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Acetylcholine synthesis and its dependence on nervous activity

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Summary. The findings discussed in this paper mainly derived from studies on salivary glands, serving as model organs, indicate that the capacity to form the neurotransmitter acetylcholine, as judged by the activity of choline acetyltransferase, is influenced by the traffic of nerve impulses, as a long term effect. In the glands, choline acetyltransferase seems to be exclusively localized to the cholinergic nerves. In the postganglionic parasympathetic nerves of the glands, the activity of choline acetyltransferase decreases when the flow of secretory impulses in these nerves is abolished or reduced either by isolating the nerves from the central nervous system, surgically or pharmacologically, or by diminishing the reflex activation of the glands from the mouth. The opposite occurs when the reflex activation of the salivary glands is enhanced, i.e. the activity of choline acetyltransferase increases. Observations on various other organs are quoted in support of the view that the traffic of nerve impulses is of importance for the activity of the enzyme. An increase in choline acetyltransferase activity also occurs in some salivary glands after sympathetic denervation. This puzzling observation is discussed in relation to impulse traffic. Increased nerve impulse traffic and collateral sprouting seem to be responsible for the rapid restitution of choline acetyltransferase activity from a low level in an organ partially deprived of its cholinergic nerve supply.

Synthesis of the neurotransmitter acetylcholine is dependent on the presence of choline acetyltransferase (EC. 2.3.1.6), the enzyme that transfers the acetylgroup from acetyl-coenzyme A to choline. This enzyme is in general confined to the cholinergic nerves; it is manufactured by the nerve cell body and transported along the axon, mostly at a slow rate of a few mm per day with the bulk of axoplasma to the nerve endings, where it accumulates and the transmitter is formed[1-8]. The present paper deals with the influence of nervous activity, as a long term effect, on the activity of choline acetyltransferase at the nerve endings in the effector organs. When it was originally put forward by Nordenfelt[9] in 1964 that the activity of the transmitter synthesizing enzyme depended on the traffic of nerve impulses, this hypothesis was based on findings in a study on salivary glands, and in the following sections, experiments on salivary glands are often referred to, a favourite model organ of this laboratory in studies on the function of the neuro-effector system which also seemed suitable for the present purpose (see Emmelin[10]).

In those experiments described here, which originate from this laboratory, a bioassay method for the determination of the activity of choline acetyltransferase devised by Catherine Hebb[11,12] has been routinely used: virtually all choline acetyltransferase is extracted from an acetone dried powder prepared from the tissue under study and then incubated in a system continuously generating acetyl-coenzyme A, and under such conditions that only the concentration of the enzyme and not its substrates is rate limiting for

the production of acetylcholine; the incubate is then assayed on the eserinized frog rectus muscle. The method of bioassay is no doubt somewhat tedious and, in contrast to radioactive methods now so commonly in use, very time-consuming. However, by the use of bioassay a serious pitfall is avoided: it has recently been realized that unless certain precautions are taken in connection with the radioactive methods, i.e. the use of adequate blanks, other acetylated compounds than acetylcholine, notably acetylcarnitine, are recognized as acetylcholine[13,14]. The lack of specificity appears to be particularly marked when tissues other than pure nerve tissue are analysed[15]. Acetylcarnitine (and also carnitine) does not interfere in the bioassay of acetylcholine[16-18].

Unless otherwise stated, the results to be presented in the following sections refer to adult animals and to total activity of choline acetyltransferase i.e. acetylcholine formed per organ or per pool of organs.

Localization of choline acetyltransferase

Section or avulsion of the auriculo-temporal nerve, aiming at a parasympathetic denervation of the parotid gland, causes the activity of choline acetyltransferase of the gland to decrease profoundly and rapidly, thereby most likely indicating a neuronal localization for the enzyme. However, in all the species examined some activity remains in the glands: in rabbits[19] and rats[20] about 3%, in cats[11,21] about 10% and in dogs[22] as much as 30% of the normal activity. The enzyme activity is not further reduced by sympathetic denervation; nor does a sympathetic denervation reduce the enzyme activity of a gland with an intact parasympathetic nerve supply[11,23-25]. The finding of a residual enzyme activity does not necessarily mean that there is also a non-neuronal source for the enzyme. Another explanation would be that some cholinergic nerves escape the denervation procedure, that this is the case is suggested by several findings. In dogs and cats, reflex secretion from such a denervated gland can still be evoked by, for instance, pouring citric acid onto the tongue of the animals or by electrical stimulation of afferent salivary nerves[21,26]; a secretion that is abolished by the administration of atropine. In all the species, injection of the cholinesterase inhibitor, eserine, into the salivary duct towards the 'denervated' gland has been found to evoke secretion of saliva[21,26-28], thus revealing leakage of the transmitter from cholinergic nerve endings; and further cholinesterase positive nerves can still be demonstrated histochemically[27-30]. In the dog, secretory cholinergic nerve fibres for the parotid gland outside the classical secretory pathway, the auriculo-temporal nerve, were actually traced along the internal maxillary artery[26], and, when these fibres were cut in combination with section of the auriculo-temporal nerve, the enzyme activity fell to about 10%[22]; by also including in the denervation procedure section of the facial nerve, which passes through the gland, the choline acetyltransferase activity fell even further, in most cases to values below the limit of detection[22]. Also in the cat, secretory cholinergic nerve fibres have been found to travel to the parotid gland along the internal maxillary artery[21].

In the rabbit parotid gland, the choline acetyltransferase activity fell to its lowest value already 3 days after section of the auriculo-temporal nerve[31], while in the cat this occurred between 5 and 7 days after the operation[21]. The difference in time may not only depend on species differences but may also reflect the level at which the nerve was severed. The denervated diaphragm muscle has been shown to retain choline acetyltransferase activity for a longer period of time when the nerve stump left in connection with the muscle is long than when it is short[32]. Using the phrenic nerve, it was also shown that choline acetyltransferase still moves in axons disconnected from their nerve cell body[5]. Thus, the conclusion seems to be that the longer the nerve stump, the more choline acetyltransferase is available for transport to the nerve endings. As to the denervation of the parotid glands, the nerve was cut close to the gland in the rabbit, whereas in the cat it was severed at the point where it emerged from the base of the skull.

In contrast to the parotid gland, the submaxillary gland has its relay of pre- and postganglionic parasympathetic nerves close to hilus or within the gland[33,34]. Therefore this gland can only be partially denervated; after nerve dissection into hilus, a decrease in choline acetyltransferase activity to 14% was found in the cat[11].

In denervated skeletal muscles, a few per cent of the acetylcholine synthesizing capacity usually remains[35]. Tentatively, it has been suggested that the Schwann cells are responsible for this production[3]. Another possibility, recently discussed[36], is an unspecific formation of acetylcholine due to the presence of the enzyme carnitine acetyltransferase in the tissue extract, which under favourable conditions may utilize choline as a substrate[37].

Parasympathetic decentralization: the starting point

The activity of choline acetyltransferase was found by Nordenfelt to decrease in the submaxillary[11] and parotid gland[9] of the cat after section of the preganglionic parasympathetic nerves: in the submaxillary gland, the enzyme activity was reduced by 40% and in the parotid gland by 26–30%. In the submaxillary gland, the reduction in enzyme activity could at least be partly due to the degeneration of preganglionic nerves within the gland, but in the parotid gland this could not be the case, since the synapses are located

outside the parenchyma. A decentralized gland atrophies and develops a supersensitivity to secretagogue substances, phenomena attributed to loss of that part of acetylcholine that is released from the postganglionic nerve endings at the arrival of secretory impulses from the central nervous system (see Emmelin[10]). In analogy, it thus seemed reasonable to Nordenfelt to suggest that the fall in the choline acetyltransferase activity in the postganglionic nerves disconnected from the central nervous system was due to loss of traffic of impulses[9]. However, the parotid gland of cats has certain drawbacks: when the preganglionic parasympathetic nerve fibres are destroyed in the middle ear, postganglionic sympathetic nerves are also damaged to a varying degree[38], which in itself may cause the choline acetyltransferase activity to increase[23] as discussed in a later section. Furthermore, as mentioned before, the anatomy of the salivary nerves is imperfectly known and some nerves often escape a denervation or a decentralization procedure. The parotid gland of the dog was turned to, since this gland offered some advantages over that of the cat: the sympathetic nerve supply of the gland is not injured by the decentralization procedure[39] and furthermore, the reflexly induced salivary response could be used to assess to what degree the attempted operation had been successful[26]; the secretory rate obtained by pouring citric acid onto the tongue of dogs was found in the decentralized glands to be 0–6% of that of the contralateral glands, thus indicating that all or nearly all preganglionic nerves had been cut[40]. 1 week postoperatively, the choline acetyltransferase activity in the decentralized parotid gland of the dog was reduced to a similar degree as in cats, i.e. by 26%[40]. It should also be mentioned that decentralization in the dog does not damage postganglionic cholinergic nerve fibres[30].

'Pharmacological' decentralization

Prolonged ganglion blockade is also followed by a fall in the choline acetyltransferase activity of the postganglionic nerves, similar in magnitude to that found after section of the preganglionic nerves. In rats it seemed more difficult to cut the preganglionic parasympathetic nerves of the parotid gland than in cats and dogs. As an alternative procedure, the ganglion blocker chlorisondamine was given repeatedly over a period of 13 days[41,42]. The 'pharmacologically' decentralized gland was found to have lost weight and to have developed a supersensitivity to chemical stimuli, thus indicating that the traffic of secretory impulses in the postganglionic nerves of the gland was reduced or abolished. The decrease in choline acetyltransferase activity was 24%. In the submaxillary gland, the enzyme activity was reduced only by 12%. In this gland, the situation is complicated by the fact that some preganglionic nerves are included in the sample, since these nerves synapse with the postganglionic ones within the gland. An explanation for the small decrease in choline acetyltransferase activity of the submaxillary gland may be that in the gland 2 phenomena occurred, the effects of which opposed each other: a diminished traffic of impulses in the postganglionic nerves; and an enhanced traffic in the preganglionic nerves due to an increased reflex stimulation from the mouth in response to the dryness of the mucosa caused by the ganglion blockade (see later section). That the ganglion blocking drug did not exert any general toxic action on cholinergic nerves is shown by the fact that the choline acetyltransferase activity did not decline in the preganglionic sympathetic (cholinergic) nerves within the adrenals. Also, in the heart ventricles of the rat, a fall in the enzyme activity was observed, thought to have occurred in the postganglionic parasympathetic nerves[43].

Botulinum toxin

In the submaxillary gland of the cat, a marked fall in choline acetyltransferase activity, by about 40%, was found 2–8 weeks after a previous injection of the toxin retrogradely via the duct. In the parotid gland, the enzyme activity was little or not at all affected[44]. Botulinum toxin impairs the release of acetylcholine from the nerve endings[45]. It is of interest to combine these observations with the knowledge that in the submaxillary gland there are both pre- and postganglionic parasympathetic nerve endings on which the toxin acts, whereas in the parotid gland there are only postganglionic nerve endings to act on, and further, that the toxin blocks the effect of electrical stimulation of the preganglionic nerves more easily than that of stimulation of the postganglionic nerves[46,47]. Thus, it is possible that the marked fall in choline acetyltransferase activity in the submaxillary gland would depend on a reduced or abolished traffic of secretory impulses in the postganglionic parasympathetic nerves caused by the interference of the toxin with the ganglionic transmission. The choline acetyltransferase activity in the somatomotor nerves of the mouse skeletal muscle has been found to be unchanged after a local injection of botulinum toxin[48].

Decreased reflex stimulation

It is not necessary to isolate the postganglionic nerves by surgical or pharmacological means from the central nervous system in order to lower their activity of choline acetyltransferase; it is also possible to do so in animals with intact nervous pathways. By feeding rats on a liquid diet instead of their normal pelleted one, it seemed likely that the reflex activation from the mouth would be diminished, an assumption support-

ed by the finding of a marked fall in gland weight; and under this condition the choline acetyltransferase activity in the parotid glands was reduced by 22–28%, 4–18 days after the start of the liquid regime[49]. Another way of decreasing the activation of oral and pharyngeal receptors would be to treat the rats with the parasympathomimetic drug pilocarpine, which causes a profound salivation; 10 days after the start of the treatment, the enzyme activity in the parotid gland was reduced by 7%[50]. The relatively small decrease is probably due to the fact that the pilocarpine treatment did not cause a continuous flow of saliva throughout the whole interval between 2 injections of the drug.

Procedures aiming at reduction of the intensity of nervous stimulation of skeletal muscles in rats, such as skeletal fixation[51,52] or tenotomy[53], lowered the enzyme activity in the disused muscle by as much as 30–50%. Tetrodotoxin, applied locally on the nerve trunk and blocking the nerve conduction, failed to do so[15].

Increased reflex stimulation

In salivary glands, an increase in the activity of choline acetyltransferase can be brought about in several ways, all of which seem to have in common that the traffic of secretory impulses is increased above the ordinary. Rats were fed on a pelleted diet with a high content of cellulose, forcing the animals to increase their food intake[20]. The oral and pharyngeal mucosa of the animals was made dry by prolonged treatment with an atropine-like drug[20]. Another way of producing dryness was to prevent saliva from reaching the oral cavity by ligating most of the salivary ducts[54]. The pulp of the tooth was irritated by cutting the incisors repeatedly[55].

Support for the assumption that stimulus for salivation was enhanced was the finding of gland enlargement. As to atropinization, it cannot be expected that the gland weights would be affected in the same way as by the other procedures, since atropinization prevents the secretory impulses from reaching the gland cells; nevertheless, a slight increase in the weight of the parotid gland occurred probably due to incomplete blockade of the transmitter action on the gland cells. However, an increase in gland mass as such does not produce a similar change in choline acetyltransferase activity. The 2 phenomena can be separated from each other. Isoprenaline, given repeatedly, is very effective in increasing the size of salivary glands in rats. Treatment over a period of 3 weeks caused the parotid gland to increase 10fold in weight, whereas the choline acetyltransferase activity was unchanged. The submaxillary gland, which increased 5fold, did, in fact, show a marked decrease in the activity of the enzyme[56], a finding discussed in a later section. In this context, a further indication that the degree of stimulation to which the glands were subjected varied, may be mentioned: the parotid glands of rats fed on the bulk diet were not as sensitive in their secretory responses to cholinomimetics as those of the rats fed on the liquid diet[57].

In parotid glands, the increase in choline acetyltransferase activity was 17% after bulk diet for a period of 3 weeks, 21% after atropinization for the same period of time, and 32% after duct-ligations for 4 weeks, i.e. when the ducts of the submaxillary and sublingual glands on both sides and the duct of the contralateral parotid gland were tied; during the 1st week no increase in the enzyme activity occurred. When it was the ducts of the submaxillary and sublingual glands on one side that were left intact, in an otherwise duct-ligated animal, the enzyme activity was found to have increased in these glands: in the submaxillary gland by 32% and in the sublingual gland by 13%. With regard to the other procedures (bulk diet and atropinization), the submaxillary and sublingual glands were less affected and it was particularly the parotid gland that responded. In very young rats a rapid increase in choline acetyltransferase activity occurs in both the parotid and the submaxillary glands during the 4th week of life[58]; at the end of the 3rd week, the suckling period is terminated and the rats have to change their diet habits leaving the liquid consistency of their food for a dry, pelleted one. After teeth amputations, the enzyme activity was increased in the submaxillary gland by 13 and 16%, 2 and 3 weeks after the beginning of the experiment; in the sublingual glands, the increase was 18% after 3 weeks. The enzyme activity did not increase in response to teeth amputations in parasympathetically decentralized submaxillary glands, indicating that for the increase to occur an intact connection with the central nervous system is necessary[55].

Some other organs have also been examined when it seemed likely that they had for some time been exposed to an increased impulse stream in cholinergic nerves. Enhanced parasympathetic (vagal) activity is usually made responsible for the bradycardia found after physical training; and in the atria of trained rats, the choline acetyltransferase activity was increased by 19%[59]. As to the effect of overuse on the enzyme activity in skeletal muscles, opinions seem to differ. A 19% increase in the rat plantaris muscle was reported in one study[51] after the tendon of the synergistic muscles had been cut, a procedure aiming at overuse of the plantaris, while in another study[53] no increase could be demonstrated in the gastrocnemius muscle, exposed to overuse in a similar way. In another experiment the diaphragm muscle of the rat was used[54]. The phrenic nerve was cut on one side, and 4 weeks later the respiratory frequency of the animal was found to be increased, reflecting enhanced traffic of motor impulses in the intact phrenic nerve; in the

innervated half of the diaphragm muscle the choline acetyltransferase activity was increased by 13%.

There are, in addition, some further papers relating the intensity of nervous stimulation to the activity of choline acetyltransferase. The normal increment of the enzyme activity in some visual structures of the central nervous system was prevented by keeping newborn rats in darkness[60]. In this connection it may also be mentioned that the increase in enzyme activity of the levator ani muscle of growing rats failed to appear after castration[61], although the authors seemed more inclined to attribute this failure to lack of retrograde influence of this muscle on the nerves[62]. In the adrenals of mice made aggressive an increase of 25–50% has been found[63]. In the optic lobe of chicken, an increased enzyme activity was found after injection of various drugs, among them reserpine[64]. Reserpine was also reported to have increased the activity of choline acetyltransferase in adrenals and sympathetic ganglia of young rats markedly and rapidly[65]. However, objections may be raised to the use of reserpine[36,66]; and the effect of the drug could not be observed when whole homogenates of ganglia were analyzed[65,67] instead of a supernatant fraction[65], suggesting that the increase in choline acetyltransferase activity in response to reserpine was merely due to a change in the distribution of enzyme, from a particulate bound form to a soluble form, rather than to a true increase in the total enzyme activity[67].

Sympathetic denervation

An increase in choline acetyltransferase activity can occur in salivary glands after sympathectomy. This puzzling observation was first made on the parotid and submaxillary glands of cats, provided the connection between pre- and postganglionic parasympathetic nerves was left intact[23]. The effect of sympathectomy is not obtained in all salivary glands, and in the same species, one type of gland may show the increase in enzyme activity, whereas the activity remains unchanged in another type of gland[24,25]. In the rat, the enzyme activity increased by 40% in the submaxillary gland, i.e. by more than in any other gland investigated, but no increase in enzyme activity occurred in the parotid and sublingual glands.

For the phenomenon to develop, some time has to pass: the increase is not obvious until about 2 weeks after sympathectomy[23,25]. In the cat's salivary glands it did not progress further, neither did the activity decline during a period of 4 months. In the rat's submaxillary gland (showing the largest increase) the enzyme activity continued to rise during the 3rd week.

Sympathetic denervation of the heart and the urinary bladder of the rat[68] caused by repeated injections of 6-hydroxydopamine, which selectively destroys adrenergic nerve endings[69], did not affect the activity of choline acetyltransferase. Chemical sympathectomy was used, since extensive surgery is needed to achieve cardiac sympathetic denervation[70], and surgical interruption of the sympathetic nerve supply of the urinary bladder would also involve section of cholinergic nerves[71]. In salivary glands of rats, 6-hydroxydopamine treatment had the same effect as removal of the superior cervical ganglion on the choline acetyltransferase activity[25].

The effect of sympathectomy on choline acetyltransferase in salivary glands seems to occur particularly in glands where the initial concentration of the enzyme is fairly low, and further where the density of adrenergic nerves is high. In parotid glands, in contrast to the submaxillary glands, some adrenergic nerves may persist after removal of the superior cervical ganglion[30,72]. However, a complete disappearance of the adrenergic nerves is not necessary for the effect to occur[25]. At first sight it might seem as if the increase in choline acetyltransferase activity is a consequence of the loss of secretory impulses in the sympathetic nerves. The highest increase in choline acetyltransferase occurred in the rat submaxillary gland; this gland has a rich supply of sympathetic nerves[72,73], and these nerves take part in the digestive reflexes[74]. Further, the lack of rise in enzyme activity in an organ such as the heart would become understandable, considering that here the 2 sets of autonomic nerves do not act in a similar way as they do in salivary glands but in an antagonistic way. However, the view that loss of sympathetic impulses from the central nervous system is responsible for the increase in enzyme activity found in some salivary glands after sympathetic denervation becomes untenable in the light of the fact that no increase in the enzyme activity occurred in the submaxillary gland of cats[23] and rats[75] after section of the preganglionic sympathetic nerves.

The mechanism by which sympathectomy, surgical or chemical, affects the level of choline acetyltransferase in some glands is at present unknown. Nordenfelt suggested that some agent from the degenerating sympathetic nerves might affect the postganglionic parasympathetic nerves, possibly inducing collateral regeneration[23]. So far no histological investigation of the glands has dealt with this specific possibility. When searching for a mechanism responsible for the effect of sympathectomy, it may perhaps be worth considering that in salivary glands there is not only a leakage of acetylcholine from the parasympathetic nerve endings, as pointed out earlier, but also a leakage of noradrenaline from the sympathetic nerve endings[10]. This leakage of transmitters, normally below the threshold for secretion, is thought to keep the gland cells in a state of readiness, facilitating the action of the nerve impulses. When the action on the cells of noradrenaline that leaks from the endings is abolished by the administration of an adrenoceptor

blocking agent, parasympathetic nerve stimulation, or an injection of a cholinomimetic drug, does not cause as much secretion of saliva as before[76]. Maybe loss of adrenergic transmitter leakage causes a compensatory enhancement of the impulse traffic in the parasympathetic nerves, and, as a consequence of this, an increased choline acetyltransferase activity. The observation that intense stimulation of the β-adrenoceptors of the submaxillary gland of the rat by prolonged isoprenaline treatment lowers the activity of choline acetyltransferase, may be of interest in this context[56].

Partial parasympathetic denervation

Restitution of choline acetyltransferase activity in organs partially denervated seems to be explained by increased impulse traffic in the remaining nerves and collateral sprouting. In the cat's submaxillary gland, decentralized and partially denervated, evidence for a collateral regeneration from persisting postganglionic nerves was not found until the preganglionic nerves had made contact with these nerves, suggesting that a flow of secretory impulses in the parasympathetic pathway may be a necessary condition for sprouting; at the same time, the activity of choline acetyltransferase began to rise from a low level[77]. In the rat's urinary bladder, a very rapid restitution in the activity of choline acetyltransferase occurred after removal of parts of the cholinergic nerve supply. On the 3rd day after cutting the hypogastric nerve bilaterally, the enzyme activity was lowered by 16%, whereas on the 8th day after the surgery, the activity tended to be higher than in control bladders[78]. The main pathway for the cholinergic nerves of the bladder is via the pelvic ganglion, indicated by a reduction in choline acetyltransferase activity to 5% or less of the normal activity after bilateral removal of the ganglion[78]. Removal of the pelvic ganglion unilaterally lowered the enzyme activity to 58% on the 3rd day. After the initial fall a rapid recovery occurred: on the 6th day the enzyme activity was 75% and on the 25th day 86%[79]. The finding that in such a partially denervated bladder supersensitivity to chemical stimuli is only a transient phenomenon[80], and that the response of the bladder to electrical stimulation of the intact nerve increases[81], may indicate both increased traffic of motor impulses in the intact pathway enhancing the bombardment of the transmitter onto the cells and collateral regeneration. This rapid restitution in choline acetyltransferase activity is in contrast to the slow reappearance of the enzyme activity after procedures aiming at a complete denervation. In parotid glands of rabbits[82] and cats[83], the enzyme activity 1–2 years after severance of the auriculo-temporal nerve was only 60–70% of that of the contralateral glands. Morphological and functional studies on partially denervated skeletal muscles[84] and sympathetic ganglia[85] have shown that there is a favourable condition for sprouting when degenerating and intact nerves intermingle with each other, and that the tendency to sprout is less when a great number of nerves is caused to degenerate; e.g. in skeletal muscles of the monkey, sprouting did not occur when the loss of nerves was as big as 90%[86]. The fact that, in the urinary bladder, the pelvic nerve of each side distributes bilaterally[87,88], may thus have been favourable for sprouting. In salivary glands some cholinergic nerves persist after procedures aiming at a complete parasympathetic denervation, as mentioned earlier, and at least some of these nerves are in contact with the central nervous system. In analogy with the experiment on the monkey muscle related above, the reduction in the number of nerves in the salivary glands may have been too big to result in a response of sprouting; the slow rise in enzyme activity is probably mainly due to a conventional nerve regeneration.

Concluding remarks

The changes in choline acetyltransferase activity in response to variations in the intensity of the flow of impulses in the cholinergic nerves reported in this paper, and mainly based on findings from salivary glands, seem on the whole to be relatively small. This may be due to the high safety margin that seems to be at work in the synthesis of acetylcholine. The in vitro formation of acetylcholine in the cat's submaxillary gland is 15[11,89] and in the dog's parotid gland[40,90] 50 times higher than the maximal release of acetylcholine from these glands at nerve stimulation in vivo; in the cat's superior cervical ganglion the in vitro synthesis was calculated to be 45 times higher than that in vivo[4]. For the changes in enzyme activity to appear at the nerve endings, a certain period of time has to pass; this is particularly evident for the increases. Since the endings are dependent for their supply of choline acetyltransferase on the nerve cell body, phenomena of importance in this context are presumably adjustment of both the enzyme synthesis in the soma and the axonal transport; there is no evidence for the presence of activators or inhibitors of choline acetyltransferase activity[8,91]. Protein synthesis in the soma[92] as well as axonal transport[93,94] have been coupled to the intensity of nervous activity. The findings of increased choline acetyltransferase activity in the hemi-diaphragm muscle but not in the phrenic nerve trunk after cutting the contralateral nerve in advance[54] may possibly indicate that it is the transport velocity of choline acetyltransferase, rather than the amount of enzyme under transport, that increases in response to enhanced impulse traffic. Recently, the transport velocity of choline acetyltransferase belonging to the slow component of axoplasmic flow was found to vary with age[8].

In the case of adrenergic nerves, a relation between the intensity of impulse traffic and the activity of enzymes involved in the formation of the transmitter has also been considered. In sympathetically decentralized salivary and pineal glands, Zieher and Pellegrino de Iraldi observed a reduced capacity to form noradrenaline from l-Dopa, which they attributed to a fall in the dopamine-β-hydroxylase activity[95]. Many studies have been devoted to the effect on the catecholamine synthesizing enzymes of various experimental set-ups, involving stress and understimulation[96-98]. As was the case in the studies on choline acetyltransferase, peripheral model organs were also chosen in the studies on the adrenergic transmitter enzymes; in the central nervous system the situation can be expected to be very complex.

1 C.O. Hebb and G.M.H. Waites, J. Physiol. *132*, 667 (1956).
2 C.O. Hebb and A. Silver, Nature *189*, 122 (1961).
3 C.O. Hebb, K. Krnjević and A. Silver, J. Physiol. *171*, 504 (1964).
4 C.O. Hebb, Physiol. Rev. *52*, 918 (1972).
5 J. Ekström and N. Emmelin, J. Physiol. *216*, 247 (1971).
6 F. Fonnum, M. Frizell and J. Sjöstrand, J. Neurochem. *21*, 1109 (1973).
7 S. Tuček, Brain Res. *86*, 259 (1975).
8 C. Jablecki and S. Brimijoin, J. Neurochem. *25*, 583 (1975).
9 I. Nordenfelt, Acta univ. lund. sectio II, *16*, 1 (1964).
10 N. Emmelin, Experientia *21*, 57 (1965).
11 I. Nordenfelt, Q.Jl exp. Physiol. *48*, 67 (1963).
12 C. Hebb and A. Silver, in: Neuropathology: Methods and Diagnosis, p. 665. Ed. C.G. Tedeschi. Little, Brown and Co., Boston 1970.
13 F. Welsch, J. Biochem. Pharmac. *26*, 1281 (1977).
14 H. Banns, J. Ekström and S.P. Mann, to be published.
15 J.J. Butler, D.B. Drachman and A.M. Goldberg, J. Physiol. *274*, 539 (1978).
16 E. Strack and K. Försterling, Arch. exp. Path. Pharmak. *185*, 612 (1937).
17 W.-D. Thomitzek and E. Strack, Acta biol. med. german. *13*, 447 (1964).
18 C. Hebb and D. Morris, in: The Structure and Function of Nervous Tissue, p. 25. Ed. G.H. Bourne. Academic Press, New York, London 1969.
19 I. Nordenfelt, Acta univ. lund. sectio II, *9*, 1 (1964).
20 J. Ekström, Q.Jl exp. Physiol. *59*, 191 (1974).
21 J. Ekström and N. Emmelin, Q.Jl exp. Physiol. *59*, 11 (1974).
22 J. Ekström and J. Holmberg, Acta physiol. scand. *86*, 353 (1972).
23 I. Nordenfelt, Q.Jl exp. Physiol. *50*, 57 (1965).
24 I. Nordenfelt, Acta univ. lund. sectio II, *10*, 1 (1964).
25 J. Ekström, Acta physiol. scand. *86*, 539 (1972).
26 J. Holmberg, J. Physiol. *219*, 463 (1971).
27 P. Alm and J. Ekström, Q.Jl exp. Physiol. *61*, 127 (1976).
28 P. Alm and J. Ekström, Archs oral Biol. *21*, 417 (1976).
29 J.R. Garrett, Jl R. microsc. Soc. *86*, 1 (1966).
30 J.R. Garrett and J. Holmberg, Z. Zellf. mikrosk. Anat. *131*, 451 (1972).
31 I. Nordenfelt, Q.Jl exp. Physiol. *49*, 103 (1964).
32 N. Emmelin, I. Nordenfelt and C. Perec, Experientia *22*, 725 (1966).
33 J.N. Langley, in: Textbook of Physiology, vol. 1, p. 482. Ed. E.A. Schäfer. Edingburg & London, Pentland 1898.
34 J. Lichtman, J. Physiol. *273*, 155 (1977).
35 S. Tuček, Exp. Neurol. *40*, 23 (1973).
36 J. Rossier, Int. Rev. Neurobiol. *20*, 283 (1977).
37 H.L. White and J.C. Wu, Biochemistry *12*, 841 (1973).
38 I. Nordenfelt, Q.Jl exp. Physiol. *50*, 62 (1965).
39 J. Holmberg, unpublished observations.
40 J. Ekström and J. Holmberg, J. Physiol. *222*, 93P (1972).
41 J. Ekström and B. Lindmark, J. Physiol. *271*, 31P (1977).
42 J. Ekström and B. Lindmark, Acta pharmac. tox., in press.
43 J. Ekström, Acta physiol. scand. *102*, 116 (1978).
44 J. Ekström, S.K. Kemplay, J.R. Garrett and L.W. Duchen, Experientia *33*, 1458 (1977).
45 D.B. Drachman, in: Neuropoisons, p. 325, Ed. L.L. Simpson. Plenum Press, New York, London 1971.
46 N. Ambache, J. Physiol. *113*, 1 (1951).
47 S.M. Hilton and G.P. Lewis, J. Physiol. *128*, 235 (1955).
48 D.A. Tonge, T.J. Gradidge and R.M. Marchbanks, J. Neurochem. *25*, 329 (1975).
49 J. Ekström, Q.Jl exp. Physiol. *58*, 171 (1973).
50 J. Ekström, Acta physiol. scand. *99*, 110 (1977).
51 D.H. Snyder, D.H. Riefenberick and S.R. Max, Exp. Neurol. *40*, 36 (1973).
52 S. Tuček, D.K. Koštířová and E. Gutmann, J. neurol. Sci. *27*, 363 (1976).
53 I. Diamond, G.M. Franklin and D. Milfay, J. Physiol. *236*, 247 (1974).
54 J. Ekström, Acta physiol. scand. *93*, 525 (1975).
55 J. Ekström, Acta physiol. scand. *99*, 48 (1977).
56 J. Ekström, Acta physiol. scand. *92*, 272 (1974).
57 J. Ekström and D. Templeton, Acta physiol. scand. *101*, 329 (1977).
58 J. Ekström, Acta physiol. scand. *98*, 185 (1976).
59 J. Ekström, Q.Jl exp. Physiol. *59*, 73 (1974).
60 G.J. Maletta and P.S. Timiras, J. Neurochem. *15*, 787 (1968).
61 E. Gutmann, S. Tuček and V. Hanzlíková, Physiol. bohemoslov. *18*, 195 (1969).
62 S. Tuček, D.K. Koštířová and E. Gutmann, J. neurol. Sci. *27*, 353 (1976).
63 A.M. Goldberg and B.L. Welch, Science *178*, 219 (1972).
64 A.J. Mandell and S. Knapp, Neuropharmacology *10*, 513 (1971).
65 F. Oesch, J. Pharmac. exp. Ther. *188*, 439 (1974).
66 F.C. MacIntosh and B. Collier, in: Handbook of Experimental Pharmacology, vol. 42, p. 99. Ed. G.V.R. Born, O. Eichler, A. Farah, H. Herken and A.D. Welch. Springer Verlag, Berlin 1976.
67 I.A. Hendry, Brain Res. *107*, 105 (1976).
68 J. Ekström, Acta pharmac. tox. *36*, 284 (1975).
69 H. Thoenen and J.P. Tranzer, A. Rev. Pharmac. *13*, 169 (1973).
70 D.E. Donald, Circulation Res. *34*, 417 (1974).
71 P. Alm and M. Elmér, Acta physiol. scand. *94*, 36 (1975).
72 P. Alm and J. Ekström, Experientia *33*, 523 (1977).
73 K.-A. Norberg and L. Olson, Z. Zellforsch. *68*, 183 (1965).
74 P. Ohlin, Q.Jl exp. Physiol. *53*, 19 (1968).
75 J. Ekström, unpublished observations.
76 N. Assarson and N. Emmelin, Br. J. Pharmac. *22*, 119 (1964).
77 N. Emmelin and C. Perec, Q.Jl exp. Physiol. *53*, 10 (1968).
78 J. Ekström and M. Elmér, Acta physiol. scand. *101*, 58 (1977).
79 H. Banns, J. Ekström and S.P. Mann, Acta physiol. scand. *102*, 47A (1978).
80 J. Ekström, M. Elmér and H. Banns, Acta pharmac. tox., in press.
81 J. Ekström and M. Elmér, to be published.
82 I. Nordenfelt and C. Perec, Q.Jl exp. Physiol. *52*, 139 (1967).
83 J. Ekström and N. Emmelin, Q.Jl exp. Physiol. *59*, 1 (1974).
84 M.V. Edds, Jr, Q. Rev. Biol. *28*, 260 (1953).
85 J.G. Murray and J.W. Thompson, J. Physiol. *135*, 133 (1957).
86 M.V. Edds, Jr, and W.T. Small, J. exp. Med. *93*, 207 (1951).
87 F.G. Carpenter and R.M. Rubin, J. Physiol. *192*, 609 (1967).
88 M. Elmér, Experientia *31*, 814 (1975).
89 N. Emmelin and A. Muren, Acta physiol. scand. *20*, 13 (1950).
90 J. Holmberg, Acta physiol. scand. *86*, 115 (1972).
91 F. Fonnum, Norwegian Defence Research Establishment, Report No. 58, 1970.
92 B. Jakoubek and B. Semiginovský, Int. Rev. Neurobiol. *13*, 255 (1970).
93 R. Lasek, Int. Rev. Neurobiol. *13*, 289 (1970).
94 B. Grafstein, M. Murray and N.A. Ingoglia, Brain Res. *44*, 37 (1972).
95 L.M. Zieher and A. Pellegrino de Iraldi, Life Sci. *5*, 155 (1966).
96 P.B. Molinoff and J. Axelrod, A. Rev. Biochem. *40*, 465 (1971).
97 A. Pletscher, Pharmac. Rev. *24*, 225 (1972).
98 H. Thoenen, Life Sci. *14*, 223 (1974).