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The afferent and efferent myelinated fibres of the avian cervical vagus*

A. B. ABDALLA AND A. S. KING

Department of Anatomy, Faculty of Veterinary Science, University of Khartoum, Khartoum, Sudan, and Department of Veterinary Anatomy, University of Liverpool, Liverpool, L69 3BX, England

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INTRODUCTION

Many papers have been written on the fibre composition of the mammalian vagus and its branches, but very little is known about the avian vagus. Dahl, Samson & Balfour (1964) assumed that the cervical vagus of the domestic fowl consisted predominantly of unmyelinated fibres. Jones (1969) observed that in the duck most of the myelinated fibres were less than 5 μm in diameter, and that fibres of 6–10 μm were rare. Apparently only Brown, Molony, King & Cook (1972) have studied the myelinated fibre spectrum of the normal vagus and some of its branches in the domestic fowl. Subsequently the topic was extensively re-investigated in a doctorate thesis by Abdalla (1974).

Our main objectives were to confirm the counts of myelinated fibres in the right and left cervical vagi, and to estimate the numbers of afferent and efferent myelinated fibres in the cervical vagus by degeneration experiments.

MATERIALS AND METHODS

Altogether 16 hens of the Shaver Starcross type 585 (*Gallus gallus domesticus*) were investigated. The birds were 6–9 months old and weighed 1–1·5 kg.

The right and left cervical vagi were studied in the normal bird, and after midcervical vagotomy. The right vagus was also studied after intracranial vagotomy (N.B. The cervical vagus has no branches. The proximal vagal ganglion is in the head but the distal vagal ganglion is in the thoracic inlet).

Removal of normal nerves

Segments 1 cm long were removed either under halothane anaesthesia or within about 5 minutes of death caused by intravenous barbiturate.

Midcervical vagotomy

In four birds 1 cm of the midcervical vagus was removed on one side under halothane–oxygen anaesthesia and the wound was sutured. All birds recovered uneventfully. After varying survival periods (Table 1) the birds were killed by intravenous barbiturate, and segments of the central and peripheral stumps of the cervical vagi, 1·5–2·0 cm from the cut ends, were removed for histological study.

* Reprint requests to Professor A. S. King, University of Liverpool.

Intracranial vagotomy

This operation was done on the right side only. General anaesthesia was induced with pentobarbitone sodium. After reflexion of the skin the periosteum was scraped from the dorsolateral aspect of the temporal and parietal bones; a hole was then drilled in this area. Pieces of bone were removed until the combined rootlets of the glossopharyngeal, vagus and spinal accessory nerves were exposed. The rootlets were either picked up in single bundles and cut, or detached from the medulla oblongata by pulling them peripherally. Haemorrhage was controlled with bone wax and adsorbable gelatin sponge. The hole was sprayed with an antibiotic and the skin was sutured. For 2–3 days the birds were ataxic and slightly anorexic, but then recovered fully. After varying survival periods (Table 1) segments of the cervical vagus were removed for histology.

At the end of every experiment involving intracranial vagotomy the rootlets of the vagal, glossopharyngeal and spinal accessory nerves were inspected *post mortem*. All the roots were found to have been cut.

Total isolation of a segment of the cervical vagus

In one bird an experiment was made to ascertain whether or not after a survival period of 14 days all fibres cut off from their cell bodies had completely degenerated. The cervical vagus was cut at two levels on one side so that a 3·5 cm segment of the nerve was isolated. After 14 days pieces of the isolated segment were removed and fixed in Millonig's (1961) fixative. These pieces were examined with the electron microscope. Other pieces were subjected to light microscopy following the Flemming–Wolter technique as described by Williams & Wendell-Smith (1960).

Examination of the proximal and distal ganglia

The proximal vagal ganglion was examined in three birds, one normal, another 30 days after intracranial vagotomy, and a third 6 days after cutting the glossopharyngeal nerve proximal to the vago-glossopharyngeal anastomosis. In yet another bird the distal vagal ganglion was examined 30 days after midcervical vagotomy. The cervical vagus was examined for aberrant ganglion cells with the light microscope.

Fixation and staining

The procedure adopted for fixation and staining was that described by Williams & Wendell-Smith (1960).

Photography

The sections were photographed with a Zeiss photomicroscope at a final magnification of $\times 800$. A montage of the complete cross section of each nerve was prepared. The diameters of a few selected fibres on the print were checked on the original section with an eyepiece graticule calibrated by a micrometer slide; this showed that photography had introduced no variations.

Measurements and counting

The external diameter of every myelinated fibre of each nerve was measured on the montage with a Perspex sheet in which a series of holes calibrated to show increases in diameter of 1 μm had been drilled. As each fibre was measured it was pricked with a needle attached to a tally counter which registered the number of

Table 1. *Myelinated fibre counts of the cervical vagus of the adult domestic fowl in normal birds and in birds studied after vagotomy*

(R, right; L, left; CV, cervical vagus; cs, central stump of cervical vagus; ps, peripheral stump of cervical vagus; N, normal; McV, midcervical vagotomy; IcV, intracranial vagotomy.)

Nerve	Bird no. and method	Survival period (days)	2 μm and less	2–3 μm	3–4 μm	4–5 μm	5–6 μm	6–7 μm	7–8 μm	8–9 μm	9–10 μm	10–11 μm	Total
R CV	B1 N	—	5316	2634	866	215	26	6	—	—	—	—	9063
L CV	B2 N	—	5213	2232	682	170	30	4	—	—	—	—	8331
L CV	B3 N	—	4078	2040	1069	780	341	148	5	4	—	—	8465
R cs	B3 McV	21	747	1534	1325	918	414	132	16	25	8	1	5120
R ps	B3 McV	21	2358	1163	251	52	1	—	—	—	—	—	3825
R cs	B10 McV	58	3076	1473	550	413	91	6	2	—	—	—	5611
R ps	B10 McV	58	1471	1469	421	175	44	—	—	—	—	—	3580
L cs	B11 McV	37	1272	1648	1107	535	160	34	—	—	—	—	4756
L ps	B11 McV	37	2791	1250	345	90	5	—	—	—	—	—	4481
L cs	B12 McV	90	1724	1259	666	470	163	39	3	—	—	—	4324
L ps	B12 McV	90	1700	1452	381	198	50	1	—	—	—	—	3782
R CV	B13 IcV	14	2486	1285	442	53	5	2	—	—	—	—	4273
R CV	B15 IcV	30	1147	1385	487	266	21	6	—	—	—	—	3312
R cs	B14 IcV+McV	14	84	303	121	10	2	—	—	—	—	—	520

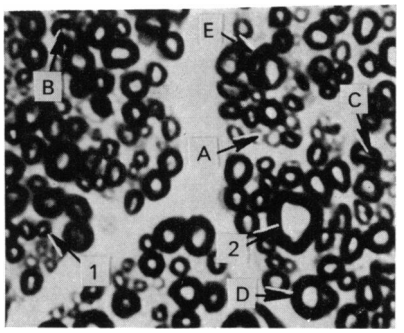

Fig. 1

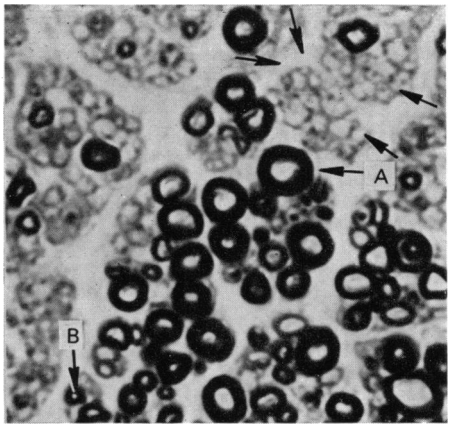

Fig. 2

Fig. 1. Normal right cervical vagus. The field contains many small myelinated fibres, some being only about 1·5 μm in external diameter, but with dark myelin sheaths (axon 1) and some larger myelinated fibres, a few of which attain about 7 μm in external diameter (axon 2). There are also many small paler, greyish structures (A), which are interpreted as Schwann cells containing unmyelinated fibres; these were excluded from all counts. Some of the sections of myelinated fibres were oval (axon B) or crenated (axon C); these were measured by taking the mean of the greatest diameter and that at right angles to it. Slight disruption of the myelin sheath is occasionally seen (axon D); such fibres were included in the counts, but fibres with a redundant sheath (e.g. axon E) were excluded from all counts. Prepared by the Flemming–Wolter technique of Williams & Wendell-Smith (1960). × 1000.

Fig. 2. The central stump of the right cervical vagus 21 days after right midcervical vagotomy. The largest myelinated fibre (A) has an external diameter of about 7 μm. The smallest fibres (e.g. B) are about 2 μm. Most of the field is occupied by large areas of greyish sheaths (e.g. zones indicated by arrows), resembling the occasional grey sheaths seen in normal nerves (e.g. A in Fig. 1); some of these are interpreted as Schwann cells of fibres which have lost their axons as a result of the vagotomy, others as normal unmyelinated fibres. Prepared by the Flemming–Wolter technique of Williams & Wendell-Smith (1960). × 1000.

fibres. The smallest fibres, up to and including 2 μm in diameter, were grouped together. The next group contained fibres larger than 2 μm and up to and including those of 3 μm, and so on in steps of 1 μm.

Only fibres with distinctly black sheaths were counted. Greyish sheaths and fibres with disrupted sheaths were excluded from the counts (see Figs. 1 and 2). Only about 1–2% of fibres were excluded because of disrupted sheaths.

RESULTS

Normal nerves

The right vagus of bird B1 contained 9063 myelinated fibres (Table 1). The left vagus contained 8331 fibres in bird B2 and 8465 in bird B3. The great majority of the fibres were of small external diameter (Fig. 1); fibres of 3 μm or less comprised 88% of the total in the right vagus (B1) and 89% and 72% respectively of those in the two left vagi studied (B2 and B3). The number of fibres in the other groups decreased as the diameter increased. There were no fibres wider than 7 μm in diameter in birds B1 and B2; but in bird B3 the largest fibres were in the 8–9 μm group; in this bird the count was made on the left vagus 21 days after midcervical vagotomy on the right side, suggesting a possible contralateral reaction to injury.

Midcervical vagotomy

After midcervical vagotomy the central stump consisted of efferent fibres and those afferent fibres with cell bodies in the proximal vagal ganglion (perhaps also in the distal glossopharyngeal ganglion); the peripheral stump consisted only of afferent fibres with cell bodies in the distal vagal ganglion. The details of the counts of these fibres are summarized in Table 1. In both the central and the peripheral stumps of the cervical vagus, increased numbers of greyish 'sheaths' occupied the areas in which myelinated axons had evidently degenerated (Fig. 2, arrowed areas); these sheaths were interpreted as Schwann cells. Afferent and efferent fibres were not apparently located in particular regions of the vagus, but were found throughout the nerve.

In the right cervical vagus the sum of the myelinated fibres in the central and peripheral stumps was 8945 in bird B3 and 9191 in B10. Of these, 3825 (43%) in bird B3, and 3580 (39%) in bird B10 lay in the peripheral stump and were therefore afferent, with their cell bodies in the distal vagal ganglion; the mean of these values is 3703. In the left cervical vagus the total number in the two stumps was 9237 in bird B11 and 8106 in bird B12. Of these, 4481 (49%) in bird B11 and 3782 (47%) in bird B12 (mean 4132) occupied the peripheral stump and therefore were afferent with cell bodies in the distal vagal ganglion.

Changes in the diameter of fibres

It was observed that with the shortest survival period (21 days) the central stump contained fibres up to 11 μm in diameter (e.g. bird B3, Table 1). In this bird (B3) the total number of fibres of the smallest size (2 μm and less) in the combined right central and peripheral stumps was only 3105, as compared to a total of 5316 in the normal right cervical vagus of bird B1 (Table 1). In bird B10 (survival period 58 days) the count was similar to the normal right cervical vagus, with a sum of 4547 fibres of the smallest size in the combined central and peripheral stumps (see Table 1).

Intracranial vagotomy

Intracranial vagotomy interrupted the efferent fibres in the rootlets of the glossopharyngeal, vagus and spinal accessory nerves. The myelinated fibres surviving in the cervical vagus should therefore represent its total afferent myelinated component (assuming that there are no sympathetic fibres in the cervical vagus).

In the *right cervical* vagus the total number of surviving myelinated fibres was 4273 in bird B13 and 3312 in bird B15. The mean of 3793 fibres could represent the total number of afferent fibres in this nerve. But the value of 3312 for bird B15 seems rather low and suggests that the proximal vagal ganglion might have been damaged during vagotomy. In bird B14, where midcervical vagotomy was combined with intracranial vagotomy, only 520 fibres survived in the right central stump of the cervical vagus, i.e. in the segment lying between the two vagotomy sites; these must have been afferent fibres with cell bodies in the proximal vagal ganglion (or perhaps in the distal glossopharyngeal ganglion).

The ganglia

Dissections of the origins of the glossopharyngeal, vagus, and spinal accessory nerves showed that the rootlets of all three nerves joined a single ganglionic enlargement (the proximal vagal ganglion) which in the bird is deeply embedded in a bony fossa in the lateral wall of the skull. This ganglion is formed by the fusion of the

proximal vagal ganglion with the proximal glossopharyngeal ganglion. Histological examination of this ganglion, and of the distal vagal ganglion after midcervical and intracranial vagotomy revealed no abnormalities in the ganglion cells. No isolated ganglion cells were found in the cervical vagus.

The isolated segment of the cervical vagus

Electron microscopy showed that after a survival period of 14 days there were only Schwann cells in the isolated segment of the cervical vagus. In light microscopy these Schwann cells appeared as greyish sheaths. The presence of normal blood vessels in sections of the nerve indicated that the segment had an active blood supply.

DISCUSSION

Total counts: afferent and efferent fibres

From a knowledge of the numbers of myelinated fibres in the normal nerves, and in the nerves after vagotomy, an attempt can be made to work out the approximate disposition of the myelinated fibres in the cervical vagus.

The total number of myelinated fibres in the right cervical vagus was found to be 9063 in one bird by a direct count, and 8945 and 9191 in two other birds by summing the fibres in the central and peripheral stumps after midcervical vagotomy; the mean of the counts for the right cervical vagus in these three birds is 9066. In the left cervical vagus direct counts in two birds gave totals of 8331 and 8465, and the sums of the stumps after mid-cervical vagotomy in two other birds yielded totals of 9237 and 8106; the mean for the left cervical vagus in these four birds is 8535.

Combined midcervical and intracranial vagotomy in one bird indicated that only 520 of the myelinated afferent fibres in the right cervical vagus had their cell bodies in the proximal vagal ganglion (or distal glossopharyngeal ganglion). In two birds, 3825 and 3580 myelinated fibres survived in the peripheral stump of the right cervical vagus after midcervical vagotomy; therefore about 3703 (the mean of these two values) myelinated afferent fibres apparently had their cell bodies in the right distal vagal ganglion. The number of afferent myelinated fibres in the right cervical vagus should therefore be about 520+3703, i.e. 4223; this means that about 12% (520) of fibres have their cell bodies in the proximal vagal ganglion (or distal glossopharyngeal ganglion) and about 88% (3703) in the distal vagal ganglion. However, this total of 4223 myelinated afferent fibres is essentially an indirect estimate. A more direct estimate of the total number of myelinated afferent fibres in the right cervical vagus was obtained by intracranial vagotomy: in two birds this gave totals of 4273 and 3312 surviving fibres, the mean being 3793. The difference between the indirect estimate of 4223 and the mean direct estimate of 3793 may be within the range of normal variation for this species. However, one of the direct counts (3312) seems rather low, possibly because of some disturbance to the blood supply of the proximal vagal ganglion during surgery, and therefore we propose to adopt the indirect estimate (4223 myelinated afferent fibres) for calculating the numbers of myelinated efferent fibres in the right cervical vagus (see below); this decision is supported by the fact that the other direct count (4273 fibres surviving intracranial vagotomy) is close to the adopted indirect estimate (4223).

In the left cervical vagus, 4481 and 3782 myelinated fibres survived in the peripheral stump after midcervical vagotomy; therefore about 4132 myelinated afferent

fibres (the mean of these two values) evidently had their cell bodies in the left distal vagal ganglion. If the number of afferent myelinated fibres with cell bodies in the left proximal vagal ganglion (or distal glossopharyngeal ganglion) is assumed arbitrarily to be about the same as in the right vagus, then the total number of myelinated afferent fibres in the left cervical vagus may well be about 520+4132, or 4652.

From these estimates of the total numbers of afferent myelinated fibres it is possible to estimate the total number of efferent myelinated fibres by subtracting the total number of afferent myelinated fibres from the total number of myelinated fibres. This would give 9066 minus 4223, i.e. 4843, efferent myelinated fibres in the right cervical vagus, and 8535 minus 4652, i.e. 3883, in the left cervical vagus. Thus in the right cervical vagus about 47% of the myelinated fibres would be afferent and about 53% would be efferent. In the left cervical vagus about 55% would be afferent and 45% would be efferent.

The preceding estimates of the afferent and efferent fibres depend on the full integrity of the ganglia of the vagus. Histological examination revealed no obvious damage to the ganglion cells, but such a possibility cannot be entirely ruled out.

Comparison with other fibre counts in the avian vagus

As in the duck (Jones, 1969), myelinated fibres of 6–10 μm are rare in the cervical vagus of the domestic fowl. We agree with Brown *et al.* (1972) that the largest fibres seldom exceed 7 μm in this species. On the other hand, all our counts are somewhat lower than theirs. Their values for the total number of myelinated fibres in the left cervical vagus in three birds ranged between 9056 and 10236 with a mean of 9262, whereas our mean for this nerve was 8535. They may well have included some small fibres which we excluded as being unmyelinated, for they used osmium tetroxide, and this is considered to impart a greyish stain to Schwann cells containing bundles of unmyelinated fibres (Addison, 1950; see also Duncan, 1934). We were able to show that the Flemming–Wolter technique also stains Schwann cells, since greyish sheaths were clearly visible in the isolated segment of the vagus in which the electron microscope proved only Schwann cells to be present. Similar greyish sheaths were present in increased numbers in the cervical vagus after vagotomy, and these too were probably the Schwann cells of degenerated axons. We believe that these observations justify us in interpreting the similar greyish sheaths in normal nerves as the Schwann cells of unmyelinated fibres, and so we excluded all such sheaths from our counts of myelinated fibres.

Comparison with mammalian vagus

The disposition of the myelinated fibres in the cervical vagus of the bird is somewhat different from that of the mammal as established by Heinbecker & O'Leary (1933), DuBois & Foley (1936), Jones (1937), Daly & Evans (1953), Evans & Murray (1954), Agostoni, Chinnock, Daly & Murray (1957), Hoffman & Kuntz (1957) and Schnitzlein, Rowe & Hoffman (1958). For example, the cervical vagus of the cat contains only about 5000 myelinated fibres (Agostoni *et al.* 1957), not much more than half the number in the fowl. Also the diameters of the efferent myelinated fibres differ greatly in the two classes; in the fowl very few of those in the cervical vagus exceed about 7 μm in diameter, but the cervical vagus of the cat has fibres up to 18 μm in diameter. On the other hand, the external diameters of the afferent myelinated fibres are similar in these two species.

Agostoni *et al.* (1957) reported the presence in the mammalian cervical vagus of about 96 myelinated fibres of sympathetic origin. Such a possibility also exists in the avian cervical vagus, but it has not been investigated in this study.

SUMMARY

The numbers and diameters of the myelinated fibres in the cervical vagus have been studied in normal birds and after midcervical and intracranial vagotomy. The mean total number of myelinated fibres was about 9066 in the right cervical vagus and 8535 in the left. In the right nerve about 4223 of these fibres were afferent and 4843 were efferent; of the afferent fibres about 520 had their cell bodies in the proximal vagal ganglion (or distal glossopharyngeal ganglion) and 3703 in the distal vagal ganglion. In the left cervical vagus, about 4132 afferent fibres had their cell bodies in the distal ganglion, and there were probably about 3883 efferent fibres. The largest fibres in the cervical vagus were usually about 7 μm in diameter, fibres of 3 μm or less comprising from 72% to 89% of the total.

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