VII. TRANSMISSION IN THE CENTRAL NERVOUS SYSTEM AND SENSORY TRANSMISSION

CENTRAL AND SENSORY TRANSMISSION

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In considering the mechanism of central and sensory transmission we are faced with the problem that, whatever our bias, we cannot state with certainty whether the transmission is chemical or electrical. But, according to which view we hold, our approach will be different. Anyone who takes it for granted that transmission is electrical, assumes that the phenomenon can be fully dealt with by analyzing the electrical changes in the central nervous system, and such an analysis forms the main subject of his research. On the other hand, the adherents of the chemical theory believe that a fruitful analysis of this kind requires accurate knowledge of the nature of the central synaptic transmitters. They like to draw attention to what happened with neuromuscular transmission. Its analysis by electrical methods took a new turn after it had become known that acetylcholine is the transmitter.

In my view central synaptic transmission is chemical, and there is no fundamental difference between the transmission processes in the central nervous system and those in the peripheral nervous system. Therefore, the first question which has to be answered, and which unfortunately cannot be answered yet, is: what are the pharmacologically active substances involved in central synaptic transmission?

It is natural that we should turn to the known transmitter substances in the peripheral nervous system, particularly to acetylcholine, the transmitter to structures closely resembling that of the central synapse, i.e. to the motor endplates of skeletal muscles and to the ganglion cells of the autonomic nervous system. Acetylcholine is a natural constituent of the tissue of the central nervous system, so is choline acetylase, the enzyme which synthesizes acetylcholine, and true cholinesterase, the enzyme responsible for its hydrolysis and inactivation in the body. In addition, acetylcholine is a substance with pronounced central actions. It causes a discharge of motor impulses leading to muscular contractions, and it alters the reflex excitability of the central nervous system. Depending upon the dosage it stimulates or depresses the activity of the respiratory centre. It has an action on centres of the autonomic nervous system, whereby changes in blood pressure, heart rate and intestinal movements are produced. Injected into the region of the supra-optic nucleus, it causes release of the antidiuretic hormone. When injected into the ventricular system of the brain, for instance in cats, the following sequence of events occurs. Immediately after the injection the cat retches, shrieks once or twice and then it sits down motionless for a minute or two. The condition resembles that of an akinetic seizure. During the following hours the cat is subdued, drowsy and dazed, although acetylcholine does not produce a condition resembling natural sleep.

The central effects observed with the various anticholinesterases represent a further series of observations perhaps even more pertinent to the question of whether acetylcholine is a central synaptic transmitter or not. Are they the result of accumulation of undestroyed acetylcholine? We must apply this question to each anticholinesterase effect we encounter. Some probably are direct effects, independent of cholinesterase inhibition, but I think it is difficult to avoid the conclusion that many are accounted for by cholinesterase inhibition. Once, however, we accept this interpretation, the question is no longer is acetylcholine a central synaptic transmitter, but, is acetylcholine the universal central transmitter or does it exert this function at certain synapses only?

There are also some observations on the release of acetylcholine during increased central activity caused by stimulation of sensory nerves or after the injection of adrenaline and potassium. A particularly striking observation has recently been made by MacIntosh and Oborin (26). They used the following method. A small volume of saline containing eserine was placed in contact with the exposed cerebral cortex of an anesthetized cat. Minute amounts of fluid were found to transude from the cortex to this pool, and the acetylcholine content of the pool increased progressively. This "weeping" of acetylcholine from the cortex decreased when the depth of anesthesia increased; at the same time there was a decrease in electrical activity recorded from the cortex; and, when the cortex was undercut the acetylcholine output ceased. We know from the experiments of Burns (3) that undercutting the cortex leads to a "silent" cortex. Thus the acetylcholine output is directly proportional to the degree of cortical activity. It is interesting to correlate these findings with the acetylcholine content of the brain tissue. In deep anesthesia, when activity is decreased and the output of acetylcholine is reduced, the content is high; it falls in the waking state and is lowest during and after convulsive activity (6, 30, 31, 32). There is thus no longer any doubt about the close relation between electrical cortical activity, acetylcholine content and release of acetylcholine from the cortex.

In an autonomic ganglion all synapses are confined to a small region and changes in content, release or action of acetylcholine can be attributed to effects on the preganglionic nerve endings or the ganglion cells, but in the central nervous system the neural arrangement is complex, and it is usually difficult to determine which synapses are actually affected by the changes observed. Recent ingenious experiments of Eccles, Fatt and Koketzu (5) have overcome this difficulty. They found in the cat's spinal cord a system of neurones where the evidence that acetylcholine acts as the transmitter is about as good as for the neuromuscular junction. It is the system of neurones which accounts for the various effects of antidromic volleys in motor axons described by Renshaw—both the depression of motoneurones (28) and the repetitive discharge of interneurones (29). The effect is attributed to collaterals given off by the motor axons in the spinal cord. The collaterals synapse onto interneurones which are caused to fire repetitively, and which in turn synapse back onto neighboring motoneurones. Since acetylcholine is liberated at the peripheral terminals of motoneurones, and since branches of the same axon are unlikely to have different

transmitter substances (8), it was to be expected that these central axon collaterals also release acetylcholine at their terminals when they synapse to the interneurones.

The evidence in favor of the cholinergic nature of these central axon collaterals was as follows: Eserine, but not prostigmine, greatly increased the repetitive discharge produced in the interneurones by an antidromic volley and could even produce a background of spontaneous discharge. Neither atropine nor curare had a significant antagonistic action. However, dihydro-β-erythroidine was found drastically to reduce the discharge. This substance may, therefore, be an antagonist for central actions of acetylcholine. Furthermore, a rapid injection of a few micrograms of acetylcholine into the aorta caused a burst of impulses in the interneurones; the response was greatly increased by eserine and was antagonized by dihydro-β-erythroidine.

It is easy to show that acetylcholine cannot be the universal central transmitter. We only need to consider conditions in the posterior roots and the synaptic transmission at their endings in the spinal cord. These nerve fibres lack the properties of cholinergic neurons; they contain very little or no acetylcholine and choline acetylase. Therefore transmission at their endings can scarcely be effected by acetylcholine. Consequently, cholinergic and non-cholinergic neurones must be assumed to exist in the central nervous system, and the question naturally arises, how they are arranged. On purely statistical grounds we should expect that there is often an alternation between cholinergic and non-cholinergic neurones. Miss Vogt and I (12) came to the same conclusion in trying to interpret the results of an investigation of the distribution of choline acetylase in the central nervous system.

We studied the synthesis of acetylcholine, i.e. the choline acetylase content of a number of relatively well-defined areas of the central nervous system. The results seemed at first sight confusing. High and low values occurred in regions belonging to the same efferent or afferent pathway. However, by applying to the results our knowledge of the properties of cholinergic neurones in the peripheral nervous system an interpretation became possible. With two kinds of neurones, cholinergic and non-cholinergic, four variations of synaptic connections are possible: (a) cholinergic—cholinergic, (b) cholinergic—non-cholinergic, (c) non-cholinergic—cholinergic, and (d) non-cholinergic—non-cholinergic; (a) and (b) correspond to the situation in the parasympathetic and sympathetic ganglia respectively. The following conclusions appear justified. 1. A cholinergic neurone is characterized by the fact that it synthesizes acetylcholine not only at its ending but along the whole course of its axon. Thus a tract composed of cholinergic fibres will be identified by a high concentration of choline acetylase. 2. On the other hand, a bundle of non-cholinergic fibres will be identified by the nearly complete absence of choline acetylase. 3. Since the enzyme is apparently located in the axon and not in the myelin sheath, the thickness of the latter may affect the result. Thus, intermediate values may not necessarily mean a mixture of cholinergic and non-cholinergic neurones. This introduces a difficulty, since it makes interpretation dependent largely on those less fre-

quent regions with either high or low values. 4. Concerning the grey matter, we must realize that the greyness is not so much determined by the nerve cells as by the abundance of non-myelinated fibres. The myelin sheath does not start at the very beginning of an axon, nor does it extend right to the end. A high value for synthesis of acetylcholine may thus indicate the arrangements (a), (b) and (c). For this reason more information can, therefore, be obtained if, in addition to the synthesizing power of the region of a nucleus, that of the tracts passing to and from the nucleus is known as well.

When these considerations were applied to our results, they suggested that often, although by no means always, the arrangement of neurones in the efferent and afferent nervous pathways was such that cholinergic and non-cholinergic neurones alternated with each other.

The voluntary motor pathway yielded the following results. High values were found in the anterior horns and in the anterior roots, suggesting that the lower motor neurone is cholinergic. On the other hand, low values were encountered in the pyramidal tracts, indicating that the upper motor neurone is non-cholinergic. In the motor area of the cerebral cortex, an intermediate value was found, which might be due to cholinergic neurones converging on the pyramidal cells. This suggestion is strengthened by the fact that both acetylcholine and eserine, when applied locally to the motor cortex, initiate impulses in the pyramidal tracts.

A succession of alternating non-cholinergic and cholinergic neurones probably also occur in the afferent pathway. Low values were found in the posterior roots and in their central continuation, the funiculi gracilis and cuneatus. This is in agreement with the view that the first afferent neurone of the sensory pathway is non-cholinergic. For the nuclear masses in which these sensory fibres end, relatively high values were obtained. This was taken to indicate the cholinergic nature of the second neurones in the sensory pathway. In the meantime, Harris, Lin and I (10) have examined regions which contain a high proportion of such second neurones, for instance the lateral filament and the region of the spinal cord which contains the spino-cerebellar tract. These regions gave intermediate values. The third ascending neurone is probably again non-cholinergic, as suggested by the low values found for the fibres in the posterior part of the internal capsula, which, at least in man, is said to contain the thalamic radiation.

Another instance is provided by the optic pathway. The retina contains large amounts of choline acetylase, whereas the optic nerve is free of it. This suggested that acetylcholine is the chemical transmitter at one or more of the synaptic junctions in the retina, and that the optic nerve is non-cholinergic. On the assumption of a succession of alternating cholinergic and non-cholinergic neurones, one would like to think that the first neurone, which consists of the rods and cones and of the nerve fibres originating from these visual elements, is non-cholinergic; the second neurone, which impinges on the ganglion cells in the inner synaptic layer, is cholinergic, whereas the third neurone, the optic nerve, again is non-cholinergic.

If we could find a retina with the first synaptic interruption outside the eye,

we could test whether the first neurone contains no choline acetylase and is non-cholinergic. This could be done in Sepia. In this species the retina consists of only one kind of visual element, which may correspond to the rods; the nerve fibres originating from them pass, without synaptic interruption, as retinal nerves out of the eye into the optic ganglion. Harris, Lin and I (10) found that the retina and the retinal nerves of Sepia contained no choline acetylase, while it was highly concentrated in the optic ganglion.

We have as yet no method for devising a decisive experiment to prove or disprove that there is often an alternation of cholinergic and non-cholinergic neurones. There are, however, two other observations which appear to support our theory. The first concerns the distribution of true cholinesterase in the retina and provides the evidence for the non-cholinergic nature of the first neurone of the mammalian retina. Both Anfinsen (1) and Francis (13) found the cholinesterase located in the inner synaptic layer, and their results have been confirmed by Hebb, Silver and Swan (16). The second observation concerns the action of acetylcholine on the cervical cord. Drs. Gray, Perry and I (9) injected small amounts of acetylcholine into the basilar artery of anesthetized cats and obtained a burst of impulses when records were taken from the cervical nerves. The question was, did the acetylcholine stimulate the motoneurones or the interneurones in the cord? A careful analysis, with records from the ventral roots and tests of reflex excitability, suggested that acetylcholine acted on the interneurones. For instance, when recording was from the ventral roots, acetylcholine caused fluctuations of the base line which one would expect from excitation of interneurones. Furthermore, we examined the reflex discharge when stimulating an ipsilateral, polysynaptic reflex. Acetylcholine caused a number of changes; an increase in the area of the reflex and of the ventral root potential; in addition, it shortened the reflex time and the time between stimulus and peak of ventral root potential. The time saved was about 2 to 3 msec. So great a saving of time cannot be explained by increased velocity of conduction in nerve fibres and must therefore be saved at the synapses of the interneurones. We concluded from these and other observations that the main effect of acetylcholine was to excite some part of the interneurones.

These results, therefore, suggest that the motoneurones or the anterior horn cells are rather insensitive to acetylcholine, and I cannot imagine that a cholinergic neurone should impinge upon a nerve cell which is rather insensitive to acetylcholine. Thus the interneurone which impinges on the motoneurone very probably is non-cholinergic. We know further that the sensory fibre which enters the cord is non-cholinergic. If we assume that this neurone impinges on a cholinergic neurone, then the nerve cell of the subsequent non-cholinergic neurone, which makes synaptic connection with the motoneurone, is affected by the arterially injected acetylcholine.

Thus in its simplest case we should have the following order of alternation, starting with the sensory roots and ending with the motor roots: non-cholinergic, cholinergic, non-cholinergic, cholinergic. But we may naturally have a greater number of interneurones involved in the build-up of a reflex. On the

other hand, if we consider the monosynaptic reflex, the scheme would fit likewise: the non-cholinergic sensory root fibre would impinge upon an acetylcholine insensitive cell, the motoneurone.

Before leaving this problem of alternation of cholinergic and non-cholinergic neurones, I want to stress once more that this arrangement is not considered to be uniform. We encounter a synaptic linkage of two cholinergic neurones in direct succession in the peripheral nervous system in the parasympathetic pathway, and similar linkages may occur in the central nervous system, for instance at synapses of the caudate nucleus and thalamus. I mentioned that a frequent alternation was to be expected purely for statistical reasons. But what is the functional importance of such an alternation? I must confess I have no suggestions to offer.

What is known about the transmitter substances of adrenergic nerves? Have we adrenergic central neurones? In other words, has adrenaline or noradrenaline central synaptic transmitter functions? To answer this question, or a similar question if other substances are proposed, we want to know first whether these substances are natural constituents of the central nervous system. Have they central actions and do we encounter in the central nervous system enzymes for their synthesis and inactivation?

Central actions of adrenaline and noradrenaline are known. Of interest are the following recent observations. Leimdörfer (28, 29) has stated that adrenaline injected intracisternally causes a condition of analgesia, sleep and anesthesia and long-lasting hyperglycaemia. Sherwood and I (11) have found that, even in doses of 20 to 80 microgm., adrenaline or noradrenaline injected into the lateral ventricle of the cat cause a condition resembling light nembutal anesthesia. But the distribution of these substances in the central nervous system does not favour the view that they are central transmitter substances. Miss Vogt has told us that the presence of sympathin in the hypothalamic region suggests to her that these amines may have some function in relation to the sympathetic centres, but not as transmitters.

There are two other substances, histamine and substance P, which both occur in posterior roots. The possibility that histamine may be the transmitter of the monosynaptic reflex has recently been envisaged by Häusler (15). He found that minute amounts of histamine injected into the perfused spinal cord of the frog caused excitation of the motoneurone, and the venous effluent collected during reflex excitation produced similar effects when re-injected. However, histamine is present in the brain in very low concentration, except in the cerebellum (22) and in parts of the hypothalamus (14), so that, without further evidence, one hesitates to attribute a transmitter function to this amine. In this connection it is worth noting that histamine has once been proposed by Ungar as a transmitter in the peripheral nervous system. He spoke of histaminergic fibres, but the evidence for this hypothesis was never convincing and it has not found acceptance.

Substance P certainly occurs in brain tissue (7, 27); in addition it is present in relatively high amounts in posterior roots (25, 27). However, no central ef-

fects of this substance are known. It is polypeptide or a group of polypeptides. At present there is not sufficient evidence in favour of a transmitter function of this constituent of nervous tissue.

The suggestion has recently been made that adenosine triphosphate (ATP) is a central synaptic transmitter. In 1935 Dale (4) pointed out that if we knew the transmitter of antidromic vasodilatation we might know the transmitter of the central synapse of sensory neurones as well, because of the fact that the nature of a neurone remains uniform throughout its whole length. If we assume a neurone with transmission from both ends, the same transmitter must be involved in both transmission processes. But are we justified in making this assumption?

We have to be careful when drawing conclusions from antidromic vasodilatation to central synaptic transmission, because we do not know for certain, as Dale himself has pointed out on several occasions, whether we are really dealing with neurones that transmit impulses from both ends, in other words, whether the fibres which conduct the afferent sensory nerve impulses are the same as those responsible for antidromic vasodilatation. There is a high probability that this is so, and therefore any information about the nature of the transmitter of antidromic vasodilatation touches at the root of the problem of central synaptic transmission. Hellauer and Umrath (17, 18) were the first to approach central synaptic transmission of sensory impulses from this point of view, but Holton and Holton (19, 20, 21) have made the greatest advance in the field. Their results indicate that ATP is the transmitter of antidromic vasodilatation.

They worked on the rabbit's ear and found that extracts prepared from spinal roots produced a vasodilatation which resembled in two characteristic features that obtained on antidromic nerve stimulation. Both vasodilatations were prolonged effects and both were reduced by anticholinesterases. When Holton and Holton examined a number of known vasodilator substances, only the vasodilatations produced by ATP and by ADP were found to show these features. Further, the chemical and biochemical properties of the vasodilator substance of spinal root extracts resembled those of ATP.

In order to find out whether ATP appears in the venous effluent from the rabbit's ear during antidromic vasodilatation, they examined the absorption spectrum of the outflowing fluid. ATP and other substances containing a purine and pyrimidine nucleus have an intense ultra-violet absorption band in the region 255 to 265 mμ. And they found a definite increase in this region of the absorption spectrum when they examined the outflowing fluid collected whilst the sensory nerve to the ear was stimulated antidromically. Holton and Holton therefore suggest that stimulation of sensory nerves liberates ATP or some other substance with a similar ultra-violet absorption spectrum, and that this occurs not only in the skin but also at the other end of the sensory neurone at the central synapse.

So far we have dealt with the problem of central synaptic transmission by non-cholinergic neurones as if the search were for a single transmitter substance, the transmitter of the non-cholinergic neurones. However, recent results

obtained by Brock, Coombs and Eccles (2) with intracellular recordings raise the question whether more than one transmitter substance is released at non-cholinergic central synapses. Eccles and his co-workers succeeded in inserting microelectrodes into the large motor cells of the anterior horns of the cat's spinal cord, so that they could record the potential changes between the surface and the interior of these motoneurones when they were either activated or inhibited.

Monosynaptic excitation of the motoneurone by impulses originating in the muscle spindles of the muscle caused depolarization. Monosynaptic inhibition of the motoneurone by impulses originating in the muscle spindles of the antagonistic muscles caused hyperpolarization. It was, in fact, these results which caused Eccles to abandon the electrical theory of central synaptic transmission.

There are two possible explanations of these results: 1. We can postulate two transmitter substances, one excitatory and the other inhibitory. Since sensory fibres are neither cholinergic nor adrenergic, acetylcholine, adrenaline or noradrenaline are excluded. 2. We can postulate one transmitter substance and a specialization of subsynaptic areas of the motoneurone, so that under the excitatory synaptic knobs, the substance would depolarize and under the inhibitory synaptic knobs the same substance would hyperpolarize the membrane. In my opinion either alternative creates great difficulties when we try to explain reciprocal innervation. What we have to explain is that afferent impulses from the muscle spindles of a muscle monosynaptically depolarize the motoneurones of that muscle and its synergists, but monosynaptically hyperpolarize the motoneurones of its antagonists. If excitation and inhibition are both *really* monosynaptic, the concept of separate excitatory and inhibitory transmitter substances would presuppose that collateral branches of a neurone can release different transmitter substances. This I find difficult to accept, but the concept of two transmitter substances, one excitatory and the other inhibitory to explain the monosynaptic excitatory and inhibitory processes, although possible, has not been proved. There is the alternative concept of sub-synaptic areas reacting differently to the same transmitter substance. This presupposes the specialization of sub-synaptic areas on the surface of a nerve cell, which, at least at present, is hypothetical. The results with intracellular recordings have created a number of as yet unsolved problems for the adherence of the chemical theory of central synaptic transmission.

When the organizers of this symposium asked me to speak on central, and especially on sensory, transmission, they probably did not realize how little we really know about the underlying transmission processes.

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