PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Summing Up: Some Implications of the Neuron as a Secreting Cell

A. D. Smith

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Summing up: some implications of the neuron as a secreting cell

By A. D. Smith
Department of Pharmacology, University of Oxford

'Believing that the nervous system is something more than a mere system of conducting paths, I formed the hypothesis that nerve cells are true secreting cells, and act upon one another and upon the cells of other organs by the passage of a chemical substance of the nature of a ferment or proferment' (Scott 1905).

'The most striking morphological feature of the neuron is the tremendous accumulation within its cytoplasm of small granules associated with a well developed endoplasmic reticulum. The same type of association is found in the ergastoplasm of glandular cells... cells which sustain an intense protein production. In the nerve this activity is implicit in chromatolysis and to certain types of generalized stress. The rapid regeneration of axons and the peculiar damming up of axoplasm proximal to a ligature are also reflexions of a continuous and rapid protein synthesis in the perikaryon.... The fact that the structure of the Nissl substance is the same as that of the ergastoplasm in glandular cells means that future analysis... in such readily available cells as those of the pancreas and liver can be profitably applied to the nerve cell' (Palay & Palade 1955).

'Neurosecretion should not perhaps be used as a term to describe only the histochemically demonstrable secretory processes of nerves such as those of the hypothalamo-pituitary system. The analogies are sufficient to indicate that similar processes are involved in the production, transport and secretion of acetylcholine from other nerve endings. So these, too, may also be called neurosecretory nerves' (Hebb 1959).

'All neurons have a secretory function by which active substances are synthesized and released. Secretion may act over a short distance on specific chemical receptors or on distant receptors by way of the blood stream. Intermediary examples are the adrenergic neuro-effectors ending on smooth muscle. Neurosecretion may be produced all along the neuron or at the nerve endings. In all cases, it is stored within a membrane in vesicles which represent multimolecular quantal units of neurosecretion' (De Robertis 1964).

The interest of the Royal Society in the neuron is nearly as old as the society itself. In 1674, fourteen years after its foundation, the Society received the first of several letters from the great Dutch microscopist Antony van Leeuwenhoek in which he described cross-sections of the optic nerve. Leeuwenhoek found that the nerve was not hollow, as Galen had supposed, but was made up of a large number of vessels or fibres. This is, in fact, the first clear description of the nerve fibre and Leeuwenhoek was able to discount Galen's view that light passed through the eye into the optic nerve and thence to the brain (see Leeuwenhoek 1693).

Rather than attempt to summarize the contributions of each speaker and demonstrator to the Discussion Meeting, I should like to consider some of the wider implications of what we have learnt in the past two days. The symposium has been mainly concerned with the storage, intracellular transport, and release of neurotransmitter substances. There can be few now who doubt that, in many nerves, the transmitter is stored in membrane-limited particles, or vesicles. What is the significance of the storage of transmitters in vesicles?

One obvious advantage of this mode of storage is that the transmitter is protected from destruction by enzymes in the cytoplasm. However, although this is important for noradrenaline and dopamine, which are attacked by the monoamine oxidase present in mitochondria, it may not be so significant for other transmitters. There is, for example, evidence that some acetylcholine may normally be stored outside the vesicles in the cytosol (for review see Marchbanks 1969). A second advantage of this mode of storage is that it enables the transmitter to be transported over large distances in the cell, along the axon. However, this type of transport is only important for the transmitter in some neurons, such as the neurosecretory neurons of the

pituitary gland, since in other nerves the local synthesis of transmitter substances occurs in the nerve terminals. The major significance of the packaging of transmitter substances in vesicles lies not so much in the storage itself, but in the purpose for which the transmitter is stored: for secretion from the cell. Here, in the vesicle, is a source of highly concentrated transmitter, poised and ready for secretion.

Katz and his colleagues have often pointed out the advantages of the 'vesicle hypothesis' and of release by exocytosis (for review see Katz 1969). It seems to me that, today, we have stronger grounds than ever before for adopting this hypothesis. We have heard at this meeting some of the biochemical evidence for the release of proteins from vesicles in the adrenal medulla, splenic nerve, and posterior pituitary gland. We have seen evidence from the microscopists that vesicle membranes can indeed fuse with the cell membrane, such that the contents of the vesicle are exposed to the extracellular space. Finally, we have heard of the electrophysiological evidence which shows that the release of acetylcholine involves an increase in the frequency of discharge, but not in the size, of the quantal packet.

I should like to discuss four implications of secretion by exocytosis from a neuron: these concern (i) the transmitter substance, (ii) the coupling between the stimulus to the cell and secretion, (iii) the cellular dynamics of the neuron, and (iv) the tissue which is innervated.

The transmitter substance

Perhaps the best way to see the implications of exocytosis for the transmitter substance is to consider the disadvantages of release by diffusion across the cytosol. Some of the problems that face the transmitter if it is released from the vesicle into the cytosol are expressed diagram-

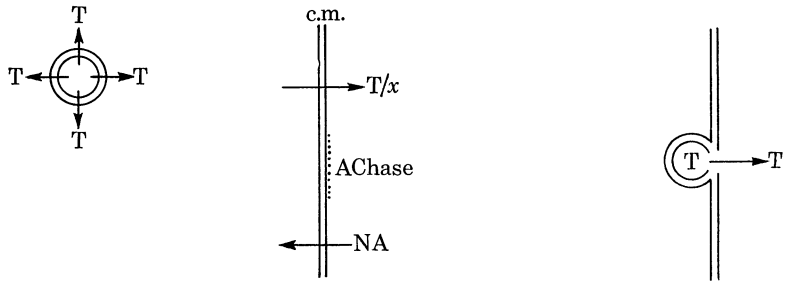

Figure 1. Disadvantages of release of a transmitter by diffusion. If a transmitter (T) stored in a vesicle diffuses out across the vesicle membrane into the cytosol, the amount reaching the postsynaptic tissue will be only a fraction (T/x) of that in the vesicle because of losses due to diffusion and enzymic destruction. Furthermore, if T is acetylcholine it will have to cross a cell membrane (c.m.) which often contains acetylcholinesterase (AChase); if T is noradrenaline (NA) it will have to cross a cell membrane specialized for the uptake of noradrenaline from the extracellular space. These disadvantages do not occur if the transmitter is secreted by exocytosis (right part of the figure).

matically in figure 1. First, before it leaves the cell, the transmitter will have to pass across two membranes; secondly, the transmitter will diffuse in all directions in the cytosol and so only a fraction of the original store will reach the cell which is innervated; and thirdly, the transmitter may be destroyed by enzymes in the cytoplasm. If the transmitter is acetylcholine, it will have to cross the nerve cell membrane which often contains acetylcholinesterase. Similarly, if the transmitter is noradrenaline, it will have to cross a cell membrane which is specialized for the uptake of noradrenaline from outside the cell. Secretion by exocytosis has none of these

disadvantages. In particular, the transmitter does not have to cross any intact membranes; it passes through an opening in the membrane, a process which allows the entire contents of the vesicle to be secreted without loss into the extracellular space.

Stimulus-secretion coupling

Exocytosis has another advantage when we consider how the stimulus to the cell initiates the sequence of events leading to secretion. Whether, as in the nerve terminal, the initial stimulus is electrical or, as in the adrenal medulla, it is chemical, the stimulus is located at the cell membrane. If the transmitter is released from vesicles within the cell it becomes necessary to pass a signal, from the cell membrane to the vesicle, across the cytosol. No such long-distance signalling mechanism is required if secretion takes place by exocytosis: this is because the cell membrane, the site of the stimulus, is also the site of release.

It is likely that the vesicles will be in constant Brownian motion and will, therefore, frequently collide with the cell membrane. Indeed, Brownian motion of vesicles in the corpus cardiacum has been observed by Thomsen (see Normann 1965). Katz (1969) has suggested that the frequency of collision between a vesicle and the cell membrane is high at all times but that most of such collisions are unsuccessful and do not lead to transmitter release. Release would only occur when two specific sites, one on each membrane, meet. From electrophysiological studies on the time at which external calcium ions have to be present in order to obtain release of transmitter following depolarization of the membrane, Katz & Miledi (1967) suggested that calcium is essential for the process which causes a transient fusion of axon and vesicular membranes. On this hypothesis, the calcium ions which enter the cell do not need to go any further than the inner side of the cell membrane.

The rôle of calcium in release processes is by no means confined to the motor nerve terminal: the number of such processes where calcium is recognized to be involved increases each year, so that the earlier reviews by Douglas (1968), Stormorken (1969) and Matthews (1970) are already out of date. In table 1, I have given a list of the secretion processes which require calcium. For all these tissues, except the adrenal cortex, it is known that the material to be secreted is stored in a membrane-limited particle (see Smith 1968). The diameter of these vesicles varies from about 1 μm for those in exocrine glands to 50 nm for a synaptic vesicle. The wide variety of secretion products, ranging from enzymes to polypeptide hormones and amines, was once held to be evidence against a common mechanism of secretion. However, it is remarkable that in nearly all these tissues, morphological or biochemical evidence is now available which is consistent with secretion by exocytosis (see table 1). Does the requirement for calcium in these diverse tissues reflect a common fundamental mechanism related to the initiation of membrane fusion?

Calcium ions cannot be the only factor involved in the interaction of vesicle and cell membranes: some sort of recognition process is required, perhaps in the form of specific proteins, one on each interacting membrane. The finding of specific membrane proteins in the adrenal chromaffin granule is a hint in this direction (Winkler 1971, this volume, p. 293).

Following attachment of the vesicle to the cell membrane, fusion of the two membranes takes place. The presence of a high concentration of lysolecithin in the chromaffin granule membrane provides an ideal way to convert the bimolecular leaflet structure of the membrane into a micellar form which can more easily fuse with another membrane (see Winkler 1971).

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TABLE 1. CALCIUM-DEPENDENT SECRETION AND EXOCYTOSIS

tissue	approx. diameter of storage vesicle / μm	substance secreted	exocytosis: electron microscopical evidence	exocytosis: bio-chemical evidence	references (a requirement for calcium; b electron microscopic evidence of exocytosis; c biochemical evidence of exocytosis)
exocrine pancreas	1	digestive enzymes	+	+	*a* Hokin (1966) *b* Palade (1959), Ichikawa (1965) *c* Keller & Cohen (1961) Green, Hirs & Palade (1963)
submaxillary gland	1	amylase	.	.	*a* Douglas & Poisner (1963)
parotid gland	1	amylase	+	.	*a* Rasmussen & Tenenhouse (1968), Selinger & Naim (1970) *b* Amsterdam *et al.* (1969)
PMN-leucocyte	1	lysosomal enzymes	+	+	*a*, *b*, *c* review by Woodin & Wieneke (1970)
adrenal medulla	1	lysosomal enzymes	.	+	*a*, *c* Schneider (1970)
	0.2	catecholamines and chromogranins	+	+	*a* review by Douglas (1968) *b* see Grynszpan-Winograd (1971) *c* review by Kirshner & Kirshner (1971)
pancreas (β-cell)	0.3	insulin	+	(+)	*a* Grodsky & Bennett (1966), Milner & Hales (1967) *b* Williamson *et al.* (1961), Sato *et al.* (1966) *c* Rubenstein *et al.* (1969)
adenohypophysis	0.25	LH	+	.	*a* Samli & Geschwind (1968) *b* Farquhar (1961)
	0.25	FSH	.	.	*a* Jutisz & de la Llosa (1970)
	0.3	GH	+	.	*a* MacLeod & Fontham (1970) *b* De Virgiliis *et al.* (1968)
	0.6	MH	+	.	*a* Parsons (1969), MacLeod & Fontham (1970) *b* Pasteels (1963), Farquhar (1969)
	0.2	ACTH	+	.	*a* Kraicer *et al.* (1969) *b* Yamada & Yamashita (1967)
	0.1	TSH	+	.	*a* Vale *et al.* (1967) *b* Farquhar (1969)
	0.25	MSH	+	.	*a* Hopkins (1970*b*) *b* Hopkins (1970*a*)
neurohypophysis	0.15	oxytocin	+	.	*a* Dicker (1966) *b* Nagasawa *et al.* (1970)
	0.15	vasopressin	+	+	*a* Douglas & Poisner (1964), Mikitin & Douglas (1965) *b* Nagasawa *et al.* (1970) *c* see Uttenthal *et al.* (1971)
pericardial organ (crab)	0.15	neurosecretory material	.	.	*a* Berlind & Cooke (1968)
platelets (α-granules)	0.3	β-glucuronidase	+	+	*a* Holmsen & Day (1968) *b* French & Poole (1963) *c* Holmsen & Day (1968)

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tissue	approx. diameter of storage vesicle μm	substance secreted	exocytosis: electron microscopical evidence	exocytosis: bio-chemical evidence	references (a requirement for calcium; b electron microscopic evidence of exocytosis; c biochemical evidence of exocytosis)
platelets (dense granules)	0.1	5-hydroxytryptamine	(+)	(+)	*a* Markwardt (1968), Takagi *et al.* (1968) *b* White (1968) *c* see Holmsen *et al.* (1969)
basophil leucocytes	0.35	histamine	.	(+)	*a* Greaves (1969) *c* Osler (1969)
mast cells	0.4	histamine	+	+	*a* Mongar & Schild (1958), Högberg & Uvnäs (1960), Suriyama & Yamasaki (1969) *b* Horsfield (1965), Bloom *et al.* (1967) *c* Diamant (1967), Fillion *et al.* (1970)
cholinergic neurons:					
skeletal muscle	0.05	acetylcholine	(+)	.	*a* review Katz (1969) *b* Hubbard & Kwanbun-bumpen (1968)
sympathetic ganglion	0.05	acetylcholine	.	.	*a* Harvey & MacIntosh (1940)
noradrenergic neurons	0.08	noradrenaline and chromogranins	+	(+)	*a* Smith, A. D. (1971) *b* Fillenz (1971) *c* Smith, A. D. (1971)
	0.05	noradrenaline	(+)	.	*a* review by Smith & Winkler (1971) *b* Fillenz (1971)
adrenal cortex	—	corticosteroids			*a* Birmingham *et al.* (1953), Rubin *et al.* (1969)

Secretion from these tissues depends upon the presence of calcium ions in the extracellular fluid. Evidence consistent with secretion by exocytosis from a storage vesicle is indicated if electron microscopy shows the presence of omega shaped profiles, or if biochemical studies show that other soluble components of the storage vesicle are secreted whereas substances in the cytosol are not released.

Previously, it has been argued (Whittaker 1968, 1970) that exocytosis is unlikely in nerves because the chemical composition of the synaptic vesicle membrane is so different from that of the nerve cell membrane: it was assumed that the two membranes would only fuse if they had a similar composition. However, lysolecithin is no respector of individual phospholipids; it will dissolve any membrane. The idea that lysolecithin is involved in membrane fusion and exocytosis would be supported if other secretion granules and vesicles, in addition to the adrenal chromaffin granule, were found to be relatively rich in this phospholipid. It is noteworthy, then, that data given by Burton & Howard (1967) show that, in synaptic vesicles isolated from rat brain, lysolecithin comprises nearly 10 % of the total phospholipids.

Cellular dynamics of the neuron

In considering the implications of exocytosis for the dynamics of the neuron, we shall again find it valuable to compare the neuron with other secreting cells. Some of the outstanding questions are:

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Where in the cell are the secretory substances synthesized, and where are they packaged into a vesicle?

How are the vesicles transported from their site of synthesis to the site of release?

What is the fate of the vesicle membrane following the release of its contents?

Synthesis and packaging

It is known that the neurotransmitter substances acetylcholine and noradrenaline can be synthesized in the nerve terminals. However, the same cannot be said of the other components of the vesicle, in particular the proteins. In protein-secreting cells, such as the exocrine pancreas, the site of synthesis of the secretory proteins is the endoplasmic reticulum. In 1955, Palay & Palade demonstrated that the ubiquitous Nissl substance in the cell bodies of neurons was made up of packed cisternae of rough endoplasmic reticulum. The Nissl substance was, of course, first discovered in neurons and was only later found in other cells. The discoverer of the Nissl substance in non-nervous tissue, F. H. Scott, was fully aware of the significance of his findings, as can be seen by this extract from his paper published in 1905:

> I find the only other place where the Nissl substance is formed in the cytoplasm with similar properties, in quantity comparable at all to that found in nerve cells, are those cells which form the strong proteolytic ferments for secretion, that is, in the chief cells of the fundus gland and in the pancreas cells. Do these resemblances mean anything, or is it a coincidence that similarity of function should also be associated with similarity of composition? These similarities have, I believe, a deeper meaning because all life processes in cells must have a chemical basis.... Believing that the nervous system is something more than a mere system of conducting paths, I formed the hypothesis that nerve cells are true secreting cells, and act upon one another and upon the cells of other organs by the passage of a chemical substance of the nature of a ferment or proferment from the first cell to the second.... Since discharge into other cells means the using up of formed material, it must be an exhaustible process, and the process of complete recovery at the synapse must depend on the integrity of the connexion of the synapse with the nucleus and the cell body which are the original seats of formation of the material involved in the activity. (Scott 1905.)

This is a remarkably prescient hypothesis, based upon the type of analogy we apply today between different types of secreting cells. Scott did in fact go further. By analogy with zymogen granules in the pancreas, he proposed that the proteins synthesized in the cell body of the neuron are packaged into a cell particle (Held's neurosome) before being transported to the terminals. 'When the impulse reaches the nerve ending it causes, I believe, the discharge of some of the neurosomes' (Scott 1905). The word *neurosome* might, in fact, be a suitable name for *the type of vesicle in neurons which carries proteins, that are destined to be secreted, from the cell body to the terminal.*

Where in the cell body are the proteins packaged into a particle? In most cells which secrete macromolecules, from the plant cell to the pancreas cell, the macromolecules are packaged into vesicles in the Golgi apparatus or in a closely related structure. The function of the Golgi system of membranes seems to be restricted to the packaging of materials for export from the cell (for review see Beams & Kessel 1968). This includes the lysosomal enzymes which are in a sense extracellular, being confined to what de Duve (1969) has called the exoplasmic space. The cell body of a neuron contains a readily identifiable Golgi apparatus (indeed, it was first discovered in neurons) and autoradiographic studies (see Droz 1969) have shown that it is involved in the segregation of newly synthesized proteins. Is there any evidence that the Golgi apparatus of the neuron is involved in the packaging of proteins which are destined to be secreted from the cell? In neurosecretory neurons, there is little doubt that the neurosecretory granules originate by budding off cisternae of the Golgi apparatus (see, for example, Normann 1965; Zambrano & De Robertis 1966), but in other neurons many different types of vesicle are

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found in the Golgi region, some of which are probably primary lysosomes (see Lentz 1969). One can only guess at the functions of the other vesicles, but it is noteworthy that several workers have pointed out the morphological similarity between large dense-cored vesicles in the Golgi region of sympathetic nerve cells and this type of vesicle in axons and terminals (Grillo 1966; Geffen & Ostberg 1969; Lentz 1969). As we have learnt at this meeting, the electron microscopical, fluorescence histochemical and biochemical evidence so far obtained from studies on axons of sympathetic nerves suggests that the particle carrying secretory proteins from the cell body to the nerve terminal is the large dense-cored vesicle. This is a particle whose function has long puzzled microscopists. Taxi (1961) was one of the first to draw attention to this type of vesicle, which he found not only in adrenergic nerves but in preganglionic (presumably cholinergic) nerves. Because of its morphological similarity to the neurosecretory granule, Taxi called it a 'neurosecretory vesicle'. In cholinergic nerves, these dense-cored vesicles (diameter about 80 nm) have been seen in cell bodies near the Golgi apparatus, and they accumulate above a ligature in preganglionic axons to the superior cervical ganglion (De Iraldi & De Robertis (1968). The proportion of large dense-cored vesicles relative to typical synaptic vesicles in cholinergic nerve terminals varies widely: only occasional ones are found in motor-nerve terminals, but they are more common in sympathetic ganglia (Taxi 1961; Grillo 1966) and in the splanchnic nerve (Coupland 1965; Grynszpan-Winograd 1969). What is the function of this type of vesicle in cholinergic nerves? We do not even know whether it contains acetylcholine. In view of the studies on adrenergic neurons, it will be interesting to see whether the large dense-cored vesicle also carries secretory proteins in cholinergic neurons.

Transport

Although there is now evidence that noradrenaline is transported along axons of sympathetic nerves within vesicles (see Dahlström 1971, this volume, p. 325), and that neurophysin (a component of the neurosecretory granule) is transported along axons from the hypothalamus to the posterior lobe of the pituitary gland (see Livett, Uttenthal & Hope 1971, this volume, p. 371) we know very little about the fundamental mechanisms involved. In sympathetic nerves, the vesicles (diameter about 0.08 μm) move about 1.4 μm in 1 s, which is of the same order as the root mean square displacement of a particle this size by Brownian motion. However, the migration of a noradrenergic vesicle at this rate takes place over a large distance (up to several centimetres) and in one direction; it cannot, therefore, be accounted for by random Brownian motion. The discovery of an association, and even of physical contact, between vesicles and microtubules in axons of lamprey nerve cord (D. S. Smith 1971, this volume, p. 395), as well as the finding that the transport of noradrenergic vesicles is prevented by the local application of colchicine (Dahlström 1971), support the idea that the microtubules are involved in the unidirectional movement of the vesicles.

In the lamprey nerve cord, D. S. Smith (1971) found that the vesicles in non-synaptic areas of the nerve process were morphologically indistinguishable from the vesicles in synaptic foci of the same cell. Thus, in this type of neuron it is likely that the synaptic vesicles originate in the perikaryon and are transported along the axon to the terminals. However, as already point out, the vesicles transported along the non-terminal axons of peripheral adrenergic nerves are large dense-cored vesicles, which form only a minority of the vesicles in the terminals. The vesicle typical of the terminals, the small dense-cored vesicle, probably originates locally (Geffen & Ostberg 1969). Likewise, there is no convincing evidence for the transport of typical electron-

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lucent synaptic vesicles along the axons of peripheral cholinergic neurons; indeed, as mentioned above, perhaps it is the large dense-cored vesicle found in cholinergic terminals which is transported along the axon. These findings raise the question: how are the macromolecular components of typical synaptic vesicles carried to the nerve terminals if the synaptic vesicles themselves are not transported along the axon? Perhaps the vesicles originate by budding off the cell membrane or off cisternae of smooth endoplasmic reticulum in the terminal. Another possibility is that the synaptic vesicles are derived from the larger (often dense-cored) vesicles which originate in the perikaryon. The latter hypothesis provides a convenient answer to the next question I want to discuss.

Fate of the membrane of the neurosome

From work reported at this meeting (Uttenthal, Livett & Hope 1971, p. 379) we know that the neurosecretory neurons of the posterior pituitary gland secrete not only the polypeptide

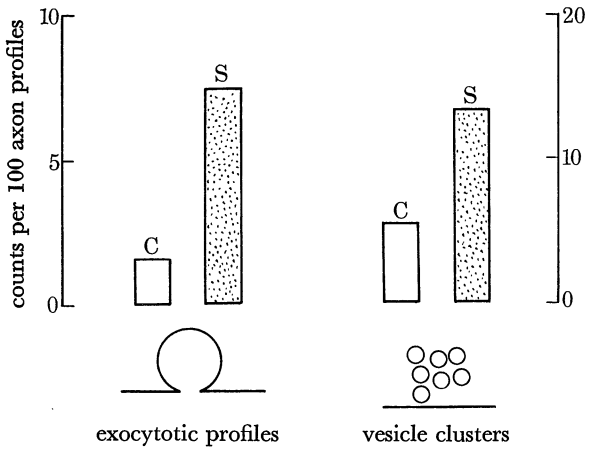

FIGURE 2. Exocytosis in the corpus cardiacum. Electron micrographs of neurosecretory nerve terminals in the corpus cardiacum of the blowfly were examined for the occurrence of exocytosis and clusters of small vesicles in control (C) flies and in flies which had been stimulated electrically by electrodes placed in the brain (S). The increased frequency of exocytosis after stimulation was significant ($P < 0.01$). Drawn from the results reported by Normann (1969).

hormones, but also a protein, neurophysin, present in the neurosecretory granules. Evidence has also been presented that proteins, as well as noradrenaline, are secreted from the splenic nerve and it was suggested that the proteins are released from the large dense-cored vesicles present in the terminals (A. D. Smith 1971, this volume, p. 363). These two particles, neurosecretory granules and large dense-cored vesicles of sympathetic neurons, are, according to the definition made above, neurosomes. After the neurosome has secreted its soluble contents by exocytosis, what happens to its membrane?

Morphological studies on neurosecretory neurons have provided valuable clues which help us to answer this question. The nerve terminals in neurosecretory organs are characterized by the presence of two types of vesicle: first, the typical neurosecretory granule and, secondly, much smaller vesicles. The function of the small vesicles has puzzled microscopists, and their similarity to synaptic vesicles led to speculations that they contained acetylcholine. However, Holmes & Knowles (1960) pointed out that the small vesicles need not contain acetylcholine, and that they might be derived from the neurosecretory granules. In confirmation of the first report by Palay (1957), several microscopists have commented on the increased number of small vesicles

relative to neurosecretory granules following stimulation. Normann (1969) studied the neurosecretory terminals in the corpus cardiacum after electrical stimulation and, as shown in figure 2, he found an increase in the number of profiles indicative of exocytosis and also an increase in the number of clusters of small vesicles. This led to the suggestion that the membrane of the neurosecretory granule disintegrates into small vesicles following exocytosis (Normann 1969), an idea which is consistent with observations on the corpus cardiacum (U. Smith 1971, this volume, p. 391) and sinus gland (Bunt 1969) which show that the small vesicles in the nerve terminals have at some time been exposed to the extracellular space. Bunt (1969) proposed that the small vesicles arose by micro-pinocytosis from membranes of the neurosecretory granules

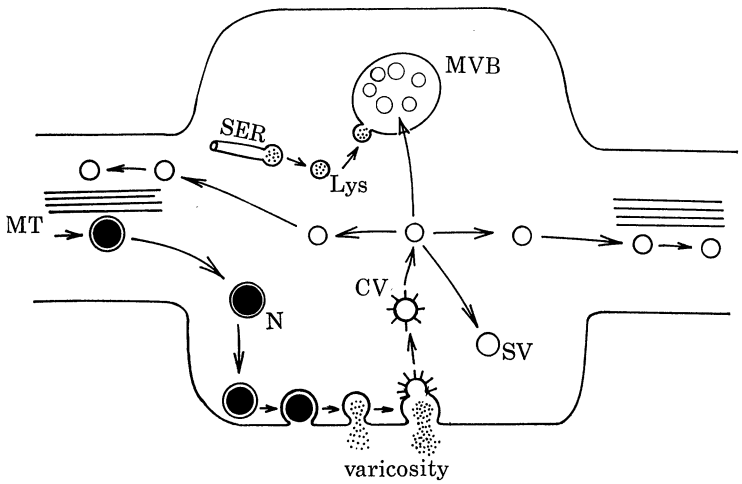

FIGURE 3. Fate of the membrane of the neurosome. The neurosome (N), a particle carrying secretory proteins from the cell body to the nerve terminal, releases its contents by exocytosis. The empty membrane may either pinch-off the plasma membrane intact (not shown) or may pinch-off to give rise to several smaller, coated vesicles (CV). The coated vesicles lose their coating, and the small vesicles so formed may either return to the cell body (towards the left), or pass on to the other variscosities (towards the right), or be degraded by lysosomal (Lys) enzymes in multivesicular bodies (MVB), or they may take up (or synthesize) the neurotransmitter substance and so become synaptic vesicles (SV). Other abbreviations: MT, microtubules; SER, smooth endoplasmic reticulum.

that were in the process of secreting their contents by exocytosis: this is illustrated schematically in figure 3. Morphological evidence consistent with this proposal had earlier been provided by Diner (1967), who found coated pits on the membranes of adrenal chromaffin granules undergoing exocytosis (see Grynszpan-Winograd 1971, this volume, p. 291). Coated pits on the membranes of secretory granules undergoing exocytosis have also been found in the crustacean sinus gland (Bunt 1969), rat anterior pituitary (Farquhar 1969, Fig. 23) and posterior pituitary glands (Nagasawa, Douglas & Schulz 1970). The presence of a coating on a pit is indicative of micro-pinocytosis, which leads to the formation of a coated vesicle. Perhaps these vesicles soon lose their coat and so become morphologically indistinguishable from the small vesicles of neurosecretory neurons. Such a mechanism of membrane retrieval may also account for the fate of the membranes of large dense-cored vesicles in the terminals of cholinergic and adrenergic neurons, since empty membranes the same size as the original vesicle are rarely found.

What happens to the small vesicles which are derived from the membrane of the neurosome? Four possible fates are shown in figure 3: (i) the vesicles might pass on to other varicosities, or

(ii) they might return to the perikaryon to be degraded, or, (iii) the vesicles might be degraded locally by incorporation into multivesicular bodies, which would become autophagic vacuoles after fusion with primary lysosomes derived from the endoplasmic reticulum (see Holtzman 1971, this volume, p. 407). Finally (iv) is it possible that the morphological similarity between the small vesicles and typical synaptic vesicles means that the two particles are identical? If the ability of a synaptic vesicle to synthesize and store the transmitter depends only on properties of its membrane, there is no need for these particles to contain soluble proteins. It has already been pointed out that some of the properties of the small noradrenergic vesicles in sympathetic nerve terminals are consistent with their origin from the membrane of the large noradrenergic vesicles (Smith 1970, 1971).

The postsynaptic cell

The implications of exocytosis from the neuron for the tissue that is innervated concern not only the neurotransmitter substance, but the other substances released from the nerve. In particular, we want to know what the fate and functions are of the proteins released from the nerve. As we have heard at this meeting, there is evidence that nerve terminals can take up exogenous proteins (Holtzman 1971; U. Smith 1971) and this raises the question whether, in the close confines of some synapses, a proportion of the protein secreted returns to the nerve terminal. If so, the protein might be taken up into typical synaptic vesicles (Brightman 1968) or into coated vesicles which bud off the plasma membrane. As Holtzman (1971) showed, the proteins taken up by coated vesicles are ultimately digested by lysosomal enzymes. Before dismissing this as of little physiological significance, we should remember that biologically active substances (thyroid hormones) are secreted from the thyroid gland following the digestion in lysosomes of thyroglobulin taken up by endocytosis (see Wollman 1969).

We can only speculate about the possible extraneuronal functions of the proteins released from the neuron. These proteins could influence the postsynaptic cell by interaction with the cell surface, or, following uptake into the cell by endocytosis, they may possibly influence events deeper in the cell (see figure 4). Electron microscopical studies have shown that coated invaginations are a general feature of postsynaptic membranes in the central nervous system and that these coated pits can take up exogenous colloidal material which is recovered intracellularly in coated vesicles (Waxman & Pappas 1969). The fate of these coated vesicles will determine what influence the extracellular material may have on the cell: the most likely fate is digestion in lysosomes, but other subcellular pathways may also exist. At present we cannot correlate these ultrastructural observations with any known function of the neuron. However, the fact that a neuron can markedly influence cells which it innervates, and the surrounding Schwann cells, is well known from the results of denervation experiments. These experiments led to the concept of the trophic function of the neuron, and to the idea that a trophic substance is released from the nerve (see review by Guth 1968). There are reasons why the trophic effect at neuromuscular junctions cannot be due solely to the neurotransmitter (Gutmann 1970) and there is some evidence that the trophic substance involved in the nerve-dependent regeneration of the newt's limb may be a protein (Lebowitz & Singer 1970). Denervation of receptor organs is often followed by degeneration of the sensory cells (for a review see Zelená 1964) and here, of course, the trophic material liberated from the sensory nerve is most unlikely to be the transmitter substance.

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Have our discussions on the neuron any relevance to these trophic phenomena? I suspect that they may have, since we are no longer bound by the view that the nerve only secretes a transmitter substance; it can also secrete proteins, whose biological activities are likely to be no less specific than those of the neurotransmitter but may be more subtle. We should be aware of the possibility that secretion of a protein by a nerve may be unrelated to neurotransmission. It is noteworthy that vesicles, with and without dense cores, are found in nerve terminals where transmission is solely electrical (Pappas & Bennett 1966) and in the terminals of sensory neurons

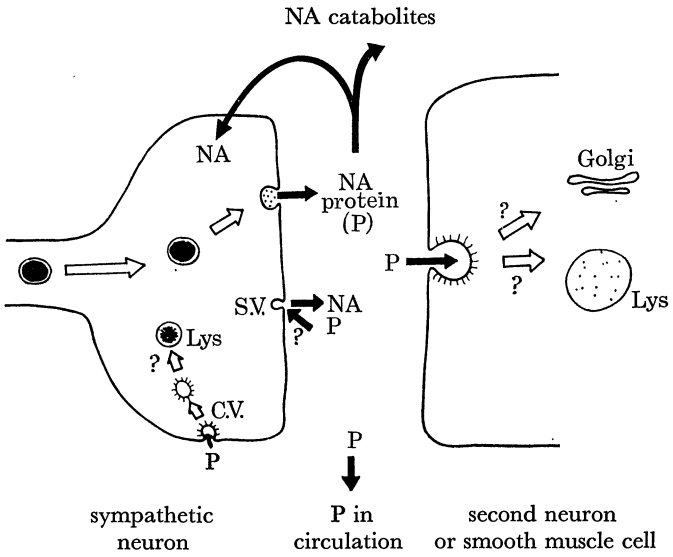

FIGURE 4. Fate of the protein secreted from a sympathetic neuron. Proteins (P) released from the neurosome (in this case, the large dense-cored vesicle) may either be taken up into nerve terminals or into postsynaptic cells, or they may pass into the circulation. Whereas uptake of noradrenaline (NA) probably takes place across an intact membrane, uptake of protein occurs by endocytosis into coated vesicles (CV). (From Smith 1970.)

in several receptor organs, e.g. in cutaneous receptor organs (Szamier & Wachtel 1970) and in muscle spindles (Adal 1969). Are some of these vesicles neurosomes, or the empty membranes of neurosomes? It should soon be possible to answer this question, since it has been found that sensory neurons transport newly synthesized proteins at a rapid rate (41 cm/day) away from the cell body, i.e. in a direction opposite to that of the nerve impulses (Ochs, Sabri & Johnson 1969). Much of this rapidly transported protein was found to be present in the microsomal fraction of nerve homogenates, and so may have been stored in vesicles. Where does this vesicle-bound protein go when it reaches the nerve endings? As we have heard at this meeting, two of the rapidly transported, vesicle-bound, proteins in sympathetic neurons are chromogranin A and dopamine β-hydroxylase; the same two proteins are released from the terminals of the splenic nerve. Perhaps the rapidly transported, vesicle-bound proteins of sensory neurons are released from the nerve endings in the receptor organ.

CONCLUSIONS

The realization that neurons have several features in common with other secretory cells has raised many new questions concerning the dynamics of the nerve cell and the role of the

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secreted substances. However, there is every chance that further application of the biochemical, ultrastructural and electrophysiological approaches to subcellular and molecular interactions will eventually lead to a deeper understanding of the many-sided activities of the nerve cell.

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