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Biochemical Specificity in Neuronal Function

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Our present-day knowledge of the specific biochemical abilities of neuronal tissue can be traced back to two historical sources, the theory of the humoral transmission of nervous impulses, first formulated by O. Loewi in 1921, and the concept of neurosecretion, i.e. release of hormones from neurons, introduced by E. Scharrer & W. Bargmann (see Scharrer & Scharrer, 1963).

Both these concepts have in common the idea that certain kinds of neurons differ from others in being able to produce and to release specific effector substances, mediators or hormones. The element common to these two concepts is that of biochemical diversity of neurons.

The catecholamines as effector substances are of particular interest to the student of these specific abilities. In the neurons that we now call adrenergic, among the first studied by O. Loewi, the catecholamines serve as mediators, and these amines are also functioning as hormones, released from the chromaffin cells, e.g. those of the adrenal medulla.

For the study of the specific biochemical abilities of the adrenergic neurons, their close relationship to the chromaffin cells of the adrenal medulla has been of particular importance. Many of the specific proteins, to be discussed below, were first isolated and characterized from the adrenal medulla, where it is relatively easy to obtain a homogeneous material for biochemical studies. The experience gained with material from the adrenal medulla could be used in the analysis of the adrenergic neurons, where anatomical complexity and scarcity of material makes the isolation of pure proteins much more difficult. This relationship explains why today our knowledge of the specific biochemical abilities of the adrenergic neurons is much better than that of any of the other specific systems present in neuronal tissues (see also Smith, 1972).

The specific biochemical properties of neurons that I want to discuss here are related to two phenomena, the reception of impulses and the release of transmitters.

Since in the nervous system there occurs a great number of transmitters, the neurons differ in their equipment with mechanisms for receiving specific chemical stimuli; they also differ in mechanisms that enable them to terminate the effects of the various transmitters. Since different kinds of neuron release different transmitter substances, these neurons differ in the presence of various catalysts essential in the elaboration of these transmitters.

In the present Lecture I shall restrict myself to the discussion of specific proteins present in adrenergic neurons. These proteins exert their function in the main in those areas where stimuli are received and where the transmitter is released. These regions may be far removed from the sites of protein synthesis.

Thus there are two main locations of these proteins in the tissues. We can distinguish
"soma-related" and 'ending-related' proteins. The proteins that we call soma-related occur close to the cell membrane, including the membrane of the dendrites. In the chief ganglion cells of the superior cervical ganglion, most of the endings of the pre-ganglionic neurons seem to lie on the dendrites (see Tamarind & Quilliam, 1971). In mammalian neurons release is usually restricted to the axonal endings, and it is in this area that the ending-related proteins become operative. It is true that catecholamine synthesis also occurs in other parts of the neuron, but quantitatively it is the ending-related formation of catecholamine that is significant.

The classical picture of the sympathetic ganglion was established about 40 years ago. This picture was a simple one, in which the ganglion cell was activated by the release, from the endings of the preganglionic neuron, of acetylcholine. An impulse was set up that travelled down the axon to the nerve endings, where it resulted in the release of the transmitter.

This picture accounted for the presence of a number of proteins in the postganglionic neuron. At the level of the cell soma the impulse was received by a receptor, the classical nicotinic receptor. Also, there was present in the neighbourhood of these receptors the catalyst responsible for the biological inactivation of the preganglionic transmitter, acetylcholinesterase (EC 3.1.1.7). At the level of the axonal endings this picture accounts for the presence of all the catalysts responsible for the formation of the postganglionic transmitter, noradrenaline.

In recent years this simple picture has been complicated by new discoveries. These have added to our knowledge of the organization of these neurons and at the same time they have contributed to our knowledge of the presence and distribution of specific proteins in the postganglionic neurons. Among these new findings there are some that relate to the cell soma (and dendrites) and others that relate to the axonal endings.

**New Findings Relating to the Cell Soma**

At the level of the cell soma, the chief new finding is the recognition that acetylcholine is not the only transmitter that acts on the chief ganglion cell. It has been known for a long time that the ganglion contains cells that have variously been described as small ganglion cells or as chromaffin cells. There is reason to believe that these cells may represent a heterogeneous mixture. However, one kind of cell that has been particularly studied in recent years is a cell described by Eränkö & Eränkö (1971) as a 'small intensely fluorescent' cell. The fluorescence, characteristic of a catecholamine-containing cell, is due to an amine identified as dopamine (3,4-dihydroxyphenethylamine) in some locations. It is presumably this type of cell that has been studied also by electron microscopy (Williams & Palay, 1969; Matthews & Raisman, 1969; Taxi et al., 1969). These investigations have shown that the cells have synaptic contacts, both afferent and efferent ones. The afferent synapses are between the endings of the preganglionic neurons and the cell bodies of these cells; these endings contain accumulations of clear vesicles. The efferent synapses are between the small cells and the chief ganglion cells and their dendrites, and in these synapses one sees, within the axoplasm of the small cells, an accumulation of vesicles characterized by the presence of a dense core. These dense-cored vesicles are of a size intermediate between that of the dense-cored vesicles of postganglionic adrenergic neurons and that of the granules found in chromaffin cells, e.g. in the adrenal medulla. From this description one concludes that these small cells are in some respects intermediate between true ganglion cells and true chromaffin cells. The efferent synapse may be located either on a very short axon or on the cell itself. Thus these cells are ganglion cells that direct their message not indiscriminately but towards an adjacent effector cell. So for the purpose of this discussion we can consider them as internuncial nerve cells which provide a second route for an impulse reaching the chief ganglion cell. The postganglionic neuron receives messages not only directly from the preganglionic endings, mediated by acetylcholine, but also from the internuncial neurons, mediated by a catecholamine, presumably dopamine. There is evidence that the activation of these dopaminergic neurons mediates an effect that can be considered as opposed to the depolarizing action of acetylcholine on the same postganglionic
neuron. This effect may be related to an increase in cyclic AMP (see Greengard & McAfee, 1972).

It seems worth while mentioning here that these cells may have actions that are more in keeping with 'neurosecretory' cells. Various authors have seen fibres emanating from the 'small intensely fluorescent' cells that seem to end in the vicinity of blood capillaries in the ganglion (Siegrist et al., 1968; Matthews & Raisman, 1969; Eränkö & Eränkö, 1973). In other words, it is possible that some of the catecholamine is released into the blood stream, where it may reach also the preganglionic endings. Dun & Nishi (1974) have adduced evidence according to which dopamine also affects the rate of firing at the level of the preganglionic endings. As far as is known, the internuncial cells do not establish any synaptic contacts with the preganglionic endings that would transmit towards the latter; it seems possible, therefore, that any dopamine may reach the preganglionic endings by way of the blood stream.

New Findings at the Level of the Postganglionic Axonal Endings

A multitude of new findings has added to our knowledge of the proteins present and active in the axonal endings.

1. The factors that terminate the response of the effector cell to the released transmitter have been recognized in the last decade. It is known that some of the noradrenaline is disposed of by enzyme action, and in particular, the inhibition of the enzyme catechol O-methyltransferase (EC 2.1.1.6) may cause prolongation and enhancement of the response of the effector organ. These effects are not yet fully analysed (see Trendelenburg, 1972). However, the adrenergic neuron differs from the cholinergic neuron in that destruction by enzyme action is not the main process by which the effective concentration of the transmitter in the neighbourhood of the effector cell is decreased. This is chiefly brought about by a removal of the transmitter, most of which is taken up, some of it by the effector cells, but in many locations the chief means of removal is by 're-uptake', that is by an uptake of the amine released into the nerve endings. The noradrenaline is actively taken up into the axonal cytosol, and the noradrenaline thus taken up into the cytosol is then removed from it by a second uptake process into the storage organelles for noradrenaline.

2. It is now known that the release of transmitter is not the only event that occurs when the neuron is stimulated. The transmitter is accompanied into the extracellular space by a number of satellite compounds that are released at the same time. These compounds include ATP and its hydrolysis products, and also a number of soluble proteins. These soluble proteins are all present also in the chromaffin granules of the adrenal medulla, and their release from the adrenal medulla either after administration of acetylcholine or carbachol (Banks & Helle, 1965; see Kirshner & Kirshner, 1971) or on stimulation of the splanchnic nerve (Blaschko et al., 1967), preceded observations on their release from stimulated sympathetic nerves (Geffen et al., 1969; De Potter et al., 1969).

3. In many tissues with autonomic innervation there occurs a close juxtaposition of adrenergic and non-adrenergic, presumably cholinergic, neurons. The electron microscopists have described 'tight junctions' between the adrenergic and the non-adrenergic endings (Thoenen et al., 1966; Ehinger et al., 1970a). There are sites where the origin of the cholinergic fibres has been traced to the parasympathetic ganglion, e.g. in the iris, where acetylcholine disappears after the removal of the ciliary ganglion (Ehinger et al., 1970b). However, Burn (1974) has pointed out that this juxtaposition has not been demonstrated in all autonomically innervated tissues.

4. It is now known that the nerve endings not only release the transmitter, but that the amount of transmitter released can be modified by substances that act on the endings from the extracellular space. These effects are believed to be mediated by receptors situated on the axonal membrane, but the mechanism by which this modulation of transmitter release is brought about is not yet clear. Three classes of such nerve-ending receptors have been described, acting, it seems, independently of each other: receptors for catecholamines, for acetylcholine, and for prostaglandin. Two distinct receptors for acetylcholine have been described, of which the one better studied appears to be a
muscarinic receptor acting by diminishing transmitter output. Likewise the catechol-
amine and the prostaglandin receptors act by diminishing transmitter output.

Specific Proteins

The carriers of the specific neuronal functions are a number of specific proteins. It
must be emphasized that specificity is not necessarily absolute. For instance, acetyl-
cholinesterase occurs also in cholinergic neurons, and monoamine oxidase (EC 1.4.3.4)
and aromatic amino acid decarboxylase both are known to be present in tryptaminergic
neurons.

All the specific proteins are, as far as we know, synthesized in the cell soma, under the
influence of nuclear genes, in the perikaryon. They then travel to the sites where they
become functional, and these are mainly the cell membrane and the axonal endings.
Thus, there appears to be a control mechanism within the ganglion cells that directs
proteins, which may be the cell organelles that they are associated with, either towards
the membrane of the cell and the dendrites or down the axon. That the proteins which
reach the axonal endings travel down the axon can be demonstrated by ligating the
nerve. The proteins then accumulate above the ligature. The speed of travel of different
proteins differs; it depends on the rate of movement of the cell compartment or cell
organelle in which they are present.

The axon therefore has a dual function. It serves the common function of conducting
the impulse and it also serves as the channel that connects the site of synthesis of the
specific proteins with the axonal endings.

(A) Biosynthetic Enzymes

Early work on catecholamine synthesis began with the finding that the enzyme
L-dopa (3,4-dihydroxyphenylalanine) decarboxylase (EC 4.1.1.28) discovered in the
mammalian kidney (Holtz et al., 1938) was unable to accept N-methyl dopa as substrate
(see Blaschko, 1973, 1974a). This pointed to the occurrence of primary amines, such as
dopamine or noradrenaline, as precursors in the biosynthesis of adrenaline, and it also
gave a reasonable interpretation for a role, which had already been discussed, of
noradrenaline as a possible transmitter substance in the sympathetic nervous system.

The biochemical explanation for the inability of the decarboxylase to act on N-methyl
dopa was discovered when it was found that the enzyme was a pyridoxal enzyme. Up to
the present time, no interaction of a pyridoxal enzyme with a secondary amine has been
described. L-Dopa decarboxylase was discovered in high concentration in chromaffin
tissue by Langemann (1951) and subsequently also in neuronal tissue (see Holtz &
Palm, 1966).

The main pathway of catecholamine biosynthesis is well established.
L-Tyrosine → L-dopa → dopamine → noradrenaline → adrenaline

Subsidiary pathways have been discussed on several occasions, but they have not yet
been securely established.

Each step in the catecholamine pathway is catalysed by its own enzyme, and all these
catalysts have been characterized and extensively studied. It is not proposed to go over
this well-reviewed field again.

In neuronal tissue it appears that each neuron contains those enzymes that are
required for the formation of its transmitter from the amino acid precursor L-tyrosine.
The distribution of the different biosynthetic enzyme differs in the three kinds of 'cate-
cholaminergic' neuron that can be distinguished: 'dopaminergic', 'noradrenergic'
and 'adrenergic'. The term 'adrenergic', generally employed for neurons that release
either noradrenaline or adrenaline, is here used in a narrower sense, to describe those
neurons that make use of adrenaline as the transmitter.

These 'adrenergic' neurons contain all the enzymes of the biosynthetic pathway; these
include the peripheral sympathetic fibres of amphibia where adrenaline serves as
the transmitter. Also, there have recently been detailed studies of the 'adrenergic'
neurons in the central nervous system and their enzyme content. Small amounts of
adrenaline were already discovered by Vogt (1954) when ‘brain sympathin’ was first described. Recently the occurrence of the enzyme phenylethanolamine N-methyltransferase (EC 2.1.1.28) in the rat brain has been studied, by using the method of immunofluorescence microscopy (Hökfelt et al., 1973a, 1974). In this work, as in most other immunohistochemical studies of the biosynthetic enzymes of the catecholamine pathway (see Hökfelt et al., 1973b), the bovine adrenal medulla served as the starting material for purification. This is possible, since the antisera against the purified bovine enzymes cross-react with enzyme from other species. The presence of phenylethanolamine N-methyltransferase was demonstrated in the region of the pons and the medulla oblongata. The fluorescence was restricted to a relatively small number of neurones, with the cell bodies, dendrites, axons and endings clearly delineated by fluorescence. The occurrence of phenylethanolamine N-methyltransferase in the rat brain has since also been studied by a conventional enzymological technique (Saavedra et al., 1974); the results obtained are in satisfactory agreement with the immunohistochemical findings.

The pictures shown by Hökfelt et al. (1974) are of particular interest to the student of biochemical specificity. They show that it is possible, by morphological as well as by biochemical techniques, to obtain information on the distribution of a macromolecule specific to one class of neuron.

The immunohistochemical method gives no evidence of the presence of the N-methylating enzyme in the postganglionic sympathetic neurones in which noradrenaline is the transmitter, but the enzyme that converts dopamine into noradrenaline, dopamine β-hydroxylase (EC 1.14.17.1) is present (Fuxe et al., 1971). According to Fuxe et al. (1971), dopamine β-hydroxylase also occurs in the central noradrenergic neurones, but it is absent from the central dopaminergic neurones.

There has been much work on the overall regulation of catecholamine biosynthesis. This work has been repeatedly reviewed in recent years (see Cotten, 1972; Thoenen, 1972). Since it is known that the bulk of the catecholamine is synthesized in the axonal endings, there is a time-lag between the formation of new enzyme in the cell soma and its arrival at the neuronal endings. The rate of migration of such enzyme appears to depend on the cell compartment in which it travels. Thoenen et al. (1973) have studied the rate of migration of the three biosynthetic enzymes in a peripheral adrenergic nerve, the rat sciatic nerve. It is interesting that dopamine β-hydroxylase migrated faster than either tyrosine hydroxylase (EC 1.14.16.2) or dopa decarboxylase. It is known that dopamine β-hydroxylase is associated with the storage organelles for noradrenaline, the only enzyme for which such a location has been established. These organelles exhibit the fastest rate of transport in the axon. Interestingly, in these studies the amount of enzyme that sedimented with the cell organelles was greater at the level of the endings than at the levels of either the cell soma or the axons, suggesting the possibility that the association of the enzyme with the cell organelle is still incomplete in the region of the cell soma and remains so until the endings are reached. This interpretation is supported by the observation that when the nerve is ligated, the bulk of the enzyme accumulating above the ligature is sedimentable, as at the axonal endings. Thus, the central stump is in some ways similar to a normal axonal ending.

(B) Satellite Proteins

The physiological significance of this second class of neuronal protein is not yet clear. In Oxford, we became interested in the protein chromogranin A, because it represents the largest fraction of the soluble proteins in the chromaffin granules, and because we assumed that it was involved, together with ATP, in the retention of the catecholamine within the chromaffin granule. Although much work has been carried out on this protein, no catalytic activity has been found that is associated with it. A complicating factor is that, in contrast with the biosynthetic enzymes, the interspecies reactivity of chromogranin A as an antigen is rather restricted. Most of the observations on protein release from adrenergic nerves therefore have been carried out on the soluble fraction of the enzyme dopamine β-hydroxylase. This enzyme occurs both in insoluble form, in the membranes of the storage organelles, and in soluble form, within these organelles.
The release of the satellite proteins has received much study in recent years, because their appearance in the extracellular space has supported the idea that their liberation occurs by a process of exocytosis. This is true notably for the chromaffin cells but also for the axonal endings, where these proteins are released, together with the mediator (see Blaschko & Smith, 1971).

Release of catecholamines from adrenergic nerve endings cannot readily be assessed because some of the amine released is removed by uptake. Therefore the liberation of satellite protein has received much attention in the past few years, in the hope that it might be useful as an index of sympathetic tone. For practical reasons, already discussed, it is the enzyme dopamine β-hydroxylase that has been mainly used in the study of sympathetic tone in man (Freedman et al., 1973; Weinshilboum et al., 1973; Geffen, 1974).

(C) Inactivating Enzymes

Acetylcholinesterase

This enzyme has long been known to be associated with membranes, which is in keeping with the idea that acetylcholine is inactivated at the level of the cell membrane. In the postganglionic sympathetic cell the enzyme can be shown to be present in the membranes of the dendrites and the cell soma, a location that is close to the receptors for acetylcholine. Kreutzberg et al. (1973) have pointed out that the enzyme makes its way from the perikaryon to the membranes of cell and dendrites. In these locations, where it must be presumed to become operative, it appears to be present on the outer surface of the membrane, and this seems to be the area where acetylcholine is hydrolysed. If the internuncial neurons receive innervation from the preganglionic neurons one would expect these cells also to have acetylcholinesterase close to the synapses formed between the preganglionic endings and these internuncial neurons, but I am not aware of any study of the enzyme in this location.

However, it is also known that in the postganglionic sympathetic neurons not all of the acetylcholinesterase is soma-related. The esterase also travels down the axon. The accumulation of the enzyme above a ligature has been studied in peripheral neurons. In recent years the presence of an ending-related acetylcholinesterase in postganglionic sympathetic neurons has also been described (see Eranko et al., 1970). Here again the location of the enzyme is in the membrane of the axonal endings.

It seems to me that the presence and location of the esterase fits in well with what is known about receptors for acetylcholine on the axonal endings. Where a juxtaposition of cholinergic and adrenergic endings occurs it would appear that the acetylcholine that would be hydrolysed by the nerve-ending acetylcholinesterase is that released from the nearby cholinergic neurons. It would be of interest to know if the fine localization is such as to suggest that the esterase is close to the sites where acetylcholine could act upon the axonal endings from the outside.

We can say that acetylcholine belongs to those proteins for which two locations exist where it is functional. We distinguish soma-related and ending-related acetylcholinesterase in the postganglionic neurons.

Monoamine oxidase

The other inactivating enzyme I wish to discuss is monoamine oxidase. I have been interested in this enzyme for almost 40 years; in collaboration with Derek Richter and Hans Schlossmann I described its action upon adrenaline, noradrenaline and dopamine.

Monoamine oxidase has, in contrast with acetylcholinesterase, an intracellular location, on the outer membrane of the mitochondria. For a long time it remained a puzzle as to how to reconcile the knowledge of such a localization with the idea of an inactivating role for the oxidase. This situation was not resolved until it was recognized that uptake and re-uptake of amines were important in their biological inactivation. The oxidase, we now know, acts upon amine that has been taken up, chiefly by the axonal endings but to some degree also by the effector cells. The preponderance in many
tissues of re-uptake into the axons accounts for the fact that, in contrast with acetylcholinesterase, monoamine oxidase is primarily a pre-synaptic enzyme. The oxidase acts in the cytosol of the axonal endings upon amine before the latter has reached the shelter offered by the storage organelles.

The study of monoamine oxidase has been actively pursued for over 40 years (for a recent review, see Blaschko, 1974b). During this period there has been an enormous expansion of our knowledge of the role and distribution of biogenic amines in the nervous system. Adrenaline, noradrenaline and dopamine as well as 5-hydroxytryptamine are all substrates of monoamine oxidase, and all these amines occur in both the central and the peripheral nervous system.

There has, in recent years, been much discussion on the isoenzymes of monoamine oxidase. It is generally agreed that there are multiple forms of the enzyme (see Youdim, 1974; Houslay & Tipton, 1974). However, it is not yet known if these multiple forms represent different chemical entities or different arrangements of one and the same protein molecule. Either explanation could account for the differences in substrate specificity that have been observed.

If we say that monoamine oxidase is specifically associated with adrenergic neurons we must remember that specificity is relative; other neurones also contain the enzyme. However, there is satisfactory evidence to show that sympathetic denervation lowers the monoamine oxidase content in a number of tissues (see Jarrott & Iversen, 1971). There is also histochemical evidence for such an association, namely monoamine oxidase accumulation in the central stump of a ligated nerve (see Dahlström, 1972), and this is paralleled by an accumulation of the mitochondria (Banks & Mayor, 1972). McLean & Burnstock (1972) have found that accumulation of the oxidase did not occur after the sympathetic chain had been removed.

There have been observations that suggest that the neuronal monoamine oxidase differs from that of the tissue cells that are sympathetically innervated (Jarrott, 1971). Also, there may be more than one kind of the enzyme in the central nervous system (Eiduson, 1972; Neff et al., 1973; Youdim, 1974). This brings me to a discussion of the different locations of monoamine oxidase. In the peripheral tissues that receive sympathetic innervation the enzyme occurs in both the effector cells and the axonal endings. These two locations are presumably co-ordinated to extraneuronal and neuronal uptake respectively. In the postganglionic neurone there is the possibility that, in addition to the axonal endings, there may be another location where the oxidase functions. We know now that the ganglion cells receive messages not only from the pre-ganglionic neuron but also from the internuncial cells, and the latter release a catecholamine. In other words, the ganglion cell is also an 'aminoceptive' effector cell. One would expect, therefore, the cell also to have a soma-related monoamine oxidase that deals with any amine, such as dopamine, that is taken up. Thus there may be in the postganglionic neurons, two locations where this mitochondrial enzyme is operative, and a mechanism must exist that directs enzyme-carrying mitochondria to these two locations.

In addition to these sites of the oxidase, it seems very probable that the internuncial neurons also contain the enzyme. Such an enzyme is likely to act upon catecholamine that has entered the cell by the counterpart of what is known as re-uptake at the peripheral endings.

To make the list of possible sites of monoamine oxidase complete, it should be added that the oxidase is also found in glia or satellite cells. All these sites of monoamine oxidase are listed as follows.

1. Effector-cell enzyme.
2. Ending-related enzyme.
3. Soma-related enzyme.
4. Internuncial-cell enzyme.
5. Glial (or satellite-cell) enzyme.

Most of these various kinds of enzyme occur in different cells; they might therefore show
slight differences, since differences are known to exist between enzymes from different organs. However, the two enzymes listed under (2) and (3) occur in one neuron, although the substrates of the ending-related oxidase would be noradrenaline, and that of the soma-related oxidase presumably dopamine.

A study of the type of monoamine oxidase in the superior cervical ganglion of the rat has been carried out by Neff & Goridis (1972); according to these authors about 90% of the total amount of the activity in the ganglion represents isoenzyme type A in Johnston's (1968) nomenclature.

I should like to add one general remark that relates to both acetylcholinesterase and monoamine oxidase. It is clear from what has been said that it is unsafe to base conclusions as to the chemical specificity of a neuron on the presence of a transmitter-inactivating enzyme. The postganglionic neurons are not cholinergic, and yet they contain acetylcholinesterase. Similarly, there is no reason why monoamine oxidase should be restricted to aminergic neurons.

(D) Uptake Proteins

Nothing is known about the chemistry of these proteins or about the way by which they reach the axonal endings. Pharmacological evidence strongly suggests that the uptake at the level of the axonal membrane, the 'Uptake 1' of Iversen (1974), is distinct from the uptake of amine into the storage organelles. To what extent any differences are due to the presence of different molecules or due to the fact that similar compounds have different properties imposed upon them by different environments, we cannot tell.

There is as yet no evidence of a soma-related transport mechanism for catecholamines in adrenergic neurons. However, if the ganglion cell is exposed to dopamine, there might be an uptake of it, analogous to 'Uptake 2' (Iversen, 1974) at the level of the effector cell.

(E) Receptor Proteins

The biochemical study of receptors is still in its initial stages. In the main, receptors are defined in pharmacological terms only.

As already mentioned, the postganglionic sympathetic neuron has two distinct receptor areas. Both the cell soma and the axonal endings have receptors for acetylcholine as well as for catecholamines.

Soma-related receptors for acetylcholine are of two kinds, muscarinic and nicotinic, and the same is true for the acetylcholine receptors of the axonal endings (see Kosterlitz & Lees, 1972). However, at the level of the cell soma the nicotinic receptors are the more prominent ones, whereas at the axonal endings the muscarinic receptors are more readily demonstrable. If the receptor proteins originate in the perikaryon of the ganglion cell, it seems likely that there exists a mechanism that directs these substances either to the cell membrane or to the nerve endings.

Similarly one distinguishes two kinds of receptors for catecholamines, the \( \alpha \)-receptors and the \( \beta \)-receptors. These receptors were also at first defined by pharmacological criteria, but there is much evidence that connects the \( \beta \)-receptors with the activation of the enzyme adenylate cyclase (EC 4.6.1.1) (for a recent review see Lefkowitz, 1974). In the soma-related receptors of the sympathetic neurons this is also the mode of action of dopamine; the amine increases the amount of cyclic AMP in the ganglion (Greengard & McAfee, 1972). On the other hand, at the axonal endings the pharmacological evidence suggests that the receptors for catecholamines are similar to \( \alpha \)-receptors, since the effects of noradrenaline (and related amines) are blocked by typical \( \alpha \)-receptor blocking agents, e.g. phenoxybenzamine (see Langer, 1974).

How the activation of the ending-related receptors modifies transmitter output is not known. Also, the source of the substances that activate these receptors is still under discussion. It seems most likely that these sources are different for the three substances for which receptors have been found at the endings: for catecholamine it is the axonal adrenergic endings that are the source, for acetylcholine it has been suggested that it is...
the adjacent cholinergic endings, and for prostaglandins it is the effector organ that synthesizes and releases them when it is active.

A discussion of the receptors specific to the adrenergic sympathetic neurons would be incomplete without mentioning the nerve growth factor (see Levi-Montalcini & Angeletti, 1958). The factor specifically accumulates in the sympathetic ganglia (Angeletti et al., 1972), and it has been found that the factor is selectively bound to a crude microsomal fraction derived from the ganglia (Banerjee et al., 1973). However, the exact location of these ‘receptors’ for the growth factor in the neuron remains to be established.

(F) General Remarks

The brief account of the various specific proteins has shown that a few common features are shared by all, or at least many, of these substances. The proteins exert their functions chiefly in two widely differing situations, and there are several of them, e.g. monoamine oxidase, that are receptors for both acetylcholine and catecholamines, and which display differences in the two locations. In each instance the question arises: are these differences due to an inherent difference in chemical composition, or are they due to different influences exerted by the micro-environment in which these substances become functional? These questions still remain unanswered.

Morphology and biochemical function

As the results of both fluorescence and immunofluorescence studies have shown, a biochemical mapping of specific neurons is now an established tool of neurochemical study. There is little doubt that the successes of these two methods in the study of adrenergic mechanism will soon be followed by similar work on systems exhibiting different methods of biochemical specificity. Also, in the study of adrenergic mechanisms there are possibilities that have not yet been fully exploited. For instance, the study of monoamine oxidase by electron microscopy (Boadle & Bloom, 1969) is only in its beginnings.

The specific biochemical properties have not only functional correlates, but they may also determine morphological features. This is due to the fact that each of the specific proteins is linked to one characteristic cell compartment, the cell membrane, the cytosol, the reticulum, the mitochondria or the storage organelles. The specific biochemical properties also determine morphology in a more general way. For instance, Arnold & Holtzman (1973) have reported that the adrenergic sympathetic neurons are specifically rich in peroxisomes, cell organelles which are scarce in other ganglion cells. The peroxisomes are the carriers of the enzyme catalase (EC 1.11.1.6) and it is suggested that the adrenergic neurons are so rich in this organelle because they contain a number of specific enzymes that catalyse reactions in which hydrogen peroxide arises as one of the products of the enzymic reaction. Such enzymes are monoamine oxidase and dopamine β-hydroxylase. Thus the specific biochemical ability here finds its counterpart in a specific morphological feature.

Common and specific biochemical abilities of neurons

At the outset I defined what I call the specific biochemical abilities of neurons. The adrenergic neurons carry a multitude of these abilities. In addition, these neurons possess all those abilities that they share with all other neurons. These common abilities, connected with the conduction of the impulse along the axon and with the recovery processes, must be integrated in some way with the specific biochemical events, but of this integration little is known at present. At the level of the receptors I have mentioned the link between β-receptors and adenylate cyclase; here one may see the beginnings of an analysis of such a connection. At the level of the axonal endings, we must postulate a link between the activated axonal membrane the organelles that contain the transmitter; Ca2+ ions are known to be involved in that link.

The integration of common and specific functions also becomes obvious when we
look at the cell compartments or cell organelles present in the neuron. Of the morphologically distinct organelles, I should like to take the mitochondria as an example. The mitochondria are the sites of synthesis of ATP, and the latter serves common functions, e.g. in maintaining ionic disequilibria at the axonal membrane. ATP also serves as the source of energy for the specific uptake processes at the axonal endings. Although this is not yet certain, the ATP found in the storage organelles for catecholamines may be of mitochondrial origin. Also, the mitochondria are the carriers of the specific enzyme, monoamine oxidase, which exerts its function at the axonal endings and possibly also at the level of the cell soma. Thus, at different stages of their life the mitochondria serve different functions.

The picture that arises of the close interdigitation of functions in the various neuronal compartments is in keeping with modern ideas on the composition of membranes in living tissue. The membrane has a multitude of differing protein molecules built into it, and such a mosaic would account for the fact that a great number of different functions are carried out in close proximity.

Conclusion

This lecture is devoted to a pioneer in neurochemistry. When one looks at Thudichum's achievements, one is impressed by their great number, and by the fact that so many of his discoveries have been of permanent value.

I have one common link with Thudichum. We both came from Germany, and we both settled in England to escape from an authoritarian regime to a liberal country. However, here the analogy ends. Thudichum remained unknown, or at best a controversial figure until long after his death. I have been more fortunate, in many ways. I have been working in a field that has been of interest to others ever since I started my work in Cambridge in 1934, and later in Oxford I have had the great satisfaction of having a stream of colleagues in my laboratory. To these we owe many of the gains that I have discussed, and they are now carrying on where I left off.

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