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Specific patterns of immunoreactivity in neuronal elements of the anterior major pelvic ganglion of the male guinea-pig

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INTRODUCTION

The anterior major pelvic ganglion (AMPG) of the male guinea-pig is a mixed autonomic ganglion which has been shown to contain both cholinergic and adrenergic neurons (Yokota & Burnstock, 1983). The organisation of the AMPG is similar to that reported by Dail, Evan & Eason (1975) for the homologous ganglion, called the hypogastric ganglion, in the male rat. The AMPG is a bilateral structure located in the angle between the ductus deferens and the seminal vesicle (Merrilees, Burnstock & Holman, 1963; Wakade & Kirkepar, 1971; Costa & Furness, 1973). Each AMPG receives two main inputs: the hypogastric nerve and the pelvic nerve (Crowcroft & Szurszewski, 1971), and the AMPG is responsible for supplying sympathetic and parasympathetic nerve fibres to the pelvic viscera and vasculature (Costa & Furness, 1973).

In addition to the classical neurotransmitters, autonomic ganglia, in general, contain neuropeptides which may function as neurotransmitters or neuromodulators (Snyder & Innis, 1979). Novel peptides have been demonstrated in autonomic ganglia, e.g. atrial natriuretic factor and enkephalin in the rat (Papka, Traurig & Wekstein, 1985) and guinea-pig (Mitchell & Stauber, 1990) paracervical ganglion and somatostatin in the guinea-pig inferior mesenteric ganglion (Hökfelt *et al.* 1977). The coexistence of neuropeptides with classical neurotransmitters seems to be a standard feature of neurons of the central and peripheral nervous system (Hökfelt *et al.* 1980; Hökfelt *et al.* 1987). In order to further the understanding of the role of the AMPG in pelvic visceral innervation the phenomenon of coexistence requires investigation. Apart from the work of Schultzberg *et al.* (1983) which briefly outlined the neuropeptide content of the male guinea-pig AMPG, including enkephalin, substance P, vasoactive intestinal peptide, bombesin, cholecystokinin and avian polypeptide, this ganglion has not been the subject of a study concerning coexistence. In the present investigation the occurrence of classical neurotransmitters (demonstrated by tyrosine hydroxylase, dopamine-β-hydroxylase or acetylcholinesterase) and several neuropeptides in the AMPG are investigated.

MATERIALS AND METHODS

Tissue preparation

Twenty prepubertal male Hartley guinea-pigs (each weighing approximately 250 g) were injected with an overdose of Euthatal (sodium pentobarbitone) prior to perfusion with ice-cold heparinised isotonic saline followed by 4% paraformaldehyde in 0·1 M phosphate buffer (pH 7·4). Small intestine and anterior major pelvic ganglion tissues were dissected out and fixed for a further 6 hours in the same fixative. The tissues were

transferred to a sucrose solution for 18 hours in preparation for sectioning on a cryostat. All sections were cut at a thickness of 5 μm. For investigation of the coexistence of neuropeptides serial sections of ganglia were taken. In order to investigate the pattern of distribution of tyrosine hydroxylase (TH)-immunoreactive neuronal perikarya, left and right ganglia from three animals were serially sectioned at 40 μm intervals at a thickness of 5 μm. Sections were mounted on slides coated with either 0·1 % poly-L-lysine, or with 0·5 % gelatin, and air dried.

The acetylcholinesterase method

Sections of AMPG and small intestine were reacted for acetylcholinesterase activity according to the method of Karnovsky & Roots (1964) using acetylthiocholine iodide as a substrate. Omission of this substrate served as a negative control while its substitution with butyrylthiocholine iodide served as control for non-specific esterase activity.

The indirect immunofluorescence method

Immunoreactivities to tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH), neuropeptide Y (NPY), vasoactive intestinal peptide (VIP), substance P (SP), atrial natriuretic factor (ANF), Leu-enkephalin (ENK) and somatostatin (SOM) were demonstrated using an indirect immunofluorescence method. Primary antisera were diluted according to the manufacturers' recommendations: α-TH and α-DBH at 1:200, α-ANF and α-SOM at 1:400, α-NPY and α-ENK at 1:800, α-SP at 1:1000 and α-VIP at 1:4000. The primary antibodies (raised in rabbits) were obtained from Cambridge Research Biochemicals, UK, for the neuropeptides and from Eugene Tech International, US, for the catecholamine synthesising enzymes. The secondary labelling antibody, a fluoresceinisothiocyanate (FITC) sheep anti-rabbit IgG conjugate (Nordic Immunological) was used (diluted 1:60) to reveal sites of immunoreactivity. The primary labelling antibodies were applied to sections for 18 hours at room temperature followed by an FITC-IgG conjugate for one hour at room temperature. Immunofluorescence preparations were mounted in AF 1 glycerol/PBS (Citifluor) to prevent fading of fluorescence, and viewed using an Olympus fluorescence microscope. For the indirect immunofluorescence method controls included omission of the primary labelling antibody, and its substitution with phosphate buffered saline. Since the presence of neuropeptides is well-documented in the enteric nervous system (Furness & Costa, 1980), positive controls consisted of sections of guinea-pig small intestine which were exposed to primary antibodies followed by the FITC-IgG conjugate.

RESULTS

Acetylcholinesterase activity

The AMPG was found to exhibit acetylcholinesterase activity as indicated by the dark brown reaction product in the cytoplasm of neuronal perikarya and in plexuses of nerve fibres (Fig. 1*a*, *b*, respectively). In sections of each AMPG examined many neuronal perikarya were devoid of acetylcholinesterase activity (Fig. 1*a*). Clear acetylcholinesterase activity was present in sections of small intestine which, therefore, acted as a reliable positive control.

Catecholamine synthesising enzymes distribution

Antibodies raised against two enzymes required for noradrenalin synthesis (TH and DBH) were used to identify postganglionic noradrenergic neuronal perikarya in the

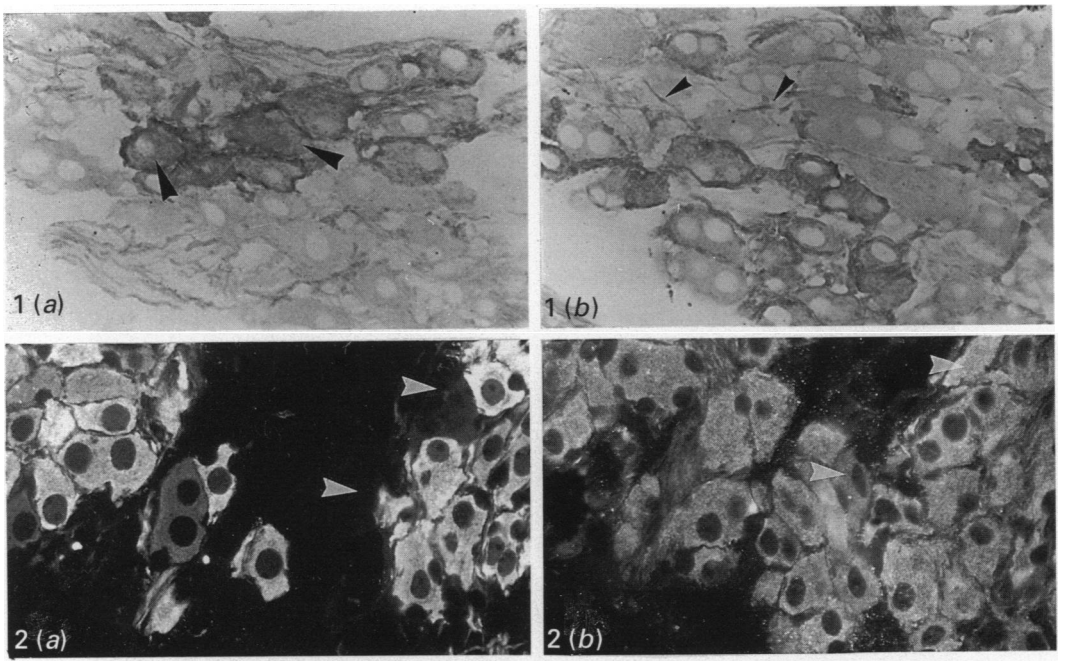


Fig. 1 (*a–b*). Photomicrographs of a preparation for acetylcholinesterase in the AMPG of the male guinea-pig showing (*a*) positive neuronal perikarya (arrowed). × 288. (*b*) Positive plexus of fibres (arrowed). × 288.

Fig. 2 (*a–b*). Photomicrographs of fluorescence preparations of serial sections of the AMPG of the male guinea-pig showing immunoreactivity to (*a*) TH in neuronal perikarya. Some neuronal perikarya, indicated by arrowheads, lack TH. × 288. (*b*) DBH in neuronal perikarya. Some neuronal perikarya (arrowed) lack TH (arrowheads in *a*). × 288.

AMPG. TH-immunoreactivity (IR) (Fig. 2*a*) and DBH-IR (Fig. 2*b*) were clearly demonstrable in neuronal perikarya and non-varicose nerve fibres in some sections taken from the male guinea-pig AMPG. An observation particularly associated with TH-IR was its presence in different regions of the ganglion. Further analysis of serial sections from six whole ganglia confirmed distinct pools of neurons immunoreactive for TH while other regions lacked immunoreactivity.

Neuropeptide distribution

Immunoreactivities for NPY, VIP, SOM, ANF, ENK and SP were evident in the ganglion. NPY-IR was present in neuronal perikarya (Fig. 3*a*) and in varicose nerve fibres and nerve terminals (Fig. 3*b*). VIP-IR occurred in both neuronal perikarya (Fig. 4*a*) and varicose nerve fibres (Fig. 4*b*). VIP-IR varicosities abutted both VIP-IR and unlabelled neuronal perikarya. SOM-IR (Fig. 5) was present in neuronal perikarya; however, no nerve fibres or nerve terminals were labelled. ANF-IR occurred in a small number of neuronal perikarya and in varicose nerve fibres which coursed between unlabelled and ANF-IR neuronal perikarya (Fig. 6). ENK-IR was only seen in varicose nerve fibres that abutted unlabelled neuronal perikarya (Fig. 7). Similarly SP-IR occurred in varicose nerve fibres and nerve terminals, the latter appearing as fluorescent varicosities abutting unlabelled neuronal perikarya (Fig. 8*a*). On a rare occasion SP-IR was also present in neuronal perikarya (Fig. 8*b*) but this was not the

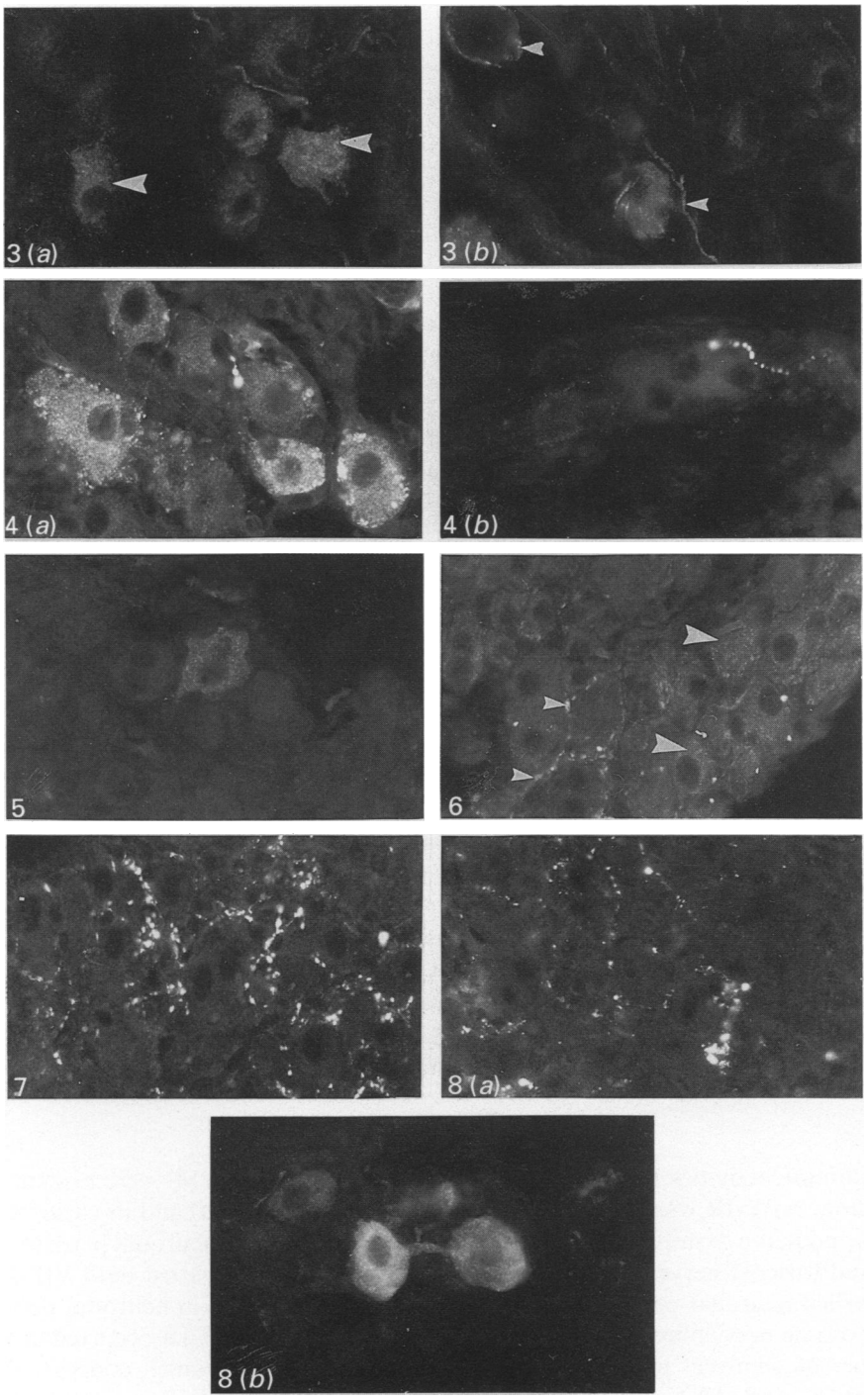

3 (a)
3 (b)
4 (a)
4 (b)
5
6
7
8 (a)
8 (b)

Table 1. *Colocalisation*

	TH	NPY	VIP
NPY	+		
VIP	−	+	
AChE	−	−	+
DBH	+	−	−

+, colocalisation
−, no colocalisation

usual pattern of labelling. Catecholamine-synthesising enzymes and neuropeptides were found in submucous and myenteric plexuses of guinea-pig small intestine (positive control).

Washing with a buffer of high sodium chloride concentration (Grube, 1980) did not abolish the immunoreactivities of the catecholamine synthesising enzymes or the neuropeptides, which indicates method specificity was satisfactory.

Colocalisation (Table 1)

The analysis of adjacent serial sections allowed specific enzyme–enzyme or enzyme–neuropeptide combinations to be determined. Immunoreactivities of the noradrenalin synthesising enzymes TH and DBH can be seen in the same neuronal perikarya in Figure 9(*a*, *b*), respectively. On the whole, TH-IR and DBH-IR were found to coincide; however, some neuronal perikarya immunoreactive for DBH were negative for TH (Fig. 2*a*, *b*). The colocalisation of TH-IR and DBH-IR was not confined to neuronal perikarya; small intensely fluorescent cells were found to exhibit this enzymne pairing (Fig. 9*a*, *b*).

Figure 10(*a*) shows intense TH-IR in three neuronal perikarya coinciding with cytoplasmic NPY-IR which is granular in appearance (Fig. 10*b*). NPY-IR did not always coincide with TH-IR; NPY-IR was observed in non-noradrenergic neuronal perikarya. In this case NPY-IR (Fig. 11*a*) coincides with VIP-IR (Fig. 11*b*) in certain neuronal perikarya. A third section (Fig. 11*c*), reacted for TH-IR, shows this specific neuronal perikaryon to be lacking TH-IR. Likewise TH-IR neuronal perikarya (Fig. 11*c*) were negative for NPY-IR and VIP-IR. VIP-IR (Fig. 12*a*) was also colocalised in particular neuronal perikarya exhibiting AChE activity (Fig. 12*b*).

Figs. 3–8. Photomicrographs of fluorescence preparations of the AMPG of the male guinea-pig showing immunoreactivity to various neuropeptides.

Fig. 3(*a–b*). (*a*) NPY in some neuronal perikarya (large arrowheads). × 288. (*b*) NPY in nerve fibre elements (small arrowheads). × 288.

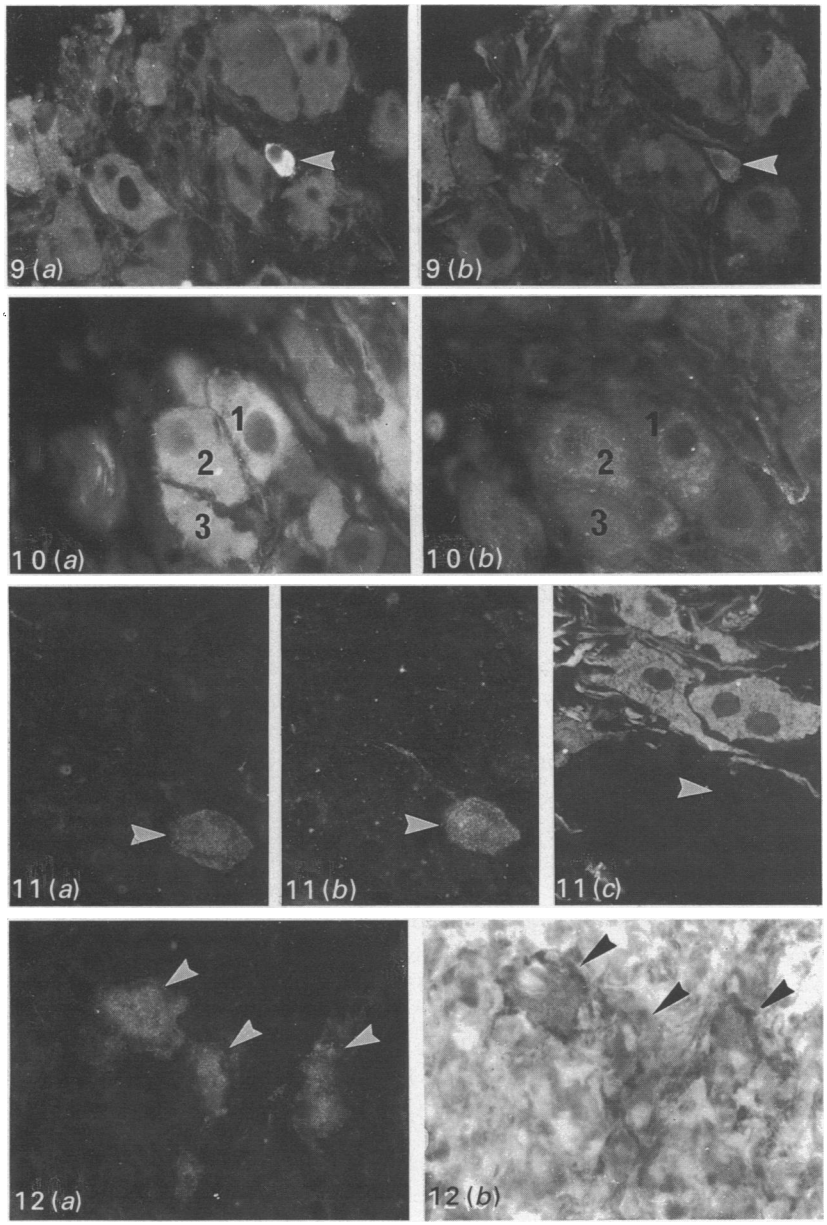
Fig. 4(*a–b*). (*a*) VIP present as granular immunoreactivity in some neuronal perikarya. × 288. (*b*) VIP in a fine varicose nerve fibre. × 288.

Fig. 5. SOM present in the cytoplasm of a neuronal perikaryon. × 288.

Fig. 6. ANF in some neuronal perikarya (large arrowheads) and varicose nerve fibres (small arrowheads). × 288.

Fig. 7. ENK in varicose nerve fibres coursing between unlabelled neuronal perikarya. × 288.

Fig. 8(*a–b*). (*a*) SP present in nerve fibre varicosities/terminals. × 288. (*b*) SP in three neuronal perikarya. × 288.



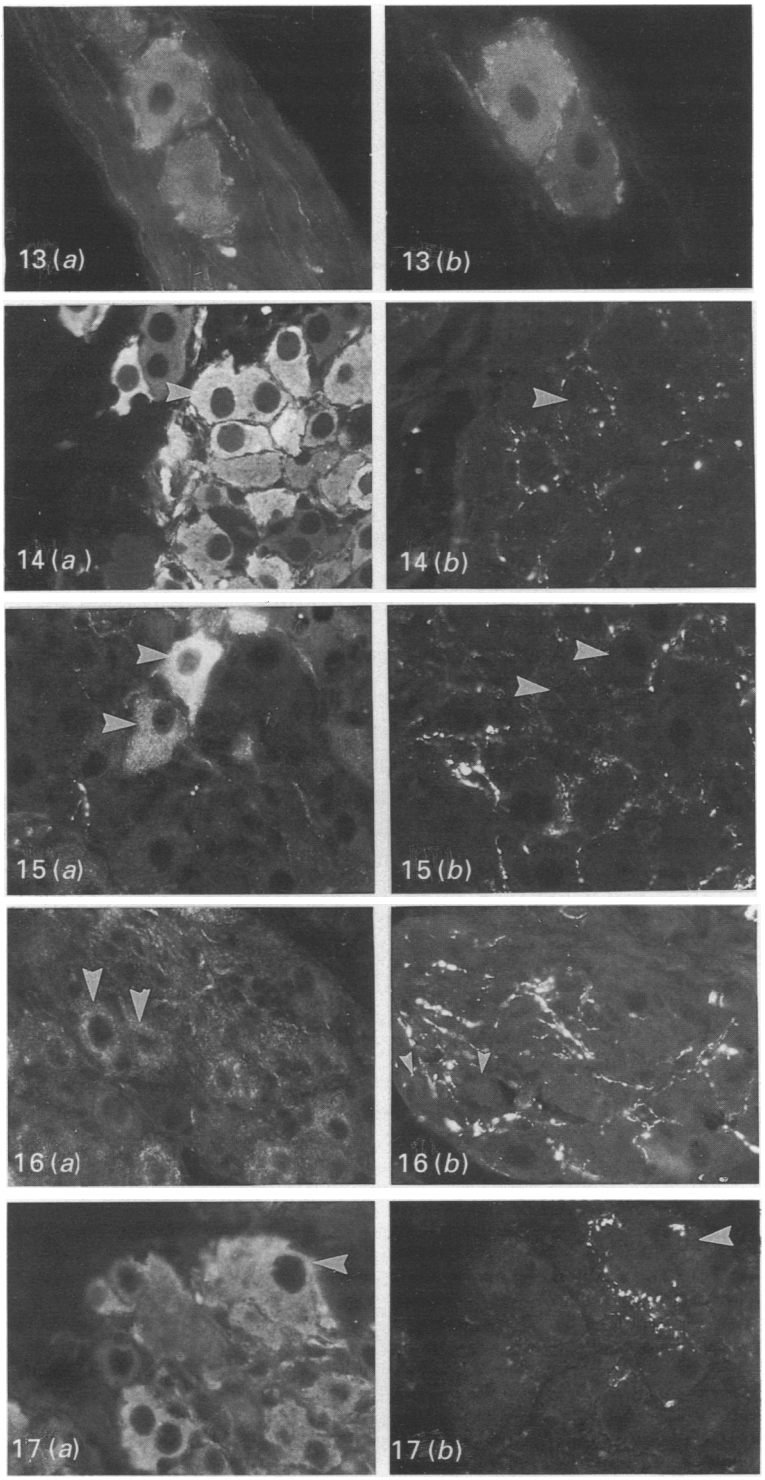
Figs 9–12. Photomicrographs of fluorescence preparations of serial sections taken from the AMPG of male guinea-pigs showing colocalisation of immunoreactivities for various neuropeptides and/or classical transmitter synthesising enzymes.

Fig. 9(*a–b*). (*a*) TH and (*b*) DBH occur in many neuronal perikarya and in a single small intensely fluorescent cell (arrowed). × 288.

Fig. 10(*a–b*). (*a*) TH and (*b*) NPY are colocalised to the cytoplasm of three neuronal perikarya (numbered 1 to 3). × 330.

Fig. 11(*a–c*). (*a*) NPY and (*b*) VIP are visible in the same neuronal perikaryon, indicated by arrowheads, which appears to be devoid of TH (*c*). Equally TH-IR is present in some neuronal perikarya devoid of NPY (*a*) and VIP (*b*) immunoreactivities. × 288.

Fig. 12(*a–b*). (*a*) VIP and (*b*) AChE are present in three neuronal perikarya (arrowed) which also receive AChE positive varicosities (*b*). × 288.



Figs. 13–17. For legend see p. 204.

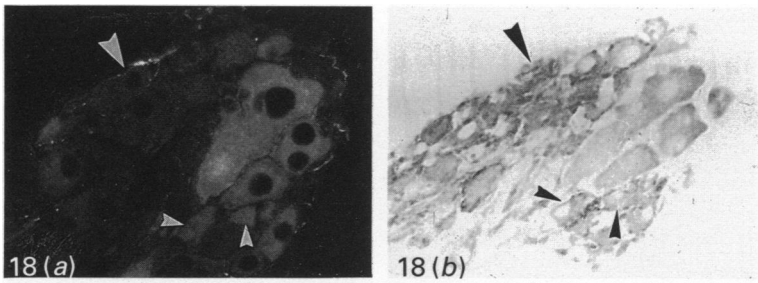


Fig. 18(*a–b*). (*a*) DBH is present in neuronal perikarya and varicose nerve fibres. × 288. (*b*) AChE-positive neuronal perikarya and nerve fibres. × 288. DBH-IR neuronal perikarya (indicated by small arrowheads in (*a*) receive AChE-positive nerve fibre terminals/varicosities (*b*). An AChE-positive neuronal perikaryon (indicated by a large arrowhead in *b*) is seen to be associated with a DBH varicose nerve fibre (*a*).

Relationship of immunoreactive neuronal perikarya and nerve fibres

Analysis of adjacent serial sections, reacted for different enzyme–neuropeptide or neuropeptide–neuropeptide combinations, revealed specific immunoreactive neuronal perikarya to be in close relationship with nerve terminals/varicosities immunoreactive for the same or a different neuropeptide.

Nerve terminals/varicosities IR for NPY (Fig. 13*a*) and VIP (Fig. 13*b*) can be seen abutting immunoreactive neuronal perikarya exhibiting NPY-VIP colocalisation. Therefore, in this case, nerve terminals/varicosities immunoreactive for either NPY or VIP both abut VIP-NPY-IR neuronal perikarya. SP-IR varicose nerve fibres are abundant in the AMPG. As a result many neuronal perikarya possessing different enzyme/neuropeptide-IR appear to be abutted by SP-IR nerve terminals/varicosities. In Figure 14(*a*) the large area of TH-IR neuronal perikarya appears to be associated with a moderately dense network of SP-IR varicose nerve fibres (Fig. 14*b*). Figure 15(*a*) clearly shows VIP-IR neuronal perikarya which appear to be abutted by SP-IR nerve terminals/varicosities (Fig. 15*b*). SP-IR nerve terminals/varicosities (Fig. 16*b*) are closely related to NPY-IR neuronal perikarya (Fig. 16*a*). TH-IR neuronal perikarya are shown in Figure 17(*a*) which appear to be related to ENK-IR nerve terminals/varicosities in the adjacent section (Fig. 17*b*). It was noticed that ENK-IR nerve fibres were not associated with neuronal perikarya immunoreactive for any of the neuropeptides. Certain DBH-IR neurons (Fig. 18*a*) were found to be associated

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Figs 13–17. Photomicrographs of fluorescence preparations of 5 μm serial sections taken from the AMPG of male guinea-pigs showing the relationship between certain immunoreactive neuronal perikarya and nerve terminals/varicosities with different immunoreactivities.

Fig. 13(*a–b*). (*a*) VIP and (*b*) NPY occur in neuronal perikarya which are abutted by nerve terminals/varicosities immunoreactive for VIP (*a*) and NPY (*b*). × 288.

Fig. 14(*a–b*). (*a*) TH is present in the majority of neuronal perikarya. The neuronal perikaryon, arrowed in (*a*) is abutted by SP-IR nerve terminals/varicosities as seen in (*b*). × 288.

Fig. 15(*a–b*). (*a*) VIP is present in some neuronal perikarya (arrowed). (*b*) SP-IR nerve terminals/varicosities are seen to abut the VIP-IR neuronal perikarya, arrowed in (*a*). × 288.

Fig. 16(*a–b*). (*a*) NPY is present in neuronal perikarya (arrowed). (*b*) SP-IR nerve terminals/varicosities are seen to abut the NPY-IR neuronal perikarya (arrowed) seen in (*a*). × 288.

Fig. 17(*a–b*). (*a*) TH in a neuronal perikaryon (arrowed) is abutted by (*b*) ENK-IR nerve terminals/varicosities. × 288.

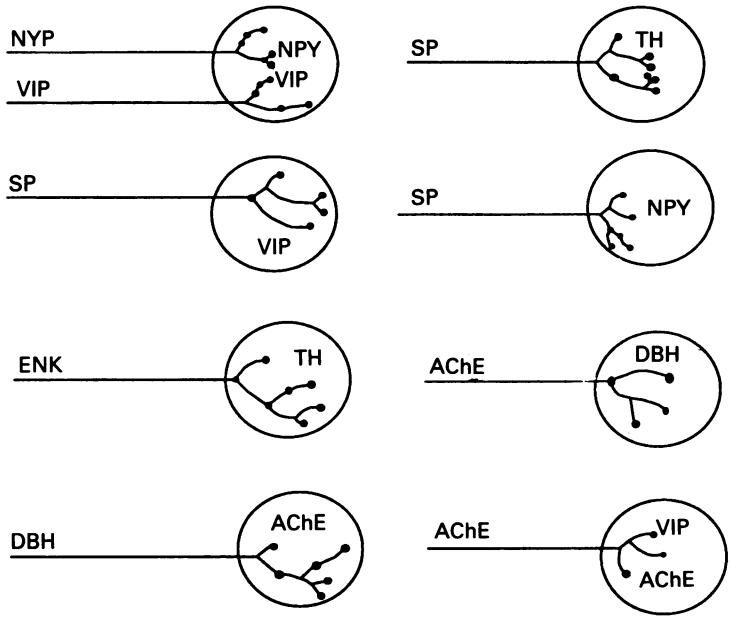

Fig. 19. Relationships between specific classes of neuronal perikarya and varicose nerve fibres.

with AChE-positive varicosities (Fig. 18*b*). The converse relationship was also observed between AChE positive neuronal perikarya (Fig. 18*b*) and DBH-IR varicose nerve fibres (Fig. 18*a*). Certain neuronal perikarya possessing VIP-IR and a positive reaction for AChE appeared to receive cholinergic terminals (Fig. 12*a*, *b*). These observations are summarised in Figure 19.

DISCUSSION

The AMPG of the male guinea-pig has a cholinergic and a catecholaminergic (probably noradrenergic) component (Bell & McLean, 1967). The acetylcholinesterase activity in certain neuronal perikarya, and in plexuses of nerve fibres, is similar to that seen in the rat major pelvic (hypogastric) ganglion (Dail, Evan & Eason, 1975). The paracervical ganglion of the female guinea-pig, the analogous structure to the AMPG in the male, also possesses demonstrable acetylcholinesterase activity (Mitchell & Stauber, 1990). It is important to remember that, even though a positive reaction for AChE activity is taken to indicate the presence of a cholinergic component, the specificity of the reaction is still questionable (Silver, 1974).

The presence of TH- and DBH-IR suggests that a proportion of the neuronal perikarya in the AMPG is noradrenergic. These immunoreactive neuronal perikarya probably represent the 'short' adrenergic neurons (Sjöstrand, 1965) previously shown by formaldehyde-induced fluorescence for catecholamines (Costa & Furness, 1973). Generally those neuronal perikarya immunoreactive for DBH also possessed TH-IR; however, for some neurons this was not the case. It is possible that these TH-IR-lacking neurons are cholinergic neurons that contain an inactive form of DBH (Morris & Gibbins, 1987). Neurons of the AMPG may initially be uncommitted to either noradrenalin or acetylcholine synthesis and contain the necessary enzymes for both pathways. This situation would be similar to that for the developing neurons of the rat

superior cervical ganglion (Hill & Hendry, 1977). It is therefore conceivable that inactive forms of enzymes persist. The fact that a proportion of the neuronal perikarya in the sections analysed was lacking in either AChE activity or TH-IR may indicate an element of functional regionalisation in these ganglia. Indeed, our personal observations in six ganglia from three guinea-pigs demonstrated that the AMPG possesses regions of TH-IR and non-TH-IR neurons. This suggests that the 'short' adrenergic neurons occur in large groups, separate from other neuronal populations comparable to the neuronal pools seen in the superior cervical ganglion (Jacobowitz & Woodward, 1968). This occurrence of regionalisation accords with the studies carried out on the major pelvic (hypogastric) ganglion of rats by Keast & DeGroat (1989) and Keast, Booth & DeGroat (1989).

In addition to the classical transmitters, the AMPG contains a number of neuropeptides (NPY, VIP, ANF, SOM, SP and ENK) in the various neuronal elements of the ganglion. These neuropeptides have been visualised in other autonomic ganglia using immunohistological techniques, e.g. the paracervical ganglia of the rat (Papka, Traurig & Wekstein, 1985) and guinea-pig (Mitchell & Stauber, 1990). The localisation of SP and ENK immunoreactivities to varicose nerve fibres concurs with the findings of Schultzberg *et al.* (1983), while the occurrence of ANF-IR in neuronal perikarya, as well as in nerve fibres, differs from that in the rat paracervical ganglion (Papka, Traurig & Wekstein, 1985). The presence of VIP in the AMPG is to be expected as VIP is associated with the genital tracts of cats (Alm, Alumets, Håkansson & Sundler, 1977), rats (Dail, Trujillo, de la Rosa & Walton, 1989) and rabbits (Ottesen & Fahrenkrug, 1981).

The localisation of NPY-IR to AMPG neuronal perikarya resembles the granular pattern of NPY-IR in the paracervical ganglion (Inyama *et al.* 1985). If TH is assumed to be a reliable marker for sympathetic postganglionic neurons, the colocalisation of NPY-IR to a number of TH-IR neuronal perikarya suggests that NPY coexists with noradrenalin. This would agree with work carried out on other ganglia (Lundberg *et al.* 1982; Macrae, Furness & Costa, 1986). The role of NPY in neurons of the AMPG is unclear. NPY is a powerful vasoconstricting agent in vascular beds (Allen *et al.* 1986) and so may be involved in the regulation of local blood flow to pelvic viscera, a similar role to that which it plays in the myometrium (Tenmoku *et al.* 1988). Alternatively NPY may act as a neuromodulator at sympathetic neuro-effector junctions (Edvinsson, Håkanson, Wahlestedt & Uddman, 1987) possibly contributing to 'transmitter conservation' (Stjärne, Lundberg & Åstrand, 1986). A sub-population of cholinergic neurons of the AMPG appeared to possess VIP. VIP in cholinergic neurons has been shown to be responsible for mediating atropine-resistant responses in the exocrine organs of the cat (Lundberg, 1981) and so may fulfil a similar role in the target organs of the AMPG, e.g. ductus deferens (Birmingham, 1966) and urinary bladder (Gesher & Thorp, 1965). The AMPG also exhibits NPY-VIP coexistence in a few of the non-TH-IR neuronal perikarya. The coexistence of these neuropeptides is not uncommon and is observed in a small percentage of neurons of the paracervical ganglion (Morris & Gibbins, 1987) and the coeliac ganglion (Macrae, Furness & Costa, 1986). Nevertheless it is surprising to find two neuropeptides with opposing functions (VIP vasodilates whereas NPY vasoconstricts) coexisting in the same neuron. If a role in the control of local blood flow is assumed it is probable that one of these neuropeptides acts as a neuromodulator in this situation since Grunditz *et al.* (1988) found that NPY enhanced the VIP-induced release of thyroid hormone secretion. The lack of TH-IR in the NPY-VIP neuronal perikarya suggests that these neurons may be cholinergic. It is equally conceivable that these were non-

noradrenergic, non-cholinergic, peptidergic neurons (Baumgarten, Holstein & Owman, 1970; Burnstock, 1986). It therefore follows that in these peptidergic neurons at least one of the neuropeptides acts as the neurotransmitter as no 'classical' transmitter would be present to fulfil such a role. Another possibility is that these neurons are purinergic, releasing purine nucleotides such as ATP (Burnstock, 1975; Burnstock, Cocks, Crowe & Kasavok, 1978).

The guinea-pig AMPG possesses an extensive network of SP-IR nerve fibres which is a typical feature of guinea-pig sympathetic ganglia (Hökfelt *et al.* 1977). The abundance of SP-IR nerve fibres sharply contrasts with the patchy appearance of SP-IR nerve fibres in the rat hypogastric ganglion (Dail & Dziurzynski, 1985). As the SP in guinea-pig sympathetic ganglia is depleted by capsaicin treatment it is likely to be present in sensory nerve fibres (Gamse, Wax, Zigmond & Leeman, 1981). SP may be present in the collaterals of sensory nerves which possibly modulate ganglionic activity by operating via a reflex loop involving the target organs and their associated ganglia (Matthews & Cuello, 1982). The fact that SP-IR nerve varicosities were seen to abut specific immunoreactive neuronal perikarya of the AMPG may implicate SP in such a role. The presence of ENK in varicose nerve fibres was in accord with work previously carried out on the guinea-pig AMPG (Schultzberg *et al.* 1983) and the paravertebral ganglia of the rat and guinea-pig (Schultzberg *et al.* 1979). The function of the ENK-IR nerve varicosities/terminals is unknown. However, ENK is known to inhibit the presynaptic release of acetylcholine (Kawatani *et al.* 1983) from cholinergic synapses which are prevalent in the guinea-pig AMPG (Yokota & Burnstock, 1983). Therefore, it may be speculated that ENK is capable of influencing ganglionic neural activity, this role having already been suggested for the paracervical ganglion of the rat (Papka, Traurig & Wekstein, 1985).

The immunoreactive neurons and AChE-positive neurons of the AMPG are closely associated with preganglionic/intraganglionic immunoreactive nerve fibre varicosities. Neuronal perikarya of other ganglia, e.g. the feline para- and prevertebral ganglia (Heym, Reinecke, Weihe & Forssman, 1984) and the toad anterior sympathetic ganglion (Morris *et al.* 1986), are also associated with varicose nerve fibres of different immunoreactivities. It appears that the neurons of the AMPG conform to the proposal that neurons are chemically coded by virtue of peptide and non-peptide putative transmitter combinations within their perikarya and by their relationship to preganglionic/intraganglionic nerve fibre varicosities (Morris & Gibbins, 1987). It is likely that the neurons of the AMPG can be subclassified on the basis of their neuropeptide content as can the submucous ganglia of the small intestine and the coeliac ganglion of the guinea-pig (Furness *et al.* 1984; Macrae *et al.* 1986). For the notion of chemically coded neurons to be meaningful it must ultimately be linked to the distribution of their axons to specific target organs (Keast & DeGroat, 1989; Keast *et al.* 1989).

The many classes of neurons exhibiting patterns of coexistence also receiving neuropeptide and amine (classical transmitter) encoded nerve varicosities present a new level of complexity to the innervation of pelvic viscera and vasculature.

SUMMARY

The anterior major pelvic ganglion (AMPG) of the male guinea-pig has been found to consist of three principal components. The presence of a cholinergic component was determined by the demonstration of cytoplasmic and nerve fibre acetylcholinesterase activity. A noradrenergic component was demonstrated by immunoreactivity (IR) of

the catecholamine-synthesising enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) in neuronal perikarya. The AMPG also had a peptidergic component which may or may not sub-classify the cholinergic and noradrenergic components. Neuropeptide Y (NPY)-, vasoactive intestinal peptide (VIP)-, and atrial natriuretic factor (ANF)-immunoreactivities were seen in neuronal perikarya, nerve fibres and nerve terminals/varicosities, while somatostatin (SOM)-IR was restricted to neuronal perikarya. Substance P (SP)-IR was present in a dense network of varicose nerve fibres. However, on a rare occasion SP-IR was observed in neuronal perikarya. Enkephalin (ENK)-IR occurred in a sparsely distributed plexus of varicose nerve fibres.

The analysis of adjacent serial sections demonstrated distinct patterns of neuropeptide coexistence in AMPG neurons. NPY-IR was colocalised to a sub-population of TH-IR neuronal perikarya. NPY-IR was also colocalised with VIP-IR in non-TH-IR neuronal perikarya. VIP-IR occurred together with AChE in particular neuronal perikarya.

The relationship between immunoreactive neuronal perikarya and immunoreactive nerve terminals was investigated. SP-IR nerve terminals were closely related to neuronal perikarya exhibiting VIP-, NPY-, or TH-IR. TH-IR neuronal perikarya were also abutted by ENK-IR nerve terminals. VIP- and NPY-immunoreactive neuronal perikarya were abutted by two nerve terminal types: one immunoreactive for VIP, the other for NPY. DBH-IR neuronal perikarya received AChE-positive varicosities while AChE-positive neurons were abutted by DBH-IR varicose nerve fibres. AChE-positive varicosities were also closely related to neuronal perikarya possessing VIP-IR and AChE activity.

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