cette méthode à l'hypothalamus de la lapine, tous neurones étudiés d'animal non couplé furent identiques quant à leur

(Suite à la page 54)

I. Introduction

Neurons with gland-like features were first identified within the central nervous system (CNS) of certain elasmobranchs Dahlgrén (1914). This specialized neuron, now commonly known as the neurosecretory cell, has since been observed within the CNS of many other vertebrates, as well as a variety of invertebrate species. Their widespread occurrence within the hypothalamus of the mammalian brain is no exception Bern (1963), Sachs (1969), Stutinsky (1970), Smith (1971), Pawlikowski and Karasek (1971). The functional significance of the neurosecretory cell as a centre of hypothalamic hormone synthesis is presently widely accepted. For example, the site of origin of the neurohypophyseal hormones oxytocin and vasopressin has long been associated with the supraoptic (SO) and paraventricular (PV) hypothalamic nuclei Bissett Clark and Errington (1971), Dyball (1971), Kalimo (1971), Kalimo and Rinne (1972), Norstrom (1972). However, the precise localization of hypothalamic nuclei responsible for the synthesis of those hormones associated with the control of adenohypophyseal activity remains more uncertain.

The notion of a 'humoral' link in the control of adenohypophyseal function probably emerged from the early studies of Hinsey and Markee (1933) and Harris (1937). However, it was not until 1947 that Green and Harris fully recognized the significance of the hypophyseal portal system of veins and proposed the 'neurohumoral Hypothesis' for the control of anterior pituitary gland function. Many of these neurohormones (now referred to as the hypothalamic releasing and inhibiting factors) have since been isolated from the mammalian hypothalamus and are now known to be polypeptide in nature.

To date, however, surprisingly few electron microscopic (EM) studies have been carried out with the specific aim of elucidating the hypothalamic synthesis sites of the releasing and inhibiting factors. Unquestionably, the ultrastructural changes that have been observed among other protein-producing cells have been consistent and repreatedly interpreted as reflecting enhanced synthetic activity. One cannot help but wonder, therefore, why similar ultrastructural changes would not represent the hypothalamic synthesis of the neurohormones, should they occur within hypothalamic neurons following specific endocrine manipulation.

II. Definitions, the Neurosecretory Neuron and the Concept of Neurosecretion

According to Scharrer and Scharrer (1963), Scharrer (1969) (1971), all neurons within the mammalian hypothalamus do not qualify as true neurosecretory cells. Throughout the text of the present paper, the terms 'neurosecretory neuron' and 'synthetically active neuron' will be considered synonymous and used interchangeably. Neurons not showing discrete morphological evidence of neurosecretory activity, will be referred to as 'synthetically inactive' or 'conven-

From the Department of Anatomy, Health Sciences Centre, The University of Western Ontario, London, Canada.

Reprint Address, Dr. R. E. Clattenburg, Dept. of Anatomy, University of Western Ontario, London 72, Canada.

tional' neurons. The 'neurohormones' are the secreted products of these neurons since they, like conventional hormones, regulate the activity of target organs only after their release into and transport via the blood. In addition, the neurohormones associated with the hypothalamo - neurohypophyseal axis will be referred to as the 'neurohypophyseal hormones' to distinguish them from the neurohormones (e.g., the releasing and inhibiting factors) known to influence the activities of the adenohypophysis.

According to Bern (1963), the neurosecretory neuron is a cell having the basic properties of both a nerve and a gland cell. The morphological and cytophysiological criteria that serve to identify the neurosecretory neuron have been the subject of many recent reviews Knowles (1965), Sloper (1966) Knowles (1967), Hofer (1968), Picard (1969), Sachs (1969), Stutinsky (1970), Smith (1971). Only the basic morphological features that separate the neurosecretory neuron from the more conventional or synthetically inactive neuron are included in Fig. 1. As clearly illustrated: (1) the somata of neurosecretory neurons usually lie in close spatial relationship with capillaries. This association has been observed at both the light (Scharrer and Scharrer (1940) and EM levels Zambrano and DeRobertis (1966) (1967) Clattenburg, Singh and Montemurro (1971) (1972a), Clattenburg (1972). Although the functional significance of such relationships are in doubt, they may provide a more direct route by which nutrients and/or hormonal feed-back may influence the metabolism or synthetic state of the neurosecretory neuron. In addition, it may also serve as a pathway for the release of neurosecretory products directly into the blood. According to Senchik (1971). merocrine or aprocrine types of secretion have been observed in lower vertebrates (teleosts, amphibia, and reptiles). However, to my knowledge, similar findings have not been made within the CNS of mammals. (2) The terminals of neurosecretory neurons usually do not form true synaptic junctions with other neurons. Although such contacts have been suggested Hofer (1968) there is presently little discrete ultrastructural evidence to support this view. (3) The relatively large terminals of neurosecretory neurons always lie in close spatial relationship with the perivascular spaces of capillaries. Areas of the brain where such neuro-vascular contacts occur have been termed 'neurohaemal organs' Knowles and Carlisle (1956) and are adapted to serve two functions: a) the storage of neurosecretory material, and b) the site from which they are released into the systemic circulation. (4) The axons of neurosecretory neurons are never myelinated.

The concept of neurosecretion as originally established by Bargmann and Scharrer (1951), and which is presently widely accepted as being accurate for all metazoa, is also illustrated in Fig. 1. According to this concept, the neurosecretory material is synthesized within the neuron soma, transported via axons to axon terminals where it is temporarily stored, or immediately released into the vasculature of a neurohaemal organ. Although it is generally agreed that the typical electron-dense secretory granule may represent the carrier of the neurohormones Zambrano and DeRobertis (1968) Ishii Iwata and Kobayashi (1969), Kobayashi Matsui and Ishii (1970), Scharrer et al

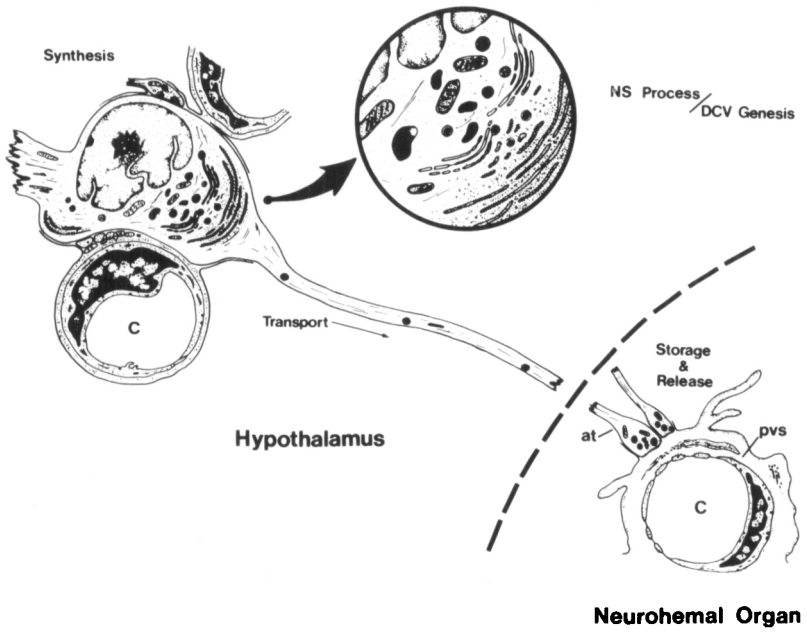

Figure 1. Schematic drawing showing the relationship between a neurosecretory neuron soma and capillaries (c) of the hypothalamus, the neurosecretory process (insert), and the relationship between axon terminals (at) and the perivascular spaces (pvs) surrounding fenestrated capillaries (c) of a neurohaemal area.

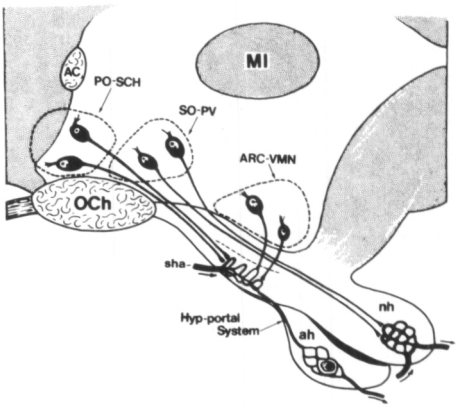

Figure 2. Schematic diagram of the rabbit hypothalamus and pituitary gland showing the localization of neurosecretory neurons implicated in the control of neurohypophyseal (nh) and adenohypophyseal (ah) activity. AC anterior commisure; ARC-VMN arcuate-ventromedian area; MI massa intermedia; OCh optic chiasma; PO-SCH preoptic-suprachiasmatic area; sha superior hypophyseal artery; SO-PV supraoptic-paraventricular area.

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

(1963) cautioned that all neurons containing electron-dense granules should not be considered neurosecretory. Only those neurons whose secreted products can be demonstrated to fluctuate with changes in the physiological state of the animal, and whose secreted material effects target organs after their release into and transport via the blood, are true neurosecretory neurons. For this reason, many neurons of the arcuate nucleus, are excluded from the category of 'neurosecretory'. Although many neurons of this hypothalamic nucleus do contain electron-dense granules, their frequently suggested content (dopamine) does not have a direct effect upon anterior pituitary gland activity Porter and Mical (1969) (1970), Porter Kamberi Goldman Mical and Grazia (1970), Kamberi Mical and Porter (1969) (1970).

III. Distribution of Neurosecretory Neurons within the Mammalian Hypothalamus

Based on numerous studies that have employed a variety of neuroendocrinological techniques, the somata of neurosecretory neurons associated with the many functions of the pituitary gland, are located within three primary areas of the hypothalamus (Fig. 2). It is presently well established that the fibers emanating from large neurosecretory neurons within the (SO) and (PV) hypothalamic nuclei form the hypothalamo-neurohypophyseal tract — the functional link between the hypothalamus and the posterior lobe.

There is now evidence that at least some of the neurons within the two remaining areas, e.g., the preoptic (PO) — suprachiasmatic (SCH) area, and the arcuate — ventromedian (VMN) area, send their axons toward the ME external layer where they terminate in contact with the perivascular spaces of the capillaries that drain into the hypothalamo-hypophyseal portal system of veins Fuxe and Hökfelt (1967), Szentágothai Flerkó Mess and Halász (1968), Zambrano and DeRobertis (1968), Tejasen and Everett (1967) Mess (1969), Tima (1971), Motta Piva Tima Zanisi and Martini (1971). Although it is generally agreed that these two hypothalamic areas influence the activity of the adenohypophysis, the precise nature of this control remains uncertain.

IV Ultrastructure of Synthetically Inactive Neurons

The ultrastructural features of a synthetically inactive neuron are illustrated in Fig. 3. With the exception of the SO and PV nuclei, this type of neuron is the most prevalent within all hypothalamic nuclei studied to date with the electron microscope. As far as can be determined, the synthetically inactive neurons referred to in the present paper are ultrastructurally identical to the conventional neuron as described by Peters, Palay and Webster (1970). The typically large, eccentric, vesicular nucleus occupies most of the central area of cytoplasm. Prominent indentations or nuclear clefts are common. The single, prominent nucleolus usually lies free within the karyoplasm, although contacts between this organelle and the inner member of the nuclear envelope are frequently observed. The filamentous intranuclear rodlet is also a common finding among the synthetically inactive neurons of the hypothalamus Clattenburg, Singh and Montemurro (1972b).

The thin ring of cytoplasm surrounding the nucleus is typically pale due to the sparsity of cell organelles (Fig. 3). Only the occasional ribosome-studded cistern of rough endoplasmic reticulum (RER) can be identified. The Golgi apparatus is likewise not well developed. Both the saccular and vesicular components of this organelle appear empty; accumulations of electron-dense material within the Golgi were never observed. The other organelles such as mitochondria, lysosome-like bodies and neurotubules are sparsely represented and generally homogeneously distributed throughout the cytoplasm. The occasional electron-dense vesicles (DCV) observed within this type of neuron are small, with mean diameters not exceeding 110 nm. The DCV is clearly identifiable on the basis of its

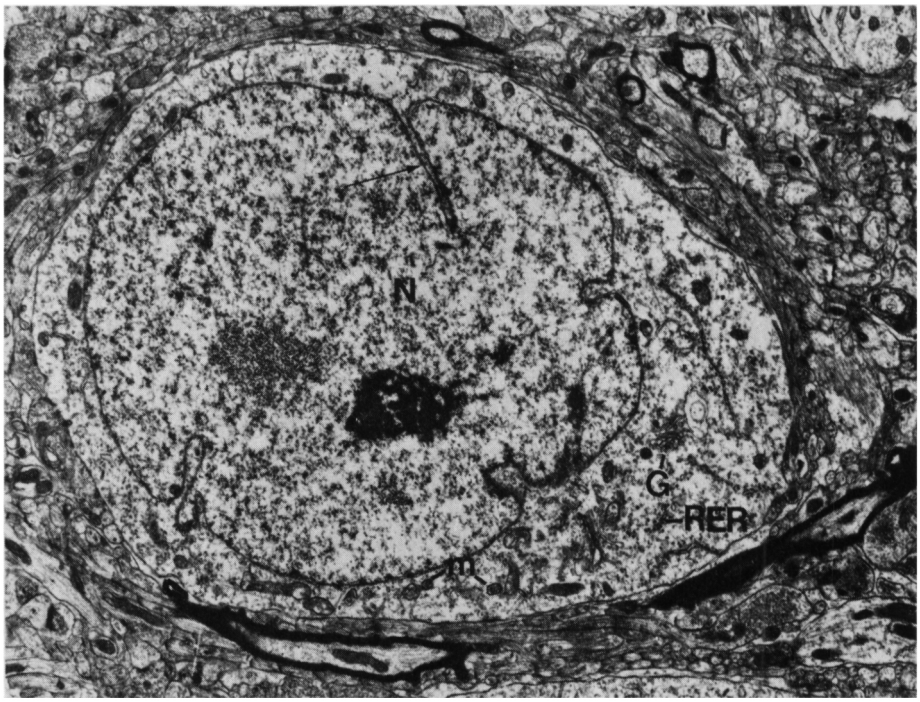

Figure 3. Electron micrograph of a synthetically inactive neuron. Most of the cell area is occupied by the large eccentric vesicular nucleus (N). Infoldings of the nuclear envelope (arrow) are common. The rough endoplasmic reticulum (RER) and Golgi apparatus (G) are not well developed. Other organelles, e.g., mitochondria (m) are sparsely represented. PO nucleus, control female rabbit. X 7,200

spherical shape and central core of electron-dense material that is characteristically separated from a limiting membraine by a clear area or 'halo'. Although the nature of these DCV is uncertain, it is generally agreed that they morphologically represent the biogenic amines Rinne (1966), Matsui (1967), Knowles (1967), Zambrano and DeRobertis (1968), Zambrano (1969).

V. Ultrastructural Features Suggesting Enhanced Neurosecretory Activity

Few EM studies on the mammalian hypothalamus have been purely descriptive in nature. Considerable effort has been made to correlate changes in ultrastructure with known functional or physiological states of the experimental animal. To date the ultrastructural changes observed among neurons of the various hypothalamic nuclei in response to specific stimuli have been consistent and, in all cases, directly associated with increases in the levels of synthetic activity. These findings, as they occurred among neurons associated with the hypothalamic control of both neurohypophyseal and adenohypophyseal function, may be summarized as follows:

1. Hypothalamo-Neurohypophyseal Axis, Zambrano and DeRobertis, (1966), (1967).

Increased:

(a) Neuronal, nuclear and nucleolar diameters.
(b) RER membrane and associated ribosomes.
(c) Amount of macromolecular material within the RER cisterns.
(d) Size of the Golgi apparatus.
(e) Number of mitochondria and lysosomes.

Decreased:

(a) Number of neurosecretory granules within the neuron soma.

2. Hypothalamo-Adenohypophyseal Axis, Clattenburg et al (1971) (1972a)

NB: Except for no significant increases in nucleolar diameters, increases were the same as those listed (a-e) above.

In addition — the presence of large (130-150 nm) DCV within the certain neuron soma following endocrine manipulation. Similar large DCV were never observed within the neuron somata of our control animals.

VI. Ultrastructure of Synthetically Active (Neurosecretory) Neurons

Many of the ultrastructural features observed within hypothalamic neurons, and which have been repeatedly interpreted as suggesting enhanced neurosecretory activity in response to specific stimuli, are evident in Fig. 4. These neurons typically lie in close spatial relationship with capillaries and are of large diameter due primarily to the elaboration of cell organelles, Sloper and Bateson (1965) Zambrano and DeRobertis (1966) (1967) Clattenburg et al (1971) (1972a).

In contrast to a synthetically inactive neuron, the nucleus of a neurosecretory cell is larger, more eccentric and shows an increase in the degree of nuclear membrane indentation. As observed in the smaller conventional neurons, contacts between the prominent nucleolus and the nuclear envelope are frequent. However, the filamentous intranuclear rodlet, a common finding among conventional neurons, was never seen in synthetically active neurons Clattenburg et al. (1972b). The elaborate infoldings of the nuclear envelope suggest an increase in the cytoplasmic-nuclear interface and have been interpreted as evidence of elevated activity among neurosecretory neurons Scharrer and Scharrer (1940), Zambrano and DeRobertis (1966) (1968), Clattenbrug (1972).

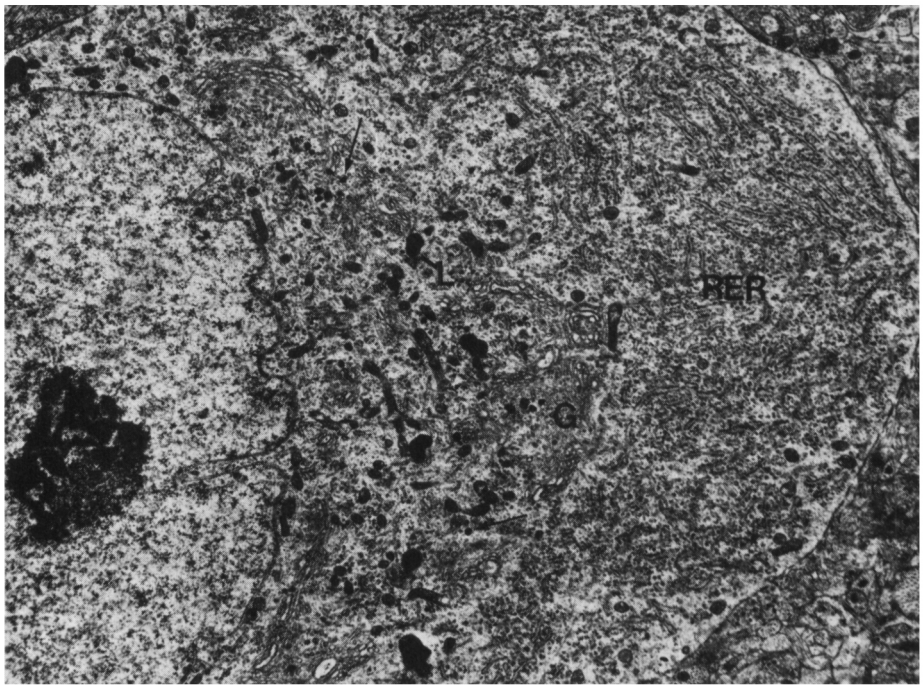

Figure 4. Electron micrograph showing the perinuclear zone of a large synthetically active neurosecretory neuron. Cell organelles normally associated with enhanced protein synthesis are well developed (compare with Fig. 3). Several large dense-core vesicles (arrows) are visible in the 'central' zone of cytoplasm. G Golgi apparatus; L lysosome; RER rough endoplasmic reticulum. PO nucleus, female rabbit — 10 hours post-coitus. X 6,300

Within the neurosecretory neuron, the elaborate development and distribution of certain cell organelles clearly provides a morphological basis for dividing the cytoplasm into "marginal" and "central" zones (Fig. 4). The "marginal" cytoplasm is largely occupied by RER and dense populations of intercisternal polysomes. The macromolecular content of the RER cisternae, which suggests

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

newly-synthesized protein, Zambrano (1966), Zambrano and DeRobertis (1966), can be seen to better advantage in Fig. 8. The "central" zone of cytoplasm clearly contains multiple well-developed Golgi profiles, numerous mitochondria, electron-dense lysosome-like bodies, a variety of "empty" vesicles and scattered neurotubules. In addition, vesicles with mean diameters exceeding 120 nm and containing a core of electron dense material, have been a consistent finding within the cytoplasm of all hypothalamic neurosecretory neurons.

VII. The Hypothalamo-Neurohypophyseal Axis

A) Neurons of the SO and PV nuclei

There is now unquestionable evidence to support the view that the hormones, oxytocin and vasopressin, are synthesized within the SO and PV nuclei of the mammalian brain. See Section I above.

a) Light microscopy. As seen with the light microscope, the SO and PV nuclei of the normal female rabbit (Figs. 5 and 6) are similar to those of the rat, Sokol and Valtin (1965), Zambrano and Mordoh (1966). Zambrano and DeRobertis (1966), Flament-Durand (1971) and to the dog, Sloper and Bateson (1965), Zambrano and DeRobertis (1967).

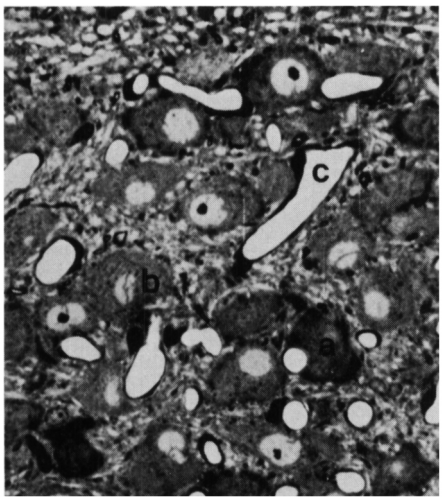

Figure 5. An area of the SO nucleus of a normal female rabbit. Variations in density reflect functional states among the neurons shown. a)—type 1 or 'resting' neuron, b)—type 2 or 'active' neuron. Note the close spatial relationship between neuron somata and capillaries (c). Thick Epon section, methylene blue-Azure II stain. X 1,000

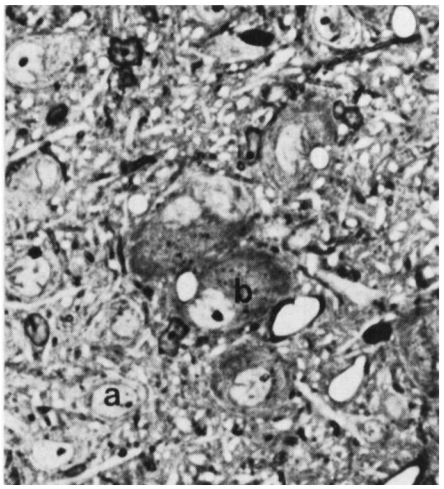

Figure 6. Light micrograph of the PV nucleus of a normal female rabbit. Two morphological types of neurons are shown: a)—small, conventional appearing neurons and, b)—larger, neurosecretory neurons. The latter are morphologically identical to the neurosecretory neurons of the SO nucleus (see Fig. 5). Thick Epon section, methylene blue-Azure II stain. X 1,200

Most neurons of the SO nucleus are noticeably active and located in close spatial relationship with one or more capillaries (Fig. 5). The variation in density among SO neurons has been interpreted by Zambrano and DeRobertis (1966) to reflect 'functional states' within neurosecretory neurons. According to these investigators, the darker neurons have been designated as type 1 or 'resting' neurosecretory cells; the lighter ones, type 2 or neurosecretory neurons in a more 'active' state of synthesis. The smaller, conventional type neuron is not a common finding within the hypothalamic SO nucleus.

In contrast, two distinct morphological types of neurons (Fig. 6) are clearly identifiable within the rabbit PV nucleus. The smaller neurons are identical to the conventional type of neuron seen in the PV nucleus of the rat, Flament-Durand (1971), Kalimo and Rinne (1972). The larger neurons are morphologically identical to the neurosecretory neurons of the SO nucleus. Furthermore, as among the SO neurons of the rabbit, rat and dog, differences in density among PV neurons have been previously observed and interpreted as changes in the functional states of these cells, Flament-Durand (1971).

b) *Electron microscopy.* Ultrastructural changes associated with neurosecretory rhythms within SO and PV neurons have been widely observed in a variety of mammalian species Sloper and Bateson (1965), Zambrano and Mordoh (1966), Zambrano and DeRobertis (1966) (1967), Zambrano (1968) Flament-Durand (1971) Kalimo and Rinne (1972). Under normal conditions, the type 1 and type 2 neurons (with intermediate forms), are also identifiable at ultrastructural levels within both the rat SO, Zambrano and DeRobertis (1966) and PV, Flament-Durand (1971), Kalimo (1971) nuclei. Following specific stimulation such as dehydration, Zambrano and DeRobertis (1966) and castration, Matsui and Engehardt (1960), there is general hypertrophy of these neurons, with a greater number of neurons showing morphological features of the type 2 or 'synthetically active' neurons.

To date, the ultrastructure of SO and PV neurons of the rabbit following various stimuli have not been reported. However, in the normal female rabbit the two types of neurons suggesting states of functional activity are clearly evident (Figs. 7, 8, and 9). A portion of the perinuclear zone of a type 1 (resting or storage) neuron is shown in Fig. 7. As far as can be determined, this neuron is ultrastructurally identical to the type 1 neuron observed within the SO nucleus of the rat, Sloper and Bateson (1965), Zambrano and DeRobertis (1966), and dog, Sloper and Bateson (1965). In general, the cell organelles normally associated with protein synthesis are not well developed within the type 1 neurons of both the SO and PV nuclei. Only the occasional cluster of flattened RER cisternae is identifiable; the Golgi apparatus is likewise not well developed. Mitochondria and lysosomes are sparsely represented.

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

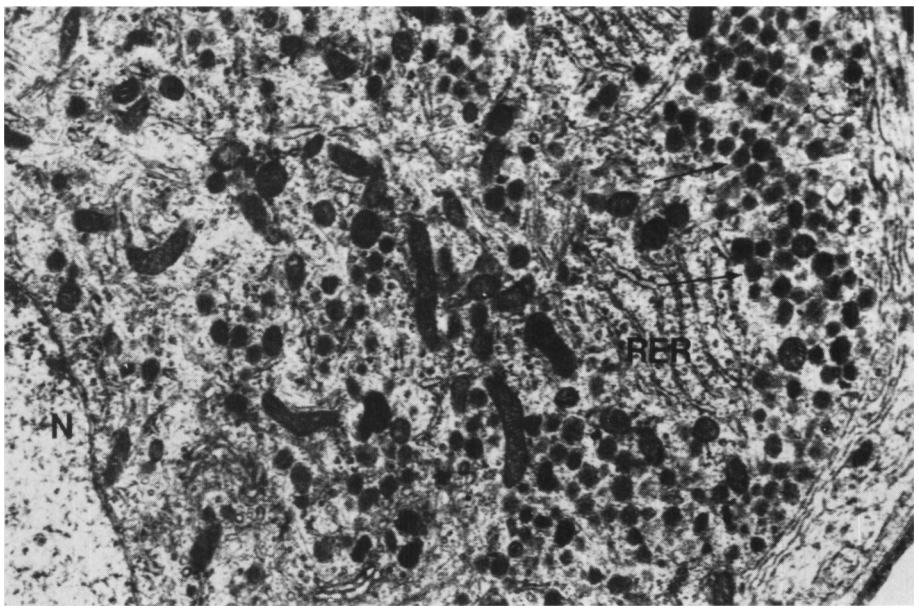

Figure 7. Electron micrograph showing an area of the perinuclear zone of a type 1 or 'resting' neuron. Note the accumulation of secretory granules (arrows) within the marginal cytoplasm. Cisternae of the rough endoplasmic reticulum (RER) are flattened and generally not well developed. SO nucleus, normal female rabbit. X 16,000

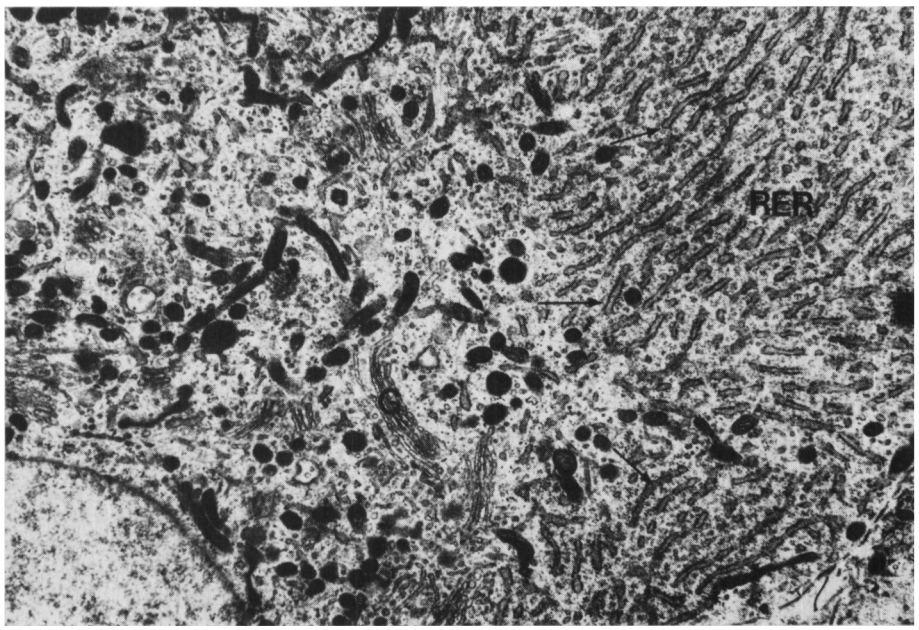

Figure 8. Part of the perinuclear area of a type 2 or synthetically 'active' neuron (compare with Fig. 7). The rough endoplasmic reticulum (RER) and Golgi apparatus (G) are well developed. RER saccules are dilated and clearly contain a granular macromolecular material (arrows). Note the sparsity of secretory granules within the marginal cytoplasm. SO nucleus, normal female rabbit. X 14,400

Within the SO and PV nuclei, a consistent feature of the type 1 neuron is the accumulation of numerous neurosecretory granules at the perephery of the neuron soma (Fig. 7). These granules clearly contain a core of electron lucent material that is not always clearly separated from a limiting membrane or 'halo'. Their mean diameters of 206 ±30 nm and 170 ±32 nm for the SO and PV neurons respectively compare favorably with those reported for the same nuclei of other mammalian

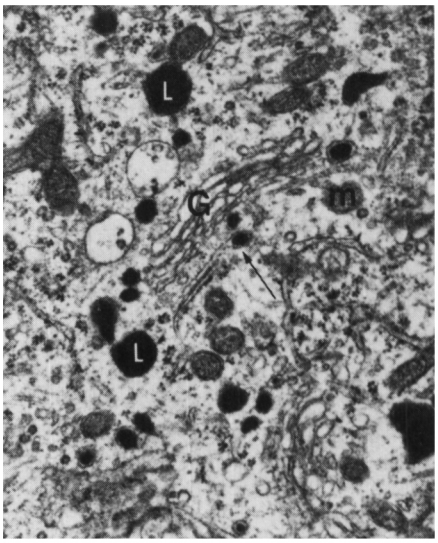

Figure 9. Electron micrograph of the Golgi apparatus (G) observed within a type 2 neuron. A secretory granule in the process of 'pinching-off' from a Golgi saccule, is clearly shown (arrow). L lysosome; m mitochondrion. PV nucleus, normal female rabbit. X 21,000

species. In contrast, the occasional DCV seen within the smaller conventional neurons of the rabbit PV nucleus, like those of the rat, Flament-Durand (1971) had diameters in the order of 90-100 nm.

The perinuclear zone of the neuron shown in Fig. 8 is representative of the type 2 or 'synthetically active' neurons of both the SO and PV nuclei of the rabbit and is ultrastructurally identical to the type 2 neurons seen within the SO and PV nuclei of other mammals, Sloper and Bateson (1965), Zambrano and DeRobertis (1966), Zambrano and Mordoh (1966), Flament-Durand, (1971), Morris (1971), Kalimo and Rinne (1972). In contrast with the type 1 neurons of these hypothalamic nuclei (Fig. 7), the cell organelles are now well developed and show a more discrete distribution within the neuron soma. The peripheral area of cytoplasm is largely occupied by the elaborate RER and numerous free polysomes; only the occasional elementary neurosecretory granule is seen (Fig. 8). The cisternae of the RER are characteristically dilated and clearly contain a fine granular or macromolecular material. Similar findings within the SO and PV type 2

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

neurons of other mammalian species have been previously reported under both normal and experimental conditions Sloper and Bateson (1965), Zambrano (1966), Zambrano and DeRobertis (1966), Kalimo and Rinne (1972). According to Zambrano and DeRobertis (1966) (1967), the macromolecular content of the RER cisternae of these cells represents newly-synthesized protein.

The 'central' zone of cytoplasm contains several well developed Golgi profiles, dense populations of mitochondria, numerous lysosome-like bodies and a variety of vesicular structures (Fig. 8). The role played by the Golgi apparatus in the 'packaging' of neurosecretory material into the neurosecretory granule is well documented Palay (1960), Scharrer and Brown, (1961), Dalton (1961), Jamieson and Palade (1966), Zambrano and DeRobertis (1966) Zambrano (1968), Beams and Kessel (1968), Holtzman and Dominitz (1968), Clattenburg (1972). The 'pinching-off' of a large neurosecretory granule from the Golgi apparatus of a large type 2 neuron of the normal rabbit PV nucleus is shown in Fig. 9.

The scope of the present paper does not permit a detailed discussion of the nature of the elementary neurosecretory granules synthesized within the somata of SO and PV nuclei. According to Kalimo and Rinne (1972) . . . "there is now substantial evidence to support the view that oxytocin and vasopressin are synthesized in separate neurons as well as in separate nuclei; oxytocin mainly in the PV nuclei and vasopressin in the SO nuclei, although there is overlapping between the nuclei." For more detailed discussion and references see Bissett et al. (1971), Dyball (1971), Kalimo (1971) and Norstrom (1972).

VIII. The Hypothalamo-Adenohypophyseal Axis

A) *Neurons of the PO and SCH Nuclei*

Since Green and Harris (1947) first suggested the presence of a 'neurohormonal link' between the hypothalamus and the anterior lobe of the mammalian pituitary gland, many investigators have attempted to localize the hypothalamic synthesis sites of these chemical mediators. As a result of these studies, two hypothalamic centres of RF synthesis have been frequently suggested: a) the anterior hypothalamus — in which lie the PO and SCH nuclei, and b) the medial basal hypothalamus (MBH) which, according to definition, includes the entire arcuate, and at least part of VMN nucleus Halász, Pupp, and Uhlarik (1962). Our studies to date on the rabbit brain have been directed toward providing discrete ultrastructural evidence for the presence of true neurosecretory neurons within these areas of the brain and, if present, do they in fact represent the hypothalamic synthesis sites of RF.?

a) *Light Microscopy*. The nature and distribution of neurons within the SCH nucleus of a non-mated, control female rabbit brain, as evident with the light microscope, are shown in Fig. 10. As far as can be determined, all the neurons are of the same basic type with no morphological evidence of neurosecretory activity. Similarly, synthetically active neurons were never observed within the PO nuclei

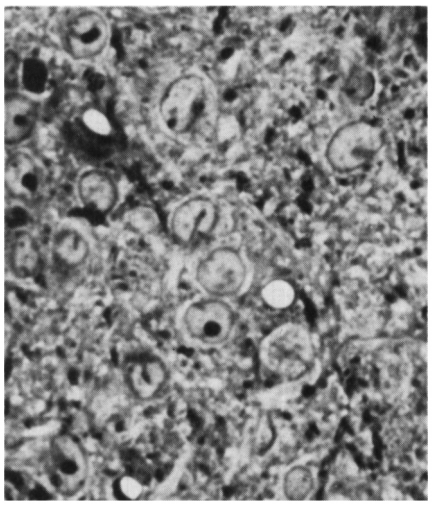

Figure 10. Light micrograph showing the nature and distribution of neurons within control SCH nuclei. All neurons are small with no morphological evidence of neurosecretory activity. Thick Epon section, methylene blue-Azure II stain. X 1,000

of non-mated, control female rabbits Clattenburg et al. (1971).

In contrast, evidence of enhanced neurosecretory activity (Fig. 11) was observed in approximately 19 and 30

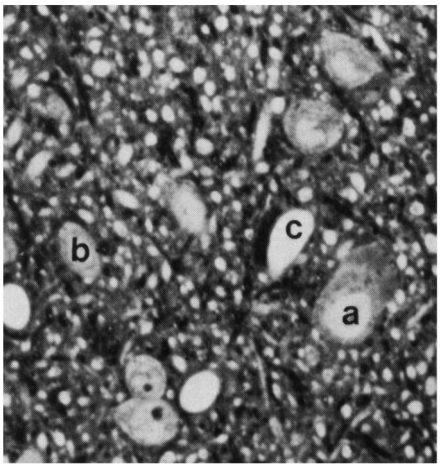

Figure 11. Light micrograph showing a large neurosecretory neuron (a). Compare with the smaller conventional neurons (b). Note the close spatial relationship between neuron (a) and the capillary (c). SCH nucleus, female rabbit, 8 weeks castration. Thick Epon section, methylene blue-Azure II stain. X 1,000

percent of the neurons located near capillaries of the PO and SCH respectively following coitus Clattenburg et al (1971), (1972a). As determined from thick epon sections stained with methylene blue—Azure II, Richardson, Jarett and Finke (1960), approximately 11 and 4 percent of the total neuron population within the SCH nucleus showed evidence of synthetic activity following castration and laparotomy respectively (Fig. 12). Similar data for the PO nu-

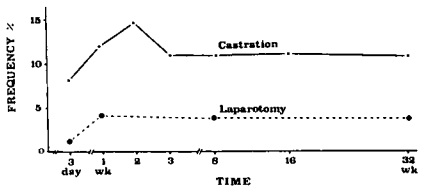

Figure 12. Frequency percent of synthetically active neurons observed within SCH nuclei of the female rabbit following castration and laparotomy.

cleus of the rabbit following castration and laparotomy are presently being compiled. Since all neurons within the PO and SCH nuclei of our

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

non-mated controls were of the same morphological type (Fig. 10), these findings clearly show that a number of PO and SCH neurons of the rabbit do undergo structural changes in response to endocrine manipulation. Such changes should also be clearly evident at ultrastructural levels.

b) *Electron Microscopy*. Ultrastructurally, all the neurons observed within the PO and SCH nuclei of the non-mated, control female rabbit, were morphologically identical to the synthetically inactive cell shown in Fig. 3. Discrete untrastructural changes suggesting enhanced synthetic activity within these neurons were never seen. The occasional DCV identified within the somata of the neurons were small, with mean diamters of 104 ±18 nm and 106 ±22 nm for the PO and SCH respectively, Clattenburg (1972). Unfortunately the present, and other similar ultrastructural data, do not establish the chemical nature of the small DCV. It is generally accepted, however, that the 100 nm DCV do contain the biogenic amines. Whether or not the DCV observed within the PO and SCH neurons of the female rabbit carry noradrenalin (NA) and/or 5-Hydroxytryptamine (5-HT) as previously reported for other mammalian species Pellegrino de Iraldi, Duggan, and DeRobertis, (1963), Fuxe (1965), Fuxe and Hökfelt (1968) (1970), Aghajanian, Bloom and Heard, (1969), cannot be concluded.

The absence of synthetic activity within the vast majority of PO and SCH neurons following mating, castration, and laparotomy, as evident with the light microscope, was readily confirmed with the EM (compare Figs. 3 and 13). In addition, a number of the neurons located near capillaries of these hypothalamic nuclei did clearly present ultrastructural features reflecting enhanced synthetic activity (Fig. 14). Since similar large, neurosecretory neurons were never seen within the PO and SCH nuclei of the control rabbits, their presence as a response to the above endocrine changes, is strongly suggested. Similar neurosecretory neurons were also observed within the PO nucleus of the rabbit following castration as well as within both the PO and SCH nuclei in response to coitus Clattenburg et al (1971) (1972a) and laparotomy (Clattenburg, unpublished data). In general these cells, like the neurosecretory neurons of the mammalian SO and PV nuclei, were characterized by their large size and development of

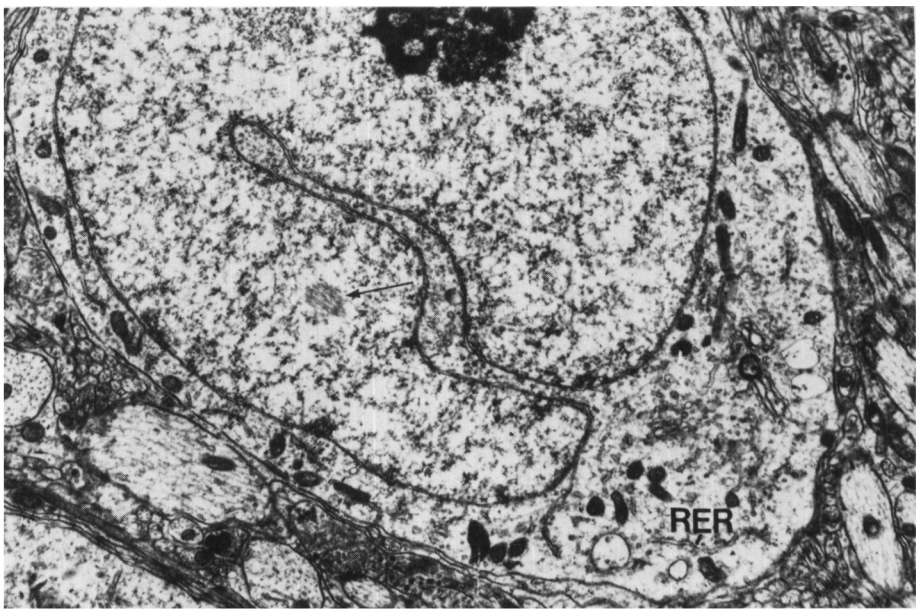

Figure 13. Electron micrograph showing ultrastructural features representative of all neurons observed within the PO and SCH nuclei of the non-mated, control female rabbit (compare with Fig. 3). The organelles normally associated with protein synthesis are not well developed. An intranuclear filamentous rodlet, sectioned transversely, is also shown (arrow). G Golgi apparatus; RER rough endoplasmic reticulum. X 6, 700

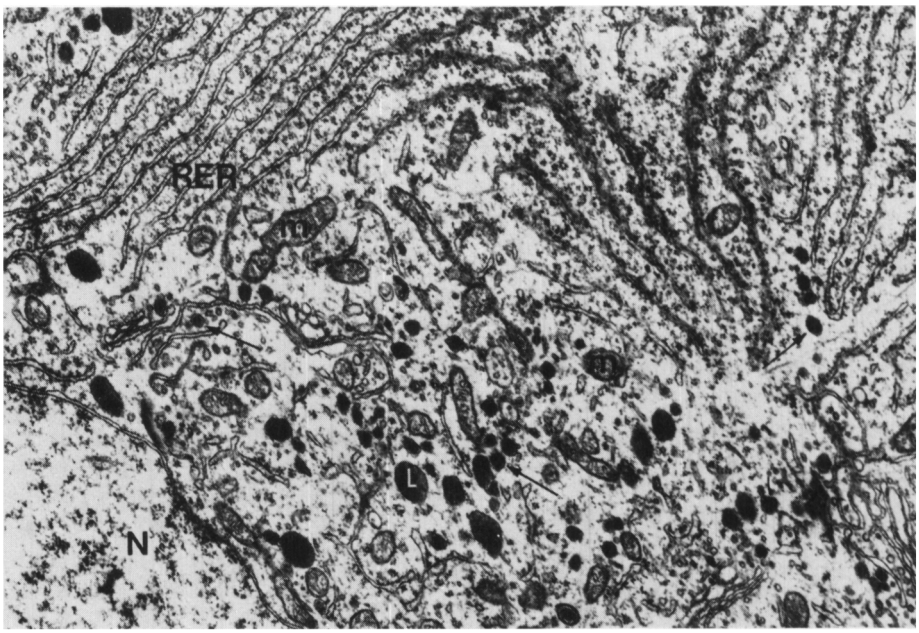

Figure 14. Portion of the perinuclear zone of a large synthetically active neuron observed within the SCH nucleus (compare with Fig. 13). Note the elaborate rough endoplasmic reticulum (RER) and dense populations of mitochondria (m) and lysosomes (L). The large dense-core vesicles shown (arrows) were never seen within neuron somata of non-mated female rabbit PO and SCH nuclei. SCH nucleus, 8 weeks castration. X 18,000

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

cell organelles commonly associated with enhanced protein synthesis (compare Figs. 13 and 14). A consistent finding within the somata of these synthetically active neurons was the presence of large DCV with diameters of 130 nm - 150 nm. It is significant that similar large DCV were never observed within the somata of inactive neurons of either the PO or SCH nuclei. The origin of the large (130 nm-150 nm) DCV from the Golgi apparatus was also a common finding Clattenburg et al (1971), (1972a).

Although the precise chemical nature of the 130 nm-150 nm DCV is uncertain, these findings do strongly support their synthesis in response to endocrine manipulation and are therefore associated with adenohypophyseal activity. Certainly, it is known that coitus, Hilliard, Hayward and Sawyer (1964) and castration, Goldman and Porter (1970), Gay, Niswender and Midgley, (1970), Dierschke, Bhattacharya, Atkinson and Knobil (1970) elevates the circulating levels of luteinizing hormone (LH). Assuming that the release of LH from the pituitary gland is mediated by the hypothalamic principal LH-RF, our findings to date within the PO and SCH nuclei of the female rabbit may well represent hypothalamic LH-RF synthesis in an attempt to replenish lost stores from the ME. Furthermore, it is also known that coitus, Desjardins, Kirton and Hafs (1967) and the surgical stress of laparotomy induces the release of ACTH from the anterior lobe. As shown in Fig. 12, approximately 4 percent of the neurons within the SCH nuclei of the female rabbit were synthetically active following laparotomy. Whether this represents the hypothalamic synthesis or adrenocorticotrophic-releasing factor (CRF) in the rabbit must be placed in the realm of speculation at this time.

B) *Neurons of the Arcuate and VMN Nuclei*

Since the early transplantation and deafferentation studies of Halász and co-workers Halász et al (1962), Halász and Pupp (1965) which demonstrated the presence of a 'hypophysiotrophic area' (HTA) within the mammalian hypothalamus, many studies have been directed toward determining its precise role in the mediation of adenohypophyseal activity. Although the boundaries of this functional area of the brain are not clearly defined, the HTA or MBH has been interpreted to include as its main central component, the arcuate and at least the ventral portion of the VMN nuclei Szentágothai, Flerkó, Mess and Halász (1972).

Of the hypothalamic nuclei presently implicated in the regulation of gonadotrophic activity, the arcuate has, without doubt, received the most attention. Presumably, many of these studies were stimulated by its close spatial relationship with the ME. Furthermore, it has been shown that many neurons of the arcuate nucleus send their axons toward the ME Spatz (1951), Szentágothai, Flerkó, Mess and Halász (1962), (1968) where they contact the perivascular spaces of the hypophyseal portal capillaries, Zambrano and DeRobertis (1968) Fuxe and Hökfelt (1967), (1968) Szentágothai et al (1968).

In recent years, the VMN has received considerable attention because of its possible role in the synthesis of growth hormone releasing factor (GRF). For further detail and references see the preceeding paper by Dr. L. Bernardis. Whether or not the VMN of the mammalian brain has an extensive fiber connection with the ME has, to my knowledge, never been clearly demonstrated.

a) *Light Microscopy*. The relationship of the arcuate and VMN nuclei with the ME of the rabbit brain is shown in Fig. 15. Two morphologically distinct types of neurons identified as 'pale' and 'dark' on the basis of certain structural features are evident within the rabbit arcuate nucleus (Fig. 16). However, as far as can be determined with the light microscope, all neurons of the rabbit VMN are morphologically similar to small, conventional neurons seen within the SCH nucleus and shown in Fig. 10.

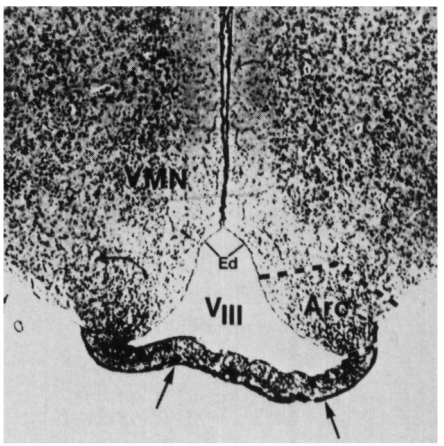

Figure 15. Coronal section of the female rabbit hypothalamus showing location of arcuate (Arc) and ventromedial (VMN) nuclei and their relationship with the median eminence (arrows). Ed ependyma; VIII third ventricle. X 100

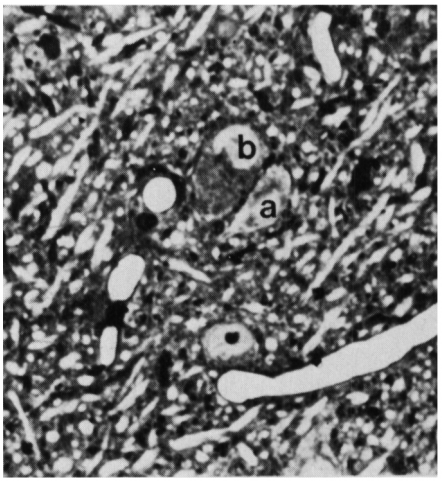

Figure 16. Light micrograph of 'pale' (a) and 'dark' (b) neurons of the rabbit arcuate nucleus. Thick Epon section, methylene blue-Azure II stain. X 650

b) *Electron Microscopy*. The ultrastructural features that serve to identify the perinuclear zones of both 'pale' and 'dark' neurons of the rabbit arcuate nucleus, are shown in Fig. 17. The 'pale' arcuate neurons are not unlike the synthetically inactive neurons described in section IV of the present paper. In general, the RER and Golgi apparatus are not well developed. Other organelles, in particular, the mitochondria, lysosomes and vesicles are also sparsely represented. In contrast, the 'dark' arcuate neurons clearly show relatively well developed RER and Golgi

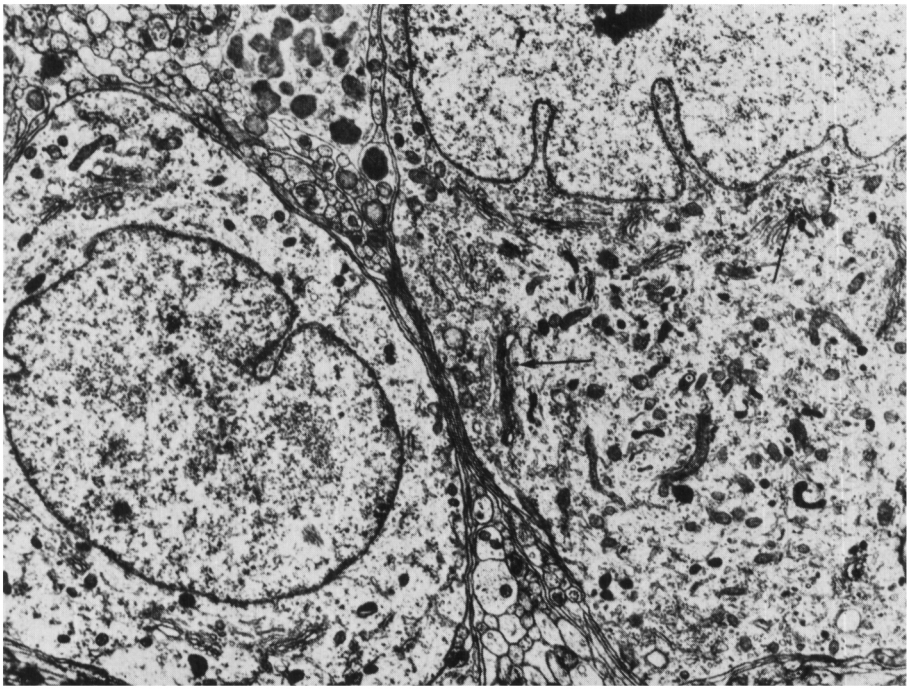

Figure 17. Electron micrograph illustrating the ultrastructural features that serve to identify 'pale' and 'dark' arcuate neurons. A 'dark' neuron occupies the the right half of the figure. Note that its overall darker appearance is due primarily to the development and/or increase in numbers of individual organelles. The occasional dense-core vesicle (arrows) observed within these neuron types were small, with the vast majority falling into the 90 - 110 nm range. Female rabbit, 34 weeks castration. X 7,500

profiles, dense populations of mitochondria and lysosomes and an increase in the number of DCV. Dense accumulations of macromolecular material, identical to those observed within the RER cisternae of neurosecretory neurons described elsewhere in the present paper, were also a common finding within the RER cisterns of the 'dark' arcuate neurons. The mean diameters of the DCV observed within the somata of the 'dark' neurons, as well as the occasional DCV seen with those of the 'pale' neuron type, did not exceed 110 nm. Evidence for their origin from the Golgi apparatus has also been shown, Clattenburg (1972). Whorled bodies similar to those observed within arcuate neurons of the rat following castration and associated with synthetic activity, Brawer (1971) were not seen within arcuate neurons of the rabbit.

The 'pale' and 'dark' neurons of the rabbit arcuate nucleus Clattenburg (1972) are ultrastructurally identical to those described for the arcuate nucleus of the rat, Jaim Etcheverry and Pellegrino DeIraldi (1968), Zambrano and DeRobertis (1968), Zambrano (1969) Ratner and Adamo (1971). According to Jaim Etcheverry and Pellegrino DeIraldi (1968), their functional significance is obscure. On the other hand, Zambrano and DeRobertis (1968), have provided rather convincing evidence that the morphological differences observed between these neurons do not represent two distinct types; rather, they represent functional states of the same kind of cell. This concept was founded on the fact that following castration in the rat, the perikarya of nearly all arcuate neurons showed a marked hypertrophy with the discrete ultrastructural changes usually associated with elevated levels of synthetic activity. This finding suggests that in response to the endocrine changes associated with castration, many of the 'pale' neurons became synthetically active and ultrastructurally appeared as the 'dark' neuron type. Similar findings within the arcuate nucleus of the rat following castration have also been reported by Brawer (1971). Our findings to date within the arcuate nuclei of the female rabbit have clearly shown that the two neuron types are common under both normal and experimental conditions. However, although the two neuron types were clearly evident in all animals studied, changes in the ratio of 'pale' to 'dark' neurons suggesting an overall increase of arcuate nucleus activity following coitus and castration, were not seen.

In recent years, the nature of the DCV observed within the neuron soma of the mammalian arcuate nucleus has received considerable attention. Do these vesicles contain the biogenic amines, in particular DA, or do they contain the RF responsible for maintaining tonic levels of the anterior lobe hormones?

To date, EM studies of the normal and experimental rat arcuate nucleus have been most consistent in demonstrating only one population of DCV, Zambrano and DeRobertis (1968), Jaim Etcheverry and Pellegrino DeIraldi, (1968) Zambrano (1969), Brawer (1971), Ratner and Adamo (1971). Although the diameters sited for these vesicles range from 70 nm to 150 nm, the vast majority show a 90 nm to 110 nm diameter range. Our finding of DCV with mean diameters of 109 ±13 nm and 104 ±14 nm within the somata of arcuate neurons of non-mated, control and mated experimental rabbits respectively, Clattenburg (1972) therefore support these EM studies on the rat. Furthermore, although it is widely accepted that the DCV of the mammalian arcuate nucleus do contain DA, Fuxe and Hökfelt (1967), Aghajanian and Bloom, (1966), Rinne (1966), Duffy and Menefee (1965) Zambrano and DeRobertis (1968), Zambrano (1969) their possible RF content cannot be disregarded, Zambrano and DeRobertis (1968) Brawer (1971). Recently, however, the density gradient centrifugation studies of Ishii and co-workers Ishii et al (1969), Ishii (1970a,b) (1972), Kobayashi and Ishii (1968) Clementi, Ceccarelli, Cerati, Demonte, Felici, Motta, Pecile (1970) have provided rather convincing evidence that the RF are

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

contained within the larger (120 nm-140 nm) DCV. Whether or not the occasional larger DCV seen within the somata of arcuate neurons do, in fact, contain the RF has not been clearly demonstrated. If so, such findings would support the view that the synthesis of both biogenic amines and the RF occurs within the same type of neuron. To my knowledge, EM findings within the mammalian arcuate nucleus, have not demonstrated the presence of at least two functionally different neurons based on differences and homogeneity of the DCV populations observed. Certainly our findings within the arcuate nucleus of the female rabbit following coitus, Clattenburg (1972), and castration do not support this view. Unlike our findings within neurons of the PO and SCH nuclei, Clattenburg et al (1971) (1972a), neurons with homogeneous populations of only the larger (120 nm to 150 nm DCV) were never seen within the arcuate nucleus of this experimental animal.

Ultrastructural changes following specific endocrine manipulation have never been observed within the mammalian VMN nucleus. As far as could be determined, all neurons observed within the VMN nucleus of the nonmated and mated female rabbits, were ultrastructurally identical to the synthetically inactive neuron shown in Fig. 3. Certainly, VMN neurons with ultrastructural features similar to those of the large synthetically active neurons reported within the PO and SCH nuclei following coitus Clattenburg et al (1971) (1972a), were never seen. Whether or not this hypothalamic nucleus is associated with the synthesis of GH-RF, Bernardis and Frohman (1970) (1971), Frohman and Bernardis (1968) has, likewise, never been supported by ultrastructural evidence. On the other hand, preliminary findings from an ultrastructural study of the rat VMN following insulin hypoglycaemia(Montemurro-unpublished data) may represent the first ultrastructural evidence to support this view.

IX. The Median Eminence

Undoubtedly, the key to a clear understanding of the functional relationships between the mammalian hypothalamus and the pituitary gland lies within our knowledge of the ME and its subcellular organization.

a) *Light Microscopy*. The coronal section of the female rabbit ME shown in Fig. 18 is representative of

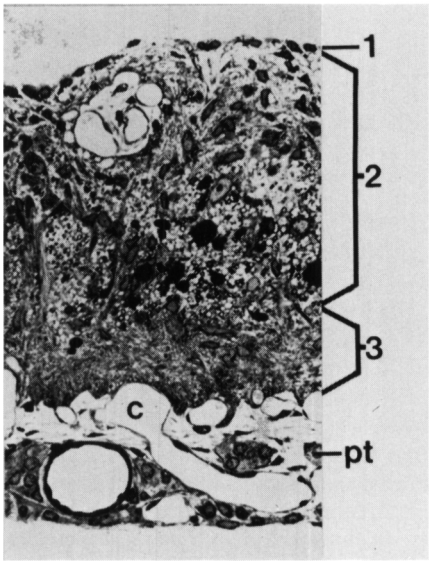

Figure 18. Coronal section of the female rabbit ME showing its basic zonation: 1)—the monolayered ependyma, 2)—the internal layer and, 3)—the external layer. Large capillaries (c) of the hypothalamo-hypophyseal portal system of veins separate deeply-staining cells of the pars tuberalis (pt) from ventral limits of the ME. X 750

all mammalian species studied to date. Three distinct zones or layers can be identified: 1) the monolayered ependyma, 2) an internal layer, and 3) the external layer, Rinne (1960).

The relatively thick ME internal layer is composed primarily of compactly arranged axon profiles of the neurohypophyseal tract, glial elements, deeply staining Herring bodies and tangentially orientated processes identified as those of glial and/or ependymal tanycytes. The large profiles of the neurohypophyseal tract emanate from the somata of SO and PV neurons, Scott and Knigge (1970), course through the ME internal layer in a rostro-caudal direction parallel to the floor of the third ventricle, and terminate upon the vasculature of the posterior lobe Monroe (1967), Knigge and Scott (1970). The vascular elements subjacent to the ependyma (Fig. 18) represent long capillary loops from the primary capillaries of the hypothalamohypophyseal system of portal veins.

The ME external layer consists of two rather distinct regions: 1) the palisade layer and 2) a contact or vascular zone. As seen with the light microscope, the palisade layer is composed chiefly of haphazardly orientated axon profiles and only the occasional glial cell. Presumably, these axon profiles emanate in part from the glial cells of the internal layer, the somata of the ependymal tanycytes and from hypothalamic neurosecretory neurons associated with the control of adenohypophyseal activity. The contact zone of the external layer is the area of the ME in which the processes of the palisade zone terminated upon the capillaries of the hypophyseal portal plexus (Fig. 18). In addition, this system of veins clearly separates the ventral limits of the ME from the thin layer of deeply staining cells of the pars intermedia.

b) *Electron Microscopy*. Many investigators have contributed to our knowledge of the subcellular organization of the normal mammalian ME Oota (1963), Seitz (1965), Duffy and Menefee (1965), Kobayashi, Oota, Vemura and Hirano (1966), Matsui (1966), Rinne and Arstila (1966), Rinne (1966), Monroe, (1967), Zambrano (1968), Scott and Knigge (1970), Voitkevich and Dedov (1972). To date, however, few EM studies have dealt with the ultrastructural changes that occur within this area of the brain in response to specific endocrine manipulation. The following brief account of ME ultrastructure will therefore deal primarily with our findings within this region of the normal female rabbit, together with some of the subcellular changes observed following coitus.

With the electron microscope, the internal layer of the rabbit ME, like that of the rat, Matsui (1966), can be further subdivided into a thin dorsal subependymal zone and a much thicker ventral fibrous zone. A detailed account of the ME subepen-

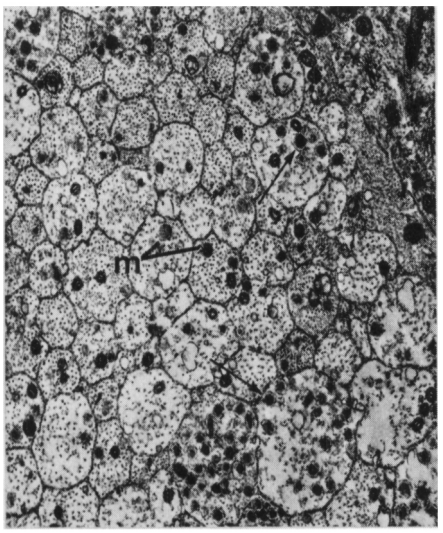

Figure 19. Electron micrograph showing the nature of large axon profiles of the hypothalamo-neurohypophyseal tract. The neurosecretory granules (arrows) have a mean diameter of 206 + 34 nm and are ultrastructurally identical to those observed within neurons of the SO-PV area. m mitochondria. ME internal layer (fiber zone), non-mated female rabbit.
X 11,700

dymal zone has already been reported Matsui (1966), Rinne (1966), Monroe (1967). A portion of the ventral fibrous zone is shown in Fig. 19. The large (1-2μ) axon profiles of the neurohypophyseal tract clearly contain a number of electron dense granules, numerous neurotubules and the occasional mitochondrion. The electron dense granules have a diameter range of 120 nm to 300 nm with a mean of 206 ±34 nm and are ultrastructurally identical to the large granules observed within the somata of both SO and PV neurons of the rabbit (see section VIIb) and the rat Zambrano and DeRobertis (1966) Flament-Durand (1971). Similar granules also characterized the large Herring bodies within the internal layer of the rabbit, Duffy and Menefee (1965), Clattenburg, Montemurro, Bruni and Singh (1973), and the rat, Rinne (1966), Scott and Knigge (1970), and the axon terminals which abut the vasculature of the rat neurohypophysis, Monroe and Scott (1966), Monroe (1967). That these granules contain the neurohypophyseal hormones oxytocin and vasopressin and their carrier neurophysins is presently well established.

In addition to the typically large axons of the neurohypophyseal tract, the occasional axon profile characterized by the presence of smaller (70 nm to 140 nm) DCV was also observed. The nature and origin of these vesicles is uncertain. Whether they contain the biogenic amine DA and originate within the somata of arcuate neurons cannot be stated. Other axon profiles orientated tangentially to large axons of the neurohypophyseal tract and characterized by their size and dense fiber content (Fig. 19) are those of glial or ependymal tanycytes.

According to the distribution and clumping of chromatin, the cell bodies observed within the ME internal layer were identified as those of astrocytes and/or oligodendrocytes.

The organization of the palisade layer is shown in Fig. 20. Clearly, the axon profiles here are smaller (0.3μ-1.0μ) than those of the adjacent fibrous zone and are characterized by their content of numerous small DCV, typical synaptic-type vesicles, neurotubules and the occasional mitochondrion. Other profiles, identified as those of glial or ependymal tanycyte processes, course through the palisade layer to terminate in contact with the vasculature of the external layer.

The ultrastructure of the contact zone is shown to better advantage in Fig. 21. The large capillaries of this zone are typically fenestrated and surrounded by a wide, irregular perivascular space (PVS). Thin (30 nm - 50 nm) basal laminae surround the elements of the capillary wall, as well as separate the PVS proper from the surrounding brain parenchyma. Fig. 21 also illustrates the intimate spatial relationship between axon terminals and ependymal tanycyte profiles with the PVS of the contact zone. Several types of axon terminals can be identified on the basis of their vesicle content: 1) those containing homogenous populations of the small (35 nm-65 nm) synaptic-type vesicles and 2) those containing both synaptic-type vesicles and larger DCV. Axon profiles or terminals containing large electron-dense granules identical to those of the fibrous zone, were not seen within the external layer of

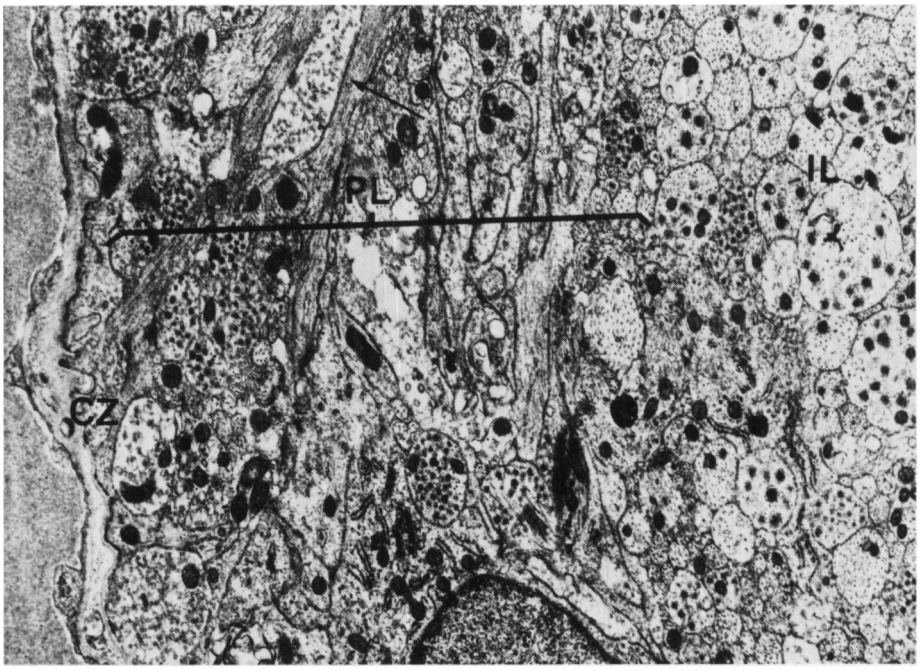

Figure 20. Portion of the palisade layer (PL) showing the nature and haphazard arrangement of cell processes. Compare with those within the small area of the internal layer (IL) included. Several processes identified as those of ependymal tanycytes of glial cells course through the PL and terminate in the contact zone (CZ). ME, female rabbit.
X 10,000

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

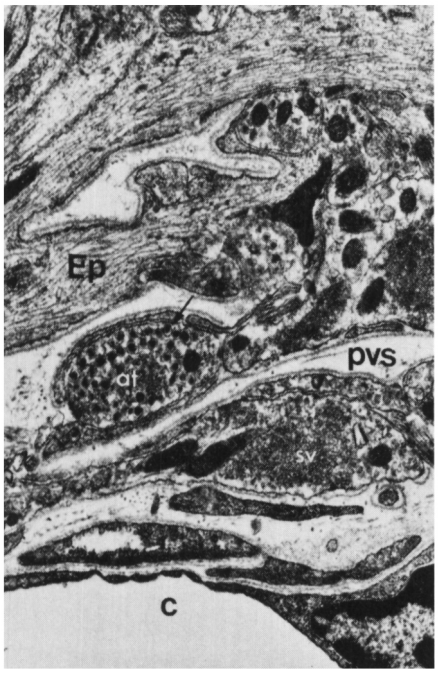

Figure 21. Electron micrograph showing a small area of the ME contact zone. Portion of a large fenestrated capillary (c), the nature of the perivascular space (pvs) and the relationship of axon terminals (at) and ependymal tanycyte processes (Ep) with the pvs are illustrated. Synaptic vesicles (sv) and larger dense-core vesicles (arrows) occurred within the same or separate axon terminals. Female rabbit, 10 minutes post-coitus. X 13,800

the rabbit ME, Duffy and Menefee (1965), Clattenburg et al (1973). Similar findings have also been reported for the rat, Monroe (1967) Scott and Knigge (1970), Rethelyi and Halász (1970).

The content and possible functional significance of the small synaptic-type vesicles within the mammalian ME has been discussed in a recent study by Scott and Knigge (1970). Whether or not they are cholinergic in nature, or are directly associated with the events surrounding the depletion of the larger DCV from the axon terminals of the ME external layer has never been clearly demonstrated at EM levels.

The nature of the DCV observed within the external layer of the mammalian ME, is not well established. Most ultrastructural studies of this ME region of both normal and experimental animals have been consistent in reporting only one population of DCV. The diameter ranges of 70 nm to 150 nm and means in the order of 100 nm, compare favourably with those sited (see section VIIb) for the DCV observed within the somata of arcuate neurons. On the basis of these data, it would seem therefore, that the RF and brain amines are contained within DCV which clearly cannot be ultrastructurally distinguished one from the other. Our recent findings within the ME external layer of the rabbit, Clattenburg et al (1973), as well as the ultrastructural findings within the ME external layer of the rat Rinne (1966), strongly oppose this view. According to Rinne (1966), the external layer of the rat ME . . . "contained such an amount of nerve fibers with only smaller (mean 1,000 Å) or only the larger (mean 1,330 Å) granules that one would be prepared to regard them as different neurosecretory products until further experiments provide evidence to the contrary". Similarly, two distinct populations of DCV were observed within the external layer of the rabbit ME, Clattenburg et al (1973), a finding which is not in keeping with the earlier studies of the rabbit ME by Duffy and Menefee (1965). Furthermore, since the two populations of DCV occurred within separate axons and axon terminals (Fig. 22), differences in their chemical nature and origin are suggested. A plot of the diameters of these vesicles showing two peaks, one at 90 nm-100 nm and the other at 120 nm-130 nm is shown in Fig. 23. These same two populations of DCV were clearly evident within separate axons and axon terminals of both non-mated control, and mated experimental rabbits Clattenburg et al (1973). Only the occasional membrane profile or 'vesicle ghost' was seen within the axon terminals abutting the PVS of the controls. However, at 10 minutes after mating, there was an obvious decrease in the number of the larger (120 nm-130 nm) DCV, together with a simultaneous marked increase in the number of 'vesicle ghosts' (Fig. 23), particularly within axon terminals and profiles lying in close spatial relationship with the PVS (Fig. 24). On the basis of their size (a mean diameter of 137 ± 14 nm) it is suggested that they represent the larger DCV of the ME that have been depleted of their content following coitus. Similar ultrastructural changes involving the smaller (90 nm-100 nm) DCV of the rabbit ME external layer, were never observed Clattenberg et al (1973).

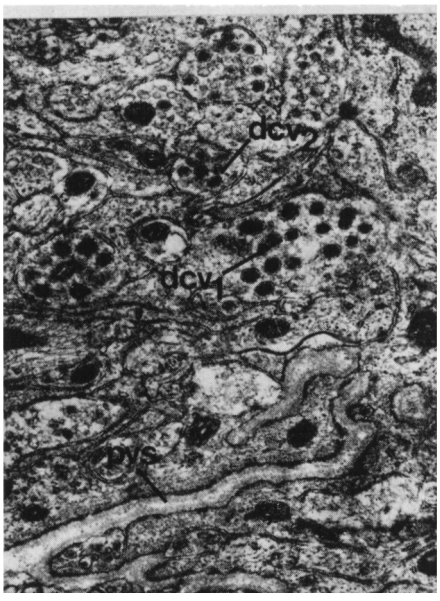

Figure 22. Portion of the female rabbit ME external layer. Several axon profiles clearly contain a number of larger dense-core vesicles (dcv1), while others contain a homogeneous population of similar vesicles (dcv2), but of smaller diameter. pvs perivascular space. X 14,700

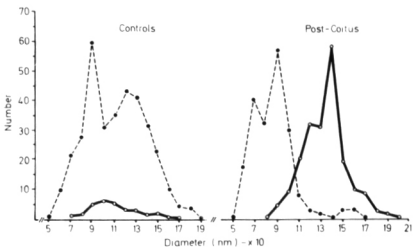

Figure 23. Distribution curves for the diameters of dense-core vesicles (DCV) and vesicle 'ghosts' within axons and terminals of control and experimental (post-coitus) animals. Controls—DCV (–•– – –•–), vesicle 'ghosts' (–o———o–); Post-coitus—DCV (–•– – –•–), vesicle 'ghosts' (–o———o–). Note the decrease in number of DCV, with peak distribution of 120 - 130 nm and the increase in number of vesicle 'ghosts' after mating.

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

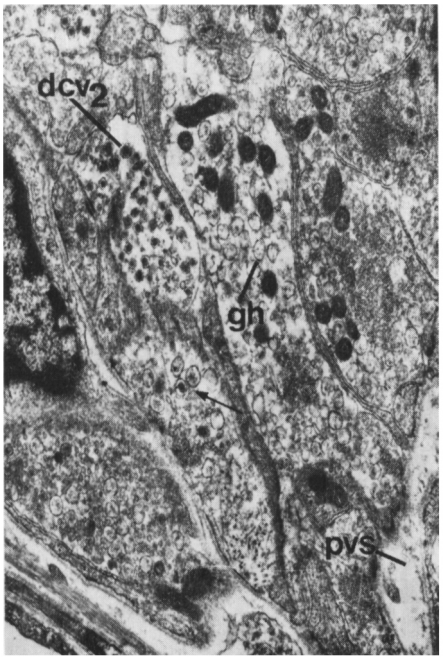

Figure 24. Electron micrograph showing several large axon terminals abutting the perivascular space (pvs) of the ME contact zone. Empty vesicles or vesicle 'ghosts' are common; several are in the process of depletion (arrows). Other terminals clearly contain a number of smaller dense-core vesicles (dcv_2).

X 18,000

The small DCV seen within the external layer of the rabbit ME, like those observed within the ME of other mammalian species, are probably DA in nature and originate from the somata of arcuate neurons Rinne (1966), Zambrano and DeRobertis (1968), Zambrano (1969), Scott and Knigge (1970). That DA does function at ME levels to control adenohypophyseal activity has already been suggested Kordon and Glowinski (1972).

The large (120 nm-130 nm) DCV of the ME external layer may contain a neurosecretion quite different from that of the smaller amine containing DCV. Certainly, it is known that coitus in the rabbit causes a rapid release of LH from the anterior lobe Hilliard et al (1964). Assuming that this release of LH is mediated by hypothalamic factors from the ME, such factors must also be released rapidly from their ME storage sites. Unquestionably, our finding of a tremendous increase in the number of 'vesicle ghosts' within axon terminals abutting the PVS of rabbits sacrificed at 10 minutes post-coitus, strongly supports this endocrinological phenomenon. Although the events surrounding this release of stored material are not known, our observations do not support release mechanisms similar to those reported recently by Stoekart, Jansen and Kreike (1972) for the rat.

We have suggested that the large (120 nm - 130 nm) DCV observed within axon terminals of the rabbit ME contain the RF, Clattenburg et al (1973). Although the specific RF content cannot be determined by the present experiments, their intimate association with the activities of the anterior pituitary gland is strongly implicated. Furthermore, studies combining ultracentrifugation and bioassay techniques have shown that the large (120 nm-140 nm) DCV within the horse ME do contain high RF activity Ishii et al (1969), Ishii (1970a), (1970b), (1972).

At the present time, the precise site(s) of synthesis of the 120 nm-140 nm DCV is not known. It is felt however, that the ultrastructural findings presented in this paper do provide further morphological evidence for the tenets that 1) the RF associated with gonadotrophic activity are synthesized, at least in part, within the somata of anterior hypothalamic neurons, and 2) that they are transported via the axons of these neurosecretory neurons directly to their storage sites within the external layer of the ME, Tejasen and Everett (1967), Mess and Martini (1968), Mess (1969), Schneider, Crighton and McCann (1969), Crighton, Schneider and McCann (1970), Gorski (1970), Tima (1971).

SUMMARY

(Continued from page 40)

the conventional neuron as described by Peters, Palay and Webster (1970). However, following coitus, castration and laparotomy, many neurons of these nuclei showed subcellular changes that have been repeatedly associated with enhanced protein synthesis. These large 'neurosecretory' neurons were usually located near capillaries and characterized by their well developed Rough endoplasmic reticulum (RER) and Golgi profiles, dense populations of mitochondria and lysosomes and by the presence of a homogeneous population of dense-core vesicles (DCV) showing a peak distribution of 120-140 nm. Since similar neurons were not observed within the PO and SCH of the normal control rabbit it is suggested that we were observing functional states of the same type of neuron and that these ultrastructural changes occur in response to endocrine manipulation.

Two types of neurons described as 'pale' and 'dark' were observed within the arcuate nucleus of both the control and experimental female rabbit. Ultrastructurally, these neuron types were identical to those described by other investigators for the rat. It has been suggested that the 'pale' and 'dark' neurons of this hypothalamic nucleus represent functional states of the same type of cell. However, increases in the ratio of 'dark' to 'pale' neurons as observed within the arcuate nucleus of the rat following castration, were not seen in the rabbit. Similar findings were also not evident within the arcuate nucleus of the female rabbit following coitus.

As far as could be determined, all neurons of the ventromedial (VMN) nuclei of both the control and experimental rabbit were morphologically identical to the smaller, conventional type neuron. Certainly, ultrastructural changes similar to those observed within the PO and SCH nuclei of the female rabbit following coitus, castration or laparotomy, were never observed.

The basic zonation and subcellular organization of the female rabbit Median Eminence (ME) is similar to that described for other mammalian species. Our EM findings within the external layer of the rabbit ME, however, are not entirely in agreement with the earlier study of Duffy and Menefee (1965). These investigators reported only one population of DCV within the axon terminals of the rabbit ME external layer. We feel that we have ultrastructural evidence for the presence of at least two distinct populations of DCV within this layer of the rabbit ME. Furthermore, since these vesicle populations occurred within separate axon profiles and terminals, differences in their content and origin are suggested.

Certainly, the relationship between releasing factors (RF) and the various populations of DCV observed within the external layer of the mammalian ME is not well established. The smaller (90 nm - 100 nm) DCV we have observed prob-

https://doi.org/10.1017/S0317167100019569 Published online by Cambridge University Press

ably contain the catecholamines, while those of larger (120 nm - 140 nm) diameters may well represent the carriers of the RF associated with gonadotropic activity. The latter view is based primarily on our finding or numerous 'vesicle ghosts' within the axon terminals abutting the perivascular space (PVS) of portal capillaries of rabbits sacrificed at 10 minutes post-coitus. The mean diameters of 137±14 nm obtained for these ghosts strongly supports the suggested depletion of only the larger of the two DCV populations. Similar changes were not apparent within the axon terminals containing homogenous populations of only the smaller DCV.

Unquestionably, the precise hypothalamic synthesis sites for the RF associated with control of adenohypophyseal function, continues to provoke comment. From the results obtained from countless studies that have employed a variety of neuroendocrinilogical techniques, two main hypothalamic centers of RF synthesis have been suggested: a) the medial basal hypothalamus (MBH) or hypophysiotropic area (HTA) and b) the anterior hypothalamus. The ultrastructural studies carried out to date within this laboratory are in favour of the latter for the following reasons:

1) — the presence of large DCV and 'vesicle ghosts' within the external layer of the rabbit ME with diameters similar to those of the large (120-150 nm) DCV synthesized within the PO and SCH nuclei of the same animal in response to coitus, castration and laparotomy.

2) — the absence of evidence for the storage of these large DCV within the somata of PO and SCH nuclei, suggesting their immediate transport toward the ME.

3) — the absence of any ultrastructural changes within neuron somata of the rabbit arcuate nuclei which might reflect enhanced neurosecretory activity in response to coitus and/or castration.

These ultrastructural findings within the rabbit hypothalamus may, therefore, provide the first evidence of a morphological nature for the actual release of RF from their ME storage sites, as well as their synthesis within certain neurons of the anterior hypothalamus.

RÉSUMÉ

(Suite de la page 40)

morphologie au neurone conventionnel, tel que décrit par Peters, Palay et Webster (1970). Cependant, à la suite de coitus, castration et laparotomie, plusieurs neurones de ces noyaux démontrèrent nettement des changements subcellulaires, tels que reliés à de multiples occasions antécédentes, à une augmentation de synthèse de protéine. Ces derniers neurones volumineux, "neurosecretoires", furent situés le plus fréquemment auprès des capillaires, étant caractérisés surtout de leur profils RER et Golgi accentués, de denses populations de mitochondres et lysosomes, ainsi que d'une population homogène de DCV, avec distribution de "peak" de 120 à 140 nm. Puisque des neurones semblables ne furent pas observés aux noyaux PO et SCH chez le lapin normal de contrôle, on suggère qu'il s'agit en effet d'observations d'états fonctionnels différents du même type de neurone, et que ces changements ultra-structurels surviennent en réponse à une manipulation endocrinienne.

Deux types de neurone, décrits "pâle" et Foncé", furent observés au noyau arcué chez le lapin de contrôle, ainsi que chez l'animal femelle expérimentaire. Quand à leur aspect ultra-structurel, ces types de neurones sont identiques à ceux décrits par d'autres chercheurs chez le rat. Que les neurones "pâles" et "foncés" de ce noyau hypothalamique représentent de différents états fonctionnels du même seul type de cellule, à déjà été suggéré. Cependant, une augmentation du ratio de neurones "foncés", par rapport à "pâles," telle que rapportée au noyau arcué chez le rat à la suite de castration, ne fut pas observée chez le lapin. Non fut tel changement évident au noyau arcué du lapin femelle à la suite de coitus.

En autant que se puisse être évalué, tous neurones du noyau VMN du lapin contrôle, ainsi que de celui expérimentaire, furent identiques quant à la morphologie à celui conventionnel, petit. Certainement, aucun changement ultra-structurel, tel que ceux aux noyaux PO et SCH, de la lapine, suivant coitus, castration et laparotomie, ne furent observés.

Organisation essentielle zonaire et sous-cellulaire de ME hypophysaire de la lapine est semblable à celle d'autres espèces mamelliennes. Nos propres observations avec microscope-électrone à la couche extérieure de ME chez le lapin cependant, ne sont pas parfaitement d'accord avec celles des études antécédentes de Duffy et Menefee (1965). Ces derniers rapportèrent une seule population de DCV à l'intérieur de terminaux axonaires de la couche externe ME chez le lapin. Nous croyons plutôt, d'après des évidences ultra-structurelles, qu'il y ait au moins deux populations différentes de DCV à cette couche de ME chez le lapin. De plus, puisque ces populations de vésicules furent apparentes à l'intérieur de profiles et terminaux axonaires indépendants, nous suggérons des différences quand à leur contenu et leur origine.

Certes, l'association de RF aux populations multiples de DCV observées à la couche externe ME de mamelles est mal connue. Les DCV plus petites (90nm à 100nm), que nous observons, contiennent probablement les catecholamines, tandis que celles de plus grande dimention (120nm à 140nm), sont possiblement porteuses du RF associé à l'activité gonado-tropique. Cette dernière impression est tirée surtout de notre observation de nombreuses vésicules déplétées ("vésicules fantômes"), à l'intérieur des terminaux axonaires, aboutant à l'espace périvasculaire (PVS), de capillaires portales chez les lapins, sacrificiés à 10 minutes post-coitus. La moyenne des diamètres de 137 plus ou moins 14nm de ces vésicules fantômes suggère fortement déplétion sélective de seulement les plus volumineuses des deux populations DCV. Des changements comparables n'étaient pas apparents aux terminaux axonaires, contenant des populations homogènes, seulement de DCV plus petites.

On sait que la question des sites précis de synthèse hypothalamique de RF, relié au contrôle de fonction adénohypophysaire, n'est pas finalement résolue. D'après les résultats de nombreuses études employant de différentes techniques neuro-endocrinologiques, deux principaux centres de synthèse hypothalamique de RF sont proposés: a) MBH ou HTA et, b) l'hypothalamus antérieur. Etudes ultra-structurelles jusqu'à date à ce laboratoire sont plutôt en faveur de synthèse à l'hypothalamus antérieur, pour les raisons suivantes:

1.—La présence de DCV volumineuses et de vésicules fantômes à la couche externe de ME hypophysaire chez le lapin, celles-ci de diamètre semblable aux grandes (120 à 150nm) DCV synthétisés à l'intérieur des noyaux PO et SCH, du même animal à la suite de coitus, castration et laparotomie.

2.—L'absence d'évidence de storage de ces DCV volumineuses aux neurones des noyaux PO et SCH, suggérant leur transport immédiat au ME.

3.—L'absence d'aucun changement ultra-structurel au soma neuronal du noyau arcué chez le lapin, tel qui pourrait refléter d'activité neurosecrétoire augmentée par coitus et/ou castration.

Ces observations ultra-structurelles à l'intérieur de l'hypothalamus chez le lapin, pourraient donc nous apporter la première évidence morphologique du relâchement actuel de différentes RF de sites de storage au ME, ainsi que de leur synthèse à l'intérieur de neurones de l'hypothalamus antérieur.

REFERENCES

AGHAJANIAN, G. K. and BLOOM, F. E. (1966). Electron-microscopic autoradiography of rat hypothalamus after intraventricular H^3-norepinephrine. Science, 153, 308-310.

AGHAJANIAN, G. K., BLOOM, F. E. and HEARD, M. H. (1969). Electron microscopy of degeneration within the serotonin pathway of the rat brain. Brain Research, 13, 266-273.

BARGMANN, W., and SCHARRER, E. (1951). The site of origin of the hormones of the posterior pituitary. American Scientist, 39, 255-259.

BEAMS, H. W. and KESSEL, R. G. (1968). The Golgi apparatus: structure and function. International Review of Cytology, 23, 209-276.

BERN, H. A. (1963). The secretory neuron as a double specialized cell. *In* General Physiology of Cell Specialization (D. Mazia and A. Tyler, eds.) pp. 349-366. McGraw-Hill, New York.

BERNARDIS, L. L. and FROHMAN, L. A. (1970). Effect of lesion size in the ventromedial hypothalamus on growth hormone and insulin levels in weanling rats. Neuroendocrinology, 6, 312-328.

BERNARDIS, L. L. and FROHMAN, L. A. (1971). Plasma growth hormone responses to electrical stimulation of the hypothalamus in the rat. Neuroendocrinology, 7, 193-201.

BISSETT, G. W., CLARK, B. J. and ERRINGTON, M. L. (1971). The hypothalamic neurosecretory pathways for the release of oxytocin and vasopressin in the cat. Journal of Physiology (London), 217, 111-131.

BRAWER, J. R. (1971). The role of the arcuate nuclei in the brain-pituitary-gonad axis. Journal of Comparative Neurology, 143, 411-446.

CLATTENBURG, R. E. (1972). Post-coital ultrastructural changes in neurons of three nuclei and the median eminence of the rabbit hypothalamus. Ph.D. Thesis. The University of Western Ontario, London, Canada.

CLATTENBURG, R. E., SINGH, R. P. and MONTEMURRO, D. G. (1971). Ultrastructural changes in the preoptic nucleus of the rabbit following coitus. Neuroendocrinology, 8, 289-306.

CLATTENBURG, R. E., SINGH, R. P. and MONTEMURRO, D. G. (1972a). Post-coital ultrastructural changes in the neurons of the suprachiasmatic nucleus of the rabbit. Zeitschrift fur Zellforschung and Mikroskopische Anatomie, 125, 448-459.

CLATTENBURG, R. E., SINGH, R. P. and MONTEMURRO, D. G. (1972b). Intranuclear filamentous inclusions in neurons of the rabbit hypothalamus. Journal of Ultrastructural Research, 39, 549-555.

CLATTENBURG, R. E., MONTEMURRO, D. G., BRUNI, J. E. and SINGH, R. P. (1973). Post-coital depletion of dense-core vesicles from the external layer of the rabbit median eminence. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 241, (in press).

CLEMENTI, F., CECCARELLI, B., CERATI, E., DEMONTE, M. L., FELICI, M., MOTTA, M. and PECILE, A. (1970). Subcellular localization of neurotransmitters and releasing factors in the rat median eminence. Journal of Endocrinology, 42, 205-213.

CRIGHTON, D. B., SCHNEIDER, H. P. G. and McCANN, S. M. (1970). Localization of LH-releasing factor in the hypothalamus and neurohypophysis as determined by an *in vitro* method. Endocrinology, 87, 323-329.

DAHLGRÉN, U. (1914). The Electric motor nerve centers in the skates (Rajidae) Science, 40, 862-863.

DALTON, A. J. (1961). Golgi apparatus and secretion granules. *In* The Cell (J. Brachet and A. E. Mirsky, eds.) Vol. 2, pp. 603-620. Academic Press, New York.

DESJARDINS, C., KIRTON, K. T. and HAFS, H. D. (1967). Anterior pituitary levels of FSH, LH, ACTH and prolactin after mating in female rabbits. Proceedings of the Society for Experimental Biology and Medicine (New York), 126, 23-26.

DIERSCHKE, D. J., BHATTECHARYA, A. N., ATKINSON, L. E. and KNOBIL, E. (1970). Circhoral oscillations of plasma LH levels in the ovariectomized Rhesus monkey. Endocrinology, 87, 850-853.

DUFFY, P. E. and MENEFEE, M. (1965). EM observations of neurosecretory granules, nerve and glial fibres and blood vessels in the ME of the rabbit. American Journal of Anatomy, 117, 251-286.

DYBALL, R. E. J. (1971). Oxytocin and Adh secretion in relation to electrical activity in antidromically identified supraoptic and paraventricular nuclei in the rat. Journal of Physiology (London), 214, 245-256.

FLAMENT-DURAND, J. (1971). Ultrastructural aspects of the paraventricular nuclei in the rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 116, 61-69.

FROHMAN, L. A. and BERNARDIS, L. L. (1968). Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. Endocrinology, 82, 1125-1132.

FUXE, K. (1965). Distribution of monoamine nerve terminals in the central nervous system. Acta Physiologica Scandinavica, 64 (Suppl.), p. 247.

FUXE, K. and HÖKFELT, T. (1967). Further evidence for the existence of tubero-infundibular dopamine neurons. Acta Physiologica Scandinavica, 66, 243-244.

FUXE, K. and HÖKFELT, T. (1968). Monoamine afferent input to the hypothalamus and the dopamine afferent input to the median eminence. *In* Progress in Endocrinology (Carlos Gual, ed.) pp 495-502. Excerpta Medica Foundation, Amsterdam.

FUXE, K. and HÖKFELT, T. (1970). Central monoaminergic systems and hypothalamic function. *In* The Hypothalamus (L. Martini, M. Motta and F. Fraschini, eds.) pp 123-138. Academic Press, New York.

GAY, U. L., NISWENDER, G. D. and MIDGLEY, A. R. (1970). Response of individual rats and sheep to one or more injections of hypothalamic extract as determined by radioimmunoassay of plasma LH. Endocrinology, 86, 1305-1312.

GOLDMAN, B. D. and PORTER, J. C. (1970). Serum LH levels in intact and castrated golden hamsters. Endocrinology, 87, 676-679.

GORSKI, R. A. (1970). Localization of hypothalamic regulation of anterior pituitary function. American Journal of Anatomy, 129, 219-222.

GREEN, J. D. and HARRIS, G. W. (1947). The neurovascular link between the neurohypophysis and

adenohypophysis. Journal of Endocrinology, 5, 136-146.

HALÁSZ, B. and PUPP, L. (1965). Hormone secretion of the anterior pituitary gland after physical interruption of all nervous pathways to the hypophysiotrophic area. Endocrinology, 77, 553-562.

HALÁSZ, B., PUPP, L. and UHLARIK, S. (1962). Hypophysiotrophic area in the hypothalamus. Journal of Endocrinology, 25, 147-154.

HARRIS, G. W. (1937). The induction of ovulation in the rabbit by electrical stimulation of the hypothalamo-hypophyseal mechanism. Proceedings of the Royal Society, London (Biology), 122, 374-394.

HILLIARD, J., HAYWARD, J. N. and SAWYER, C. H. (1964). Post-coital patterns of secretion of pituitary gonadotropin and ovarian progestin in the rabbit. Endocrinology, 75, 957-963.

HINSEY, J. C. and MARKEE, J. E. (1933). Pregnancy following bilateral section of the cervical sympathetic trunks in the rabbit. Proceedings of the Society for Experimental Biology and Medicine (New York), 31, 270-271.

HOFER, H. O. (1968). The phenomenon of neurosecretion. *In* The Structure and Function of Nervous Tissue. (G. H. Bourne, ed.) Vol. I, pp 461-517. Academic Press, New York.

HOLTZMAN, E. and DOMINITZ, R. (1968). Cytochemical studies of lysosomes, Golgi apparatus and endoplasmic reticulum in secretion and protein uptake by adrenal medulla cells of the rat. Journal of Histochemistry and Cytochemistry, 16, 320-336.

ISHII, S. (1970a). Association of luteinizing hormone-releasing factor with granules separated from equine hypophyseal stalk. Endocrinology, 86, 207-216.

ISHII, S. (1970b). Isolation and identification of secretory vesicles in the axons of the equine median eminence. Gumma Symposium of Endocrinology, 7, 1-11.

ISHII, S. (1972). Classification and identification of neurosecretory granules in the median eminence. *In* Brain-Endocrine Interaction (K. M. Knigge, D. E. Scott and A. Weindl, eds.) pp 119-141. S. Karger, New York.

ISHII, S., IWATA, T. and KOBAYASHI, H. (1969). Granular localization of corticotropin-releasing activity in horse hypophyseal stalk homogenate. Endocrinologica Japonica, 16, 171-177.

JAIM ETCHEVERRY, G. and PELLEGRINO DE' IRALDI, A. (1968). Ultrastructure of neurons in the arcuate nucleus of the rat. Anatomical Record, 160, 239-253.

JAMIESON, J. D. and PALADE, G. E. (1966). Role of the Golgi complex in the intracellular transport of secretory proteins. Proceedings of the National Academy of Sciences of the United States of America, 55, 424-431.

KALIMO, H. (1971). Ultrastructural Studies on the hypothalamic neurosecretory neurons of the rat. I. The paraventricular neurons of the non-treated rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 122, 283-300.

KALIMO, H. and RINNE, U. K. (1972). Ultrastructural studies on the hypothalamic neurosecretory neurons of the rat. II. The hypothalamo-neurohypophysial system in rats with hereditary hypothalamic diabetes insipidus. Zeitschrift fur Zellforschung und Mikrosckopische Anatomie, 134, 205-225.

KAMBERI, I. A., MICAL, R. S. and PORTER, J. C. (1969). Luteinizing hormone-releasing activity in hypophyseal stalk blood and elevation by dopamine. Science, 166, 388-390.

KAMBERI, I. A., MICAL, R. S. and PORTER, J. C. (1970). Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indolamines on LH release. Endocrinology, 87, 1-13.

KNIGGE, K. M. and SCOTT, D. E. (1970). Structure and function of the median eminence. American Journal of Anatomy, 129, 223-244.

KNOWLES, F. (1965). Neuroendocrine correlations at the level of ultrastructure. Archives d'Anatomie Microscopique et de Morphologie Expérimentale, 54, 343-357.

KNOWLES, F. (1967). Neuronal properties of neurosecretory cells. *In* Neurosecretion (F. Stutinsky, ed.) pp 8-19, Springer-Verlag, New York.

KNOWLES, F. and CARLISLE, D. B. (1956). Endocrine control in the Crustacea. Biological Reviews, 31, 396-473.

KOBAYASHI, H. and ISHII, S. (1968). The median eminence as a storage site for releasing factors and other biologically active substances. *In* Progress in Endocrinology (C. Gual, ed.) pp 548-554. Excerpta Medica Foundation, Amsterdam.

KOBAYASHI, H., OOTA, Y., UEMURA, H. and HIRANO, T. (1966). Electron microscopic and pharamacological studies on the rat median eminence. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 71, 387-404.

KOBAYASHI, H., MATSUI, T. and ISHII, S. (1970). Functional electron microscopy of the median eminence. Internal Review of Cytology, 29, 281-381.

KORDON, C. and GLOWINSKI, J. (1972). Role of hypothalamic monoaminergic neurons in the gonadotropin release-regulating mechanisms. Neuropharmacology, 11, 153-162.

MATSUI, T. (1966). Fine structure of the median eminence of the rat. Journal of the Faculty of Science, University of Tokyo, 4, 71-96.

MATSUI, T. (1967). Effects on the rat estrous cycle of implants of norepinephrin placed in the median eminence. Annotationes Zoologicae Japonenses, 40, p 74.

MATSUI, T. and ENGELHARDT, F. (1960). Die hypothalamo-hypophysaren systeme nach kastration und thyreoidictomie bei der ratte. Proceedings from the Deutsche Gesellschaft fur Endokrinologie, Kiel, S. 343-357.

MESS, B. (1969). Site and onset of production of releasing factors. *In* Progress in Endocrinology (C. Gual, ed.) pp 564-570. Excerpta Medica Foundation, Amsterdam.

MESS, B. and MARTINI, L. (1968). The central nervous system and the secretion of anterior pituitary hormones. *In* Recent Advances in Endocrinology (V. H. T. James, ed.) 8th Ed. pp 1-49. J. and A. Churchill Ltd., London.

MONROE, B. G. (1967). A Comparative study of the ultrastructure of the median eminence, infundibular stem and neural lobe of the hypophysis of the rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 76, 404-432.

MONROE, B. G. and SCOTT, D. E. (1966). Fine structural features of the neural lobe of the rat hypophysis during lactation and suckling. Journal of Ultrastructural Research, 14, 497-517.

MORRIS, J. F. (1971). Electron microscopical study of the paraventricular nucleus of the rat hypothalamus. Journal of Anatomy, 108, 592-593.

MOTTA. M., PIVA, F., TIMA, L., ZANISI, M. and MARTINI, L.

(1971). Intrahypothalamic localization of the nuclei synthesizing the gonadotropin releasing factors. Journal of Neuro-Visceral Relations (Suppl X), 32-40.

NORSTROM, A. (1972). Axonal transport and turnover of neurohypophysial proteins in the rat. Ph.D. Thesis. University of Gothenburg, Sweden.

OOTA, Y. (1963). Fine structure of the median eminence and the pars nervosa of the mouse. Journal of the Faculty of Science, University of Tokyo, Section IV, 10, 169-170.

PALAY, S. L. (1960). The fine structure of the secretory neuron in the preoptic nucleus of the goldfish *(Carassius auratus)*. Anatomical Record, 138, 417-444.

PAWLIKOWSKI, M. and KARASEK, M. (1971). Ultrastructural basis of neurosecretion. Acta Physiologica Polonica, 22 (suppl. 3), 755-766.

PELLEGRINO DE IRALDI, A., FIRINI DUGGAN, H. and DE-ROBERTIS, E. (1963). Adrenergic synaptic vesicles in the anterior hypothalamus of the rat. Anatomical Record, 145, 521-531.

PETERS, A., PALAY, S. L. and WEBSTER, H. deF. (1970). The fine structure of the nervous system. The Cells and their Processes. pp 32-43. Harper and Row Publishers, New York.

PICARD, D. (1969). The neurosecretory cell of vertebrates. Journal of Neuro-Visceral Relations. (suppl. 9), 23-62.

PORTER, J. C. and MICAL, R. S. (1969). Description of a method for the microcannulation of perfusion of a hypophyseal portal vessel in the rat. Federation Proceedings, 28, p 317.

PORTER, J. C., KAMBERI, I. A., GOLDMAN, B. D., MICAL, R. S. and GRAZIA, Y. R. (1970). The role of substances in the affluent blood supply to the adenohypophysis and in the third ventricular fluid and the release of luteinizing hormone. Journal of Reproduction and Fertility, Suppl. 10, 39-49.

RATNER, A. E. and ADAMO, N. J. (1971). Arcuate nucleus region in androgen sterilized female rats. Ultrastructural observations. Neuroendocrinology, 8, 26-35.

RETHELYI, M. and HÁLASZ, B. (1970). Origin of the nerve endings in the surface zone of the median eminence of the rat hypothalamus. Experimental Brain Research, 11, 145-158.

RICHARDSON, K. C., JARETT, L. and FINKE, E. H. (1960). Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technology, 35, 313-323.

RINNE, U. K. (1960). Neurosecretory material around the hypophysial portal vessels in the median eminence of the rat. Studies on its histological and histochemical properties and functional significance. Acta Endocrinologica (Kbh.), 35, Suppl. 57, 1-108.

RINNE, P. K. (1966). Ultrastructure of the median eminence of the rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 74, 98-122.

RINNE, U. K. and ARSTILA, A. U. (1966). Electron microscopic evidence on the significance of granules and vesicular inclusions of neurosecretory nerve endings in the median eminence of the rat. I Ultrastructural alterations after reserpine injection. Medicina et Pharmacologia Experimentalis.

SACHS, H. (1969). Neurosecretion. Advances in Enzymology, 32, 327-372.

SCHARRER, B. (1969). Neurohumors and neurohormones: Definitions and terminology. Journal of Neuro-Visceral Relations. Suppl. 9, 1-20.

SCHARRER, B. (1971). Concepts of neurochemical mediation. Neurocirugía, 29, 257-262.

SCHARRER, E. and BROWN, S. (1961). Neurosecretion. XII The formation of neurosecretory granules in the earthworm *Lumbricus terrestris L.* Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 54, 530-540.

SCHARRER, E. and SCHARRER, B. (1940). Secretory cells within the hypothalamus. Research Publications. Association for Research in Nervous and Mental Diseases, 20, 170-194.

SCHARRER, E. and SCHARRER, B. (1963). Neuroendocrinology. 289 p. Columbia University Press, New York.

SCHNEIDER, H. P. G., CRIGHTON, R. G. and McCANN, S. M. (1969). Suprachiasmatic LH-releasing factor. Neuroendocrinology, 5, 271-280.

SCOTT, D. E. and KNIGGE, K. M. (1970). Ultrastructural changes in the median eminence of the rat following deafferentation of the basal hypothalamus. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 105, 1-32.

SEITZ, H. M. (1965). Zur electronenmikroskopischen morphologie des neurosekrets im hypophysenstiel des schweins. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 67, 351-366.

SENCHIK, Y. I. (1971). Relationship of neurosecretory cells of the supraoptic nucleus and capillaries. Doklady (Proceedings) of the Academy of Science of the U.S.S.R., 200, 622-624.

SLOPER, J. A. (1966). Hypothalamic neurosecretion. British Medical Bulletin, 22, 209-215.

SLOPER, J. C. and BATESON, R. G. (1965). Ultrastructure of neurosecretory cells in the supraoptic nucleus of the dog and rat. Journal of Endocrinology, 31, 139-150.

SMITH, A. D. (1971). Summing up: some implications of the neuron as a secreting cell. Philosophical Transactions of the Royal Society (London), B, 261, 423-437.

SOKOL, H. and VALTIN, H. (1965). Morphology of the neurosecretory system of rats homozygous and heterozygous for hypothalamic diabetes insipidus (Battleboro strain). Endocrinology, 77, 692-700.

SPATZ, H. (1951). Neues uber die verknupfung von hypophyse und hypothalamus. Acta Neurovegetativa (Wein), 3, 5-49.

STOECKART, R., JANSEN, H. G. and KREIKE, A. J. (1972). Ultrastructural evidence for exocytosis in the median eminence of the rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 131, 99-107.

STUTINSKY, F. S. (1970). Hypothalamic neurosecretion. *In* The Hypothalamus (L. Martini, M. Motta and F. Fraschini, eds.), pp 1-23. Academic Press, New York.

SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B. and HALÁSZ, B. (1962). Hypothalamic control of hypophyseal gonadotrophic function. *In* Hypothalamic Control of the Anterior Pituitary. (J. Szentágothai, B. Flerkó, B. Mess and B. Halász, eds.) VI, pp 192-264. Akadémiai Kiadó, Budapest.

SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B. and HALÁSZ, B. (1968). Hypothalamic control of hypophyseal gonadotrophic function. *In* Hypothalamic Control of the Anterior Pituitary. (J. Szentágothai, B. Flerkó,

B. Mess and B. Halász, eds.), Ch. VI, pp 249-342. Akadémiai Kiadó, Budapest.

SZENTÁGOTHAI, J., FLERKÓ, B., MESS, B. and HALÁSZ, B. (1972). The trophic dependence of the anterior pituitary on the diencephalon; the hypophysiotrophic area of the hypothalamus. *In* Hypothalamic Control of the Anterior Pituitary (J. Szentágothai, B. Flerkó, B. Mess and B. Halász, eds.), Ch. III, pp 110-155. Akadémiai Kiadó, Budapest.

TEJASEN, T. and EVERETT, J. W. (1967). Surgical analysis of the preoptico-tuberal pathway controlling ovulatory release of gonadotropins in the rat. Endocrinology, 81, 1387-1396.

TIMA, L. (1971). On the site of production of releasing factors. Memoirs. Society for Endocrinology, 19, 895-901.

VOITKEVICH, A. A. and DEDOV, I. I. (1972). Ultrastructural study of neurovascular contacts in the median eminence of the rat. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 124, 311-319.

ZAMBRANO, D. (1966). Intracisternal filamentous material in neurosecretory cells of the supraoptic nucleus. Anatomical Record, 154, p 446.

ZAMBRANO, D. (1968). On the presence of neurons with granulated vesicles in the median eminence of the rat and dog. Neuroendocrinology, 3, 141-155.

ZAMBRANO, D. (1969). The arcuate complex of the female rat during the sexual cycle. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 93, 560-570.

ZAMBRANO, D. and DeROBERTIS, E. (1966). The secretory cycle of supraoptic neurons in the rat: a structural-functional correlation. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 73, 414-431.

ZAMBRANO, D. and DeROBERTIS, E. (1967). Ultrastructure of the hypothalamic neurosecretory system of the dog. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 81, 264-282.

ZAMBRANO, D. and DeROBERTIS, E. (1968). The effect of castration upon the ultrastructure of the rat hypothalamus. II Arcuate nucleus and outer zone of the median eminence. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 87, 409-421.

ZAMBRANO, D. and MORDOH, J. (1966). Neurosecretory activity in the supraoptic nucleus of normal rats. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 73, 405-413.