Proposal of a Neurosecretory System in the Pancreas. 
An Electron Microscope Study in the Dog*

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Summary. The nerve fibers supplying the islets of the dog pancreas were examined by electron microscopy. Axons with swollen portions containing various types of synaptic vesicles, including a cholinergic type, two varieties of presumable aminergic type and a possible peptidergic type, rush to the pericapillary space to end there, whereas only a small part of them seem to terminate on the endocrine cells of the islet.

Schwann cell cytoplasm invests the axons and often separates these from the endocrine cells. The terminal portions filled with vesicles usually become free of the Schwann sheath on the side facing the blood capillary. Here the neuronal secretions are believed to be released into the blood stream through the pored endothelium of the capillary.

The neurosecretions, together with the islet hormones are distributed to the exocrine pancreas in high concentrations via the insulo-acinar portal system. It seems thus sufficient for the nerves to supply the islet in order to control the exocrine function of the pancreas.

During the three decades since the establishment of the hypothalamo-hypophyseal neurosecretory system by Bargmann (1949), the concept and criteria of neurosecretion have changed a great deal (Lederis, 1974; Sano, 1978). Bargmann and Scharrer (1951) regarded that stain-technologically demonstrable secretory granules are essential for a neurosecretory cell, but later this criterion had to be abandoned as newly discovered neurosecretory system producing and transporting releasing hormones from the hypothalamus to the hypophysial portal vessels revealed no stainable granules. Recent advance in the knowledge of peptidergic neurons and of amine-containing neurons occurring in different central and peripheral nervous tissues caused further confusion to the concept of neurosecretion. In agreement with Sano (1978), the last reliable criterion of neurosecretion (in higher vertebrates) must be that the neuronal secretions are released into the circulation and this is morphologically reflected in that the nerve terminals are not related to their target organ or cell but end in the perivascular (especially pericapillary) space.

In the course of our electron microscope study of the dog pancreas, we recently noticed that numerous nerve terminals occur in the pericapillary spaces of the islet. Also by re-examining the electron micrographs which we produced for our previous

* This paper is dedicated to the memory of the late Professor Wolfgang Bargmann.
publication (KOBAYASHI and FUJITA, 1969), we became convinced of our idea that nerves are concentrated in the pericapillary spaces of the islet and here they might release their secretions into the sinusoidal capillaries.

This idea seems of physiological importance as the islet blood is directly conveyed to the exocrine pancreas via the insulo-acinar portal vessels, and neurosecretions in the islet may be delivered in high concentrations to the exocrine pancreas.

MATERIALS AND METHODS

The body and tail of the pancreas of young and adult dogs were fixed either by immersion or by perfusion. The immersion fixation was performed by the use of (1) osmium dichromate solution (DALTON, 1955), (2) 1.3% osmium tetroxide in a 0.1 M phosphate buffer (pH 7.3) containing 50 ppm calcium chloride, or (3) 2.5% glutaraldehyde in the phosphate buffer with calcium chloride followed by 1.3% osmium tetroxide in the same buffer. The perfusion fixation was made through the aorta or the celiac artery with 2.5% glutaraldehyde in 0.1 M phosphate buffer containing 50 ppm calcium chloride. After 3–10 min perfusion the tissue was cut in small pieces and further fixed in the same glutaraldehyde solution for 1–4 hrs and post-fixed in osmium as indicated above.

Furthermore, the present study was reinforced by examination of the electron micrographs of dog pancreas prepared in our previous study (KOBAYASHI and FUJITA, 1969).

OBSERVATIONS

Both in young and adult dogs the islets of the pancreas are richly supplied by unmyelinated nerves. Numerous axons with distinct neurotubules are found between the islet cells—A, B and D cells. Noteworthily, they are more numerous close to the sinusoidal capillaries. Many profiles of axons are found in the pericapillary space, i.e., between the islet cell apices and the pored endothelium of the sinusoid (Fig. 1, 2).

Schwann cell perikaryon may be found among the axon bundles, between or on the islet cells, and in the pericapillary space. Axons in the islet are mostly bundled and invested by Schwann cells, thus forming structures called Schwann-axon complexes (Fig. 1). Individual axons may be separated by Schwann cell cytoplasm but, otherwise, two or three axons may be juxtaposed within a common tube of the cytoplasm. Schwann cells often invest, with their attenuated cytoplasm, single or grouped islet cells, regardless of their cell type—A, B or D (Fig. 2–4). Structures which might be called Schwann-islet cell complexes are thus formed. Furthermore, such a Schwann cell sheath may simultaneously contain some axons, thus forming a Schwann-islet-axon complex (Fig. 2).

The axons, including their portions containing synaptic vesicles (vide infra), are occasionally found in direct juxtaposition with islet cells. Very often the axons are invaginated into corresponding indentations of islet cells. The distance between the islet and nervous elements is about 30 nm, neither membrane showing any special
thickening (Fig. 5). In our previous observation (KOBAYASHI and FUJITA, 1969) occurrence of this synapse-like structure was overestimated and the nerves coming to the islet were interpreted as exclusively innervating islet cells. Thus it remained a puzzle for us at that time, that apparently superfluous amounts of axons occurred

Fig. 1. Numerous axons close to the blood capillaries (C) in dog pancreatic islet. Many of the axon profiles represent terminal swellings containing various types of synaptic vesicles. Schwann cytoplasm (S) invests the axons and forms a Schwann-axon complex. Endocrine A and B cells are also closely associated with this complex. Dalton’s fixation. ×15,000
in the islet for the innervation of its endocrine cells.

In the present study we noticed that the majority of the bundles of axons rush at the pericapillary space or its niches (Fig. 1, 2). In these pericapillary and para-capillary spaces the axons assume the structure of terminals, being filled with synaptic vesicles, and open towards the capillary: The terminals have lost their Schwann investment on the vascular side and tend to concentrate their vesicles on

Fig. 2. A structure to be called Schwann-islet-axon complex found in a paracapillary space of dog islet. Schwann cell (S) invests an endocrine A cell and numerous axons which partly contain synaptic vesicles. Dalton’s fixation. $\times 14,000$
this side (Fig. 6). The Schwann-axon complex in the perivascular space is surrounded by its own basal lamina, which may often separate the structure from the islet cell mass (Fig. 7, 8).

Fig. 3. An endocrine D cell ensheathed by a Schwann cell (S) found in a paracapillary space of dog islet. Arrows indicate thin cytoplasm of the Schwann cell, which invests also a few axons (arrowhead). Glutaraldehyde and osmium fixation. ×11,000
On the other hand, the perivascular Schwann-axon complex may involve an apparent process of an islet cell. Figure 9 shows that a D cell process is invested, together with axons, in a Schwann cell sheath which is lacking on the vascular side.

Fig. 4. A Schwann cell (S) associated with axons (arrows) and endocrine B cells. Thin cytoplasmic processes of the Schwann cell extend to invest nervous and endocrine elements and the cell apparently does not discriminate between both elements. Centrioles with ciliary base are seen in the middle. C blood capillary. Glutaraldehyde and osmium fixation. ×11,000
The sinusoidal capillary of the islet, including the sites close to the Schwann-axon complexes, is lined by ordinary pored endothelium backed by a basal lamina (Fig. 1, 6). Only a few collagen filaments and occasional thin profiles of cytoplasm (pericyte or fibroblast?) are found in the clear perivascular space, i.e., the space between the basal lamina of the capillary endothelium and that of the Schwann-axon complex (Fig. 8, 9).

Fig. 5. A portion of B cell associated with axons containing synaptic vesicles (arrows). The nerve terminals shown in this micrograph seem to fall in Type 3 of this paper, containing large-sized granules and smaller vesicles with a fine-granular substance. One of the terminals shown at the bottom contains large lysosome-like bodies. The terminals are partly juxtaposed with the endocrine cell but partly separated from the latter by a thin process of Schwann cell (S). Glutaraldehyde and osmium fixation. ×27,000
Fig. 6. Numerous axons filled with synaptic vesicles directly facing the blood capillary. The axon terminals are suggested to be adrenergic (Ad) and cholinergic (Ch) in type according to the synaptic vesicles they contain. Note that the synaptic vesicles are gathered towards the capillary (C). Small mitochondria and glycogen granules are also seen in the terminals. Schwann cell cytoplasm (S) separates the axons and endocrine D cells. Dalton's fixation. $\times 30,000$
The nerve terminals observed in the dog islet could be divided into at least four different types according to the appearance of the synaptic vesicles they contain:

1. Terminals containing numerous small vesicles (30-50 nm) with a tiny dark core. This type of terminals are usually regarded as adrenergic (Fig. 1, 6, 7).
2. Terminals containing numerous small clear vesicles (30-50 nm) and occasional large cored vesicles (100-300 nm), usually regarded as cholinergic endings (Fig. 6, 7).
3. Terminals containing numerous vesicles of different sizes ranging from 30 to 200 nm in diameter, loosely filled by a dark, fine-granular substance (Fig. 5, 10; Fig. 10, 15 in KOBAYASHI and FUJITA, 1969).
4. Terminals containing (1) large granules (150-300 nm) with fine-granular and,

Fig. 7. A Schwann-axon complex in a paracapillary space. The axons include typical cholinergic (Ch) and adrenergic (Ad) types. Some axons are contained in indentations of endocrine A cell (arrows). Dalton’s fixation. ×30,000
Fig. 8. A Schwann-axon complex in the perivascular space of an islet capillary. In this micrograph the complex is ensheathed by its proper basal lamina, thus located in a neural compartment separated from the endocrine compartment. The latter in this specimen is rich in intercellular spaces and shows only a fragment of A cell. P indicates a nerve terminal of Type 4 which may be called peptidergic in structure. Note the large dense granules and small flattened vesicles in it. E endothelial cell. Glutaraldehyde and osmium fixation. ×20,000
occasionally, fine-vesicular contents of medium to high electron density and a narrow clear space left beneath the limiting membrane, and (2) small flattened vesicles which appear either vacant or containing a faint, fine-granular substance (Fig. 8; Fig. 10 in Kobayashi and Fujita, 1969).

As reported previously, A, B and D cells all seem to have the possibility to be attached by two or three different types of nerve terminals. In addition to this finding, the present observation reveals that all of the four types of nerve terminals can

![Fig. 9. A Schwann-islet-axon complex in pericapillary space. Schwann cell cytoplasm (S) invests axons and a process of endocrine D cell. From all respects the latter appears equivalent to neuronal processes in this peculiar structure. Arrows indicate axon terminals of presumable Type 3. Glutaraldehyde and osmium fixation. ×17,000](image)
be found in the pericapillary and paracapillary spaces and they are very often devoid of Schwann cell coverage on the vascular side as mentioned above.

DISCUSSION

Islet cells as paraneurons
It has been repeatedly pointed out that the pancreatic islet cells, together with a series of peptide and amine producing endocrine cells, share cell-biological characteristics with neurons (Pearse and Polak, 1971; Fujita, 1976; Fujita et al., 1979; Niki and Niki, 1976). The islet cells thus represent one of the important members of paraneurons (Fujita, 1976, 1977; Fujita and Kobayashi, 1979; Fujita et al., 1979). Secretion of somatostatin, a neurohormone, in the islet D cells (Luft et al., 1978) and

Fig. 10. A Schwann-axon complex in a paracapillary space. Two of the terminal portions are partly denuded from the Schwann cell (S). The upper one contains granular vesicles of different sizes and seems to fall in the category of Type 3 terminal. Glutaraldehyde and osmium fixation. ×35,000
possible occurrence of insulin and glucagon in certain neurons (Tager et al., 1976; Havrankova et al., 1978) seem to symbolize the paraneuronal nature of islet cells.

The long known structure called the neuro-insular complex (Simard, 1935; Fujita, 1959) which is formed by a mixture and direct juxtaposition of neurons, Schwann cells and islet cells has called attention anew as an evidence of the paraneuronal nature of islet cells (Fujita, 1976; Fujita et al., 1979). Although it is still unclear whether in the neuro-insular complex (Type I, Fujita, 1959) the neurons and islet cells functionally are connected in series or in parallel, or both, the electron microscope observation indicates that islet cells in the complex are equivalent to neurons in their fine-topographical relation to the neurons and Schwann cells (Serizawa et al., 1979; Fujita et al., 1979).

**Schwann-islet cell relationship**

The peculiar relation of islet cells to Schwann cells is seen everywhere in the electron micrographs shown in this paper. A, B and D cells may be, singly or in groups, attached or invested by Schwann cell cytoplasm. Smith (1975) reported that in the dog Schwann cells may extend a thin cytoplasmic sheet surrounding a considerably
large group of islet cells. Although Smith was interested in the possible significance of the Schwann cell sheath to insulate the electric excitation conducted from cell to cell in the islet, we like to pay attention to the neuron-equivalent (paraneuronal) attitude of the islet cells. It is unknown whether dog islet cells need a Schwann cell taking care of them, but what is certain and important in this context is that the Schwann cell apparently cannot discriminate between the islet cell and neuron and treats the former as a neuron. Thus, in the dog pancreas a Schwann cell often embraces an islet cell or cells (Schwann-islet cell complex) exactly in the same way as it does for a neuronal soma and axons (Fig. 3). A Schwann cell sometimes invests islet cells and, simultaneously neurons, either soma (neuro-insular complex Type I, Serizawa et al., 1979; Fujita et al., 1979) or axons (Schwann-islet-axon complex, Fig. 2 and 9). It is unknown whether some other mammalian species may possess a similar structure, but a Schwann-endocrine cell-axon complex identical in composition and fine structure as seen in the dog pancreas has been demonstrated by Kataoka (1974, 1977) in the lamina propria of finch proventriculus.

**Axon-islet cell relationship**

A close relation between the islet endocrine cell and autonomic axons has repeatedly been reported in various mammals (Bencosme, 1959; Stahl, 1963; Legg, 1967; Esterhuizen et al., 1968; Watari, 1968; Kobayashi and Fujita, 1969; Woods and Porte, 1974).

When axons are found in direct contact with an islet cell as frequently demonstrated in the present study, it often is difficult to decide whether it represents a synaptic innervation (series connection) or a mere concurrence (parallel juxtaposition). Only when an islet cell is attached directly by a part of an axon filled with synaptic vesicles and, favorably, when the vesicles are concentrated towards the islet cell, we can reasonably say that the islet cell is innervated by the axon. When a thinner portion of an axon containing only neurotubules is in direct contact with an islet cell, we previously have guessed that the axon might have beaded portions somewhere outside of the section, to terminate on this or another islet cell. In our previous paper we demonstrated vesicle-filled terminal portions occurring in a pericapillary space keeping a few micra apart from islet cells, but we interpreted this finding as an “en distance” innervation to the islet cells (Kobayashi and Fujita, 1969) in agreement with the prevailing view of researchers (see the review by Woods and Porte, 1974). We thus have been preoccupied by the view that nerves coming into the islet are connected in series with the endocrine cells, the former controlling the hormone secretion of the latter.

Breaking this spell, in the present study we could discover masses of nerve terminals exposed to the pericapillary space and its niches (paracapillary spaces). Although exocytotic openings of synaptic vesicles or granules could not be demonstrated, it is most probable that the nerves there might release their secretions into those spaces and, through the basal laminae and pored endothelial wall, into the blood (Fig. 11).

This discovery leads to the idea that many of the axon-islet cell juxtapositions may represent merely a parallel juxtaposition in which both the axons and islet apices rush side by side to release their secretions to the capillary blood. Thus, we propose that the axon-islet cell relationship in the dog might be dual, i.e., series con-
nection and parallel juxtaposition. A likely possibility must be added in this context that some, if not all, axons might terminate in the pericapillary space after innervating an islet cell or cells with their beaded portions (Fig. 11).

The parallel function in secretion release of axons and islet cells may be further assured by such structures as represented by Figure 9. A cytoplasmic process of a D cell is incorporated in a pericapillary Schwann-axon complex, and one may hardly find a reason for discriminating this cell process from an axonal terminal laden with "peptidergic" type granules (Fig. 8).

**Synaptic types**

Four types of nerve terminals are classified in the present study according to the structure and combination of synaptic vesicles and granules. Two of them are supposed to be adrenergic and cholinergic by the widely accepted criteria.

The third type characterized by round vesicles of different sizes containing dark, powder-like substances (Fig. 5, 10) is unknown in nature. A possibility seems worthy to propose that this type of terminal might be dopaminergic in nature. The vesicles in this type of terminals closely resemble in fine structure the small granules in the SGC (small granule chromaffin) cell of the adrenal medulla (KOBAYASHI et al., 1978), which are suspected to contain dopamine.

The fourth type of terminals containing large, endocrine-like granules and small, flat or elongate vesicles may possibly correspond to the VIP containing neurons immunohistochemically revealed in the canine intrapancreatic ganglia by LARSSON et al. (1978) and confirmed in our research group (IWANAGA et al., unpublished). LARSSON (1977) reported that VIP containing nerves, under the electron microscope, are "peptidergic" in type, possessing large, solid-looking granules. The significance, thereby, of the flattened vesicles revealed in this type of terminals is unknown.

In the bat, dog and some other vertebrates, WATARI (1968, 1973) reported that nerve terminals in the islets were classifiable into four types by the fine structure of their synaptic vesicles. As these four types seem to correspond only partly to the four types demonstrated in the present study, it seems reasonable to propose that more than four types of nerve terminals might be present in the pancreas, representing the sites of different neurohumors.

**Insular neurosecretion and its implications**

As far as we are aware, this paper is the first to propose that nerves with different synaptic vesicles release their secretions into the capillary blood of the islet. As is well known, the dog pancreatic islet is especially rich in nerve supply, and this species characteristic apparently has favored the present result. It is unknown but seems possible that the pancreas of human and other animals might possess similar neurosecretory fibers, even though less numerously than in the dog.

Occurrence of ample nerve fibers along the vascular wall in the islet of pancreas has been noticed since the early studies using silver impregnation techniques (HONJIN, 1956; STÖHR, 1957), and later confirmed both by cholinesterase histochemistry (COUPLAND, 1958; LIBMAN and SUTHERLAND, 1965) and by Falck-Hillarp's method for monoamine detection (LEGG, 1968; CEGRELL, 1968). None of the previous authors, however, have dreamed of the possibility that nervous secretions might be released into the circulation here. LEGG (1968) and CEGRELL (1968) pointed to the possible
adrenergic innervation to the blood vessels as well as to the endocrine cells. These authors apparently suggested a vasomotor innervation. In this context, ALM et al. (1967) conceived that the vessels of the pancreatic islet, as capillaries, might be quite exceptional to receive innervation, as this usually occurs in arterioles and other larger vessels.

If the insular neurosecretion proposed in the present paper actually occurs into the sinusoidal capillaries of the islet, the neurosecretory substances are conveyed to the exocrine pancreas by the insulo-acinar portal vessels (HENDERSON, 1969; FUJITA and MURAKAMI, 1973). As in other mammals the dog has been evidenced by vascular cast scanning electron microscopy to possess distinct portal vessels radiating from the capillary net of the islet to that of the acinar tissue (Fig. 1, 2 in the paper by FUJITA et al., 1976). Thus, neurosecretions, as well as islet cell hormones, released in the islet must instantly be distributed in high concentrations to the exocrine pancreas.

The most attractive and likely candidate of the insular neurosecretions is VIP (vasoactive intestinal polypeptide), as this substance is known to have a secretin-like activity, i.e., to increase pancreatic secretion of bicarbonate and water (KONTUREK et al., 1976; SINGH and WEBSTER, 1978). LARSSON et al. (1978) and recently IWANAGA et al. (1979, unpublished) recognized that in the dog the intrapancreatic ganglia contain many neurons with VIP immunoreactivity and that some VIP-positive nerve fibers occur both in the endocrine and exocrine pancreas. These authors correlate the occurrence of VIP fibers in the islet to the glucagon releasing action of this substance. This interpretation might be valid especially for the peptidergic type axons terminating on the islet cells, but we hypothesize that a part of the VIP in the islet might be given to the exocrine pancreas by neurosecretion into the insulo-acinar portal system. LARSSON et al. (1978) pointed out that a few VIP-containing nerves terminated on the wall of blood vessels in the pancreatic lobule but did not conceive that the substance might be released into the capillary lumen.

Noradrenalin and acetylcholine are presumed to be released into the insulo-acinar portal system from the axon terminals of adrenergic and cholinergic type, respectively. The actions upon the secretion of exocrine pancreas of these transmitters have been extensively studied by physiologists (for review see SINGH et al., 1978) and we may only switch our understanding from the control by neurotransmitters to that by neurohormones. This new idea may account for the much smaller distribution of adrenergic and cholinergic nerves, especially of the former, in the exocrine pancreas as compared with the islet. It also seems to account for the hitherto repeatedly noticed discrepancy between the physiological fact that splanchnic stimulation enhances secretion from the pancreas in the dog and the morphological evidence that adrenergic fibers occur very sparsely in the exocrine pancreas in this animal (see the review by SINGH et al., 1978).

In the dog, but not in certain other species examined, dopamine is known to exert a potent secretin activity (HASHIMOTO et al., 1971). If dopaminergic axons, even if quite few, might be included in the insular neurosecretion system of the dog, their influence upon the pancreatic secretion must be great. A histochemical examination concerning this problem seems worthwhile.

Although the significance of the insulo-acinar portal system has hitherto been interpreted only on the basis of the actions of islet hormones upon the exocrine pancreas (HENDERSON, 1969; FUJITA, 1973; KANNO et al., 1976; KANNO and SAITO, 1976),
the present hypothesis of insular neurosecretion seems to shed new light on the portal vessels of the pancreas. Whatever might be the secretions concerned, the exocrine pancreas, at least in the dog, may possibly be controlled by neurohormones conveyed by blood rather than by neurotransmitters given by direct nerve termination.

The islets of Langerhans, in association with the intrapancreatic ganglia, seem to deserve the designation, “neuro-paraneuronal control centers of the pancreas.”

REFERENCES


