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Brainstem connections of vagal afferent nerves in the ferret: an autoradiographic study*

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INTRODUCTION

Many studies on the central connections of vagal afferent fibres have been carried out over a large number of years (Cajal, 1911; Cottle, 1964; Harding & Leek, 1973; Beckstead & Norgren, 1979; Ciriello, Hrycyshyn & Calaresu, 1981). Earlier studies were carried out using degeneration techniques whereas more recently anterograde (Beckstead & Norgren, 1979; Kalia & Mesulam, 1980; Norman & Bower, 1982) and retrograde (Kalia & Mesulam, 1980; Kalia & Wells, 1980; Ciriello *et al.* 1981; Kalia & Sullivan, 1982) tracing techniques have been employed. The number of studies, the number of species and the number of techniques have only served to increase and not decrease the controversy over precisely where vagal afferents terminate.

There is general agreement that the vagal afferent fibres terminate in the ipsilateral nucleus of the solitary tract (Cajal, 1911; Kimmel, Kimmel & Zarkin, 1961; Cottle, 1964; Beckstead & Norgren, 1979; Kalia & Mesulam, 1980; Kalia & Wells, 1980; Norman & Bower, 1982; Kalia & Sullivan, 1982) and the commissural nucleus of Cajal (Cajal, 1911; Allen, 1923; Cottle, 1964; Dubbeldam, Brus, Menken & Zeilstra, 1979). However, there is no general agreement over any of the other reported central connections of vagal afferent fibres. There have been widespread reports of a projection to the contralateral nucleus of the tractus solitarius (Kerr 1962; Beckstead & Norgren, 1979; Dubbeldam *et al.* 1979; Gwyn, Leslie & Hopkins, 1979; Kalia & Wells, 1980; Norman & Bower, 1982) but this projection was not found by Morita, Ito & Mabai (1980) or Rubinson & Friedman (1977). Bilateral projections to the area postrema have been reported (Cajal, 1911; Beckstead & Norgren, 1979; Gwyn *et al.* 1979) but denied by Cottle (1964), Katz & Karten (1978), Dubbeldam *et al.* (1979) and by Norman & Bower (1982). Similarly the dorsal motor nucleus of the vagus nerve has been the subject of controversy with Anderson & Berry (1956), Harding & Leek (1973) and Rhoton, O'Leary & Ferguson (1966) reporting vagal afferent terminations there while Cottle (1964), Dubbeldam *et al.* (1979), Jordan & Spyer (1978) and Norman & Bower (1982) have failed to find this connection. Other areas where the vagal afferent fibres are reported to terminate include the reticular formation (Anderson & Berry, 1956; Hino, Kanafusa & Tsunekawa, 1978; Norman & Bower, 1982), the nucleus ambiguus (Hino *et al.* 1978; Kumada & Nakajuma, 1972) the spinal nucleus of the trigeminal nerve (Anderson & Berry, 1956; Beckstead & Norgren, 1979; Kalia & Mesulam, 1980),

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Table 1. *Central connections of vagal afferent fibres as revealed by axonal transport tracing techniques. A summary of results by different authors*

Author	Species	Techniques	Ipsi TS	Ipsi NTS	Contra NTS	Contra TS	DMN	AP and subAP	RF	Spinal V	ECN
Beckstead & Norgren (1979)	Monkey	ARG+HRP	+	+	+	−	?	+	−	+ From sup. vagal gang. only	+ From sup. vagal gang. only
Kalia & Wells (1980)	Cat	HRP	+	+	+	−	+	+	−	−	−
Ciriello *et al.* (1981)	Cat	HRP	+	+	+	−	−	+	−	−	+
Kalia & Mesulam (1980)	Cat	ARG+HRP	+	+	+	−	+	+	−	+ Traversing	−
Kalia & Sullivan (1982)	Rat	HRP	+	+	+	−	+	+	−	+ Traversing	−
Norman & Bower (1982)	Hen	ARG	+	+	+	+	−	−	+	−	−
Odekunle & Bower (in this study)	Ferret	ARG	+	+	+	−	+	+	−	−	−

ARG, autoradiography;
HRP, horseradish peroxidase;
TS, tractus solitarius;
NTS, nucleus of tractus solitarius;
DMN, dorsal motor nucleus of the vagus.

AP, area postrema;
subAP, subpostrema;
RF, reticular formation;
ECN, external cuneate nucleus.

the nucleus intercalatus (Jordan & Spyer, 1978) and the cerebellum (Sobusiak, Zinney & Matlosy, 1971).

Possible reasons for the controversy are either that the work has been done in different species or that different techniques have been used in the same species. There are now a number of separate reports on the central connections in different species, all of them using retrograde or anterograde tracing techniques, which should enable species differences rather than technical differences to be elucidated. Table 1 summarises the sites of termination of vagal afferent fibres in various species as revealed by axon transport tracing techniques by a number of authors.

This report concerns the vagal afferent fibre connections of a carnivore, the ferret, investigated using autoradiography, which could confirm or refute findings using horseradish peroxidase in another carnivore, the cat, by Ciriello *et al.* (1981), Kalia & Mesulam (1980) and Kalia & Wells (1980). This may indicate whether the vagal afferent fibres of carnivores have similar central connections. Also, the ferret is an animal increasingly being used for the investigation of gastric physiology (Andrews, Lawes & Bower, 1980; MacKay & Andrews, 1983; Andrews & Lawes, 1984) and as yet there have been no reports on the central connections of the vagus nerve afferents in this animal.

MATERIALS AND METHODS

Ten adult ferrets of both sexes and ranging in weight from 800–1500 g were used. The animals were anaesthetised with sodium pentobarbitone at a dose of 60 mg kg^{-1} and the right inferior vagal (nodose) ganglion exposed through a midline incision in the neck. Tritiated leucine (Catalogue no. TRK 170, Amersham International Ltd., England) was freeze dried so that 1 mCi was concentrated into 0·1 ml of normal saline. Using a technique previously described (Bower, Parker & Molony, 1978), about 0·05 ml of the leucine was injected through multiple injections into the ganglion. After injection the wound was closed in layers and the animal allowed to survive for 24 hours. At the end of this time, the animal was anaesthetised again with sodium pentobarbitone and perfused transcardially with normal saline followed by 10 % buffered formalin. The brains and inferior vagal ganglia were removed and embedded in paraffin wax, and serially sectioned at a thickness of 10 μm. The sections were mounted on subbed slides and coated with Ilford K5 nuclear emulsion diluted 1:1 with a 1 % solution of glycerol. After being stored at 4 °C for 4 weeks (in the case of the brainstem) or 4 days (ganglia) in light tight boxes containing a dessicant, the sections were developed in Kodak D10 at 18 °C for 5 minutes and fixed in sodium thiosulphate for 4½ minutes. They were counterstained with cresyl fast violet and viewed with a Vickers M17 microscope using both incident and transmitted light. All sections of the nodose ganglia were examined to determine the completeness of the injection. All sections of the brainstem were examined between the lower pole of the inferior colliculi to the caudal part of the second cervical spinal segment. Criteria that have been generally accepted as indicating a terminal site when using autoradiography, i.e. silver grains over the interstitial tissue but not over neurons, and a higher density of silver grains than in the background, were used in this study. All sections showing a positive result were checked against the controls for positive and negative chemography to ensure that the results were due to radioactivity in the material and not due to spurious interactions between the tissue and the emulsion (Rogers, 1973, pp. 94–98).

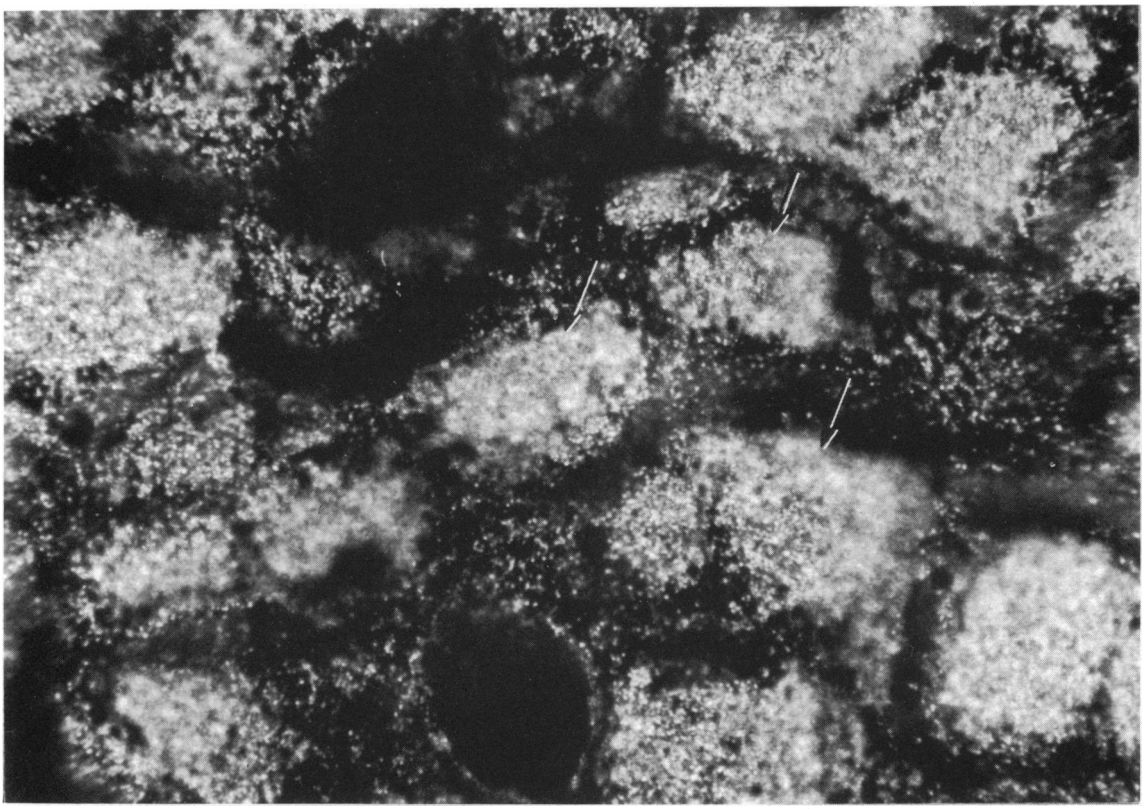

Fig. 1. A typical section of an injected nodose ganglion. The cell bodies (arrowed) can be seen to be very heavily labelled with silver indicating active uptake of the ^{3}H-leucine. Incident light photograph. × 1000.

RESULTS

In all experiments the ganglia were successfuly injected as indicated by high concentrations of silver grains over the neurons (Fig. 1). Two of the ganglia were completely labelled while all the others had small areas that were not injected. However, the combination of all the animals showed that the total extent of the ganglion was injected. The differences in the degree of labelling in the ganglia were not reflected in differences in the number or location of terminal areas in the brainstem but only in their rostrocaudal extent in the brainstem. The results given are a compilation from all ten animals.

In all ten experiments, the central processes of the afferent neurons were seen entering the dorsolateral aspect of the medulla oblongata in one or two fascicles. The labelled fascicles then travelled in a dorsomedial direction towards the ipsilateral tractus solitarius and its nucleus. Labelled fibres crossing the brainstem to the tractus solitarius or its nucleus extended from 1·31 mm to 2·51 mm rostral to the obex (Fig. 2). On reaching the tractus solitarius the fibres turned rostrally or caudally to run in the tract so that the total length of labelled tract lay between points 2·36 mm rostral, and 1·84 mm caudal, to the obex, i.e. the rostrocaudal extent of labelling in the tractus solitarius was greater than the rostrocaudal extent of labelled vagal fibres.

Labelling with an appearance consistent with that of a terminal field (heavily labelled neurophil, lightly labelled neurons) was seen in the ipsilateral nucleus of the

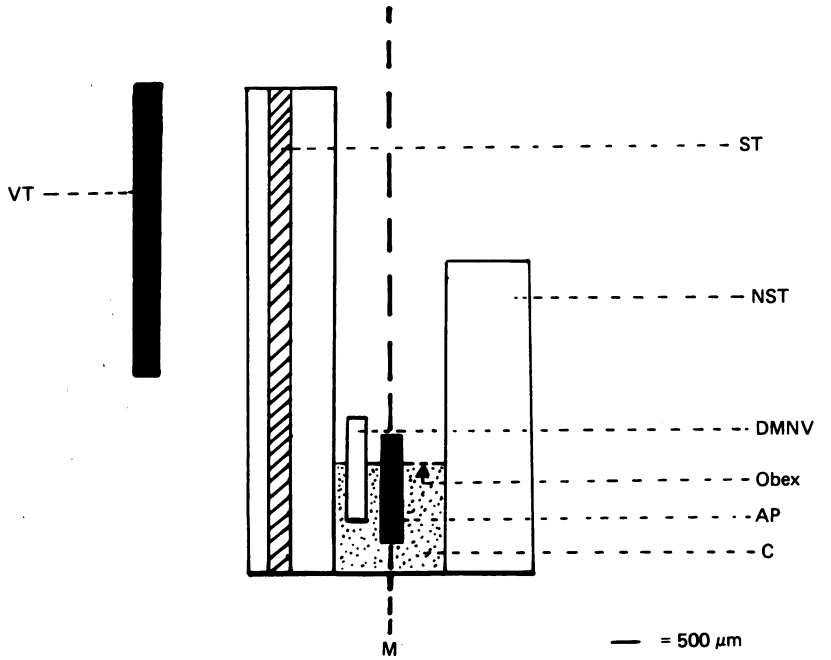

Fig. 2. Schematic diagram summarising the areas where labelled vagal afferent fibres were seen. – – – M is the midline. VT, vagal rootlets; ST, solitary tract; NST, nucleus of solitary tract; DMNV, dorsal motor nucleus of the vagus nerve; AP, area postrema; C, commissural nucleus of Cajal.

tractus solitarius. The rostrocaudal extent of this labelling was similar to that of the tractus solitarius but the density varied throughout the length of the nucleus. Rostrally, it was the lateral part of the nucleus of the tractus solitarius that was intensely labelled whereas caudally it was the medial part. Both medial and lateral parts of the nucleus were more intensely labelled in the region adjacent to the obex than rostrally or caudally. This suggested that a greater number of vagal afferent fibres terminated in the region of the nucleus of the tractus solitarius adjacent to the obex. Neither the tractus or its nucleus had labelling that extended more rostrally than the labelled vagal rootlets. The contralateral nucleus of the tractus solitarius was labelled in its medial aspect only and had a much shorter rostrocaudal extent, 0·86 mm rostral to 1·9 mm caudal to the obex, than the ipsilateral nucleus. The intensity of labelling in the contralateral nucleus was much lower than the ipsilateral nucleus which suggested that fewer fibres terminated contralaterally. The contralateral tractus solitarius was not labelled.

Low intensity labelling was seen bilaterally in the area postrema, extending from 0·22 mm rostral to 0·7 mm caudal to the obex (Fig. 3). The area between the area postrema and the nucleus of the tractus solitarius also had a low level of labelling though higher than the background. This was interpreted to mean that the area subpostrema was labelled on both sides. The commissural nucleus of Cajal had labelled fibres running across it but a second site of crossing as described by Norman & Bower (1982) was not seen.

The ipsilateral dorsal motor nucleus of the vagus had labelling in its dorsal margin that was slightly above background. This indicated that vagal afferent fibres

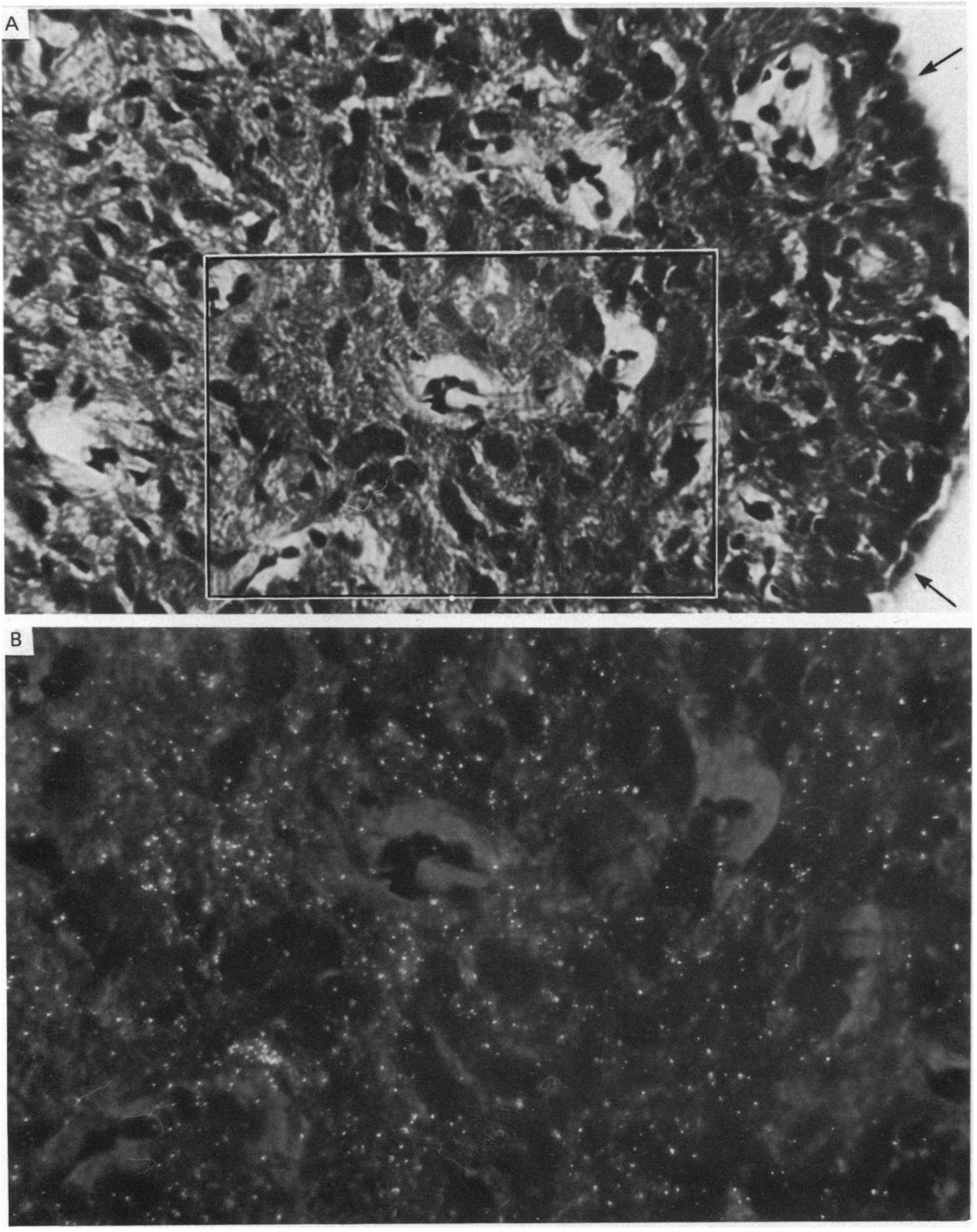

Fig. 3 (A–B). Area postrema. (A) is a bright field photograph. The black square indicates the area taken at higher power for (B). The edge of the brainstem is arrowed. × 350. (B) is an incident/transmitted light photograph. Note the silver grains over the neurophil but not the neurons. × 700.

terminated directly in the dorsal motor nucleus but that the projection was very sparse. The rostrocaudal extent of the labelled structures is summarised in Figure 2.

There were also significant negative findings. No labelling was found in the nucleus ambiguus, the medial and lateral external cuneate nuclei, the dorsal horn of the first and second cervical segments of the spinal cord, any part of the trigeminal nuclei and the reticular formation. All of these sites have had positive results reported by other authors.

DISCUSSION

The central connections of vagal afferent fibres in the ferret have been studied using an anterograde tracing technique. It is now well established that the inferior vagal ganglion contains only afferent neurons (Wakley & Bower, 1981) and that fibres of passage do not take up and transport tritiated aminoacids (Cowan *et al.* 1972). This means that in this study labelled fibres seen in the brainstem are primary vagal afferents, the results indicating that, for the ferret, the principal site of termination of such fibres is the ipsilateral nucleus of the tractus solitarius. All other studies on the central connections of vagal afferent fibres which have used axon tracing techniques have reported this regardless of the species being used (Beckstead & Norgren, 1979; Kalia & Mesulam, 1980; Kalia & Wells, 1980; Ciriello *et al.* 1981; Norman & Bower, 1982). There is also general agreement that the afferent fibres project to the medial part of the nucleus of the tractus solitarius. It therefore seems reasonable to conclude that whatever the species the medial side of the nucleus is the principal site of first analysis of information carried by vagal afferent nerves. It has not previously been reported that the lateral part of the nucleus of the tractus solitarius is labelled rostrally and it is not clear why this should be so in this investigation.

There is also general agreement that the contralateral nucleus of the tractus solitarius is a site of termination but there is only one previous study which suggests that the contralateral tractus contains fibres from the injected side and this was in the hen (Norman & Bower, 1982). It is difficult to explain why the only non-mammalian study should have made this finding in the contralateral tractus solitarius. It cannot be to convey the vagal fibres rostrocaudally because the rostrocaudal extent of labelling in the contralateral nucleus of the tractus solitarius was no greater in that study than reported here or by other workers.

The termination in the dorsal motor nucleus of the vagus is more controversial. The vagal afferent termination in the ipsilateral dorsal motor nucleus reported here agrees with Kalia & Mesulam (1980) and Kalia & Wells (1980) in the cat and with Kalia & Sullivan (1982) in the rat, but is in disagreement with Norman & Bower (1982) in the hen and with Ciriello *et al.* (1981) in the cat; Beckstead & Norgren (1979) were uncertain about this in the monkey. The negative result of Norman & Bower (1982) is probably a genuine species difference. Ciriello *et al.* (1981) had cut the vagal supply to the carotid and aortic sinuses and had found that the glossopharyngeal but not the vagus nerve projected to the dorsal motor nucleus of the vagus. The negative result from the vagus is unlikely to be due to these authors' use of horseradish peroxidase as opposed to autoradiography because Kalia & Sullivan (1982) have demonstrated vagal afferent terminals with horseradish peroxidase. This leaves the tempting conclusion that the vagal afferent fibres which terminate in the dorsal motor nucleus are those from the aortic and carotid sinuses: rapid responses to changes in blood pressure would obviously then be possible. Whatever modality

served by the vagal afferents projecting directly to the dorsal motor nucleus of the vagus, all authors agree that the projection is sparse.

Reported here is a bilateral projection to the area postrema and subpostrema, in agreement with Beckstead & Norgren (1979), Kalia & Mesulam (1980), Kalia & Wells (1980), Ciriello *et al.* (1981) and Kalia & Sullivan (1982). Again, Norman & Bower (1982) gave the only negative report of projections to these areas. This could be related to the fact that the area postrema and subpostrema have been implicated in vomiting reflexes.

The reported projection of vagal afferent fibres to the spinal tract of the trigeminal nerve, the first and second cervical segments of the spinal cord and to the external cuneate nucleus seems to be related to the technique used. Kalia & Mesulam (1980) and Kalia & Sullivan (1982) reported afferents traversing the spinal tract of the trigeminal nerve on their way to terminate in the tractus solitarius and its nucleus. Ciriello *et al.* (1981) reported afferents to the external cuneate nucleus. Beckstead & Norgren (1979), Norman & Bower (1982) and the authors of the present study have not found any such terminations of vagal afferent fibres. The explanation probably lies in the fact that the former reports all used horseradish peroxidase so that all the vagal afferent fibres were labelled whereas the latter reports used tritiated aminoacids so that only those vagal fibres with their cell bodies in the nodose ganglion were labelled. Beckstead & Norgren (1979) reported that when the superior vagal ganglion was almost completely spared, very little labelling in the spinal tract of the trigeminal nerve or the external cuneate nucleus was observed. It is therefore reasonable to conclude that the vagal afferent fibres which terminate in the spinal tract of the trigeminal nerve or the external cuneate nucleus have their cell bodies in the superior rather than the inferior vagal ganglion.

In this study there was no evidence of labelling in the contralateral reticular formation or in a more rostral commissure, as reported by Norman & Bower (1982) although no other author has been able to confirm those results which were probably due to species differences. The differences between the results of the studies shown in Table 1 are probably due to genuine species rather than technical differences.

SUMMARY

Using an autoradiographic technique the central connections of vagal afferent fibres in the ferret were studied. The results show that the principal site of termination is the ipsilateral nucleus of the tractus solitarius with smaller projections to the contralateral tractus nucleus, the dorsal motor nucleus of the vagus and the area postrema and subpostrema. The fibres cross the midline via the commissural nucleus of Cajal. No evidence of vagal afferent fibres was found in the reticular formation, the spinal tract of the trigeminal nerve, the external cuneate nucleus or the dorsal horns of the first and second cervical spinal segments. The findings from all studies using axon tracing techniques on vagal afferent fibres are summarised.

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