J. Anat. (1968), **103**, 2, pp. 225–232
With 8 figures
Printed in Great Britain

Effects of vagotomy on the cholinesterase content of the preganglionic innervation of the rat heart

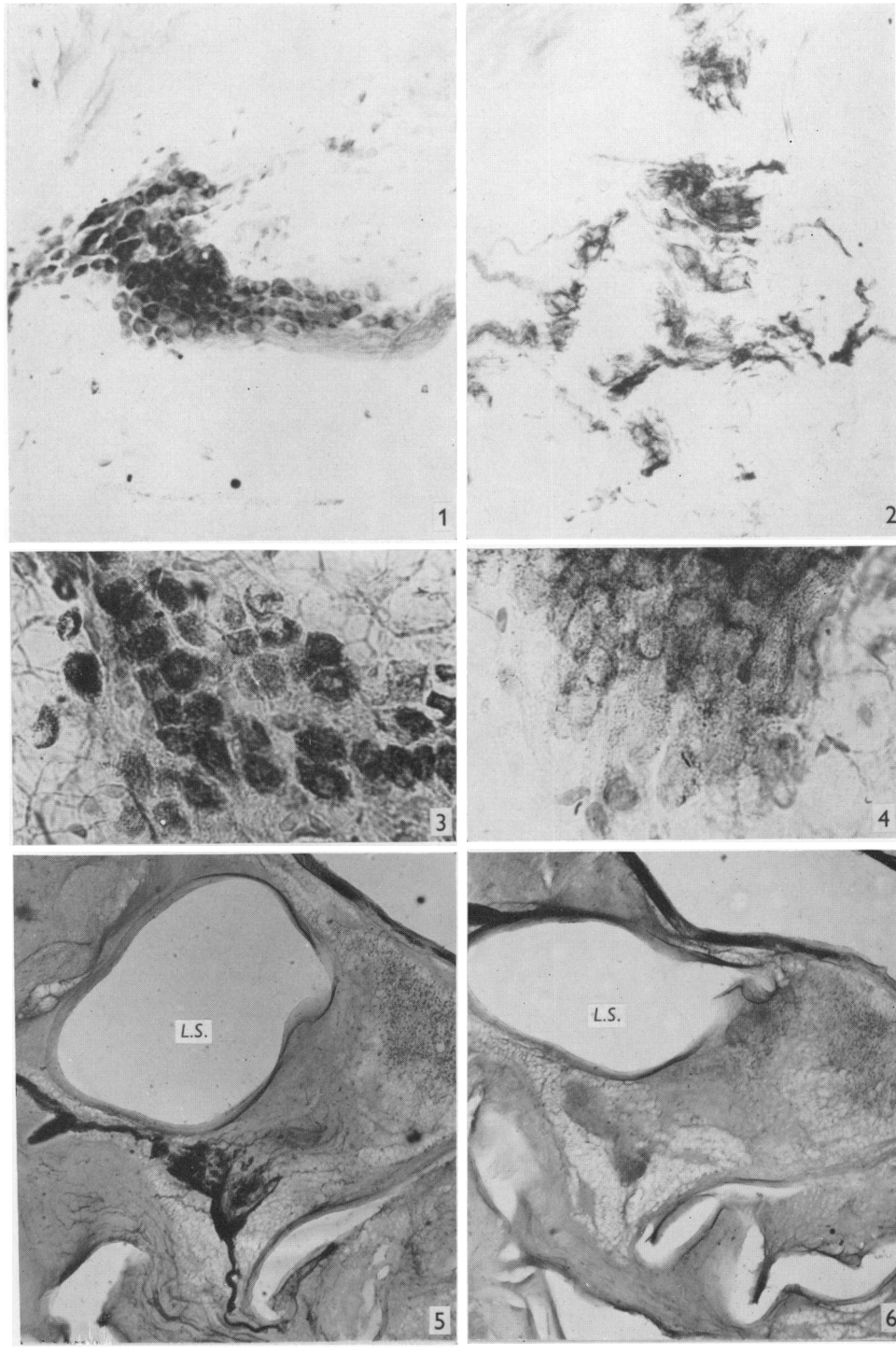
V. NAVARATNAM, P. R. LEWIS AND C. C. D. SHUTE
Department of Anatomy, University of Cambridge

In an earlier paper it was noted that, whereas the cardiac ganglion cells contain true cholinesterase (AChE) only within their cytoplasm, both AChE and pseudocholinesterase (ChE) are present in the interstices between the cells (Navaratnam, 1965*a*). It is not normally possible under the light microscope to determine whether the ChE surrounding the individual nerve cells is located within presynaptic terminals, in dendritic processes of the ganglion nerve cells or within satellite cells. Nevertheless, the presence of considerable amounts of ChE in addition to AChE within the cells of the dorsal motor nucleus of the vagus (Shute & Lewis, 1963; Lewis & Shute, 1966) suggests that much of the ChE within the cardiac ganglia may be related to the terminations of the presynaptic neurones. This hypothesis can be tested by interrupting the preganglionic nerves, when it would be expected that the ChE content of the ganglia would become depleted; similar preganglionic denervation experiments by Sawyer & Hollinshead (1945), Snell (1958) and Koelle (1962) have served to establish that most of the AChE activity within the superior cervical ganglion in the cat is related to presynaptic terminals. Moreover, if nerve section results in characteristic changes in the cardiac ganglia, it should be possible to map the distribution of the individual preganglionic nerve trunks.

A preliminary report of this investigation is available (Navaratnam, Lewis & Shute, 1964).

MATERIAL AND METHODS

Cervical vagotomy with transection of the cervical cardiac nerves was performed on twenty-three albino rats (right side—10, left side—10, bilateral—3). The animals were killed with open ether after periods ranging from 1 to 161 d and perfused with 20% formol saline. In each case the heart and great vessels were dissected out, fixed in 10% formalin at 4 °C for 8 h and embedded in Alginate (Lewis & Shute, 1963). The hind brain also was removed and fixed in formalin. The specimens were sectioned serially on the freezing microtome and cholinesterase activity was demonstrated by a modification of the thiocholine technique (Lewis, 1961). Sections were incubated at room temperature in media containing 6 mM substrate, 9 mM copper sulphate, 16 mM glycine and 50 mM acetate buffer at a pH of 5·0. After a wash in distilled water the sections were treated with a solution of sodium sulphide buffered to a pH of about 5 with acetic acid. Where necessary, calcium ions were added to the sulphide solution to prevent dissolution of the Alginate. Typically, to demonstrate AChE activity, acetylthiocholine iodide was used as substrate with 10^{-4}M ethopropazine hydrochloride present to inhibit ChE and the sections were incubated for 2–4 h. To demonstrate ChE activity, butyrylthiocholine iodide was used as substrate and the


1
2
3
4
L.S.
5
L.S.
6

sections were incubated for 4–7 h; in some experiments 5×10^{-5}M 62C47e was added to inhibit AChE (Bayliss & Todrick, 1956).

In some of the early experiments the vagal trunks were prepared as whole mounts for the demonstration of cholinesterase activity (Lewis, 1961).

RESULTS

Cardiac ganglia

In normal rats the vast majority of cardiac nerve cells contain a high concentration of cholinesterase within the cytoplasm (Figs. 1, 3). This intracellular staining, which is almost completely specific for the acetyl substrate, is hardly affected by 10^{-4}M ethopropazine but is suppressed by 5×10^{-5}M 62C47e. Apart from the intracellular staining there is obvious staining round the individual ganglion nerve cells (Figs. 2, 4). With butyrylthiocholine as substrate, ethopropazine greatly reduces the intensity of extracellular staining and the residual staining is inhibited by 62C47e. The substrate specificity and the sensitivity to inhibitors indicate that the intracellular staining is almost exclusively due to AChE, while the extracellular staining is caused by both AChE and ChE with the latter predominating.

Whereas the intracellular AChE is unaffected by vagotomy (Fig. 5), large portions of the cardiac ganglionic mass show reduction of extracellular enzyme activity after the operation. Of the extracellular enzymes, the depletion of ChE is, of course, simpler to detect (Fig. 6) because it is not masked by intracellular staining. The precise position of the ganglionic clumps exhibiting enzyme depletion depends on which side the operation was performed. After right vagotomy ChE activity is reduced in the ganglionic clumps surrounding the inlet of the right precaval vein into the right atrium, whereas after vagotomy on the left side the change is demonstrable in the clumps lying along the left precaval vein and left sinus horn (Fig. 6).

The reduction of extracellular ChE activity commences approximately 3 days after vagotomy and is evident in all animals which survive this period; of the three rats on which bilateral vagotomy was performed, two survived for 3 days and in both these animals there is a marked reduction of extracellular enzyme activity throughout the cardiac ganglia. The ChE activity persists at a reduced level for a further 4–7 days, but after that period it cannot be detected unless the incubation period is greatly prolonged. A feature of all the operated animals is that the intense AChE activity in the sinus node and atrioventricular connecting system is unaffected by section of the preganglionic nerves.

Fig. 1. A group of cardiac nerve cells in a normal rat showing the distribution of AChE. ×90.

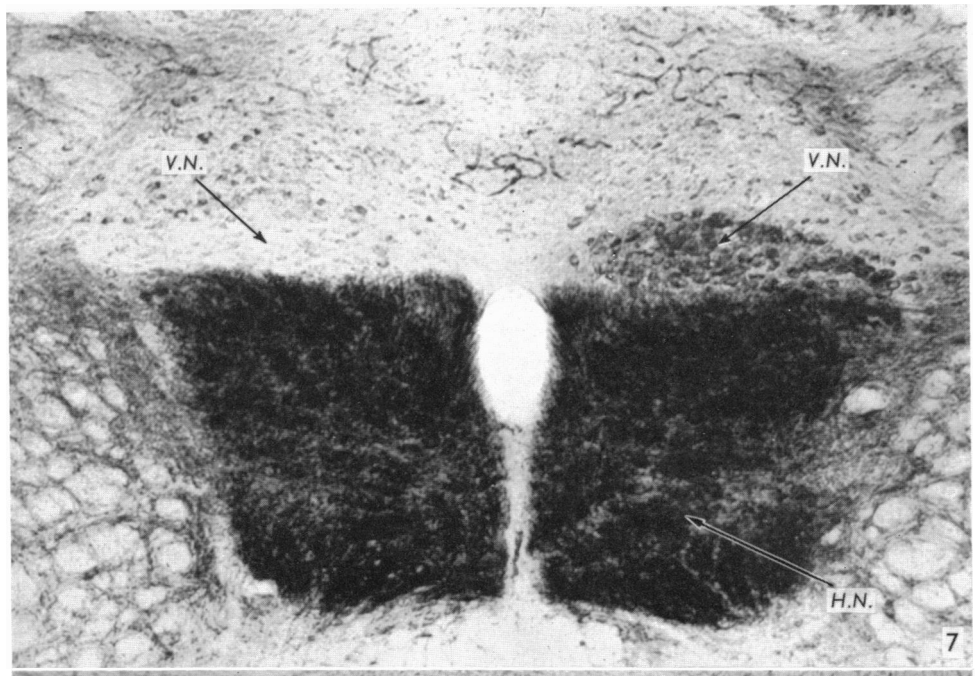
Fig. 2. Adjacent section showing the distribution of ChE in the same ganglion. The intensity of staining is approximately the same as in Fig. 1 but the distribution is quite different. ×90.

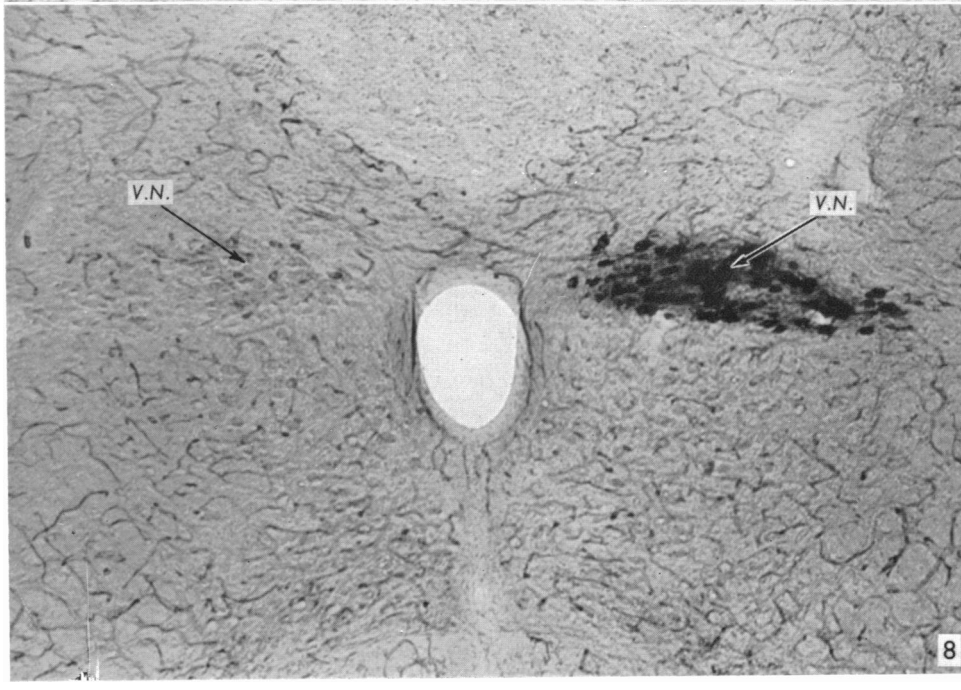
Fig. 3. Enlargement from Fig. 1 to show the preponderantly intracellular localization of AChE. ×250.

Fig. 4. Enlargement from Fig. 2 showing that ChE is extracellularly situated. ChE. ×250.

Fig. 5. A cardiac ganglion near the left sinus horn (*L.S.*), showing no appreciable loss of AChE 9 d after left vagotomy. ×40.

Fig. 6. Adjacent section showing the marked diminution of ChE activity in the same clump. ×40.


V.N.
V.N.
H.N.
7


V.N.
V.N.
8

Dorsal motor nucleus of the vagus

Under normal conditions, the cells of the dorsal motor nucleus of the vagus contain a high concentration of intracytoplasmic cholinesterase (Figs. 7, 8). With butyrylthiocholine as substrate the cells stain very intensely (Fig. 8) and this staining is abolished by 10^{-4}M ethopropazine. With acetylthiocholine as substrate, ethopropazine reduces the intensity of staining by a factor of about 4 or 5 and the residual staining is inhibited by 62C47e. Thus the histochemical evidence suggests strongly that these cells contain both AChE and ChE but with the latter predominating. Of the other visceral motor nuclei, the Edinger–Westphal nucleus possesses a high concentration of ChE but the salivatory nucleus contains AChE only. The nuclei of the branchiomotor and somatic motor columns in the brain stem contain AChE only, with the exception of a group of cells in the caudal part of the hypoglossal nucleus where ChE is also present.

Besides the undoubted intracellular staining in the dorsal motor vagal nucleus there is diffuse staining between the cells; however, at the light microscope level it is impossible to decide whether this latter staining is in presynaptic endings or in fine processes of the motor nerve cells.

Two days after unilateral vagotomy the cholinesterase activity of the cells of the dorsal motor vagal nucleus on the operated side begins to decrease rapidly and, after 5 days, it is usually not detectable in sections incubated for the normal period (Figs. 7, 8); the extracellular staining disappears similarly. No consistent difference was noted in the rates at which AChE and ChE disappeared. The cholinesterase activity in the nucleus remains negligible for several weeks after vagotomy, although the cells themselves are present and can be demonstrated by counterstains. Gradually, some intracellular staining for AChE reappears though, even after 5 months, it is considerably lower than in the control nucleus on the opposite side. There is no return of ChE activity during this period.

Although all three bilaterally operated animals in this study had to be perfused before the fourth post operational day, they all show depletion of enzyme activity within the dorsal motor nuclei on sides as judged by comparison with the staining in the neighbouring brain-stem nuclei.

Vagal trunks

In those vagal trunks which were examined there is obvious enzyme pile-up cranial to the cut. These changes are similar to those we have observed in the sciatic nerve, though in the vagus the changes occur more rapidly and axonal swelling proximal to the cut is less pronounced. The most striking difference, however, is that pile-up is seen with butyryl substrate as well as with acetyl substrate.

Fig. 7. Section through the hind brain, 9 d after unilateral vagotomy, showing the distribution of AChE in the dorsal motor nucleus of the vagus (*V.N.*) on both sides. On the control side there is intense activity in the vagal cells, while on the operated side the enzyme has almost completely disappeared. Note the marked activity in the hypoglossal nucleus (*H.N.*) on both sides. × 80.

Fig. 8. Adjacent section showing ChE activity. On the operated side, the enzyme is almost undetectable, whereas on the control side there is considerable activity within the cells of the dorsal vagal nucleus. × 80.

Some nerve fibres show enhanced staining with acetyl substrate caudal to the cut; this phenomenon could be due to the presence of ascending sympathetic fibres in the cervical vagus. The possibility that there are cholinergic fibres running in both directions in this length of the vagal trunk introduced serious uncertainties into any interpretation of the observations on pile-up; so the study of changes in enzyme activity in the region of the cut was not pursued beyond the first few animals.

DISCUSSION

The foregoing observations show that the cholinesterase content of the preganglionic nerves differs, in substrate specificity and in sensitivity to inhibitors, from that of the postsynaptic elements. Both sets of neurones contain AChE but the preganglionic neurones possess in addition a high concentration of ChE. It is not obvious why the preganglionic vagal neurones should be so rich in ChE. One possibility is that this enzyme could be important in preventing the dangerous accumulation of acetylcholine during periods of rapid stimulation (Shute & Lewis, 1963), since, unlike AChE, it is not inhibited by excess of substrate. Another possibility is that the vagal transmitter in the rat may be a higher ester of choline; ChE is able to hydrolyse the higher esters of choline (Whittaker, 1951), some of which, it has been suggested by Kewitz (1954, 1959), may be involved in synaptic transmission at certain sites.

Transection of the vagal trunk results in the rapid disappearance of cholinesterase from the cells of the dorsal motor vagal nucleus concomitant with enzyme depletion in the presynaptic terminals within the cardiac ganglia. Similar reduction in the cholinesterase content of motor cells following axotomy has been observed in the hypoglossal nucleus (Schwarzacher, 1958), in the ventral column of the spinal cord (Hard & Peterson, 1950; Söderholm, 1965) and in the superior cervical sympathetic ganglion (Härkönen, 1964). In all these situations, however, enzyme activity is restored within a matter of weeks. In the vagal cells, on the other hand, AChE activity was very slow to reappear and no return of ChE was observed even after 5 months. The fall in enzyme activity appears to be part of the chromatolytic response of the cell body to axotomy (Watson, 1966) and may reflect a massive movement of cell proteins down the axon to the site of regeneration. It is likely that return to normal activity levels occurs only when functional contact has been re-established at the periphery, a contact which may not have been fully regained in our experiments.

The disappearance of ChE from portions of the cardiac ganglionic mass after unilateral vagotomy provides an opportunity to compare the distribution to the heart of one vagus with that of the other. The results suggest that the two nerves are distributed more or less symmetrically when they are considered in relation to the left and right sinus horns. The right vagus nerve is distributed mainly to the ganglionic clumps related to the right cavo-sinus junction while the left vagus nerve supplies the ganglia lying along the left sinus horn behind the left atrium. These findings are consistent with the symmetrical distributions of right and left cardiac nerves reported in mammalian embryos by Shaner (1930), Licata (1954) and by one of us (Navaratnam, 1965*b*).

SUMMARY

1. Histochemical investigation by means of a thiocholine technique reveals that the cells in the dorsal motor nucleus of the vagus in the rat contain both true cholinesterase (AChE) and pseudocholinesterase (ChE), with the latter predominating.

2. In the cardiac ganglia, whereas intracellular staining is caused almost exclusively by AChE, extracellular staining is caused by both enzymes, particularly ChE.

3. Cervical vagotomy with transection of the cervical cardiac nerves leads to rapid loss of enzyme from the cell bodies of the corresponding dorsal motor nucleus of the vagus. There is concurrent loss of extracellular (chiefly ChE) staining in the cardiac ganglia, whereas intracellular staining is unaffected by the operation. It is thus likely that the ChE activity in the ganglia is related to the terminals of the preganglionic axons.

4. After right vagotomy there is loss of ChE activity from the ganglia surrounding the inlet of the right precaval vein while vagotomy on the left side leads to disappearance of the ChE in the ganglionic clumps along the left sinus horn.

REFERENCES

BAYLISS, B. J. & TODRICK, A. (1956). The use of a selective acetylcholinesterase inhibitor in the estimation of pseudocholinesterase activity in rat brain. *Biochem. J.* **62**, 62–67.

HARD, W. L. & PETERSON, A. C. (1950). The distribution of choline esterase in nerve tissue of the dog. *Anat. Rec.* **108**, 57–69.

HÄRKÖNEN, M. (1964). Carboxylic esterases, oxidative enzymes and catecholamines in the superior cervical ganglion of the rat and the effect of pre- and post-ganglionic nerve division. *Acta physiol. scand.* **63**, suppl. 237.

KEWITZ, H. (1954). Zur Bedeutung des Acetylcholins als Überträgersubstanz sympathischer Ganglien. *Arch. exp. Path. Pharmak.* **222**, 323–329.

KEWITZ, H. (1959). Nachweis von 4-Amino-n-butyrylcholin im Warmblütergehirn. *Arch. exp. Path. Pharmak.* **237**, 308–318.

KOELLE, G. B. (1962). A new general concept of the neurohumoral functions of acetylcholine and acetylcholinesterase. *J. Pharm. Pharmac.* **14**, 65–90.

LEWIS, P. R. (1961). The effect of varying the conditions in the Koelle technique. *Biblthca anat.* **2**, 11–20.

LEWIS, P. R. & SHUTE, C. C. D. (1963). Alginate gel; an embedding medium for facilitating the cutting and handling of frozen sections. *Stain Technol.* **38**, 307–310.

LEWIS, P. R. & SHUTE, C. C. D. (1966). The distribution of cholinesterase in cholinergic neurons demonstrated with the electron microscope. *J. Cell Sci.* **1**, 381–390.

LICATA, R. H. (1954). The human embryonic heart in the ninth week. *Am. J. Anat.* **94**, 73–125.

NAVARATNAM, V. (1965*a*). The ontogenesis of cholinesterase activity within the heart and cardiac ganglia in man, rat, rabbit and guinea-pig. *J. Anat.* **99**, 459–467.

NAVARATNAM, V. (1965*b*). Development of the nerve supply to the human heart. *Br. Heart J.* **27**, 640–650.

NAVARATNAM, V., LEWIS, P. R. & SHUTE, C. C. D. (1964). Effects of vagotomy on the cholinesterase content of the preganglionic innervation of the heart. *J. Anat.* **98**, 287.

SAWYER, C. H. & HOLLINSHEAD, W. H. (1945). Cholinesterases in sympathetic nerve fibres and ganglia. *J. Neurophysiol.* **8**, 137–153.

SCHWARZACHER, H. G. (1958). Der Cholinesterasegehalt motorischer Nervenzellen während der axonalen Reaktion. *Acta anat.* **32**, 51–65.

SHANER, R. F. (1930). On the development of the nerves to the mammalian heart. *Anat. Rec.* **46**, 23–39.

SHUTE, C. C. D. & LEWIS, P. R. (1963). Cholinesterase-containing systems of the brain of the rat. *Nature, Lond.* **199**, 1160–1164.

SNELL, R. S. (1958). The histochemical appearances of cholin-esterase in the superior cervical ganglion and the changes which occur after preganglionic nerve section. *J. Anat.* **92**, 408–418.

SÖDERHOLM, U. (1965). Histochemical localization of esterases, phosphatases and tetrazolium reductases in the motor neurones of the spinal cord of the rat and the effect of nerve division. *Acta physiol. scand.* **65**, suppl. 256.

WATSON, W. E. (1966). Quantitative observations upon acetylcholine hydrolase activity of nerve cells after axotomy. *J. Neurochem.* **13**, 1549–1550.

WHITTAKER, V. P. (1951). Specificity, mode of action and distribution of cholinesterases. *Physiol. Rev.* **31**, 312–343.