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REGULAR ARTICLE

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Calbindin immunoreactivity of enteric neurons in the guinea-pig ileum

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Abstract Previous studies have identified Dogiel type II neurons with cell bodies in the myenteric plexus of guinea-pig ileum to be intrinsic primary afferent neurons. These neurons also have distinctive electrophysiological characteristics (they are AH neurons) and 82–84% are immunoreactive for calbindin. They are the only calbindin-immunoreactive neurons in the plexus. Neurons with analogous shape and electrophysiology are found in submucosal ganglia, but, with antibodies used in previous studies, they lack calbindin immunoreactivity. An antiserum that is more effective in revealing calbindin in the guinea-pig enteric nervous system has been reported recently. In the present work, we found that this antiserum reveals the same population that was previously identified in myenteric ganglia, and does not reveal any further population of myenteric nerve cells. In submucosal ganglia, 9–10% of nerve cells were calbindin immunoreactive with this antiserum. The submucosal neurons with calbindin immunoreactivity were also immunoreactive for choline acetyltransferase, but not for neuropeptide Y (NPY) or vasoactive intestinal peptide (VIP). Small calbindin-immunoreactive neurons (average profile 130 μm^2) were calretinin immunoreactive, whereas the large calbindin-immunoreactive neurons (average profile 330 μm^2) had tachykinin (substance P) immunoreactivity. Calbindin immunoreactivity was seen in about 50% of the calretinin neurons and 40% of the tachykinin-immunoreactive submucosal neurons. It is concluded that, in the guinea-pig ileum, only one class of myenteric neuron, the AH/Dogiel type II neuron, is calbindin immunoreactive, but, in the submucosal ganglia, calbindin immunoreactivity occurs in cholinergic, calretinin-immunoreactive, secretomotor/vasodilator neurons and AH/Dogiel type II neurons.

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Introduction

In the myenteric plexus of the guinea-pig small intestine, calbindin immunoreactivity is reported to be confined to one type of neuron, AH/Dogiel type II neurons, and has come to be used as an identifier for these neurons (Iyer et al. 1988; Furness et al. 1990; Costa et al. 1996; Reiche et al. 1999; Zholos et al. 1999). AH/Dogiel type II neurons with cell bodies in myenteric ganglia are intrinsic primary afferent (sensory) neurons (IPANs, Furness et al. 1998). IPANs have also been identified in submucosal ganglia (Kirchgessner et al. 1992, 1996).

Unlike myenteric IPANs, in which calbindin immunoreactivity is generally strong (Furness et al. 1988, 1990) and is present in 82–84% of these neurons (Iyer et al. 1988; Song et al. 1991), submucosal IPANs appear to lack calbindin; in fact calbindin-immunoreactive nerve cells in the submucosal ganglia are extremely rare (Furness et al. 1988; Costa et al. 1996). In other ways, the myenteric and submucosal IPANs are similar, e.g., they have AH electrophysiological characteristics and Dogiel type II morphology (Bornstein et al. 1989; Barajas-López et al. 1991; Evans et al. 1994). The possibility that the neurons do contain calbindin, but that it is only revealed by some antibodies, is suggested by the recent data of Reiche et al. (1999), who found that an antiserum (Swant CB38), raised against recombinant rat calbindin, revealed calbindin-immunoreactive neurons in the gastric corpus of the guinea-pig, whereas the neurons were not revealed by other anti-calbindin antibodies. Unlike myenteric calbindin-immunoreactive neurons in the small intestine, few of these had Dogiel type II morphology. Immunoreactivity revealed with CB38 was abolished by equilibration of the antiserum with calbindin protein. Both CB38 and other anti-calbindin antibodies revealed positive neurons in the myenteric plexus of the small intestine, but no quantitative data were reported.

That is, it was not determined whether CB38 reveals the same population of neurons that is localised by other anti-calbindin antibodies. The specific question, whether submucosal neurons, in particular the submucosal IPANs, are revealed by CB38 was not addressed.

Materials and methods

Tissue was obtained from guinea-pigs of both genders, in the weight range 200–400 g, that were stunned by a blow to the head and killed by severing the carotid arteries and spinal cord. All procedures were approved by the University of Melbourne Animal Experimentation Ethics Committee. Segments of distal ileum were removed, opened along the mesenteric border, pinned tautly on balsa board and immersed in 2% formaldehyde plus 0.2% picric acid in 0.1 M sodium phosphate buffer, pH 7.0, at 4°C overnight. The next day, tissue was cleared of fixative with 3×10 min washes in dimethylsulphoxide, followed by 3×10 min washes in phosphate-buffered saline (PBS; 0.15 M NaCl in 0.01 M sodium phosphate buffer, pH 7.2). In order to visualise cell body immunoreactivity for neuropeptides, some preparations were incubated in colchicine, under sterile conditions, for 24 h prior to fixation, as described previously (Furness et al. 1989).

Fluorescence immunohistochemistry

Following fixation and clearing, the tissue was dissected into layers. The mucosa, submucosa and circular muscle were removed to produce whole-mounts of longitudinal muscle plus the myenteric plexus. In the second type of preparation, the mucosa and muscularis externa were removed to leave behind the intact submucosa. All preparations were incubated in a 10% solution of normal horse serum and 1% Triton X-100 in PBS for 30 min at room temperature, prior to exposure to combinations of primary antisera (Table 1).

Following incubation for one or two nights at room temperature in combinations of antisera, tissue was washed in PBS and then incubated in a mixture of secondary antibodies (see Table 2). Biotin label was revealed after washing for 30 min in PBS and incubation with streptavidin-Texas red for 90 min. A final wash in PBS was made before tissue was mounted in glycerol buffered with 0.5 M sodium carbonate buffer (pH 8.6).

Preparations were examined on a Zeiss Axioskop microscope equipped with the appropriate filter cubes for discriminating between fluorescein isothiocyanate (FITC) and Alexa 594 fluorescence. Images were recorded using a Sensys cooled CCD camera (Photometrics Ltd., Tucson, AZ) and V++ for Windows imaging software (Digital Optics Ltd., Auckland, New Zealand). Preparations were also analysed by confocal microscopy on a Biorad MRC1024 confocal scanning laser system installed on a Zeiss Axioplan 2 microscope. The system had a krypton/argon laser for differential visualisation of the fluorophores using 488 nm excitation and 522/535 nm emission for FITC and 568 nm excitation and 605/632 nm emission for Alexa 594. The images were 512×512 pixels and the thickness of the optical section was 0.5 µm. Immunoreactive cells were scanned in a series of optical sections with a centre-to-centre spacing of 0.2 µm. The images were further processed using Confocal Assistant, Corel PhotoPaint and Corel Draw software programs.

Table 2 Secondary antibodies and streptavidin complex used. Supply companies: Amersham Pty. Ltd., Melbourne, Australia; Molecular Probes, Eugene, OR; Jackson Immunoresearch Lab., PA

Antibody and label	Dilution	Source
Donkey anti-rabbit IgG FITC	1:50	Amersham
Donkey anti-sheep IgG Alexa 594	1:50	Molecular Probes
Goat anti-mouse IgG Alexa 594	1:50	Molecular Probes
Biotinylated donkey anti-rabbit IgG	1:100	Jackson
Biotinylated donkey anti-sheep IgG	1:100	Jackson
Biotinylated sheep anti-rat IgG	1:200	Amersham
Streptavidin-Texas red	1:400	Molecular Probes

Diaminobenzidine immunohistochemistry

In order to determine the shapes and sizes of calbindin- and calretinin-immunoreactive neurons in the submucosal ganglia, tissue samples (two for each antigen) were prepared in which immunoreactivity was revealed with diaminobenzidine. Whole-mounts, fixed and prepared as above, were incubated in primary antisera against calbindin (rabbit) or calretinin (goat). After washing, the preparations were incubated with biotinylated donkey anti-rabbit or anti-sheep IgG. The biotin was localised using an avidin-biotin-horseradish peroxidase (HRP) kit (Vectastain, Vector Laboratories, Burlingame, CA). The HRP was reacted with diaminobenzidine and H_2O_2 to yield a permanent deposit. Cells were observed on an Olympus BH microscope under positive-low phase-contrast optics, and drawn or measured with the aid of a camera lucida drawing tube at ×500 or ×1250 final magnification.

Quantitative analysis

The proportions of neurons immunoreactive for a particular neurochemical that were also immunoreactive for other neurochemicals were estimated by examining fluorescent double-stained preparations. In each case, double-stained preparations from several animals were used. The percentage of neurons immunoreactive for a particular marker that was also immunoreactive for another neurochemical was calculated and expressed as mean ± standard error of the mean (SEM) (n = number of preparations counted).

Results

Myenteric ganglia

Anti-calbindin antiserum CB38 revealed a single type of nerve cell in the myenteric ganglia of the guinea-pig ile-

Table 1 Characteristics of primary antibodies. Commercial suppliers: SWant, Bellinzona, Switzerland; Chemicon, Temecula, CA

Tissue antigen	Host	Dilution	Code and reference
Calbindin	Rabbit	1:2000	SWant CB38 against recombinant rat calbindin (Reiche et al. 1999)
Calbindin	Mouse	1:500	SWant 300, batch 17-F against chicken intestinal calbindin 28K (Reiche et al. 1999)
Calretinin	Goat	1:100	SWant
ChAT	Goat	1:50	Chemicon (Li and Furness 1998)
NPY	Sheep	1:400	E2210 (Furness et al. 1985)
TK	Rat	1:200	CC (Cuello et al. 1979)
VIP	Rat	1:200	FI-III (Morris et al. 1985)

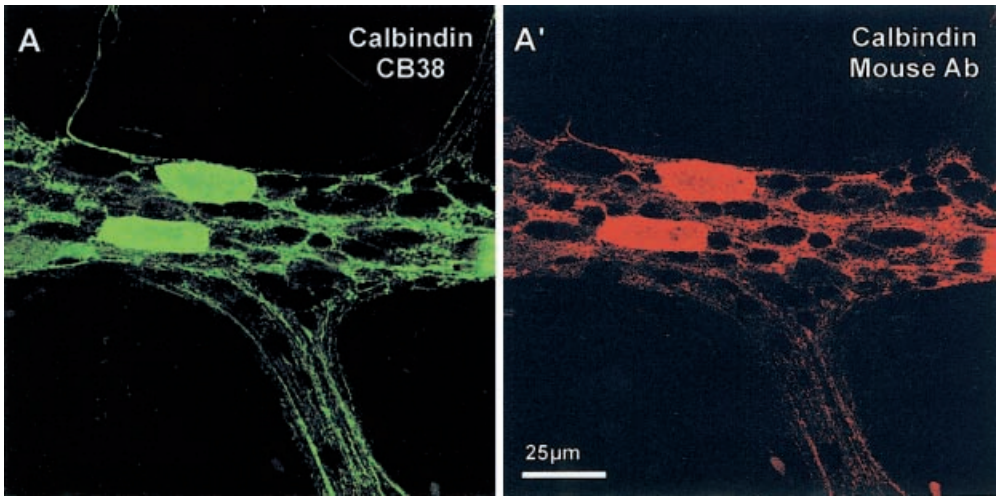


Fig. 1 Co-localisation of immunoreactivity revealed by different anti-calbindin antibodies, rabbit anti-calbindin CB38 and mouse anti-calbindin 17-F, in the myenteric plexus of the guinea-pig ileum. Despite the fact that CB38 reveals a greater number of cells elsewhere in the gut than do other anti-calbindin antibodies, in the small intestine it reveals the same nerve cells and fibres that are located by other anti-calbindin antibodies

um. These were large round or oval cells. They varied in fluorescence intensity, and immunoreactivity was observed in the cytoplasm and nucleus (Fig. 1). Similar neurons revealed by calbindin immunoreactivity have been identified as Dogiel type II neurons on the basis of shape determined immunohistochemically, after dye injection and by electron microscopy (Furness et al. 1988, 1990; Iyer et al. 1988; Pompolo and Furness 1988; Song et al. 1991; Costa et al. 1996). To confirm that this is the same population revealed by other anti-calbindin antibodies, an antibody (17-F) raised in mouse against chicken intestinal calbindin was used. The original antiserum, used to define the population of calbindin-immunoreactive neurons in the myenteric ganglia of the guinea-pig small intestine, was also against chicken intestinal calbindin (Furness et al. 1988, 1990), but was raised in rabbit, so it cannot be readily used in double staining with the rabbit antiserum CB38.

Double staining with the rabbit and mouse antibodies was examined in five preparations from two guinea-pigs (Fig. 1). Quantitative data were obtained by scanning the tissue and counting all immunoreactive cells. Of 2328 nerve cells that were immunoreactive, 2308 were reactive with both antibodies, 1 cell that was reactive with only the mouse anti-calbindin was encountered, and 19 cells were immunoreactive with only the antiserum raised in rabbit. All nerve cells had Dogiel type II morphology.

In 4 further preparations, 2 from each of 2 animals, double staining with the rabbit anti-calbindin CB38 and anti-choline acetyltransferase (ChAT) was examined; 50 calbindin-immunoreactive cells in each preparation were examined. Each of the 200 calbindin-immunoreactive cells was ChAT immunoreactive.

Submucosal ganglia

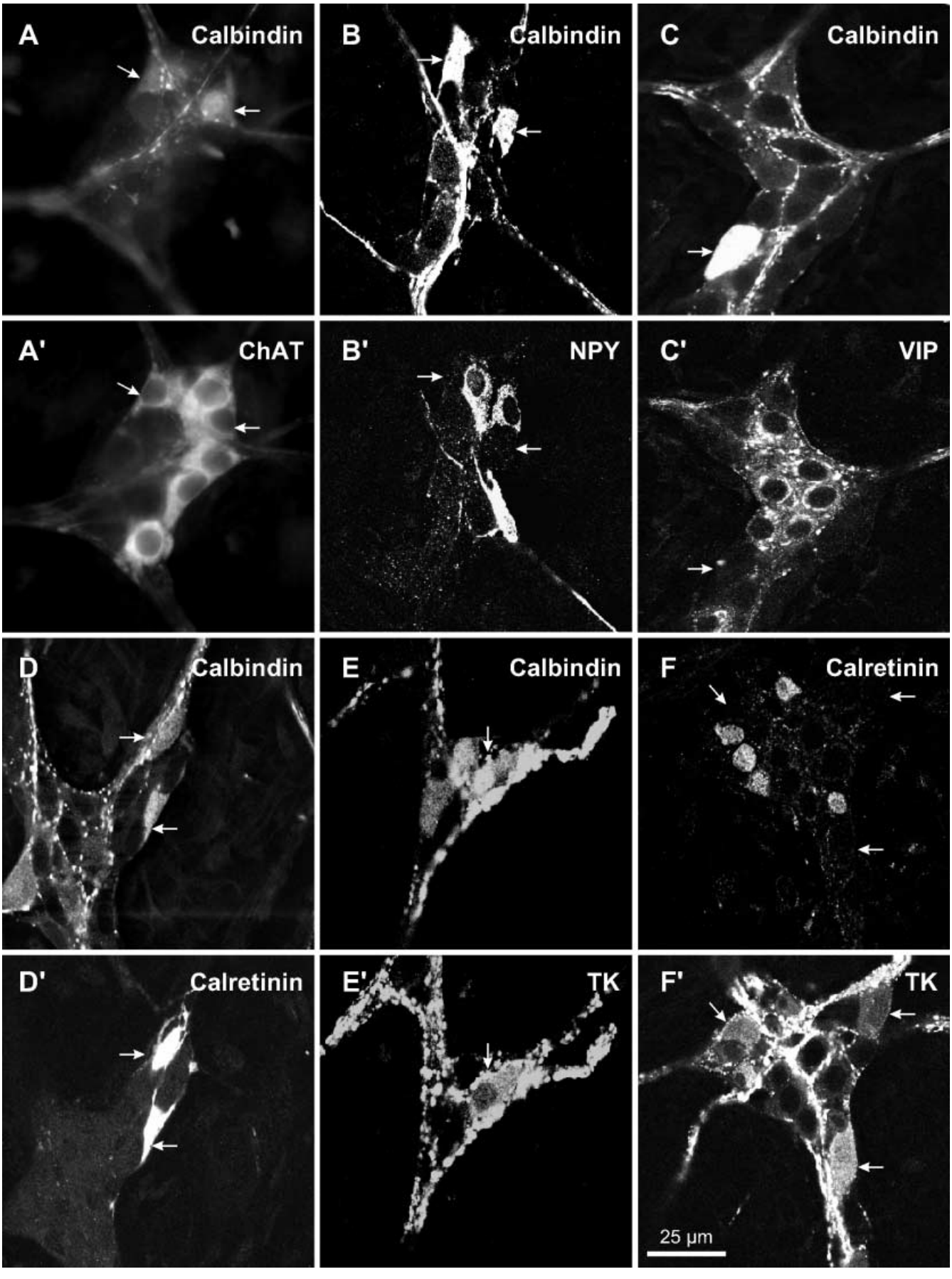
Anti-calbindin antiserum CB38 revealed small and large nerve cell bodies, but did not show their processes well enough for their shape classification to be determined. Small and large cells were also distinguished immunohistochemically (see below). Most cells were faintly stained by anti-calbindin CB38, but a few cells with strong immunoreactivity were encountered. Immunoreactivity occurred in the cytoplasm and nucleus.

Double staining revealed that almost all calbindin-immunoreactive cells were also immunoreactive for ChAT, and 50 calbindin-immunoreactive cells were evaluated from each of 5 preparations from 3 animals (Fig. 2). Of the 250 nerve cells, 248 were ChAT immunoreactive. By contrast, no calbindin-immunoreactive nerve cells were neuropeptide Y (NPY) immunoreactive (Fig. 2). Cells were counted across ganglia, so that the ratios of calbindin to NPY cells could be determined. There was about a third the number of calbindin as NPY nerve cells. Previous comprehensive counts (2032 NPY nerve cells in 870 ganglia) indicate that NPY neurons are 28.6% of all neurons (Furness et al. 1984). From counts of neuron numbers in 6 cohorts of 50 ganglia (6 preparations from 4 animals), the percentage of all neurons with calbindin immunoreactivity was calculated to be 8.8±1.0% (mean ± SEM).

There was no overlap in immunoreactivity between calbindin and vasoactive intestinal peptide (VIP) in submucosal ganglia. In 200 ganglia from 4 preparations (2 from each of 2 animals), there were 772 VIP-immunoreactive and 175 calbindin-immunoreactive nerve cells. Based on VIP neurons being 45.3% of all submucosal neurons, these data indicate that calbindin neurons are 10.1±0.8% (mean ± SEM) of all nerve cells.

All cells that were immunoreactive for calretinin were small, and some of these were calbindin immunoreactive. Counts of the numbers of neurons with only calbindin immunoreactivity, with only calretinin immunoreactivity, or with immunoreactivity for both proteins, were made in 200 ganglia, 50 in each of 2 double-stained preparations from 2 animals. Of all calbindin-immunoreactive neurons, 70.9±6.3% were calretinin positive. Conversely of all calretinin-immunoreactive neurons, 51.8±3.7% were calbindin positive.

Double labelling for tachykinin and calbindin immunoreactivity was determined in preparations that had been previously incubated with colchicine to enhance


A
Calbindin
B
Calbindin
C
Calbindin
A'
ChAT
B'
NPY
C'
VIP
D
Calbindin
E
Calbindin
F
Calretinin
D'
Calretinin
E'
TK
F'
TK
25 µm

tachykinin immunoreactivity. Large oval nerve cells with only tachykinin or with both tachykinin and calbindin immunoreactivity were observed. Of tachykinin-immunoreactive nerve cells, 42% were calbindin immunoreactive; all of these were large cells.

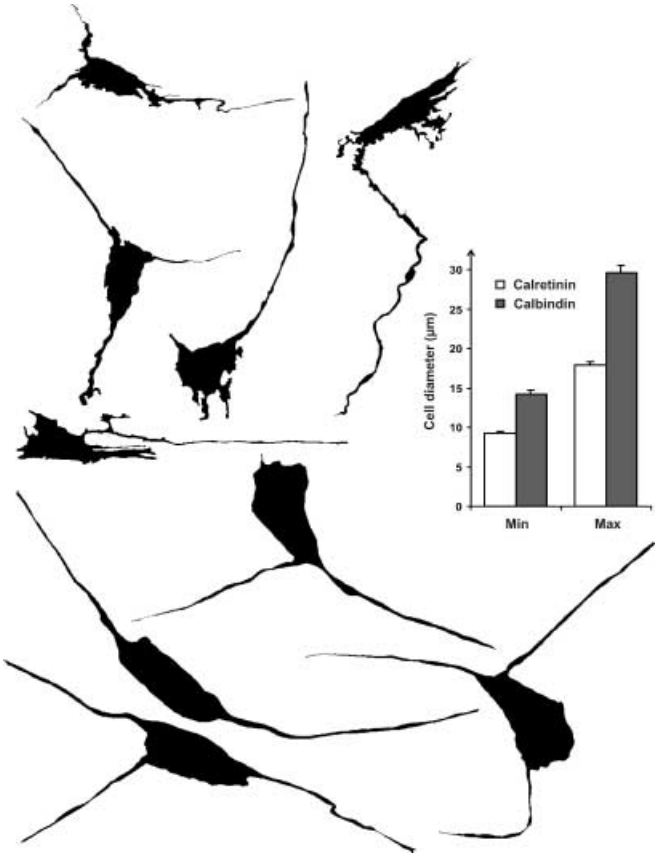


Fig. 3 Comparison of the morphologies of small secretomotor neurons (*upper five cells*), some of which are immunoreactive for calbindin, and large Dogiel type II neurons (*lower four*), many of which are calbindin immunoreactive. The cells were drawn using a camera lucida, after staining for calretinin (small cells) or calbindin (Dogiel II cells). *Inset* shows the minimum and maximum diameters of the two cell types (n=100)

◀ **Fig. 2** Patterns of co-localisation of calbindin, using antiserum CB38, with other markers in nerve cells in submucosal ganglia. **A, A'** Co-localisation of calbindin and choline acetyltransferase (*ChAT*). All calbindin neurons (examples at *arrows*) were ChAT immunoreactive. Note that calbindin immunoreactivity is in both the nucleus and cytoplasm. **B, B'** Calbindin neurons **(B)** are not immunoreactive for NPY **(B')**. **C, C'** Submucosal ganglion stained for calbindin and VIP; these are not co-localised. Note, VIP immunoreactivity is perinuclear and thus the nerve cell shapes are not revealed. **D, D'** Co-localisation of calbindin and calretinin. A proportion of, but not all, calbindin neurons have calretinin immunoreactivity (examples at *arrows*). **E, E'** Co-localisation of calbindin and tachykinin (*TK*) immunoreactivity. In order to enhance the immunoreactivity for TK, the preparation was treated with colchicine, which enhances cell body immunoreactivity and also causes the swollen appearance of nerve fibres. A cell body that is immunoreactive for both antigens is *arrowed*. Confocal image. **F, F'** Calretinin and TK co-localisation. In this confocal image, the nuclei of the calretinin neurons are seen. Large TK neurons **(F')** are not immunoreactive for calretinin

Because of the report of Song et al. (1992) that there may be a small population of nerve cells with both calretinin and tachykinin immunoreactivity, we prepared tissue for the co-localisation of these two antigens. Calretinin immunoreactivity occurred in 17% of the tachykinin-immunoreactive nerve cells. None of the small tachykinin (TK)-immunoreactive cells was calbindin immunoreactive.

To define the large and small cells more accurately, the diaminobenzidine reaction was used to locate calbindin or calretinin, in separate preparations. All calretinin cells had similar morphology, a single axon and several dendrites, which were usually fine and tapering (Fig. 3). The large calbindin cells were round or oval, and had multiple long processes, that is Dogiel type II morphology (Fig. 3). The minor and major diameters of calretinin cells were 9.3±1.7 and 17.9±4.4 µm (mean ± SD) and of the large multipolar calbindin cells they were 14.3±4.5 and 29.6±9.0 µm.

Discussion

In previous studies, it was reported that nerve cells that are immunoreactive for calbindin in the myenteric ganglia of the guinea-pig small intestine are of a single type, AH/Dogiel type II nerve cells (Furness et al. 1988, 1990; Clerc et al. 1998a), although in other regions (the stomach, proximal and distal colon) nerve cells with other shapes and functions have calbindin immunoreactivity (Furness et al. 1988; Messenger et al. 1994; Reiche et al. 1999). In the present work, using the antiserum that was reported most sensitive for revealing calbindin immunoreactivity in guinea-pig enteric neurons (Reiche et al. 1999), no further population of neurons was revealed in the myenteric ganglia, which consolidates the view that calbindin immunoreactivity is exclusive to AH/Dogiel type II nerve cells in these ganglia.

The presence of calbindin immunoreactivity in the nucleus has been previously reported in neurons in the central and enteric nervous system and in epithelial cells (Thorens et al. 1982; Furness et al. 1988; Celio 1990). Because neuronal calbindin is a cytoplasmic protein of a size (28 kDa) that will pass through nuclear pores, calbindin might be expected to be found in the nucleus. As suggested by Thorens et al. (1982), calbindin may have a role regulating nuclear Ca^{2+} levels.

Four readily defined populations of neurons occur in submucosal ganglia of the guinea-pig small intestine: neurons with VIP and galanin immunoreactivity, but not ChAT immunoreactivity (45.3%), neurons with NPY and ChAT immunoreactivity (28.6%), neurons with calretinin and ChAT (12.3%) and neurons with tachykinin (TK) and ChAT (10.8%) (Furness et al. 1984, 2000; Brookes et al. 1991; Song et al. 1992; Evans et al. 1994). It is possible that there is one or more small classes that accounts for the remaining 3% of neurons, although counting errors might be the reason that the data add to 97% rather than 100%. Small overlaps between these

populations have been discovered; Song et al. (1992) reported that 3.5% of all TK-immunoreactive neurons contain one or more of VIP, NPY or calretinin. Other markers have also been located in submucosal neurons, but VIP, NPY, calretinin, TK and ChAT are sufficient to distinguish the major classes. In the present work, we have located calbindin immunoreactivity in 9–10% of submucosal neurons, using a different antiserum than previously used; this antiserum was shown by others to be effective in revealing calbindin neurons, in the guinea-pig stomach, which had not been previously found (Reiche et al. 1999). The calbindin-immunoreactive submucosal neurons that we describe were immunoreactive for ChAT, but not for NPY or VIP. Calbindin immunoreactivity occurred in 52% of the calretinin neurons, which is about 6.4% of all submucosal neurons; these had small cell bodies. Furthermore, 40% of tachykinin-immunoreactive neurons were calbindin immunoreactive, which is about 4.3% of all neurons; calbindin/TK neurons had large (Dogiel type II) cell bodies. A proportion of small neurons are both TK and calretinin immunoreactive, about 2% in the present study; but small calbindin-immunoreactive cells were not TK immunoreactive. We can therefore divide submucosal calbindin neurons into two groups: calbindin/calretinin/ChAT neurons and calbindin/ChAT/TK Dogiel type II neurons.

The calretinin neurons in submucosal ganglia of the small intestine project to the glands at the base of the mucosa and to submucosal arterioles (Brookes et al. 1991; Clerc et al. 1998b; Li et al. 1998). Unlike the ChAT/TK Dogiel type II neurons, they never project to the myenteric ganglia (Song et al. 1998). The calretinin neurons are reactive for ChAT and the vesicular acetylcholine transporter (Brookes et al. 1991; Li et al. 1998) and are therefore cholinergic. Because they project to both the arterioles and glands, they have been classed as secretomotor/vasomotor neurons (Furness 2000; Furness et al. 2000). It was suggested that they function to dilate the arterioles in order to increase the available water and electrolyte for secretion by the mucosal glands, and to enhance the secretion. Whether there is a physiological difference between the 50% of these neurons with and the 50% without calbindin immunoreactivity is not known.

Other calbindin-immunoreactive submucosal neurons are a subgroup of the ChAT/TK neurons that have been previously identified as Dogiel type II neurons with AH electrophysiological characteristics (Bornstein et al. 1989; Evans et al. 1994). These neurons project to the mucosa (Costa et al. 1981; Song et al. 1992) and to the myenteric plexus (Kirchgessner et al. 1996; Song et al. 1998). The AH/Dogiel type II neurons in the myenteric and submucosal ganglia are intrinsic primary afferent neurons (Kirchgessner et al. 1992; Furness et al. 1998). About 30% of submucosal AH/Dogiel type II neurons and 82–84% of myenteric Dogiel type II neurons are immunoreactive for calbindin (present study; Iyer et al. 1988; Song et al. 1991). The differences between these proportions might relate to differences in the modalities of sensory stimulation detected by submucosal and myenteric IPANs. Submucosal IPANs are more sensitive than myenteric IPANs to mechanical stimulation of the mucosa, both myenteric and submucosal IPANs are sensitive to chemical stimulation of the mucosa and distension of the gut, and myenteric IPANs respond selectively to distension of the intestine and to tension in the external muscle (Kirchgessner et al. 1992, 1996; Bertrand et al. 1997; Kunze et al. 1999). Kirchgessner et al. (1996) reported that mild distortion of the villi or glucose applied to the mucosal surface is a selective stimulant of submucosal IPANs. It would be interesting to correlate calbindin immunoreactivity with the responsiveness of IPANs to different sensory stimuli. Neurochemical differences might also be related to the output connections of the neurons. Motility reflexes, secretory reflexes and vasodilator reflexes are all initiated through IPANs (Furness et al. 2000). It is possible that different IPANs feed to greater or lesser extents into different reflex-controlling pathways.

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References

Barajas-López C, Surprenant A, North RA (1991) Adenosine A1 and A2 receptors mediate presynaptic inhibition and postsynaptic excitation in guinea pig submucosal neurons. J Pharmacol Exp Ther 258:490–495

Bertrand PP, Kunze WAA, Bornstein JC, Furness JB, Smith ML (1997) Analysis of the responses of myenteric neurons in the small intestine to chemical stimulation of the mucosa. Am J Physiol 273:G422–G435

Bornstein JC, Furness JB, Costa M (1989) An electrophysiological comparison of substance P-immunoreactive neurons with other neurons in the guinea-pig submucous plexus. J Auton Nerv Syst 26:113–120

Brookes SJH, Steele PA, Costa M (1991) Calretinin immunoreactivity in cholinergic motor neurones, interneurones and vasomotor neurones in the guinea-pig small intestine. Cell Tissue Res 263:471–481

Celio MR (1990) Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35:375–475

Clerc N, Furness JB, Bornstein JC, Kunze WAA (1998a) Correlation of electrophysiological and morphological characteristics of myenteric neurons of the duodenum in the guinea-pig. Neuroscience 82:899–914

Clerc N, Furness JB, Li ZS, Bornstein JC, Kunze WAA (1998b) Morphological and immunohistochemical identification of neurons and their targets in the guinea-pig duodenum. Neuroscience 86:679–694

Costa M, Furness JB, Llewellyn Smith IJ, Cuello AC (1981) Projections of substance P-containing neurons within the guinea-pig small intestine. Neuroscience 6:411–424

Costa M, Brookes SJH, Steele PA, Gibbins I, Burcher E, Kandiah CJ (1996) Neurochemical classification of myenteric neurons in the guinea-pig ileum. Neuroscience 75:949–967

Cuello AC, Galfre G, Milstein C (1979) Detection of substance P in the central nervous system by a monoclonal antibody. Proc Natl Acad Sci USA 76:3532–3536

Evans RJ, Jiang MM, Surprenant A (1994) Morphological properties and projections of electrophysiologically characterized neurons in the guinea-pig submucosal plexus. Neuroscience 59:1093–1110

Furness JB (2000) Types of neurons in the enteric nervous system. J Auton Nerv Syst 81:87–96

Furness JB, Costa M, Keast JR (1984) Choline acetyltransferase and peptide immunoreactivity of submucous neurons in the small intestine of the guinea-pig. Cell Tissue Res 237:329–336

Furness JB, Costa M, Gibbins IL, Llewellyn Smith IJ, Oliver JR (1985) Neurochemically similar myenteric and submucous neurons directly traced to the mucosa of the small intestine. Cell Tissue Res 241:155–163

Furness JB, Keast JR, Pompolo S, Bornstein JC, Costa M, Emson PC, Lawson DEM (1988) Immunohistochemical evidence for the presence of calcium binding proteins in enteric neurons. Cell Tissue Res 252:79–87

Furness JB, Pompolo S, Murphy R, Giraud A (1989) Projections of neurons with neuromedin U-like immunoreactivity in the small intestine of the guinea-pig. Cell Tissue Res 257:415–422

Furness JB, Trussell DC, Pompolo S, Bornstein JC, Smith TK (1990) Calbindin neurons of the guinea-pig small intestine: quantitative analysis of their numbers and projections. Cell Tissue Res 260:261–272

Furness JB, Kunze WAA, Bertrand PP, Clerc N, Bornstein JC (1998) Intrinsic primary afferent neurons of the intestine. Prog Neurobiol 54:1–18

Furness JB, Clerc N, Gola M, Kunze WAA, Fletcher EL (2000) Identification of component neurons and organisation of enteric nerve circuits. In: Krammer HJ, Singer MV (eds) Neurogastroenterology – from the basics to the clinics. Kluwer Academic, Dordrecht, pp 134–147

Iyer V, Bornstein JC, Costa M, Furness JB, Takahashi Y, Iwanaga T (1988) Electrophysiology of guinea-pig myenteric neurons correlated with immunoreactivity for calcium binding proteins. J Auton Nerv Syst 22:141–150

Kirchgessner AL, Tamir H, Gershon MD (1992) Identification and stimulation by serotonin of intrinsic sensory neurons of the submucosal plexus of the guinea pig gut: activity-induced expression of Fos immunoreactivity. J Neurosci 12:235–248

Kirchgessner AL, Liu MT, Gershon MD (1996) In situ identification and visualization of neurons that mediate enteric and enteropancreatic reflexes. J Comp Neurol 371:270–286

Kunze WAA, Clerc N, Bertrand PP, Furness JB (1999) Contractile activity in intestinal muscle evokes action potential discharge in guinea-pig myenteric neurons. J Physiol (Lond) 517:547–561

Li ZS, Furness JB (1998) Immunohistochemical localization of cholinergic markers in putative intrinsic primary afferent neurons of the guinea-pig small intestine. Cell Tissue Res 294: 35–43

Li ZS, Fox Threlkeld JET, Furness JB (1998) Innervation of intestinal arteries by axons with immunoreactivity for the vesicular acetylcholine transporter VAChT. J Anat 192:107–117

Messenger JP, Bornstein JC, Furness JB (1994) Electrophysiological and morphological classification of myenteric neurons in the proximal colon of the guinea-pig. Neuroscience 60:227–244

Morris JL, Gibbins IL, Furness JB, Costa M, Murphy R (1985) Co-localisation of NPY, VIP and dynorphin in non-adrenergic axons of the guinea-pig uterine artery. Neurosci Lett 62:31–37

Pompolo S, Furness JB (1988) Ultrastructure and synaptic relationships of calbindin-reactive, Dogiel type II neurons, in myenteric ganglia of guinea-pig small intestine. J Neurocytol 17: 771–782

Reiche D, Pfannkuche H, Michel K, Hoppe S, Schemann M (1999) Immunohistochemical evidence for the presence of calbindin containing neurones in the myenteric plexus of the guinea-pig stomach. Neurosci Lett 270:71–74

Song ZM, Brookes SJH, Costa M (1991) Identification of myenteric neurons which project to the mucosa of the guinea-pig small intestine. Neurosci Lett 129:294–298

Song ZM, Brookes SJH, Steele PA, Costa M (1992) Projections and pathways of submucous neurons to the mucosa of the guinea-pig small intestine. Cell Tissue Res 269:87–98

Song ZM, Costa M, Brookes SJH (1998) Projections of submucous neurons to the myenteric plexus in the guinea pig small intestine. J Comp Neurol 399:255–265

Thorens B, Roth J, Norman A, Perrelet, Orci L (1982) Immunocytochemical localization of the vitamin D-dependent calcium binding protein in chick duodenum. J Cell Biol 94:115–122

Zholos AV, Baidan LV, Starodub AM, Wood JD (1999) Potassium channels of myenteric neurons in guinea-pig small intestine. Neuroscience 89:603–618