The effects of age on the overall population and on sub-populations of myenteric neurons in the rat small intestine

R. J. R. JOHNSON[1], M. SCHEMANN[2], R. M. SANTER[3] AND T. COWEN[1]

[1] *Department of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, London, UK*; [2] *Physiologisches Institut, Tierärztlichen Hochschule Hannover, Germany, and* [3] *Anatomy Unit, School of Molecular and Medical Biosciences, University of Wales Cardiff, UK.*

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ABSTRACT

Previous studies on ageing animal and human subjects have demonstrated a significant overall decline in neuronal numbers in the myenteric plexus of the enteric nervous system (ENS). Our study aimed to confirm this observation by counting myenteric neurons stained with the panneuronal markers PGP 9.5 and NADH-diaphorase. We also wished to examine the possibility that particular subpopulations of neurons are vulnerable. Therefore, we have immunostained and counted a number of nerve cell groups within the myenteric plexus of old and young Sprague Dawley rats using markers which reflect some of the neuronal phenotypes present, including ChAT and VIP. The number of neurons demonstrating NADH-diaphorase activity was significantly reduced ($P < 0.05$) by approximately 15% in old rats. However, the number of neurons stained for PGP 9.5 immunohistochemistry was not reduced and demonstrated larger numbers of neurons than the NADH-diaphorase method. None of the other neuronal markers studied showed any significant reductions with age. In contrast to previous work, this study has gathered little evidence for extensive cell loss in the myenteric plexus of the aged rat, either in overall populations, or in any of the principal functional groups of neurons.

Key words: Enteric nervous system; myenteric plexus; ileum; ageing; cholinergic neurons; nitric oxide.

INTRODUCTION

Ageing involves changes in certain aspects of gastrointestinal structure and function. There is a reduction in gastric emptying time, and of both frequency and amplitude of the peristaltic wave in elderly patients (Horowitz et al. 1984). Changes in digestion and absorption of foodstuffs and immune function also occur with age (for a review, see Hosoda et al. 1992). Isolated organ bath experiments on segments of intestine taken from aged rats have revealed significant reductions in the cholinergic component of neuromuscular transmission (Nowak et al. 1990), an important observation since the majority of neurons in the human intestine express choline acetyltransferase activity (Porter et al. 1996). In contrast to neurons in other autonomic nerve groups (for a review, see Cowen, 1993), quantitative studies of cell numbers within the myenteric plexus have consistently demonstrated extensive losses in old rats (Santer & Baker, 1988) and guinea pigs (Gabella, 1989), as well as in humans (de Souza et al. 1993; Meciano Filho et al. 1995). Immunohistochemical studies have demonstrated a reduced density of peptidergic fibres in the gastrointestinal tract of aged rats (Feher & Penzes, 1987). The density of extrinsic fibres to the myenteric plexus, projecting from the coeliac superior mesenteric ganglion (CSMG) and containing noradrenaline (NA), also decline significantly with age (Baker et al. 1991).

The first aim of our study was to confirm the loss of neurons in the ageing rat myenteric plexus using immunohistochemistry for PGP 9.5 (Thompson et al. 1983), which has been used in a number of descriptive studies of the ENS (Krammer et al. 1993; Eaker & Sallustio, 1994), and NADH-diaphorase histo-

Correspondence to Dr T. Cowen, Department of Anatomy and Developmental Biology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK.

chemistry (Gabella, 1971). In addition to determining the extent of cell loss with age, we also wished to examine if functionally significant neuronal subtypes were particularly vulnerable. Nitric oxide is an important neurotransmitter in the gut (Brookes, 1993). Neurons expressing NO activity have previously been shown to be selectively spared in the myenteric plexus of Wistar rats with age (Santer, 1994) and to increase as a proportion of the total in the proximal colon of aged Sprague Dawley rats (Belai et al. 1995). The relative proportions of NO bearing neurons in the myenteric plexus appear to be altered by extrinsic denervation (Yunker & Galligan, 1994). To our knowledge, other neuronal populations have not been examined in studies of the aged ENS. We have therefore studied the numbers of cholinergic, peptidergic and nitrergic myenteric neurons in the ileum of young and aged rats using appropriate immunohistochemical markers.

METHODS

Sprague Dawley rats aged 4 mo (n = 8) and 24 mo (n = 9) were used for all experiments. The old animals were kept on a restricted diet of approximately 60% of their normal daily intake (approximately 30 g) after reaching 6 mo of age, a regime which enhances the animals health and longevity. Animals were terminally anaesthetised with Expiral (Sanofi, UK), and the whole of the small intestine removed and flushed through with Krebs solution to remove gut contents. The small intestine was sealed at both ends with cotton thread and dilated with Krebs solution to a similar pressure, at which the specimen started to curl. Length and circumference were then measured. Mean values were calculated and used to derive a correction factor for growth of the intestinal wall with age (see Table 2). A 10 cm length was tied off with thread to maintain distension, resected close to the ileocaecal junction and cleaned of all traces of mesenteric attachment. Specimens were fixed in 4% paraformaldehyde (PFA) dissolved in phosphate buffered saline (PBS) at 4 °C for 2 h. After washing in Hepes buffer, they were placed in a 20% sucrose solution overnight at 4 °C. Using watchmakers forceps, a 1 cm wide strip of both circular and longitudinal muscle, with the myenteric plexus sandwiched between the muscle layers, was peeled off, and rinsed in Hepes buffer. Two preparations were usually removed from each animal.

Immunohistochemistry

The specimens were permeabilised with 0.3% Triton X-100 for 20 min prior to an incubation of 90 min in 5% swine serum. The primary antibodies were diluted in Hepes buffer containing 0.1% Triton X-100, 5% swine serum, 0.05% sodium azide and 0.05% DL-lysine. The antibodies used in this study, together with their suppliers, are listed in Table 1. Preliminary double staining using antibodies labelling neuronal nitric oxide synthase (nNOS-1) and the NADPH diaphorase method confirmed that these methods labelled identical neuronal populations, as has been reported elsewhere (Belai et al. 1995). After overnight incubation at room temperature, the specimens were washed 3 times in PBS before visualisation using secondary antibodies labelled with either fluorescein (FITC – DAKO, Denmark) or Texas Red (TR – Jackson Immunoresearch, USA) conjugated IgG for 2 h at room temperature. After 3 further PBS washes, those preparations labelled with FITC were counterstained with a 0.05% solution of pontamine sky blue (PSB) for 5 min to reduce background autofluorescence (Cowen et al. 1985). Specimens were then mounted in antifade mountant (Citifluor, UK) and coverslipped.

Preincubation. To enhance the visualisation of antibodies to ChAT, SP and VIP, tissue was pre-

Table 1. *Antibodies used and their working dilutions*

Antibody	Supplier	Dilution
Protein gene product 9.5 (PGP 9.5)	UltraClone, UK	1:400
Vasoactive intestinal polypeptide (VIP)	Incstar, USA	1:600
Somatostatin (SOM)	Incstar, USA	1:800
met-Enkephalin (met-ENK)	Peninsula Laboratories, USA	1:400
Substance P (SP)	Sera-Lab, UK	1:600
Neuropeptide Y (NPY)	Gift of Dr D. Corder	1:800
Choline acetyl transferase (ChAT)	Dr M. Schemann (Schemann et al. 1993)	1:400
Neuronal nitric oxide synthase (nNOS)	Affiniti Research, UK	1:100

incubated at 37 °C for 24 h in tissue culture medium (DMEM F12) containing 0.25 ml of a 10 mg/ml gentamycin solution, 1 ml penicillin/streptomycin solution (5000 U), 0.5 ml of a 0.25 mg/ml amphotericin B solution, 1 ml of pig serum and 7 mg of the axonal transport inhibitor, colchicine (Steele et al. 1991) (all Sigma Aldrich, UK).

NADH histochemistry. Gut segments were dilated with Krebs solution as above, and pretreated in 0.3 % Triton X-100 for 8 min. After a brief wash, specimens were incubated in 0.1 % Triton X-100, 30 mg nitroblue tetrazolium (NBT), 30 mg NADH (all Sigma), 45 ml distilled water and 15 ml phosphate buffer (Gabella, 1969) at room temperature, while being agitated gently on an orbital shaker. The progress of the staining was monitored under a dissection microscope and halted after 60–90 min. The specimens were rinsed in Krebs and fixed in 4 % PFH for 24 h at 4 °C. Muscle strip preparations were made as described and mounted in PBS glycerol (Aquamount, BDH, UK).

NADPH histochemistry. Gut segments were fixed for approximately 30 min in 4 % PFH at 4 °C, after dilation with Krebs solution. Specimens were washed and incubated in a medium consisting of 30 mg NADPH (Sigma), 30 mg NBT, 0.1 % Triton X-100 in 45 ml distilled water and 15 ml phosphate buffer, for 2 h at 37 °C. The progress of the reaction was monitored visually by colour change of the medium from yellow to rose-pink. The tissue was then removed from the medium and muscle strips were prepared and mounted in Aquamount (BDH). Orientation of specimens was maintained by clipping one corner of the tissue.

Quantification of neuron numbers

Positively stained cell bodies were counted after histochemical staining on a twin bank counter under transmitted light on a Nikon Optiphot equipped with a $\times 10$ lens, using an eyepiece graticule which framed an area of 1.03 mm² on the specimen. Following immunohistochemistry, neurons were counted using an Olympus AH-2 fluorescence microscope equipped with a $\times 20$ objective and an eyepiece graticule which defined an area of 0.06 mm². Three series of frames were counted per specimen, parallel to the longitudinal axis of the gut, 2 of which were on either side of the mesenteric aspect and 1 of which was from the antimesenteric aspect. Those neurons lying within the field area were counted, as were those intersecting the bottom and right hand edges of the graticule perimeter. As many frames as possible were counted from each specimen, and the counts pooled for subsequent statistical analysis. We chose areal counts in preference to the 'cells per ganglion' method often used in quantification of guinea pig myenteric neuron numbers (see e.g. Furness et al. 1994) as, in our experience, it was not always possible to define the boundaries of individual ganglia accurately, due to their confluent nature in the rat.

Table 2. *Derivation of correction factor* (*Corr. factor*) *from length and circumference* (*Circ.*) *measurements of small intestine of young and old rats to correct raw neuron counts for changes in size of the ileum in old rats.*

	Length (cm)	Circ. (cm)	Area (cm²)	Corr. factor
4 m (n = 9)	110 ± 3.8	1.24 ± 0.01	137 ± 5	Standard
24 m (n = 10)	145 ± 3.5	1.33 ± 0.03	194 ± 5	× 1.42

Correction for growth of the intestine

Increased length and, to a lesser extent, circumference of the rat ileum with age, has the effect of reducing areal counts of neuronal numbers by a 'dilution' effect. We have corrected for this by multiplying counts from old segments of gut by a factor derived from the ratio of areas between the young and old gut wall. Measurements of length and circumference were used to calculate a correction factor by which counts of aged neurons were multiplied to account for this dilution of cell numbers (see Table 2). Counts of neurons from old animals were thus increased by 42 % to compensate for this effect. All corrected cell counts were expressed as 'positive neurons per cm²'. Differences between counts of cells from young and old animals were compared by Student's *t* test using Statview for the Apple Macintosh computer, and neuron numbers were expressed as mean cells per cm² ± S.E.M.

RESULTS

In a preliminary study, 3-D optical sectioning on a BIORAD confocal microscope of PGP 9.5 stained whole mounts revealed little evidence for superimposition of neurons within the myenteric plexus, which we were concerned could lead to an underestimation of nerve cell numbers (Young et al. 1993). We therefore used 2-D counts made on the light microscope in all cases.

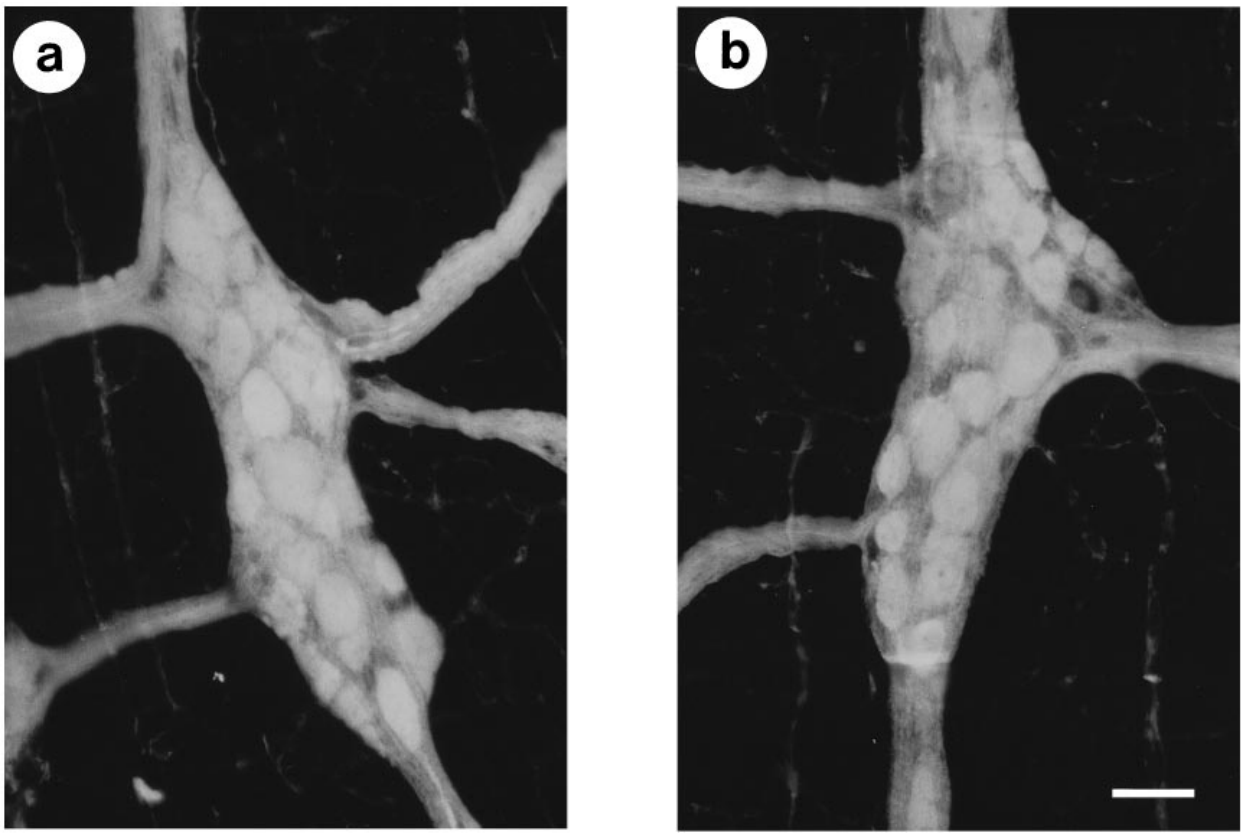

Fig. 1. PGP immunoreactivity in the myenteric plexus in the small intestine of young (4 mo) (*a*) and old (24 mo) rats (*b*). Note no obvious differences were seen between the preparations either in numbers or in the density of the tertiary plexus. Bar, 25 μm.

Counts of total neuron numbers using PGP 9.5 and NADH

PGP (Fig. 1*a*, *b*). The structure of the myenteric plexus was similar in both old and young animals, and consisted of large ganglia interconnected by thick nerve bundles. These were densely packed with nerve cell bodies, from which projections could be seen passing both up and down the plane of the plexus and perpendicularly to the mucosa and through both muscle layers. Numerous fine fibres of the tertiary plexus were apparent between the network of interconnected ganglia and it was possible to separate these fibres from nerve bundles running in the surrounding muscle layers by refocusing the microscope. No qualitative or visually obvious difference was apparent between young and old specimens in either cell numbers or fibre density. Statistical comparison of PGP positive cell numbers in the small intestine revealed no significant difference in cell numbers between the age groups (young: 19600 ± 2030 neurons per cm^2; old: 20700 ± 1290 neurons per cm^2; see Table 3).

Table 3. *Corrected mean counts per* cm^2 $\pm$ *S.E.M. of myenteric neurons from young and old rats**

Antibody	Young (n = 8)	Old (n = 9)
PGP 9.5	19600 ± 2030	20700 ± 1290
NADH	9490 ± 580	7950 ± 370
NADPH	2100 ± 90	1780 ± 420
ChAT	16300 ± 480	17500 ± 1120
SP	16700 ± 460	18600 ± 2100
NPY	2500 ± 190	2700 ± 500
VIP	4900 ± 520	4100 ± 300
ENK	650 ± 370	280 ± 100
SOM	250 ± 23	380 ± 74

* Note that there were fewer ($P < 0.05$) NADH stained neurons in old, compared with young, myenteric plexus. No other populations of stained neurons showed significant age-related changes.

NADH (Fig. 2*a*, *b*). Stained neurons were distinct due to purple formazan deposits in the neuronal perikarya, and were abundant in both old and young samples. Nuclei were always left unstained. The under- and overlying smooth muscle was very faintly stained, but this was never strong enough to obscure stained cell bodies. We did not detect a noticeable absence of neurons or atrophy of the ganglia in aged

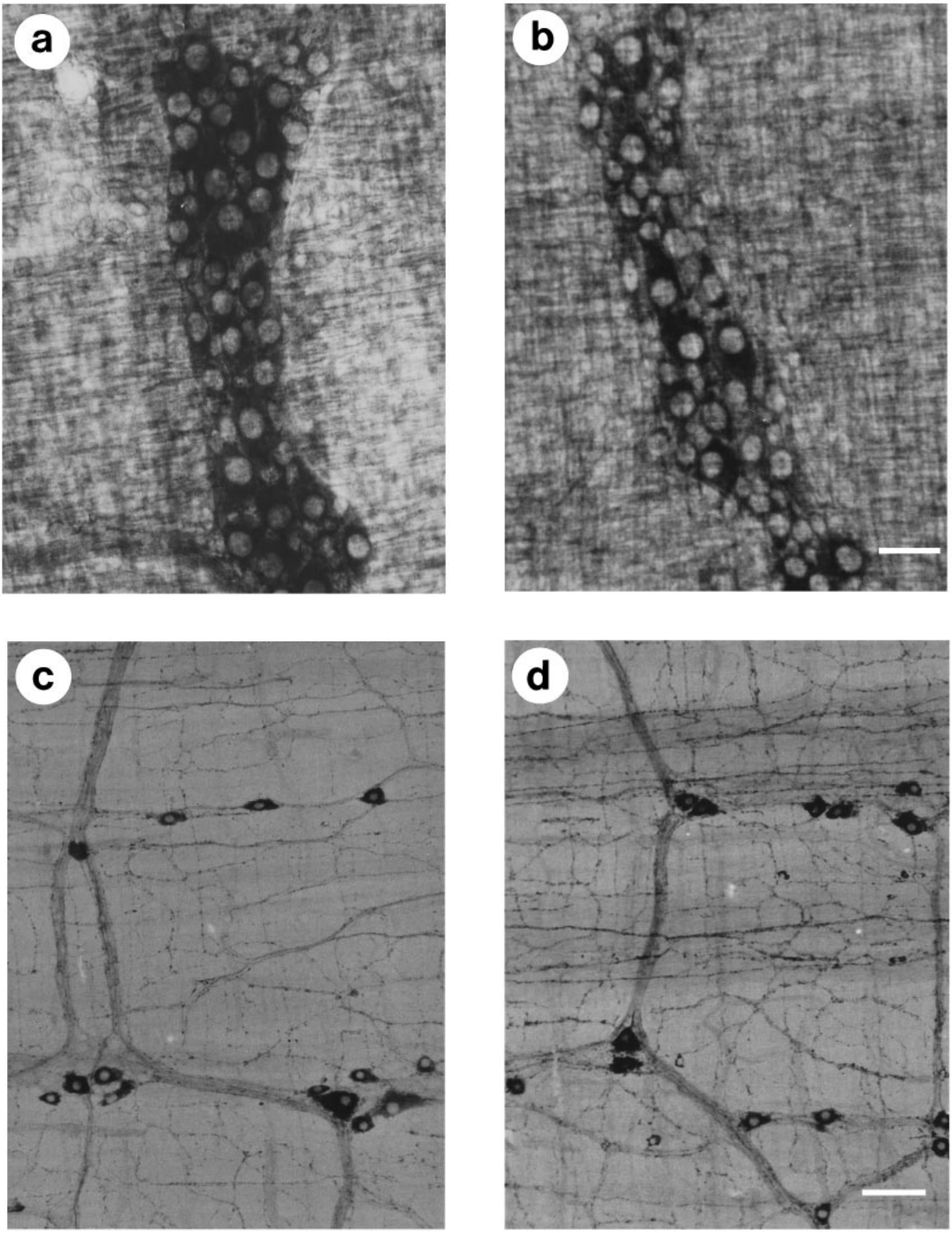

Fig. 2. NADH (*a*, *b*) and NADPH (*c*, *d*) histochemistry on whole mount preparations from young (4 mo) (*a*, *c*) and old (24 mo) rats (*b*, *d*). In the case of NADH-diaphorase histochemistry, the number of neurons stained by this method was significantly ($P < 0.05$) reduced by 15% in preparations from old animals. Bars, a = b, 25 μm; c = d, 50 μm.

specimens. However, the packing density of cells appeared to be slightly reduced. In some of the preparations some poorly stained regions were apparent, in which there may have been additional unstained neuronal profiles. These areas were discounted for quantification purposes. Stained cell

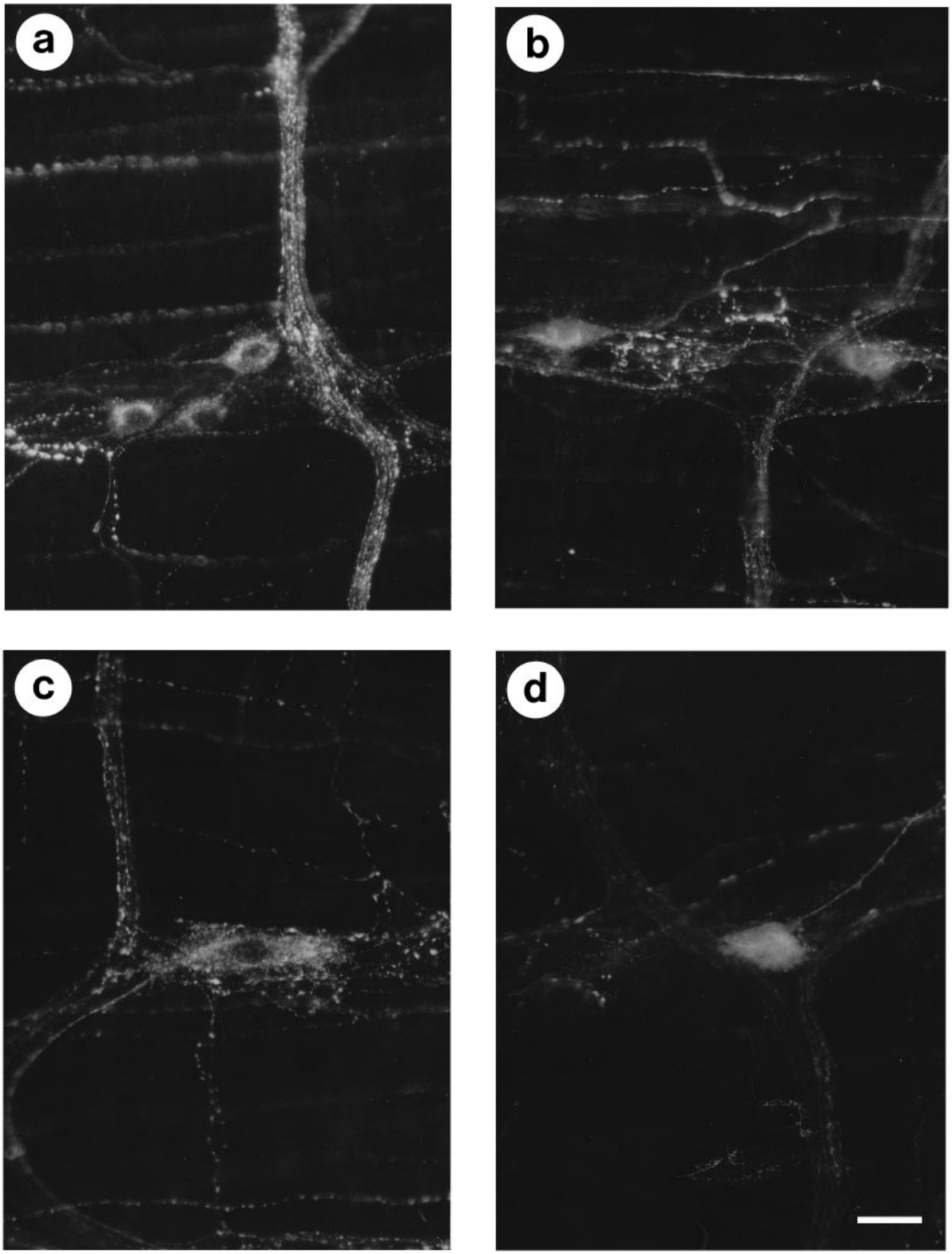

Fig. 3. Immunoreactivity for neuropeptides in the myenteric plexus of old (24 mo) rats (*a*) and young (4 mo) (*b*, *c*, *d*) rats. VIP positive neurons and fibres in old (24 mo) rats (*a*) and young (4 mo) rats (*b*). Enkephalin (*c*) and somatostatin (*d*) immunoreactivities in myenteric neurons from young (4 mo) rats. Bar, 25 μm.

numbers in young animals were 9490 ± 570 neurons per cm^2; in old animals there were 7950 ± 370 neurons per cm^2, indicating a significant reduction ($P < 0.05$) of approximately 15% in old age (Table 3).

Counts of myenteric neuron subpopulations

NADPH histochemistry/NOS immunohistochemistry (Fig. 2*c*, *d*). Stained neurons were readily identifiable

but were notably less numerous than, for instance, preparations stained with the NADH method. Again, there were intense deposits of formazan in the perikarya of positive cells. In addition, abundant stained fibres were apparent connecting the ganglia as well as in the tertiary plexus and the muscle layers themselves. As described in the methods section, antibodies to nNOS were found to stain the same neuronal populations as those cells expressing NADPH-diaphorase. Counts of neurons within sections stained by both methods agreed closely, confirming previous observations (Belai et al. 1992). We have therefore pooled the cell counts obtained from both methods to give 2100 ± 90 neurons per cm² in young and 1780 ± 420 neurons per cm² in old; see Table 3). No significant difference was found between young and old groups.

ChAT. Positive cell bodies were very numerous but difficult to distinguish without prior colchicine treatment. Quantification was therefore carried out on specimens preincubated with colchicine. We found that the specific fluorescence faded relatively rapidly on exposure to fluorescent light. However, accurate counting of stained cell bodies was possible before fading occurred. ChAT positive cell bodies were normally large and occupied a large proportion of the ganglia. Cell counts for ChAT positive neurons were: young, 16300 ± 480 neurons per cm², and old: 17500 ± 1120 neurons per cm² (see Table 3) indicating no significant change in numbers with age.

SP. A large number of neuronal cell bodies were apparent, with a substantial proportion of faint, but positive, nerve cells which were included in the counts. Most of the neurons were clearly large and uni- or bipolar and issued extensive projections both orally and anally throughout the tract within the primary bundles. Cell numbers were 16700 ± 460 in preparations from young animals, and 18600 ± 2100 from old animals, showing no significant difference with age.

NPY. There were considerable numbers of cells displaying NPY-IR. A small number of single neurons showed intense fluorescence, and it was possible to follow their projections for some distance. Most were large and issued projections in the plane of the plexus as well as through to the mucosa. Cell numbers were 2500 ± 190 neurons per cm² in young animals, and 2700 ± 500 neurons per cm² in old animals, again demonstrating no significant difference with age.

VIP (Fig. 3*a*, *b*). Without prior colchicine treatment, dense granular fibres were apparent around the ganglia, and in the tertiary plexus. Rare positive cells were found which exhibited clear but diverse morphology, and issued projections mainly perpendicularly through to the mucosa. Cell counts were made after colchicine treatment which was found to reduce the level of fibre staining. However, a substantially increased number of cells became visible, and these were quantified. Cell numbers were 4930 ± 520 neurons per cm² in young animals, and 4110 ± 310 neurons per cm² in old animals (Table 3), again showing no significant differences with age.

ENK (Fig. 3*c*). Granular fibres were visible in both primary and secondary plexus components. Numbers of cell bodies were not altered by colchicine pretreatment. Stained neuronal cell bodies were rare but strongly stained with visible nuclei. These cells issued extensive projections normally in the plane of the plexus. Cell numbers were 650 ± 370 neurons per cm² in young animals, and 280 ± 100 neurons per cm² in old. The reduction in numbers of cells with age was not statistically significant.

SOM (Fig. 3*d*). Fibres were varicose, but present in appreciable numbers in primary and secondary strands. Neuronal cell bodies were sparse with mainly unipolar morphology, and descending and ascending projections. Colchicine pretreatment failed to alter the numbers of stained neurons which were therefore counted on untreated specimens. There were 250 ± 23 neurons per cm² in young animals and 380 ± 74 neurons per cm² in old animals, showing no significant difference with age.

DISCUSSION

This study has evaluated neuronal numbers in the ageing rat small intestine using established neuron labelling techniques, combined with quantification of several neuronal populations. The accurate assessment of changes in neuron numbers within the gastrointestinal tract is a complex task. Neurons are present in huge numbers throughout the rodent gastrointestinal tract. Using the panneuronal histochemical marker, cuprolinic blue, Karaosmanoglu et al. (1996) have estimated that there are approximately 6.5 million neurons present in the guinea pig small intestine, compared to estimates of 2.75 million using NADH (Gabella, 1989). Because of the large numbers involved, it is only possible to count cells over restricted areas of the gut wall. To extrapolate from these counts to an estimate of the total number of neurons in the whole organ, we have measured the length and diameter of the small intestine under constant conditions of distension folowing the methods of Gabella (1987). Measurements of the

small intestine of young and old rats indicate a substantial age-related increase in both length and circumference to give a calculated increase in overall area of 42% in old age. In comparison, the aged guinea pig small intestine increases in length by 68% (Gabella, 1989). The effect of these areal increases is to substantially reduce areal counts of neurons in aged preparations, relative to those from young animals. Correction for this 'dilution' of cell numbers in aged animals is therefore essential in order to avoid overestimating the extent of cell loss in the aged myenteric plexus.

Previous studies have used NADH histochemistry and Giemsa staining to quantify total neuron numbers on the assumption that these methods stained the majority, if not all, the neurons present in the myenteric plexus. In the guinea pig, a loss of 40–60% of all myenteric neurons is observed with NADH staining after corrections for changes in length (Gabella, 1989). In contrast, myenteric neuron numbers in the ileum of the Wistar rat without correction for growth or size changes, showed a reduction of 43% in old age (Santer & Baker, 1988). These results may have important clinical implications, as studies of certain areas within the gastrointestinal tract of postmortem tissue from young and old human subjects have also revealed reductions in cell number with increasing age (de Souza et al. 1993; Meciano Filho et al. 1995). In contrast to these indications of substantial age-related neuronal degeneration, we have shown a far smaller, although still significant, reduction in cell numbers of only 16% using the NADH-diaphorase method, in aged Sprague Dawley rats, following correction for growth in the mature gut. Areal counts of neurons in the ileum of young rats (9490 ± 580 cells per cm^2 in the present study, 8169 cells per cm^2 (Santer & Baker, 1988)) and guinea pigs (8600 cells per cm^2 (Young et al. 1993)) were similar in all 3 studies. Comparisons between young and old suggest major species, strain or other differences, where guinea pigs may exhibit a far greater loss of myenteric neurons in old age compared with rats, and where Wistar and Sprague Dawley strains of rat may also exhibit different responses to ageing. Although few studies have examined differences in innervation between these strains of rats, there is a different pattern in the segmental organisation of pelvic innervation between these strains (Pascual et al. 1989). Since the mortality rate of ageing Wistar rats is significantly lower than that of equivalently aged Sprague Dawley rats, it may be that there is a difference in the responses of enteric neurons from these strains to ageing. In addition, it seems possible that the dietary restriction we used to maintain the aged rats in good health, as well as the different markers employed, may contribute to the contrasting results from Sprague Dawley (this study, and see Johnson et al. 1996) and Wistar (Santer & Baker, 1988) rats. Further investigations will address this possibility.

PGP 9.5 is a well established general marker for the majority of peripheral autonomic, including enteric, neurons (Thompson et al. 1983) and has been widely used in descriptive studies (Krammer et al. 1993; Eaker & Sallustio, 1994). However, our study has been the first to quantify myenteric neurons using this panneuronal marker. Neuron numbers were 19600 ± 2030 cells per cm^2 in young animals, and 20700 ± 1290 cells per cm^2 in old. Surprisingly, PGP staining revealed approximately double the numbers of neurons (1900 ± 2030 cells per cm^2) in the young rat ileum compared to those identified by NADH histochemistry (9490 ± 580 cells per cm^2), a difference which was accentuated in old age where PGP stained 20700 ± 1290 cells per cm^2, whilst NADH stained 7950 ± 370 cells per cm^2. Comparison of micrographs revealed a closer packing of PGP neurons compared with those stained with NADH, suggesting that some neurons may not be stained by the NADH-diaphorase method. Previous work using an 'anti-nerve cell body' serum suggested that only around 80% of all nerve cell bodies were identified by NADH-diaphorase histochemistry (Young et al. 1993). Our results suggest a figure nearer to 50%. The lack of an age-related reduction in PGP-stained neuron numbers, contrasting with a 16% reduction of NADH-stained neurons, indicate that this underestimation by NADH is accentuated in old age, so that NADH positive neurons may only represent some 40% of the total neuron number in the aged rat ileum. Detectable alterations in diaphorase proteins have been proposed as biochemical markers for the ageing process (Stadtman, 1988) suggesting that NADH, although a useful neuronal marker in many respects, may be unsuitable for longitudinal studies which extend into old age. The findings of this study confirm differences in NADH activity between young and old Sprague Dawley rats. Whether similar changes are found in other rat strains or species is a matter for further investigation. We propose that PGP 9.5 should therefore be the marker of choice for ageing studies of neuron numbers, although it has been suggested that, even with PGP, only 80% of myenteric neurons are stained (Eaker & Sallustio, 1994). If so, it is possible that the number of unstained cells was altered in old age. PGP is not the marker of

choice for enteric neurons in all animal species. A recent study examining the efficacy of neuronal markers in the guinea pig small intestine concluded that neurons could not be accurately counted using this marker (Karaosmanoglu et al. 1996). We were concerned that cell numbers could have been underestimated due to neurons overlying each other within the myenteric ganglia. However, examination of successive optical sections of ganglia in several fields of myenteric plexus from young and old rats with a confocal microscope failed to show a significant number of cells hidden in this way. High power confocal micrographs also provided no evidence of atrophic or degenerative neuron profiles, although packing density appeared to be reduced in the aged rat preparations.

Counts of neuronal subpopulations strengthened the conclusions from the PGP staining, showing no significant cell loss in any of the neuronal populations, as well as confirming that nitric oxide expressing neurons are spared in the ageing ENS (Santer, 1994). No significant cell loss or other evidence of neuronal atrophy or degeneration was encountered using neuronal markers colocalised with NOS, such as VIP, or in ChAT or SP-stained neurons, confirming that age-related cell death probably did not occur to any significant extent within functionally significant groups of neurons under the conditions of the present study.

In conclusion, our assessment of neuronal loss from the ageing rat myenteric plexus, using different neuronal markers, has indicated that the panneuronal marker PGP 9.5 provides a more accurate and complete measure of neuronal numbers than the NADH-diaphorase reaction, particularly in the aged ENS where NADH may underestimate neuron numbers by as much as 60%. Using PGP, and in contrast to previous studies, no evidence was found of significant neuronal loss with age. However, there were dietary and strain differences between the present and previous studies in ageing rats. Confirming the maintenance of neuron numbers in old age, no significant loss of nitric oxide synthesising, peptidergic or cholinergic populations of myenteric neurons was found, accentuating the contrast between this and previous studies of the ageing rat myenteric plexus.

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