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Invited Review

Morphology and neurochemistry of the pelvic, and paracervical ganglia

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Summary. Autonomic ganglia are relays in the distribution of nerve fibres in the peripheral nervous system. In the pelvis there are local ganglion formations in the pathway of nerve fibres to and from pelvic viscera and vasculature: in rodents these are the male anterior major pelvic ganglion and the female paracervical ganglion (Frankenhäuser's ganglion). They are unusual in that they contain both sympathetic and parasympathetic ganglion cells. Homologous formations occur in humans. Since the best studied examples of these ganglionic formations are rodent ganglia the latter are reviewed in terms of their gross anatomy, cell morphology and immunohistochemistry. The synaptology, and neurotransmitter and neuropeptide contents of the neuronal perikarya and nerve terminals, of the ganglia are discussed in relation to the concepts of coexistence and chemical coding in autonomic ganglia in general. The neuropeptide content of the nerve fibres projecting to their visceral targets is described and discussed in functional terms. Conclusions are drawn with respect to the contributions made by study of these ganglia to further understanding of the organisation of the autonomic nervous system in general. The possible link between the autonomic nervous system and the endocrine system is discussed with respect to control of pelvic visceral activities.

Key words: Autonomic, Ganglia, Pelvic, Paracervical

Introduction

The organisation of the autonomic nervous system in the pelvis has hitherto been the subject of little detailed study. Classical texts describe the contributions made by sympathetic splanchnic nerves, from the paravertebral ganglia, from the prevertebral ganglia, and from the pelvic splanchnic nerves of the parasympathetic system. Formed from all these contributions is the prevertebral plexus which continues anterior to the sacrum in the pelvis and has been described as comprising the superior and inferior hypogastric plexa.

The regulation and modulation of the activities of the pelvic viscera, and vasculature are to an extent performed by local ganglion formations within the pelvis, part of the hypogastric plexa. Much attention has been focused recently on the pelvic ganglion in the male, and its homologue in the female, the paracervical ganglion, particularly in rodents, though similar ganglia occur in the hypogastric plexa of humans (Williams et al., 1989). The ganglia are unique in the autonomic nervous system, not just because they contain both noradrenergic neurons and cholinergic neurons, but because they seem to be affected by reproductive hormones, and they display sexual dimorphism.

This review will describe the gross anatomy, sexual dimorphism, cell morphology, neurotransmitter and neuropeptide content of the pelvic and paracervical ganglia. The chemical coding of neurons in the ganglia will be examined in relation to their specific targets. The review will attempt to show how study of these pelvic local ganglion formations provides both systematic and systemic information about the autonomic nervous system.

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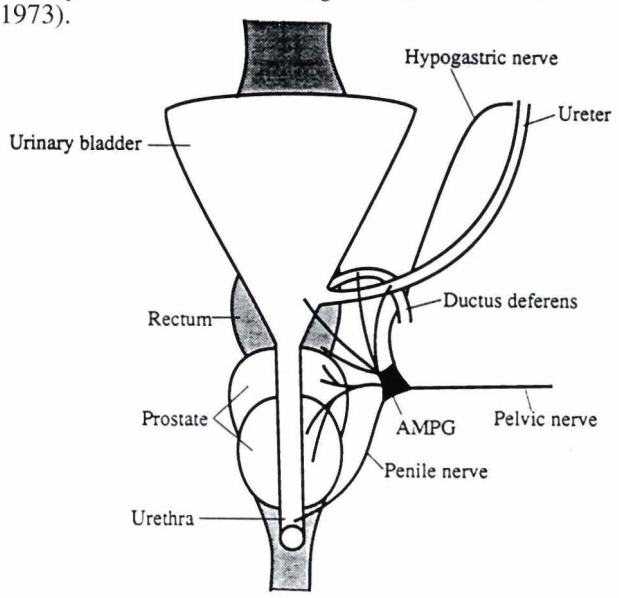
The pelvic ganglion of the male rodent

Gross anatomy

The major pelvic ganglion of a number of rodent species consists of ventro-cranial and dorso-caudal groups of neurons and their associated nerve fibres, collectively termed the pelvic plexus (Wozniak and Skowronska, 1967), though in rats only a single formation exists (Purinton et al., 1973). A variety of terms have been used to describe these autonomic formations. In general, the two groups of neurons are referred to as anterior and posterior pelvic ganglia (Costa

and Furness, 1973), though the former formation has been referred to as the terminal mesenteric ganglion (Wakade and Kirpekar, 1971), and the latter as the hypogastric ganglion (Watanabe, 1971). The anterior pelvic plexus contains the anterior major pelvic ganglion (AMPG) in the male and the paracervical ganglion (PCG) in the female which are the contemporarily accepted terms.

In guinea pigs, the AMPG consists of bilateral collections of neurons lying close to the ducti deferentes and the prostate (Fig. 1). The ganglia lie adherent to the lateral aspect of the proximal part of the seminal vesicles (Watanabe, 1971; Costa and Furness, 1973), and receive projections from, and send projections to, the inferior mesenteric ganglion (IMG) (not to be confused with the terminal mesenteric ganglion, *vide supra*). These projections form the hypogastric nerves. The paired hypogastric nerves arise from the inferior mesenteric ganglion and lie in the mesocolon of the terminal portion of the gastrointestinal tract. At the level of the bifurcation of the abdominal aorta each diverges to pass to the ipsilateral AMPG. The preganglionic fibres of the nerve originate from the first and second lumbar segments of the spinal cord, having passed through the IMG; post-ganglionic nerves arise in the IMG in guinea pigs (Aldskogius and Elfvin, 1987) and rats (Purinton et al., 1973). The AMPG also receives nerve fibres from the pelvic nerves in rats (Langworthy, 1965) and guinea pigs (Aldkogius and Elfvin, 1987). In rats at least, the pelvic nerves arise from the second to fourth sacral segments of the spinal cord (Purinton et al., 1973), mainly from the second segment (Costa and Furness, 1973).



AMPG = Anterior major pelvic ganglion

Fig. 1. Diagrammatic representation of the gross anatomical arrangement of the anterior major pelvic ganglion, and its inputs and some of the visceral targets with which it is associated. AMPG: anterior major pelvic ganglion.

Gross anatomical studies have indicated that nerve fibres from the AMPG supply the ductus deferens, seminal vesicles, urinary bladder, prostate, lower end of the ureter (Wakade and Kirpekar, 1971; Sjöstrand, 1965), and pelvic vasculature (Costa and Furness, 1973). The preganglionic axons in the hypogastric and pelvic nerves are mainly unmyelinated, of diameter between 0.5-2.5 μm, and make synaptic contact with the principal ganglion cells (the neuronal perikarya), which are postganglionic, in a convergent manner (Crowcroft and Szurszeweski, 1971).

In guinea pigs and rats, the posterior pelvic plexus is much smaller and consists of a small number (three or four) of small interconnected ganglia which project to the rectum and anal canal. The interconnections between the AMPG and its posterior neighbour pass on the lateral wall of the coagulating gland, and are part of the pelvic nerve.

Cell morphology

There are three major cell types in the guinea pig AMPG: neuronal perikarya, satellite cells and small intensely fluorescent (SIF) cells (Watanabe, 1971; Yokota and Burnstock, 1983). The neuronal perikarya are polyhedral cells ranging between 30-40 μm in diameter, and up to 150 μm^2 in area at the maximum diameter (Yokota and Burnstock, 1983). The large, ovoid nuclei are palely stained (in the electron microscope) and eccentrically placed structures, possessing 2 or 3 distinct nucleoli, and are sometimes polyploid. Approximately 70% of the neurons possess small, clear synaptic vesicles, but not large granular synaptic vesicles which allowed Yokota and Burnstock (1983) to identify them as cholinergic. The remaining 30% contained a variety of morphological types of synaptic vesicles and a large proportion could be categorised as containing large granular vesicles, possible identifying them as adrenergic. Noradrenaline-containing neurons were first demonstrated at the distal end of the hypogastric nerve in guinea pigs by Falck et al. (1965). The finding of cholinergic and noradrenergic neuronal elements in the guinea pig AMPG accorded with the work of Bell and McLean (1967).

The neurons of the AMPG are capable of extensive but reversible growth when their target organ undergoes hypertrophy. In experiments with rats where the urinary bladder outlet was obstructed it was found that there was an increase in the number of maximally sized neurons in the AMPG (Steers et al., 1990) which was reversed once the obstruction was relieved (Gabella et al., 1992). Both groups proposed that the effect might be attributable to trophic factors derived from the bladder itself, reinforcing the idea of target organs affecting their neuronal projections.

Whether cholinergic or noradrenergic, the synapses of the AMPG are of Type I morphology (Gray, 1961), and there is a relatively high proportion of axo-somatic synapses (21% Yokota and Burnstock, 1983), which

most likely is related to the relatively short dendritic spines of the neuronal perikarya: there are few axodendritic synapses in the AMPG and virtually no dendrodendritic synapses (Devine, 1967).

The satellite cells of the guinea pig AMPG surround the principal neuronal perikarya and SIF cells of the ganglion, *vide infra*. This cell type is characterised by a flattened cytoplasm which contains dense filaments arranged in parallel arrays (Watanabe, 1971) and by an elongated nucleus, typical of the arrangement in other autonomic ganglia.

The SIF cells in the AMPG occur as isolated clusters, occasionally singly, throughout the AMPG. Dail et al. (1975) classified the SIF cells of the guinea pig AMPG into two groups on the basis of their dense cored cytoplasmic vesicles. The predominant Type I SIF cells possessed large (150-200 nm in diameter) dense cored vesicles. Type II SIF cells possessed fewer and smaller dense cored vesicles (50-120 nm in diameter). In the rat, Watanabe (1971) classified the SIF cells of the AMPG into 4 types. Type 1 contained large granules (200-250 nm in diameter) with highly electron dense material, often eccentrically placed, and bound by a limiting membrane. Type 2 possessed smaller rounded synaptic vesicles (100-150 nm in diameter) containing electron dense material. Type 3 contained synaptic vesicles which are polymorphous and of high electron density. Type 4 synaptic vesicles were found to be large (200-250 nm in diameter) and of low electron density.

Neurotransmitters and neuropeptides

In guinea pigs, a noradrenergic category of neurons was demonstrated by the presence of immunoreactivity to the catecholamine-synthesising enzymes tyrosine hydroxylase (TH) and dopamine ß hydroxylase (DßH) in neuronal perikarya (Dhami and Mitchell, 1991). The presence of catecholamine-synthesising enzymes in the AMPG is not inconsistent with the notion that a proportion of the neuronal perikarya are noradrenergic, representing «short» noradrenergic neurons (Sjöstrand, 1965), as previously demonstrated by the formaldehyde induced fluorescence technique (Falck et al., 1965; Costa and Furness, 1973) in guinea pigs. It cannot be excluded, however, that other catecholamines may be present. Cholinergic postganglionic neurons and nerve fibres have been demonstrated in the guinea pig AMPG by virtue of their content of acetylcholinesterase (AChE) in the study of Dhami and Mitchell (1991), similar to the homologous ganglion of the rat (Dail et al., 1975).

Neuropeptides have been demonstrated in the AMPG by Schultzberg et al. (1983) who briefly outlined the neuropeptide content of the guinea pig AMPG, including enkephalin (ENK), substance P (SP), vasoactive intestinal peptide (VIP), bombesin (BOM), cholecystokinin (CCK) and avian polypeptide (AP) immunoreactivities in nerve fibres. A large number of AP and VIP immunoreactive neuronal perikarya were also observed. Wang et al. (1990) demonstrated the presence of methionine ENK in the rat AMPG in a few neuronal perikarya, and in SIF cells and nerve fibres. Immunoreactivity was reduced in the nerve fibres following transection of the hypogastric and/or pelvic nerves, and it was suggested, therefore, that some of the immunoreactivity in intact ganglia represented preganglionic nerve fibres, though some may have represented SIF cell terminations. Studies where dyes are injected into the wall of a viscus and are taken up by intramural nerve fibres and retrogradely transported to the neuronal perikarya in the ganglia indicated that the ENK positive principal neurons project to the urinary bladder. ENK may be responsible for the inhibition of smooth muscle, as has been shown in the cat urinary bladder (de Groat et al., 1983, 1987), and smooth muscle in preparations of human ductus deferens (Sternquivst et al., 1983). The mechanism by which ENK might affect cholinergic neurons must be by means other than the conventional synaptic one since Wang et al. (1990) mentioned that there was a lack of synaptic contact between ENKergic and presumptive cholinergic neurons in the AMPG of rats. This might imply a parasynaptic function for the ENK containing nerve terminals whereby neuropeptide reached its receptor by local diffusion. This parasynaptic mechanism may account for the action of many neuropeptides in many parts of the autonomic nervous system, and may not be unusual.

A more comprehensive immunohistochemical study of the guinea pig AMPG was undertaken by Dhami and Mitchell (1991). They demonstrated that the AMPG conformed to the general phenomenon of neurotransmitter and neuropeptide coexistence in autonomic ganglia. They demonstrated three different categories of neurally active substances, a cholinergic component, a noradrenergic component *vide supra*, and a neuropeptidergic component. Unusually, there was a very small component of SP-containing neuronal perikarya.

L-glutamate decarboxylase immunoreactivity was localised in the AMPG of rats by Karhula et al. (1988). This enzyme is involved in gamma amino butyric acid (GABA) synthesis, an inhibitory neurotransmitter. They found that the enzyme was not in the principal neurons, but in presumptive preganglionic nerve fibres, and in SIF cells. Intravenous infusion of GABA inhibits the contractions of rat urinary bladder induced by preganglionic nerve stimulation by inhibition of excitatory neurotransmission in the rat pelvic ganglia (Maggi et al., 1983, 1985). GABA inhibits the motility of guinea pig urinary bladder *in vitro* by blocking vesical ganglia (Taniyama and Tanaka, 1986). Keast (1992) detected nitric oxide synthetase in the rat AMPG; this enzyme catalyses the release of nitric oxide from arginine, and Keast suggested that it might play a role in the mechanism of penile erection. A similar finding of nitric oxide synthetase in neurons of the rat AMPG which project to the urinary bladder prompted McNeill et al. (1992) to speculate that the resulting nitric oxide might play a part in maintenance of urinary bladder detrusor

muscle tone.

Regionalisation

In the study of the guinea pig AMPG by Dhami and Mitchell (1991) it was found that there was an element of regionalisation of TH-IR, such that groups of neuronal perikarya were positive whilst adjacent regions of the ganglia were negative for TH. This resembles the situation in the cat superior cervical ganglion (Jacobwitz and Woodward, 1968). It also accords with the studies of Keast and de Groat (1989) and Keast et al. (1989) who found that there was regionalisation of target-specific neurons in the rat AMPG. To what extent regionalisation occurs with other catecholamine synthesising enzymes, or with cholinergic neurons is unknown. Whilst regionalisation of neuropeptidergic neuronal perikarya was not described by Dhami and Mitchell (1991), it was clear that such neurons do exist in clusters, as well as singly. The existence of clusters of neuronal perikarya projecting to the same targets seems reasonable even on the simple grounds that in being in a common location it is efficient for the inputs and outputs of the ganglion. Furthermore, there must be a similar convenience for incoming modulatory fibres which can affect clusters of adjacent neurons which have the same target. Further discussion of potential targets of the AMPG follows later.

Coexistence of neurotransmitters and neuropeptides

In the main the findings of Dhami and Mitchell (1991) confirmed the study of Schultzberg et al. (1983), though the former workers additionally demonstrated the presence of somatostatin (SOM), and atrial natriuretic factor (ANF) in neuronal perikarya; the latter was also detected in nerve terminals and nerve fibres. Analysis of serial sections by Dhami and Mitchell (1991) demonstrated distinct patterns of neuropeptide coexistence in AMPG neurons, and these workers discussed the possible roles for such neuropeptide and their specific combinations. Generally, those neuronal perikarya immunoreactive for DßH also exhibited TH-IR, which would accord with their identity as noradrenergic, though there were exceptions. For example, there were some TH-IR negative, DßH positive neurons which were suggested to be cholinergic neurons that contained an inactive form of DßH, as seen in the guinea pig paracervical ganglion (Morris and Gibbins, 1987). TH was frequently colocalised in neurons with neuropeptide Y (NPY), a finding frequently observed in the autonomic nervous system, and which further supports the identification of these neurons as noradrenergic. NPY-IR was also colocalised with VIP-IR in non-TH-IR neuronal perikarya. It was suggested (Dhami and Mitchell, 1991) that NPY might play a role in vasoconstriction in the targets of the AMPG. Dhami and Mitchell (1991) also postulated a neuromodulatory involvement of NPY in the conservation of neurotransmitter in the AMPG, such as suggested by Stjärne et al. (1986) for NPY-containing sympathetic nerves supplying the mouse ductus deferens. VIP-IR neuronal perikarya also exhibited AChE reactivity in some instances. The presence of VIP in cholinergic neurons has been reported in many different neuronal populations, and is not, therefore, unexpected: it may imply a modulatory role for the neuropeptide in such neurons. Dhami and Mitchell (1991) referred to a possible role in the induction of contractility of the smooth muscle of the urinary bladder and the ductus deferens. The coexistence of NPY and VIP was surprising in the light of the reported contrasting functions (VIP vasodilates, whereas NPY vasoconstricts). It was suggested that one of these neuropeptides acted as a neuromodulator in this situation, and drew analogy with other systems where NPY enhanced VIP release, citing as an example nerves in the thyroid ganglion (Grunditz et al., 1988). In rats, Keast (1991) provided similar evidence of patterns of coexistence in neuropeptides and nerve fibre associations *(vide infra)*.

Neuronal coding

The concepts of coexistence (Hökfelt et al., 1987) and of chemical coding (Gibbins et al., 1987) have been explored by Dhami and Mitchell (1991) in the AMPG. In their study, the relationship between immunoreactive neuronal perikarya and immunoreactive nerve terminals in the guinea pig AMPG revealed certain specific combinations. SP-IR nerve terminals were closely related to neuronal perikarya exhibiting VIP-IR, NPY-IR or TH-IR. SP-IR nerve terminals are typical features of guinea pig sympathetic ganglia (Dail et al., 1975), and may represent sensory nerve fibres, though Matthews and Cuello (1982) suggested that in the inferior mesenteric ganglion, SP terminals may represent collateral branches of sensory nerves thus representing a reflex loop from target organ to ganglion. TH-IR neuronal perikarya were also abutted by ENK-IR nerve terminals. ENK may influence ganglion neuronal activity and Kawatani et al. (1983) have demonstrated presynaptic inhibition of ACh release by ENK. ENK is known to be inhibitory to cholinergic neurons (Konishi et al., 1980). VIP-IR and NPY-IR neuronal perikarya were abutted by two nerve terminal types: one immunoreactive for VIP, the other for NPY. DßH-IR neuronal perikarya received AChE-positive varicosities, whilst AChE-positive neurons were abutted by DßH-IR varicose nerve fibres. AChE-positive varicosities were also closely related to neuronal perikarya possessing VIP-IR and AChE activity. The various relationships between neuropeptide-containing neuronal perikarya and neuropeptide-containing pre-/inter-ganglionic nerve fibres indicates a complexity of organisation in the ganglion which conforms to the generally accepted notion of chemical coding of neuronal projections in autonomic ganglia (Gibbins et al., 1987). It is clear that

the use of chemical coding allows considerable precision in identifying specific neural pathways. It is also the case that the effects of stimulating a particular neuronal population projecting from a ganglion to a particular target are likely to be mediated by complex pluri-chemical processes, though the precise inter-relationships of the chemical milieux in nerve terminals are still not fully understood.

Keast (1991) examined the patterns of coexistence of peptides and different nerve fibre types associated with noradrenergic and non-noradrenergic (putative cholinergic) neurons in the AMPG in the rat. Keast's findings were very similar to those of Dhami and Mitchell (1991) in the guinea pig. Keast also detected galanin (GAL), but in small neurons and SIF cells, and in nerve fibres around GAL-negative neuronal perikarya. Keast (1991) also found ENK in a small proportion of neuronal perikarya which coexisted with VIP, NPY or GAL. Keast suggested that GAL stimulates the contraction of smooth muscle, and was a candidate for neurotransmitter status.

Substance P-containing neuronal perikarya

One of the principle features of the AMPG is the presence of a SP-containing nerve plexus which surrounds SP-negative neuronal perikarya. The origins of this plexus were investigated by Dhami and Mitchell (1992) in a series of decentralisation experiments. Selective nerve transections involving the principle inputs to the AMPG, the hypogastric and pelvic nerves, indicated that the SP-immunoreactive perineuronal plexus is derived from multiple sources: an extrinsic source involving both the hypogastric and pelvic nerves, and another source (possibly the projections of SIF cells). Dhami and Mitchell suggested that the normal absence of SP-IR in neuronal perikarya of the AMPG is the result of suppression, since after transection of the hypogastric nerve cytoplasmic immunoreactivity for SP can be demonstrated readily. It was further suggested that the active suppression might be attributable to neurotrophic factors derived from the prostate. Similar findings were made by Dail and Dziurzynski (1985) after colchicine treatment of the rat AMPG, where a small population of substance P-containing neuronal perikarya were demonstrated, though no explanation of this was offered.

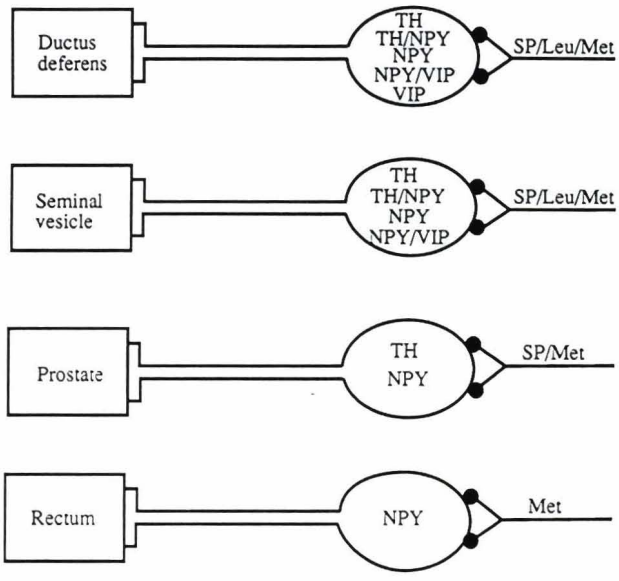


Fig. 2. Some of the targets of the postganglionic neuronal perikarya of the guinea pig AMPG with the immunoreactivities of the postganglionic neurons (ellipses), together with the immunoreactivities of associated preganglionic nerve fibres (from the work of Dhami and Mitchell, 1993). NPY: neuropeptide Y; TH: tyrosine hydroxylase; AADC: aromatic amino acid decarboxylase; DßH: dopamine beta hydroxylase; VIP: vasoactive intestinal peptide; SOM: somatostatin.

Neuronal targets

Dhami and Mitchell (1993) have investigated the targets of the neuronal projections of the guinea pig AMPG. Using retrograde tracer transport studies combined with immunohistochemistry the neuro-chemical identity of neuronal projections and their targets was established (see Fig. 2). They demonstrated projections from the ganglion to the ductus deferens, seminal vesicle, prostate and rectum, though not urinary bladder. In the case of the former three, viscera received neuronal projections from both ipsilateral and contra-lateral ganglia. Immunohistochemistry of serial sections of AMPG showed that neurons projecting to the ductus deferens and seminal vesicles exhibited either TH alone, TH and NPY, NPY alone, NPY and VIP, or VIP alone; the neurons projecting to the seminal vesicle did not contain any VIP-IR. VIP-containing nerve fibres have been demonstrated in the seminal vesicle by Lamano-Carvalho et al. (1986), which means that their origin is likely to be a source other than the AMPG. Three categories of neuronal projections were associated with these projecting neurons, namely SP, leucine and methionine ENK. Neurons whose targets were the prostate contained either TH or NPY, and were infrequently associated with the three categories of nerve terminal referred to above. Neurons projecting to the rectum contained only NPY, and in one instance, it was found that they were associated with methionine ENK-containing nerve terminals. Llewellyn-Smith (1989) listed a number of neuropeptide immunoreactivities within the gut wall; it is, therefore, curious that the rectum exhibited only NPY-IR. The absence of neuronal projections to the urinary bladder may be related to the findings of Gabella (1990) who described clusters of neuronal perikarya in the wall of the urinary bladder, which may obviate the need for AMPG neurons projecting to the urinary bladder. Dhami and Mitchell (1993) did suggest that a full examination of all regions of the guinea pig urinary bladder ought to be undertaken before any conclusions are drawn. In the rat, however, Keast et al. (1989) and Keast and de Groat (1989) have

demonstrated AMPG neuronal projections to the urinary bladder, and in the rat there are apparently no intramural urinary bladder ganglia (Sharkey et al., 1983).

Information about neuronal targets of autonomic ganglia is only useful, however, once a complete categorisation of the neuropeptide content of neurons has been ascertained.

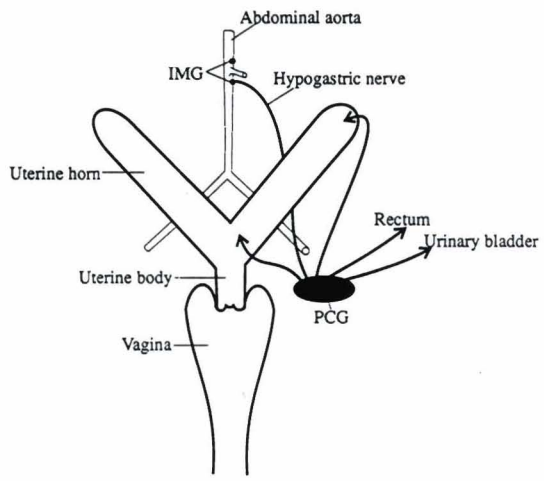
The paracervical ganglion of female rodents

Introduction

The sympathetic and parasympathetic nerves supplying the female genital organs converge at the paired paracervical ganglia which lie close to the uterine cervix (Fig. 3). In primates, Mitchell and Stauber (1990a) have given a brief description of the PCG in marmosets, and there are details of the homologous human ganglia (Williams et al., 1989), though most analyses have been carried out on rodents and in particular rats and guinea pigs. The ganglion was first described by Lee in 1841 (Knight, 1980), and later by Frankenhäuser (1867).

Gross anatomy

The studies of Langley and Anderson (1895) in the cat and rabbit were the first to describe the gross anatomy of the PCG. More recently, Baljet and Drukker (1980) provided gross anatomical evidence for the existence of anatomical connections between the PCG and its putative targets in the rat. In the study of the PCG of guinea pigs by Mitchell and Stauber (1990b) clusters of ganglion cells were detected at various levels in the paracervical tissues. There was variation in the number and size of ganglionic clusters and differing disposition of the clusters in different animals. In the study of the guinea pig PCG by Morris and Gibbins (1987) dissection revealed 6-10 ganglionic clusters around branches of the internal iliac artery consisting of a total mean number of nearly 1200 neuronal perikarya. This compares with 2200 perikarya in the study by Mitchell and Stauber (1990b). The use of serial sections in the latter study may have eliminated the possibility of missing clusters which could have occurred by using the dissection method. Compared to the study by Greenwood et al. (1965) who counted numbers of neuronal perikarya in the rat PCG *(vide infra)*, the numbers of neuronal perikarya counted by Mitchell and Stauber (1990b) seem quite low. Species differences may account for much of the range of neuronal numbers, but it is not clear whether Greenwood et al. included in their count the periureteric ganglia (which are contiguous with lower parts of the paracervical ganglion in guinea pigs at least (Mitchell et al., 1993b)), or the posterior pelvic plexus, because this would have increased the numbers significantly.



PCG = Paracervical ganglion
IMG = Inferior mesenteric ganglion

Fig. 3. Diagrammatic representation of the gross anatomical arrangement of the paracervical ganglion, and its inputs and some of the visceral targets with which it is associated. PCG: paracervical ganglion; IMG: inferior mesenteric ganglion.

Sexual dimorphism in the AMPG and PCG

Greenwood et al. (1965) showed that there was sexual dimorphism in terms of difference in neuron numbers in male and female rat pelvic/paracervical ganglion formation: male 14654 ± 936, female 5892 ± 797. Greenwood et al. suggested that this could be attributable to hormonal differences, and reflected a difference in the numbers of axons in the pelvic nerve. This most likely relates to the different viscera supplied in male and female pelves. It is generally agreed that development in mammals proceeds towards female characteristics unless fetal testicular hormones impose male characteristics early in development. Whether adult neuron numbers are achieved in females by excess proliferation followed by massive cell death, or in males simply by greater cell proliferation is not known. This difference in neuron numbers accords with the reported distribution of neurons in the hypogastric nerves of male and female guinea pigs (McLachlan, 1985). The role of hormones in influencing SIF cell development is well established (Kanerva and Hervonen, 1975), and the notion that hormones can affect neuronal function is appealing and has some substance (Bell, 1972). Indeed, studies on aging autonomic ganglia (Burnstock, 1990) have shown that oestrogen, but not progesterone, can influence noradrenergic neurons in arterial preparations (Dhall et al., 1988). Certainly, there are androgen sensitive periods in the development and organisation of the AMPG (Melvin and Hamill, 1989).

Cell morphology

In the main, the cell distribution and ultrastructure of the PCG in guinea pigs (Mitchell and Stauber,

1990b) are similar to the findings in rats (Kanerva and Teravainen, 1972), and are typical of autonomic ganglia.

Principal neurons

Amongst the conclusions of the work by Kanerva (1972) were that one third of the principal neurons (neuronal perikarya) of the ganglion in guinea pigs are noradrenergic, and two thirds are non-noradrenergic, of which one-fifth are cholinergic. The different categories are distinguishable after potassium permanganate fixation by virtue of their content of different sized synaptic vesicles. Vacuolated principal neurons in the guinea pig PCG were reported by Kanerva (1972), and they have been considered to be similar to the vacuolated neurons reported by Partenen et al. (1980) in the aging AMPG of the rat. In subsequent studies of the guinea pig PCG such cells have not been observed (Mitchell and Stauber, 1990b). Data from Kanerva concerning the ontogeny of the amine-containing neurons of the PCG in rats indicate that at the time of birth the principal neurons contain relatively low concentrations of catecholamines, but this steadily increases.

SIF cells

Using the formaldehyde induced fluorescence technique Eränkö and Eränkö (1971) demonstrated large numbers of SIF cells in the rat PCG. Kanerva (1972) found the abundant SIF cells of the guinea pig PCG were situated close to fenestrated capillaries, a site facilitating their putative paracrine role. The reaction for noradrenaline in guinea pig SIF cells, using the formaldehyde induced fluorescence technique, was more intense than that of the principal neurons (Baker et al., 1977). At the time of birth the content of catecholamine in SIF cells is in relatively high amounts (Kanerva, 1972). Mitotic SIF cells and degenerative SIF cells are encountered during the post natal period up to the 8 day stage. There seems to be a scarcity of SIF cells in the adult guinea pig PCG, compared to the adult rat ganglion (Kanerva and Teräväinen, 1972), which was confirmed by Mitchell and Stauber (1990b). It is well established that the numbers of such cells vary in the autonomic ganglia of different species (Böch, 1982; Taxi et al., 1983). Mitchell and Stauber (1990b) confirmed the findings of Morris and Gibbins (1987) in relation to the content of TH and DßH in the SIF cells in the guinea pig PCG, though they did not determine whether the two immunoreactivities coexisted in the same cells. They also demonstrated, for the first time, the presence of SP in this cell type.

Classical studies

The arrangement whereby sympathetic and parasympathetic preganglionic nerve fibres synapse in the same ganglion was first suspected by Langley and Anderson (1895) in a series of dissections and stimulation experiments. More compelling evidence was adduced from the work of Adham and Schenck (1969) who demonstrated the presence of cholinergic nerve fibres in the PCG of rats, following the discovery a little earlier of Sjöberg (1967) who confirmed the presence of noradrenergic nerves in the rat PCG in denervation experiments and formaldehyde induced fluorescence studies. These noradrenaline-containing nerves were categorised by Sjöberg (1967) as «short» noradrenergic nerves, to distinguish them from «long» noradrenergic nerves, and with which they share a number of pharmacological differences (Kanerva, 1971).

Kanerva (1972) found that the formation of synapses in the rat PCG parallels the increase in the AChE activity of the preganglionic nerve fibres and of the principal neurons. Few synapses to the principal neurons were detected, however, in the newborn rat. The cholinergic neurons of the PCG innervate the smooth muscle, glands and blood vessels of the uterus (Thorbert et al., 1977). Although Mitchell and Stauber (1990b) and Mitchell and Ahmed (1992) were able to demonstrate AChE activity in the guinea pig PCG the latter workers did not find any demonstrable activity in the uterine intra-mural innervation. This does not accord with the findings of Hammarström and Sjöstrand (1980), but is in agreement with the work of Thorbert et al. (1977). Reasons for the discrepancy are not clear, but technical differences could be a factor. Acetyl-cholinesterase-positive nerve fibres have been demonstrated in the rat uterus, irrespective of the stage of the oestrus cycle (personal observation).

The uterus receives its noradrenergic innervation by a variety of routes. «Long» noradrenergic nerves pass via the PCG, but do not synapse, to supply uterine blood vessels. These are thought to have arisen in prevertebral ganglia. «Short» noradrenergic nerves arise in the PCG, and supply uterine smooth muscle.

Morris and Gibbins (1987) found that less than 10% of principal neurons of guinea pig PCG were catecholaminergic. Another 5% contained DßH immunoreactivity, but no other catecholamine synthesising enzyme. The presence of aromatic acid decarboxylase (dopa decarboxylase) (AADC) in guinea pig neuronal perikarya in the PCG was reported by Mitchell and Ahmed (1992).

Neuropeptides

In rats, neuropeptide-like immunoreactivities detected in the PCG include VIP and NPY, as well as the catecholamine synthesising enzymes TH and DßH in principal neurons (Gu et al., 1984; Inyama et al., 1985; Papka et al., 1987), VIP, NPY, SP, calcitonin gene related peptide (CGRP), CKK, ENK, and ANF in nerve terminals (Papka et al., 1985, 1987) and NPY, CGRP and CCK in SIF cells (Papka et al., 1987). In pregnant rats, Kanerva (1972) found that there was an increase in catecholamine fluorescence, but not in AChE activity, of the principal neurons of the PCG in late pregnancy. In

guinea pigs, neuropeptides detected in PCG tissues included VIP, NPY, peptide histidine isoleucine (PHI), dynorphin (DYN), neurotensin (NT), SOM, TH, or DßH immunoreactivities in principal neurons, and TH, DßH, NPY, PHI, ENK, SP, SOM, CGRP, DYN, or VIP immunoreactivities in nerve fibres (Morris and Gibbins, 1987; Alm and Lundberg, 1988). The immunohistochemical studies on guinea pig PCG tissues of Mitchell and Stauber (1990b) have confirmed the results of Morris and Gibbins (1987) and Alm and Lundberg (1988).

The absence of ANF in the guinea pig PCG is in contrast to its localisation in the PCG of rats (Papka et al., 1985, 1987), though, at present, it is of unknown function in the PCG.

Combined immunohistochemical and dye retrograde transport studies enabled Papka (1990) to find nerve endings in the rat PCG containing CGRP which originated from dorsal root ganglia and projected to the uterine cervix. Further studies in the rat PCG by Papka and McNeill (1993) have led to the suggestion of the presence of a regulatory loop involving primary afferent nerve fibres (as identified by their content of SP, CGRP and neurokinin A (NKA)), principal neurons and effector end organs. Thus, PCG neurons may be affected not just by preganglionic nerve fibres but also by CGRP, SP and NKA released from the sensory endings. Although some synaptic connections were identified by Papka and McNeill (1993), a proportion of the release was classified as parasynaptic, a view which makes the pursuant regulation of autonomic functions an attractive alternative to conventional synaptology.

In rats, Papka et al. (1991) detected GAL in nerve fibres in the PCG. Retrograde transport studies indicated that these nerves arose in dorsal root ganglia and projected from the uterine cervix. Whether the GAL in these nerve fibres coexists with the CGRP described by Papka (1990) is not known. However, Papka et al. (1991) made the suggestion that there is the possibility of an axon reflex arc with collateral branches synapsing in the paracervical ganglion, much like the situation with the inferior mesenteric ganglion (Matthews and Cuello, 1982). Alternatively, the preganglionic nerve fibres could release their GAL to influence post-synaptic neurons. Indeed, GAL reportedly influences cholinergic activity in myenteric autonomic ganglia (Palmer et al., 1986). There is evidence for GAL as an inhibitory neuropeptide influencing myenteric cholinergic neurons in the guinea pig small intestine (Yau et al., 1986). It has also been found that GAL suppresses nicotinic synaptic transmission in the myenteric plexus of the guinea pig small intestine (Tamura et al., 1986). In addition, information is accumulating which suggests that the peripheral endings of most, if not all, sensory neurons have secretory properties, and that these sensory fibres provide synaptic collaterals to principal neurons in autonomic ganglia. This arrangement could form the basis for an axon reflex permitting sensory axon modulation of autonomic neuron activity (such as with SP and the IMG (Matthews and Cuello, 1982)), and has been described in the rat PCG by Papka and McNeill (1993).

Despite the foregoing discussion of the possibility of parasynaptic and collateral synaptic arrangements in the PCG, the majority of nerve terminals in the guinea pig PCG (Mitchell and Stauber, 1990b) appear to make axosomatic synapses with the neuronal perikarya of principal neurons. Similar findings have been made by Kanerva and Teräväinen (1972) in rats, and this accords with similar findings in the AMPG, *vide supra*. This unusual observation could indicate that the cells are not typical multipolar autonomic neurons and that they possess fewer cell processes than usual. Ultrastructural observations suggest that this may be so in the rat (Tabatabai et al., 1986) and mouse (Rogers et al., 1990). The latter group suggested that the ganglia might, therefore, function more as relays than in integrative roles.

Coexistence of neurotransmitters and neuropeptides

Morris and Gibbis (1987) carried out a comprehensive study of neuropeptide and neurotransmitter coexistence in the guinea pig PCG, and gave details of 11 classes of neurons with different combinations of neuropeptide immunoreactivities. They found that 90%-95% of neuronal perikarya in the PCG contained more than one neuropeptide. Morris and Gibbins (1987) found that the neurochemically different neuronal perikarya were associated with a large number of different combinations of neuropeptide-containing nerve fibres. It would be of interest to know whether this combination of expression of neuropeptides is immutable. Factors such as the influence of excitatory post-synaptic potentials and inhibitory post-synaptic potentials on individual neuronal perikarya, and hormones may modulate the rates of neuropeptides synthesis, or rates of neuropeptides storage and release. Different combinations of expressions may occur for a variety of regulatory functions. It may be that not all neurally active substances within a given nerve terminal are released at the same time. It would be of interest to determine the phenotypic neuropeptide expression *in vitro* to see whether it varied, or could be manipulated. Taken together with the association with different immunoreactivities of pre-ganglionic/intra-ganglionic nerve fibres the possibility for specific regulatory activity are considerable.

Recently, Mitchell and Stauber (1993a) utilised an electron microscope immunogold labelling technique to localise SP and leucine ENK in the same synaptic vesicles of the same nerve terminals in the guinea pig PCG. This observation opens up the intriguing possibility of a requirement for mechanisms to regulate differential release of the two different neuropeptides, and indicates the need for confirmatory electrophysiological studies. Together, both SP and ENK were detected in nerve terminals and varicosities comprised

mainly of large vesicles with electron dense cores. On its own, the former neuropeptide was detected in nerve terminals and varicosities comprised mainly of large vesicles with electron dense cores; the latter was detected in nerve terminals and varicosities, however, that also included small, clear synaptic vesicles. In a minority of nerve terminals or varicosities coexistence of both immunoreactivities could be demonstrated within vesicles with an electron dense core. Also present in these nerve terminals and varicosities were small, clear synaptic vesicles, though these were unreactive. The finding of such coexistence is in accord with the generally accepted phenomena as described by Hökfelt et al. (1987) and others. Similar findings have been made for the rat PCG by Papka et al. (1985) using immunogold methodology, though in this case the two neuropeptides involved were ENK and ANF. Moreover, the colocalisation did not determine whether the same synaptic vesicles were involved, merely the same terminals. The notion is consistent with the idea that patterns of neural connectivity are coded according to the particular target of the neuron(s) (Gibbins et al., 1987). Mitchell and Stauber (1993a) found that there were at least seven categories of nerve terminals coded by a combination of SP and/or ENK, considered in relation to the three morphological types of nerve terminal or varicosity observed. This categorisation of the PCG terminals is quite specific, since related studies on the IMG (Mitchell and Stauber, 1993b) and on the periureteric ganglia (Mitchell et al., 1993b) have resulted in very different neurochemical terminal profiles.

Classical studies of the autonomic nervous system have employed decentralisation experiments to determine the origin of preganglionic nerve fibres projecting to a ganglion. In the guinea pig PCG this is extremely difficult because of the diffuse nature of the ganglion. In rats, where the ganglion is reputedly a long cylindrical structure (Papka et al., 1985), parasympathetic decentralisation has been attempted by Van Orden et al. (1983) who found that such a manoeuvre had no effect on the rhythmicity of the oestrous cycle, whereas after ablation of the PCG cycling ceased. The implication of this finding is that although cholinergic nerves may not have a direct role, the ganglion is involved in the regulation of the oestrous cycle, and implies some interaction of the autonomic nervous system and the endocrine system, as discussed by Bell (1972).

Neuronal targets

Mitchell et al. (1993a) provided evidence of the identity of visceral targets of the neuronal perikarya of the guinea pig PCG by using a combined immunohistochemical/retrograde transport study. They injected either the uterine cervix, urinary bladder or rectum with the dye fast blue, and then serially sectioned the paracervical region, including the PCG. For each viscus there were dye-laden neuronal perikarya. They then reacted the sections with dye-laden neurons for TH, DßH, AADC, NPY or VIP immunoreactivities. All these neuronal perikarya exhibited the immuno-reactivities above, with the exception of the urinary bladder which did not express AADC-IR. Their findings provided direct evidence for the assertion that the PCG projects to pelvic viscera other than the uterus (Mitchell, 1988), despite its close association with the latter. Previously, Alm and Lundberg (1988) used similar retrograde tracer experiments to show that the PCG does indeed project to the uterus. Some of the non-nor-adrenergic neurons of the PCG, with immuno-reactivities to DßH, DYN, NPY or VIP, project to the uterine artery (Morris et al., 1985, 1986). In contrast, neurons exhibiting immunoreactivity to SOM alone, or in combination with TH, do not project to the uterine artery, but to the uterine horn (unpublished observations, cited by Morris and Gibbins, 1987). In the

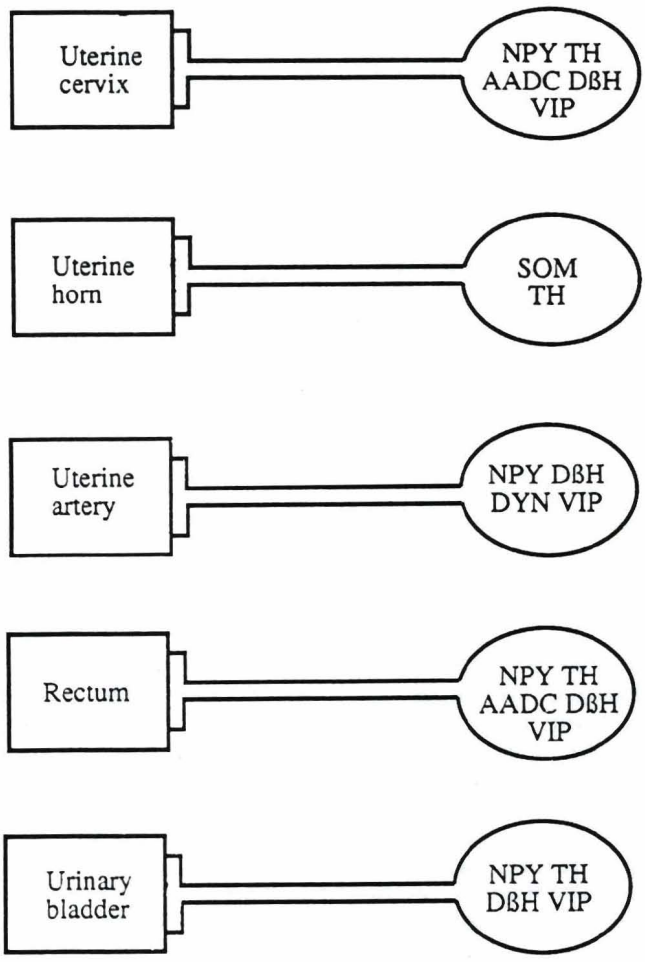


Fig. 4. This figure shows some of the targets of the postganglionic neuronal perikarya of the guinea pig PCG with the immunoreactivities of the postganglionic neurons, together with the immunoreactivities of associated preganglionic nerve fibres (from the work of Morris et al., (1985, 1986) and Morris and Gibbins (1987), and Mitchell et al. (1993a). TH: tyrosine hydroxylase; NPY: neuropeptide Y; VIP: vasoactive intestinal peptide; SP: substance P; LEU: leucine enkephalin; Met: methionine enkephalin.

rat, similar studies have shown that the PCG is the source of the VIP-IR nerve fibres in the uterus (Gu et al., 1984). Traurig et al. (1985) used immunohistochemical and retrograde transport studies to demonstrate NPY-containing neuronal perikarya in the rat PCG whose axons had originated from the reproductive organs, though they were not specific about which. The results of the work by Morris et al. (1985, 1986) and Morris and Gibbins (1987) and Mitchell et al. (1993a), on the guinea pig, have been summarised in Figure 4.

Conclusions

In conclusion, the ganglion formations described in the pelvis of rodents are suggested to play relay, rather than integrative roles in pelvic autonomic control and have been discussed in relation to the neurochemical content of the neuronal populations in the ganglion. Information has been presented which details the neurochemical coding of the neurons in the ganglion by pre- and intra-ganglionic nerve fibres and their terminals. Details of the targets of the neurons of both the AMPG and the PCG have been described, though a comprehensive analysis of the neurochemical coding and targeting of the neuronal populations of both ganglia is still awaited. Nevertheless, there is now a considerable understanding of the principles of pelvic innervation, and the role of the AMPG and PCG.

Studies of the pelvic and paracervical ganglia have demonstrated that these ganglia are organised in a very similar way to other autonomic ganglia, despite the obvious sexual dimorphism, and relay roles, though there are obvious differences in details of targeting, and associated neurochemical coding. Thus the pelvic and paracervical ganglia are ideal model systems for functional studies of the autonomic nervous system in general. Evidence is accumulating that neuropeptide products of nerve terminals in the autonomic nervous system may be released in a paracrine fashion, in contrast to the conventional manner, and this indicates a further degree of complexity to ganglion organisation and function. The interaction of the endocrine system and the autonomic nervous system is an additional factor adding further sophistication to autonomic control processes.

Despite much progress in demonstrating neuropeptide presence in the AMPG and PCG complete understanding of the functioning of the ganglia cannot be made until a full definition of the complete neuropeptide and neurotransmitter content has been obtained. Future studies in which electrophysiological investigations are coupled with quantitation of neuropeptide localisation would seem a fruitful avenue for further progress. Studies of the nervous system which deal with the mechanisms of differentiation resulting in the sexual dimorphism displayed by the AMPG and PCG should lead to further understanding of neuronal differentiation and its relationship with the developing endocrine system.

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