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REGULAR ARTICLE

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Immunohistochemical characterisation of pelvic autonomic ganglia in male mice

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Abstract Pelvic ganglia are mixed sympathetic-parasympathetic ganglia and provide the majority of the autonomic innervation to the urogenital organs. Here we describe the structural and histochemical features of the major pelvic ganglion in the male mouse and compare two different mouse strains. The basic structural features of the ganglion are similar to those in the male rat. Almost all pelvic ganglion cells are monopolar and most are cholinergic. All contain either neuropeptide Y (NPY) or vasoactive intestinal peptide (VIP), or both peptides together. The peptide coexistence varies between strains, with C57BL/6 mice having similar proportions of neurons with NPY alone, VIP alone or both peptides. In contrast, virtually all pelvic neurons in the Quackenbush-Swiss (QS) strain express NPY, i.e. the level of VIP/NPY coexistence is much higher. Cholinergic axons provide the major nerve supply to epithelia of reproductive organs, bladder smooth muscle and, as described previously, penile erectile tissue. They also provide a minor component of the smooth muscle innervation of the prostate gland, seminal vesicles and vas deferens. Virtually all non-cholinergic pelvic ganglion cells are noradrenergic and contain NPY. Their major target is smooth muscle of reproductive organs. This study shows that the male mouse pelvic ganglion bears many similarities to that in the rat, but that VIP/NPY colocalisation is much more common in the mouse. We also show that there are differences in peptide expression in parasympathetic pelvic neurons between strains of mice. These studies provide the framework for future investigations on neural regulation of urogenital function, particularly in transgenic and knockout models.

Keywords Pelvic plexus · Bladder · Reproductive tract · Sympathetic · Parasympathetic · Mouse (C57BL/6; QS)

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Introduction

Pelvic ganglia provide the majority of the autonomic innervation to the lower urinary tract and reproductive organs, and a substantial part of the extrinsic motor innervation of the lower bowel (Keast 1999). These are mixed autonomic ganglia, containing both sympathetic and parasympathetic neurons that receive synaptic inputs from preganglionic neurons located in the lumbar and sacral cord, respectively (Keast 1995a). There is considerable heterogeneity between species in the organisation and neurochemical properties of pelvic ganglion cells and their spinal inputs (reviewed in Keast 1995b, 1999). At a macroscopic level, one end of the extreme is the complicated plexus of numerous interconnected pelvic ganglia, as seen in humans, cats, dogs and rabbits. The simplest case is the rat, where the same functional classes of neurons are clustered in two large major pelvic ganglia and a small adjacent clump of accessory ganglia. An intermediate situation is seen in the guinea-pig, where there are two separate plexuses, posterior and anterior, comprising neurons innervating different pelvic organs. These levels of complexity are broadly reflected at a cellular level, with multidendritic neurons common in pelvic ganglia of most species but rare in rats. Immunohistochemical analysis of chemical coding in pelvic ganglia has been performed for few species, but also shows interspecies and gender variability.

The species in which pelvic ganglia have been best defined neuroanatomically is the rat, particularly the

male (Purinton et al. 1973; Dail et al. 1975, 1983; Dail 1976). Here, the transmitter profile of ganglion cells and their preganglionic inputs have been defined for neurons supplying each of the major visceral targets (Keast and de Groat 1989; Keast 1992, 1995a; Keast et al. 1995; Kepper and Keast 1995, 1997; Luckensmeyer and Keast 1995). This forms a valuable adjunct to physiological studies on pelvic reflexes (e.g. micturition, penile erection) and changes in pelvic function after trauma or injury (Steers et al. 1988, 1990; Kihara et al. 1996; Kihara and de Groat 1997).

The primary aim of this study was to describe the fundamental structural and chemical properties of pelvic ganglion cells and their axonal projections in male mice. This information is important for consolidating our current understanding of pelvic ganglia in different species. In particular, mice are being used increasingly in biomedical research because of the relative ease of generating transgenic and knockout animals. A thorough description of the pelvic circuitry in mice is an essential prelude to detecting effects of different genetic manipulations. A second aim of our study was to make a comparison of two mouse strains, including one that is commonly used in transgenic or knockout studies, the C57BL/6 strain.

Materials and methods

Tissue removal and immunohistochemical processing

All animal procedures were approved by the Animal Experimentation and Ethics Committee of the University of Queensland and the University of New South Wales. A total of 14 male mice were used for these experiments. Studies of pelvic organ innervation and chemistry of pelvic ganglion neuron somata were performed on both inbred C57BL/6 and outbred Quackenbush-Swiss (QS) mice. Studies on soma size in different chemical classes of neurons were only performed on QS mice. Tissues were removed from animals (7–8 weeks of age, 32–45 g), anaesthetised with ketamine (80 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.), then killed by exsanguination or cervical dislocation after tissue removal. Pelvic ganglia or organs (seminal vesicles, vas deferens, ventral lobes of prostate gland, urinary bladder) were removed and fixed overnight in cold Zamboni's solution (0.15% picric acid, 2.5% formaldehyde in 0.1 M phosphate buffer, pH 7.0). Fixative was removed by brief washes in dimethylsulphoxide, followed by phosphate-buffered saline (PBS, 0.1 M, pH 7.2). Ganglia from one side of three animals were stained and viewed as whole-mounts (see below) and the remainder were blocked in an inert mounting medium (OCT, Sakura Finetek; Torrance, CA) after equilibrating in PBS containing 30% sucrose, then cryosectioned (10 µm sections). Sections were collected on chrome-alum subbed slides, and dispersed amongst groups of three slides so that adjacent sections were not placed on the same slide and double-counting of somata avoided. Ganglia were sectioned through their entire thickness, whereas a minimum of ten non-consecutive sections per organ were examined for each substance or combination.

Ganglion and organ sections were stained with combinations of antisera, selected to identify the key substances found in pelvic ganglion cells in rats (Keast 1991, 1995a; Keast et al. 1995). Noradrenergic neurons were identified by labelling for tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) and cholinergic neurons by choline acetyltransferase (ChAT). Peptide-containing neurons were labelled for vasoactive intestinal peptide (VIP) or neuropeptide Y (NPY) as these are commonly expressed in parasympathetic and sympathetic neurons, respectively (Morris and Gibbins 1992). Sections or whole ganglia were incubated in a dark, humid chamber at room temperature with primary antisera (18–24 h), either alone or in combination; whole ganglia were first incubated for 45 min in PBS containing 0.5% Triton X-100 and 10% horse serum. Antisera were diluted with hypertonic PBS to the working dilutions shown (Table 1). After washing in PBS, secondary antisera were applied for ~2–4 h (Table 2). Binding of biotin-conjugated secondary antisera was visualised using streptavidin-Cy3 (Sigma, 1:2,000). Sections were coverslipped with bicarbonate-buffered glycerol (0.5 M, pH 8.6).

Analysis of somata and axons

For this study the proportional representation of each chemical class of neuron was important, rather than the absolute numbers of neurons of each type in the ganglion. The proportion of neurons stained with each antibody was determined by counting the total number of labelled neuron profiles cut through the nucleus and the total number of such profiles (determined using the broad UV filter cube, to visualise autofluorescence). For double-labelled preparations, the proportion of single- and double-stained neurons was determined, again restricting the analysis to nucleated profiles. For analysis of soma size for each of the main chemical classes of neurons, digital images were obtained using a CCD camera (Videoscope International, Model 200E, Herndon, VA) and LG-3 frame grabber (Scion Image Corporation, Frederick, MD). Soma profile areas of immunostained neurons sectioned through the nucleus were determined using NIH-Image (Version 1.6), manually outlining soma profiles on the computer screen.

Samples of each pelvic organ were cryosectioned (14 µm) and immunostained for combinations of two or three of the major markers of somata, to identify the key locations of noradrenergic and cholinergic axons. ChAT immunostaining of terminals within organs was much dimmer than staining of ganglion somata so was not used to identify cholinergic axons. These were instead identi-

Table 1 Primary antisera

Antigen	Host	Source	Working dilution
Choline acetyltransferase	Rabbit	Gift, M. Schemann	1:5,000
	Goat	Chemicon (Temecula, CA)	1:500
Dopamine-β-hydroxylase	Rabbit	Gift, R. Rush	1:400
Neuropeptide Y	Sheep	Auspep (Melbourne, Australia)	1:500
Tyrosine hydroxylase	Rabbit	Chemicon	1:250
	Mouse	Incstar (Stillwater, MN)	1:2,000
Vasoactive intestinal peptide	Rabbit	Incstar	1:2,000
	Guinea-pig	Eurodiagnostica (Arnhem, The Netherlands)	1:500
	Sheep	Accurate Chemicals (Westbury, NY)	1:1,000

Table 2 Secondary antisera

Antigen	Host	Conjugate	Source	Working dilution
Goat IgG	Horse	Biotin	Vector Laboratories (Burlingame, CA)	1:200
Guinea-pig IgG	Donkey	FITC	Jackson Labs (West Grove, PA)	1:100
Mouse IgG	Donkey	Cy3	Jackson Labs	1:1,000
Rabbit IgG	Donkey	Cy3	Jackson Labs	1:1,000
Rabbit IgG	Donkey	FITC	Jackson Labs	1:50
Sheep IgG	Donkey	FITC	Jackson Labs	1:50

fied by the presence of peptides (VIP or NPY) and the absence of TH. A minimum of eight sections from each organ, each section separated by at least 56 µm, were analysed for each antibody combination.

Digital images were produced from either conventional 35-mm film (digitised with a Nikon LS-1000 film scanner) or directly with a SPOT cooled CCD camera (RT Slider model, Diagnostic Instruments Inc.). Figures were produced in Adobe Photoshop, altering contrast and brightness to best resemble the immunostaining seen under the microsope.

Results

General organisation of the pelvic ganglia

The paired major pelvic ganglia in male mice resemble those of male rats (Purinton et al. 1973), in general shape, orientation and connections (Fig. 1). This did not vary between mouse strains. However, between individual mice there were some more minor variations, as indicated below. The major pelvic ganglia (for brevity referred to as pelvic ganglia) were located close to the dorsal surface of the prostate gland. They were roughly triangular in shape, with the base of the triangle being the dorsolateral edge. The hypogastric nerve could be followed from the inferior mesenteric ganglion to the rostral edge of the major pelvic ganglion. There was considerable variability between animals and also between sides of the same animal as to the site of entry of the hypogastric nerve. While it was consistently located at the rostral edge of the ganglia, in some ganglia its entry was very close to the pelvic nerve, while in others it was closer to the accessory nerves. Bundles of axons together comprising the pelvic nerve entered the dorsolateral edge. The penile (cavernous) nerve left the ganglion nearby, slightly more caudal than the pelvic nerve. The penile nerve was very thick where it joined the ganglion, but tapered after leaving the ganglion. In rats this is due to the presence of many neuronal somata in the proximal region of the penile nerve (Dail et al. 1975, 1983).This also seemed to be the case in mice. Very fine bundles of axons emanated from the ganglion at various points between the hypogastric and penile nerves, presumably supplying the various urogenital organs, as they are in the male rat (Purinton et al. 1973). The largest bundles of these emanated from the ventral edge of the ganglion and resembled the accessory nerves in rats. However, smaller branches on each side of these seemed to vary in thickness and exact location between ganglia. In some ganglia, tiny swellings could be seen along some of the bundles leaving the most ventral edge, and these contain ganglion cells, presumably equivalent to the accessory pelvic ganglia in a similar location in rats.

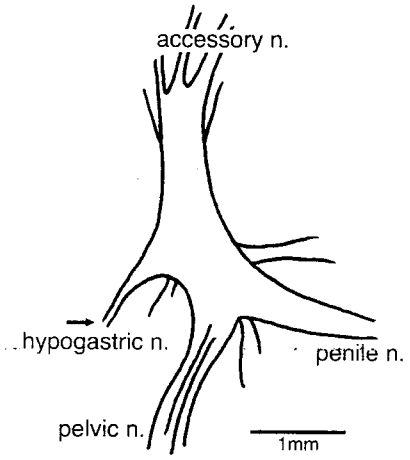


Fig. 1 Structure of the mouse major pelvic ganglion. The main features of a ganglion from the right side of an adult male mouse are shown. *Top of diagram* represents ventral and towards the midline, and the penile nerve projects caudally. The pelvic nerve consists of a number of separate nerve bundles. The number of accessory nerves varies between ganglia and between animals, as does the location of hypogastric nerve, although it consistently enters the rostral edge of the ganglia. The shape of the body of the ganglion also varies somewhat between ganglia, in some being very elongated and in others more rounded

Major chemical classes of neurons

The structural features of immunolabelled neurons and their staining properties did not appear to differ between strains, although there were some differences in the proportions of neurons in each main chemical class (Table 3). Almost all of the neurons appeared to be monopolar (Fig. 2a–d), as also seen in male rat pelvic ganglia (Dail et al. 1975; Tabatabai et al. 1986; Keast et al. 1989).

Tyrosine hydroxylase (TH) was expressed by many neurons, located in clumps and singly throughout the ganglion (Fig. 2a–d). These comprised, about one-third

Table 3 Comparison of chemical classes of pelvic neurons between two mouse strains. Data represent proportions of all pelvic ganglion cells that fall into each chemical class

Chemical class	Quackenbush-Swiss (n=4)		C57BL/6 (n=2)	
	Mean ± SE (%)	Range	Mean ± SE	Range
TH (total)	31±3	23–36	34±4	30–37
NPY (total)	93±1	88–92	67±7	60–73
VIP (total)	55±4	47–64	43±3	40–45
TH/NPY	31±3	23–36	33±3	30–37
TH/VIP	<1	0–0.2	<1	0–0.3
TH/–	0	0	<1	0–0.3
NPY/VIP	50±4	43–60	24±6	18–30
NPY/–	13±4	9–24	18±1	18–19
VIP/–	5±1	4–6	25±2	22–27

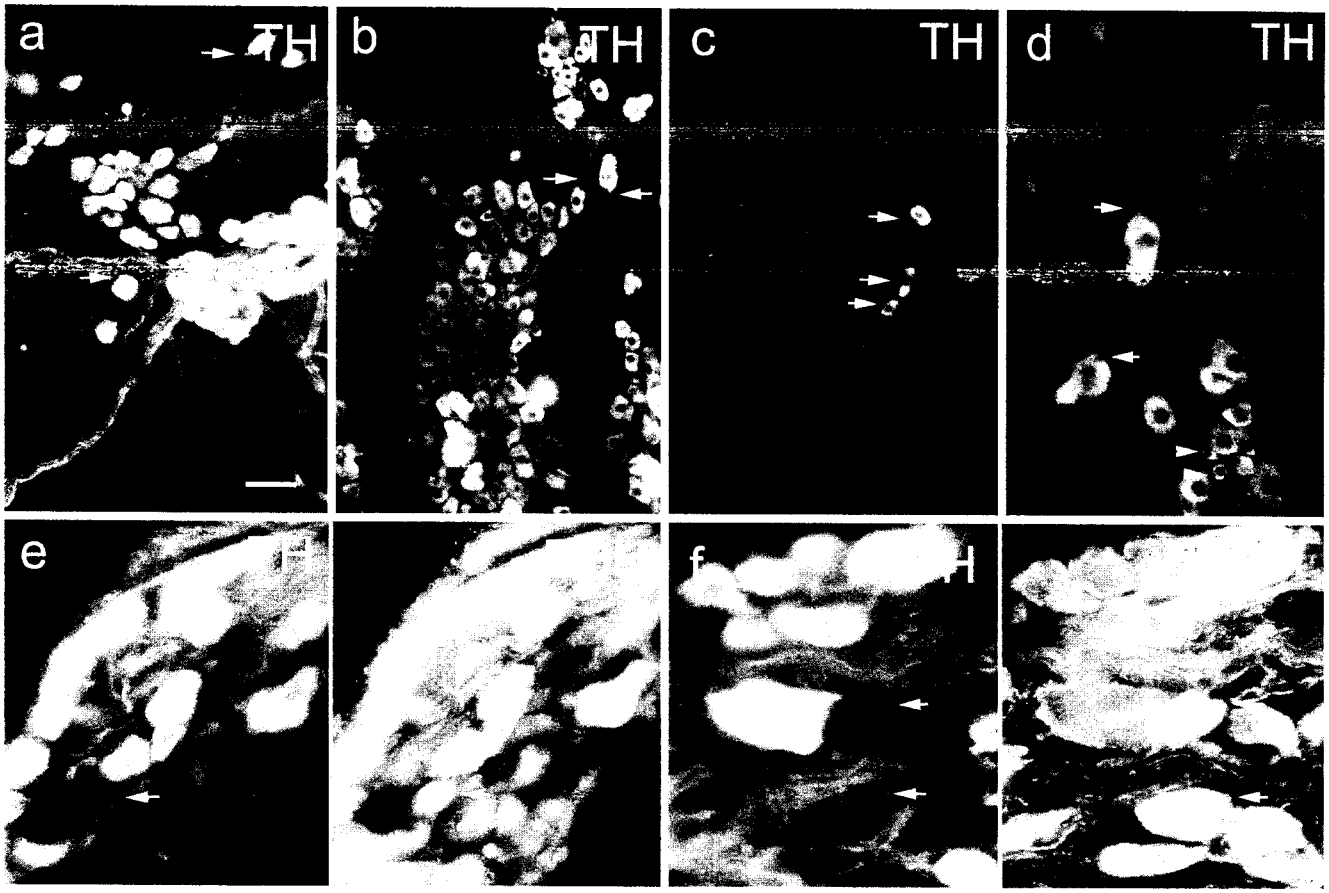


Fig. 2a–f Comparison of noradrenergic and cholinergic pelvic ganglion cells. **a–d** are single-stained whole-mount preparations, whereas **e** and **f** are double-stained cryosections. **a, e, f** QS, **b–d** C57BL/6. **a, b** Clusters of TH neurons. In many cases the axons can be traced to their cell bodies of origin (examples shown by *arrows*). **c** Small group of three positively stained TH neurons, each of which has an axon that crosses the ganglion. **d** TH neurons shown at higher magnification (sites of axons emerging shown with *arrows*). Some neurons also show small fine projections emanating from the soma (*top arrow*). SIF cells are present in this region (*arrowheads*). **e** TH/DBH double-staining. There is almost complete coexistence of the two enzymes, although there are rare examples of neurons that are DBH-positive but with no TH (*arrows*). **f** TH/ChAT double-staining. Cholinergic and noradrenergic neurons form two separate populations. All neurons are heavily innervated by cholinergic terminals, some of which lie out of the focal plane and give all neurons a "hazy" appearance. Examples of cholinergic neurons indicated by *arrows*. *Bar in* **a** represents 40 μm (**a–c**), 30 μm (**d**), 15 μm (**e, f**)

of neurons in the ganglion and this proportion was consistent between strains (Table 3). Some of the TH somata possessed very small projections, just a few micrometres in length (Fig. 2d), as also seen in rat pelvic ganglia (Dail et al. 1975). TH-positive axons could be seen emanating from individual neurons and in bundles exiting the ganglion, presumably projecting to the pelvic viscera. Numerous TH axons were present in the hypogas-

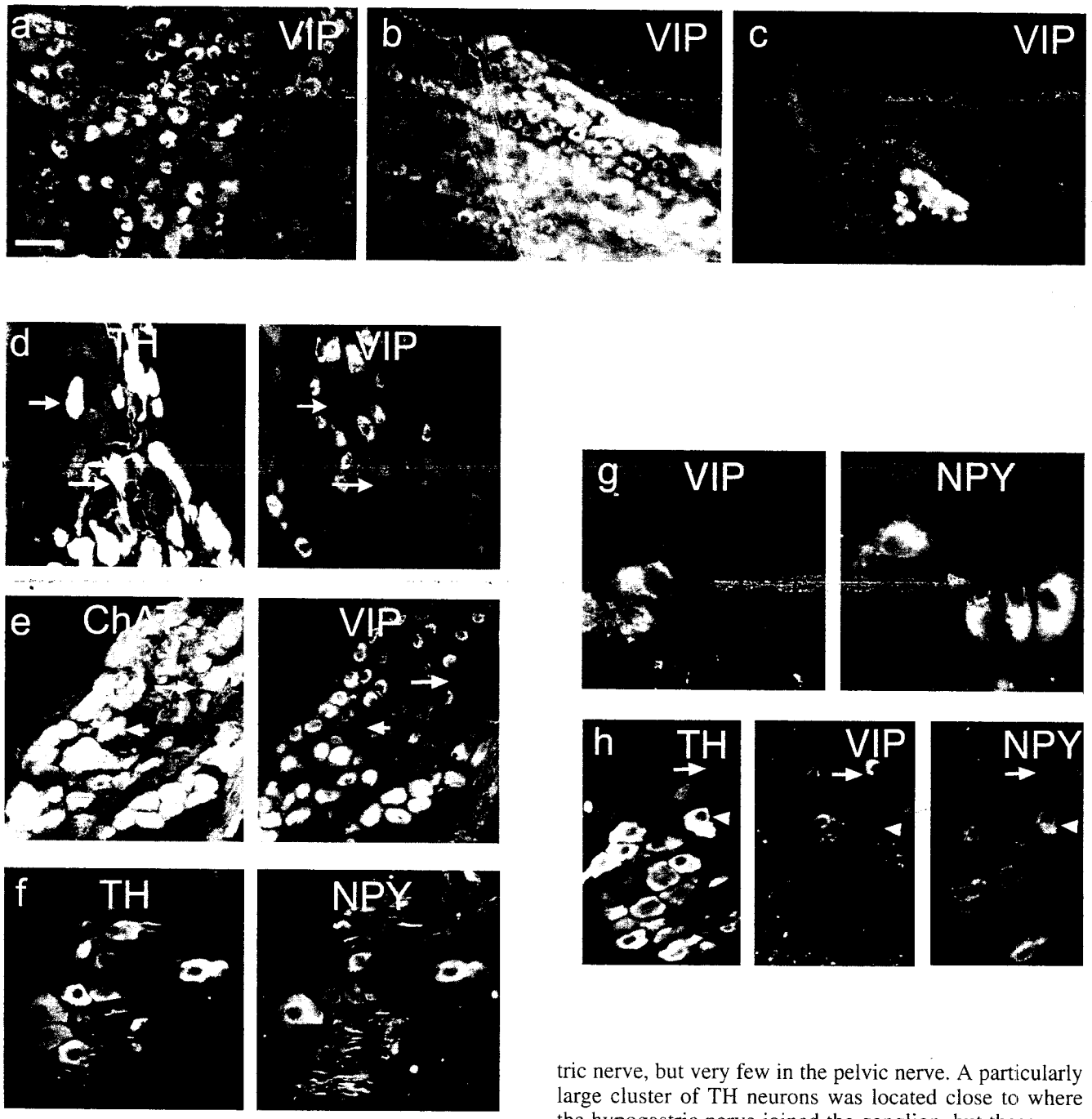


Fig. 3 Peptide distribution in noradrenergic and cholinergic pelvic ganglion neurons. **a–c** are of single-stained whole-mount preparations and **d–g** are double-stained and **h** triple-stained cryosections. **a–e** QS, **f–h** C57BL/6. **a–c** VIP neurons scattered randomly throughout the body of the ganglion(**a**), but clustered in the proximal part of the penile nerve(**b**). Further distally along the penile nerve, small clumps of VIP neurons are occasionally seen(**c**). **d** VIP neurons and TH neurons form two completely separate populations (*arrows* show TH-positive, VIP-negative neurons). **e** All VIP neurons are cholinergic (*ChAT*), but some cholinergic neurons do not contain VIP (*arrows*). **f** All noradrenergic neurons (*TH*) contain NPY. This particular field does not contain any TH-negative NPY neurons. **g** A group of neurons where there is no VIP/NPY coexistence. **h** A region containing both cholinergic and noradrenergic neurons. *Arrow* shows example of cholinergic VIP neuron and *arrowhead* a noradrenergic NPY neuron. *Bar in* **a** represents 40 μm (**a–c**), 30 μm (**d–f, h**), 15 μm (**g**)

tric nerve, but very few in the pelvic nerve. A particularly large cluster of TH neurons was located close to where the hypogastric nerve joined the ganglion, but these neurons did not appear to project into the hypogastric nerve. The TH axons in the hypogastric nerve are therefore likely to originate from other sympathetic ganglia (e.g. the inferior mesenteric ganglion) and project to the pelvic viscera via the pelvic ganglia. TH neurons were scarce or absent in the penile nerve. Brightly stained TH-positive non-neuronal cells occurred in some areas of the ganglion (Fig. 2d). These were substantially smaller than neurons (i.e. 83±4 μm^2, n =36 cells) and resembled small, intensely fluorescent (SIF) cells observed in many other autonomic ganglia (Baluk 1995). They did not contain any of the other neuronal markers examined below. Some of these cells had short projections, sometimes but not always directed towards other SIF-like cells.

We also tested if TH could be used as a marker for noradrenergic neurons in mouse pelvic ganglia by performing TH/DBH double-labelling (Fig. 2e). There was almost complete coexistence between the two enzymes, although very rarely DBH-positive neurons were found that did not contain TH, as described previously in female guinea-pig pelvic ganglia (Morris and Gibbins 1987). Because the DBH antibody caused slightly higher "background" (non-specific) staining, we performed all future studies on noradrenergic neurons using TH.

Neurons immunostained for ChAT (cholinergic neurons) had a complementary distribution to noradrenenergic neurons (Fig. 2f). Both ChAT antisera showed the same patterns of staining, but the best results were obtained for the rabbit antibody, which was used for all numerical analyses and micrographs in this paper. Virtually all neurons appeared to be immunoreactive for TH or ChAT, with <1% pelvic ganglion cells having both substances and <5% with neither substance. It is therefore appropriate to consider that virtually all neurons in the ganglion are either noradrenergic or cholinergic but not both. ChAT also stained many varicose axon terminals that were closely associated with ganglion neurons and these were likely to arise from spinal preganglionic neurons. The terminals around noradrenergic neurons appeared to form a denser plexus than those around cholinergic neurons, as described previously in rat pelvic ganglion (Keast et al. 1995).

VIP neurons were numerous and distributed throughout most of the ganglion (Fig. 3a–c). The only obvious clustering was near the origin of the penile nerve and within the proximal portion of this nerve (Fig. 3b). Further distally along the nerve (~1 mm from the ganglion), small clumps of VIP neurons were also present (Fig. 3c). Irrespective of strain, about half of the ganglion cells contained VIP (Table 3). These were all cholinergic neurons, as <1% neurons contained both VIP and TH (Table 3, Fig. 3d). This was also demonstrated directly in QS mice, where 94.7±2.0% of VIP neurons contained ChAT (Fig. 3e).

NPY neurons were numerous and apparently randomly distributed throughout the ganglion. The proportion of NPY-positive neurons varied substantially between strains, comprising around two-thirds of all pelvic neurons in C57BL/6 mice but almost 100% of pelvic neurons in QS mice (Table 3). In each strain all noradrenergic neurons contained NPY (Fig. 3f), but many NPY neurons were not noradrenergic, i.e. they were cholinergic (Fig. 3h). Many but not all of the cholinergic NPY neurons contained VIP (Fig. 3h). As expected from the different prevalence of NPY between the two mouse strains, the degree of VIP/NPY coexistence varied, with about one-half of all neurons containing both peptides in QS mice, but only a quarter of all pelvic neurons being of this type in C57BL/6 mice (Table 3). Conversely, in C57BL/6 mice there was a higher proportion of VIP neurons without NPY (25% vs 5% in QS).

Neither of the peptides were located in preganglionic terminals, although very rarely an accumulation of VIP or NPY terminals appeared around a single neuron in the section. These may arise from viscerofugal neurons in the myenteric plexus of the lower bowel (Luckensmeyer and Keast 1996). Neither peptide was present in SIF cells.

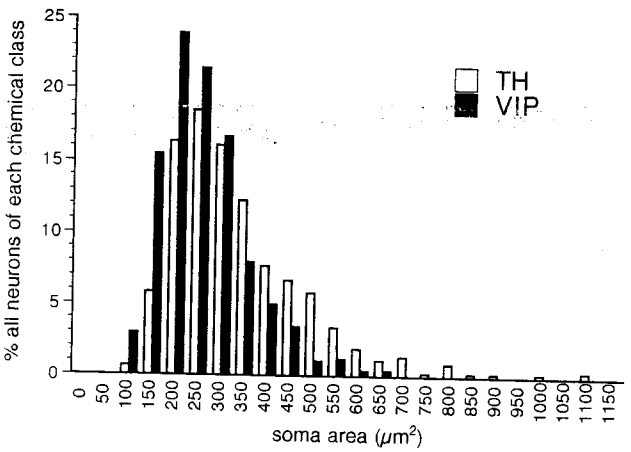
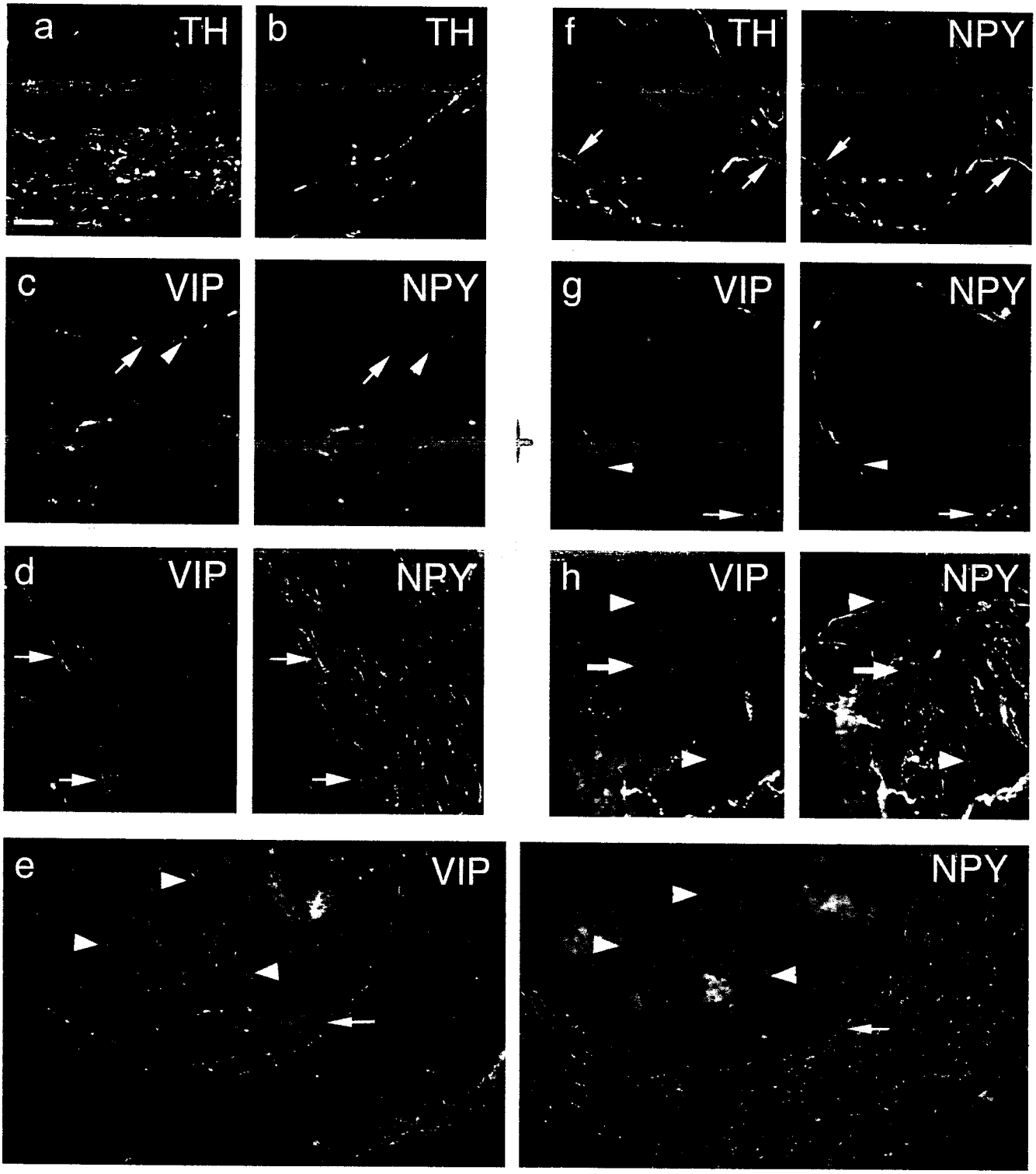


Fig. 4 Soma profile area of pelvic ganglion neurons. Neurons immunostained for TH or VIP were analysed by measuring soma profile area of all neurons sectioned through the nucleus. Neurons were measured for each substance in each of four QS animals (159–206 TH, 159–440 VIP neurons per animal) and the pooled data are shown. Noradrenergic neurons (TH; mean area 362±21 μm²) are larger than VIP neurons (mean area 255±5 μm²; P =0.003, unpaired t -test)

Neurons of different chemical classes had slightly different soma sizes. This data was obtained in four animals (159–440 neurons per substance per animal; QS), and was very similar between animals. As NPY labels almost

Fig. 5 Distribution of autonomic axons in mouse reproductive organs. **a** and **b** show single staining, whereas other panels show pairs of double-stained sections. **a, b, f, h** QS, **c, d, e, g** C57BL/6. **a, b** Noradrenergic axons are found in the muscle of the vas deferens **(a)** and seminal vesicles **(b)** but provide a much more dense supply to the vas. **c** Vas deferens, mucosa and inner muscle. Mucosa contains VIP axons, which are a mixture of NPY-positive (*arrows*) and NPY-negative axons (*arrowheads*). Many NPY-positive VIP-negative axons are also present in the muscle. **d** Vas deferens, muscle. Inner muscle, closest to the mucosa, is *on the left of the micrograph* and contains a number of NPY axons with both VIP and NPY (*arrows*). Most of the muscle is innervated by NPY axons that do not contain VIP. **e** Vas deferens. VIP axons are more prevalent in the inner muscle layers and many of these axons contain NPY (*arrow*). Rare axons with only VIP are also present. In the mucosa many VIP-positive, NPY-negative axons lie beneath the epithelium (*arrowheads*). **f** Prostate gland, acini cross sections. Noradrenergic NPY axons are prevalent (*arrows*). **g** Prostate gland, acinus cross section. VIP axons are not as numerous as NPY axons (*arrowhead* shows NPY axon with no VIP), but a few axons contain both peptides (*arrows*). **h** Prostate gland, sectioned tangentially at the base of an acinus. The different types of nerve fibres form an intermeshed network around each acinus (*arrow* shows VIP/NPY axon, *arrowheads* show NPY axons without VIP, most of which are expected to be noradrenergic). *Bar in* **a** represents 10 μm **(c, g, h)**, 30 μm **(b, d, f)**, 40 μm **(a, e)**



all neurons in the ganglion of this strain, the size of neurons labelled with this marker (316±20 μm²) represents the average size of pelvic ganglion cells. Noradrenergic neurons are larger than VIP neurons (Fig. 4; TH: 362±21 μm²; VIP: 255±5 μm²; P=0.003, unpaired t-test). This is similar to the relative sizes of noradrenergic and cholinergic pelvic ganglion in male rats (Keast and de Groat 1989).

Distribution of different axon types in pelvic organs

The main patterns of innervation were similar between strains. The only noticeable difference was in the distribution of VIP/NPY axons, which is to be expected from their variable prevalence between strains. Unless otherwise stated, the descriptions for each organ apply to both strains. Noradrenergic/NPY axons provided a dense sup-

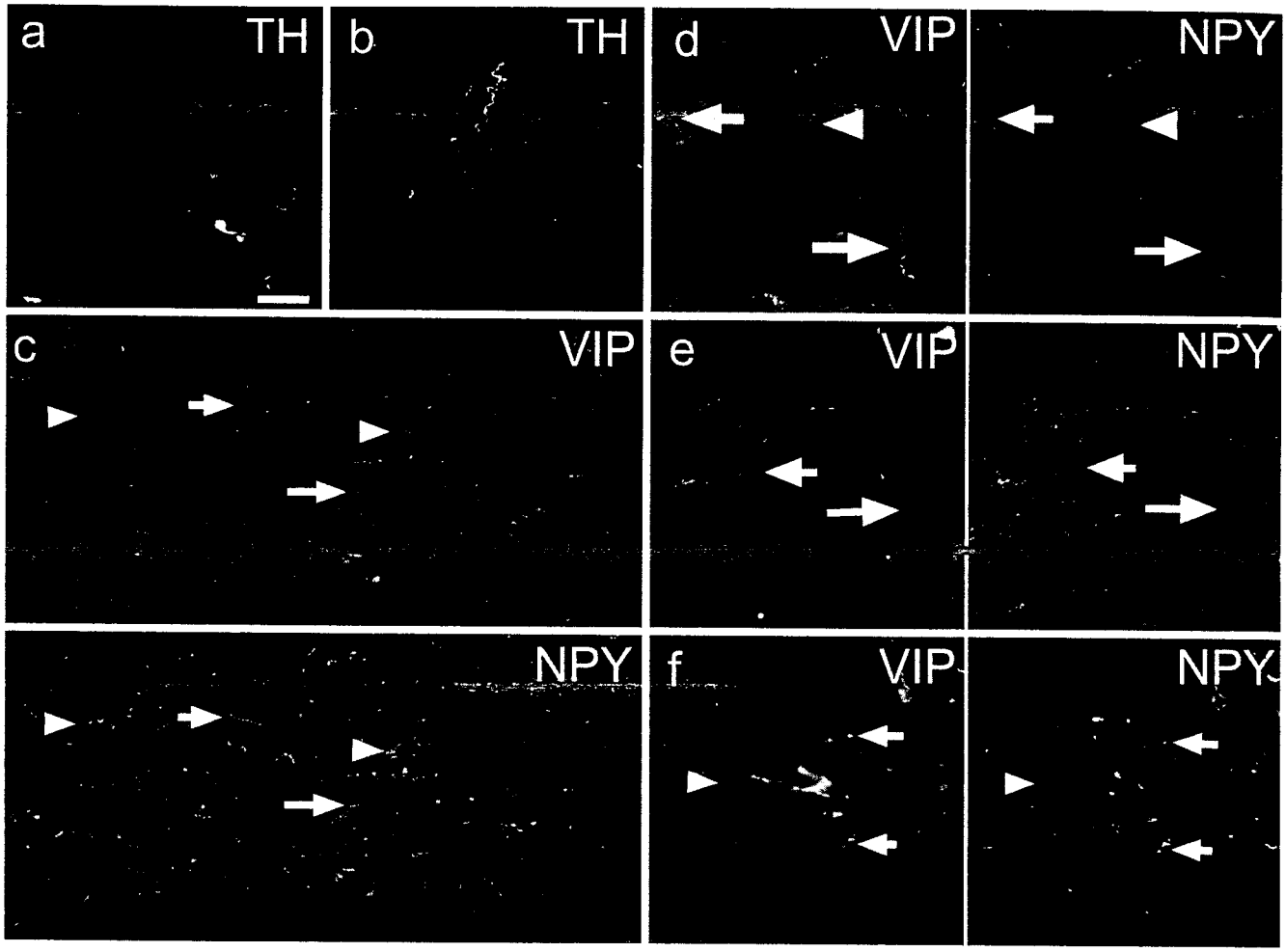


Fig. 6 Distribution of autonomic axons in mouse urinary bladder. **a, b** are single-stained sections, whereas other panels are double stained. **a, b, f** QS, **c–e** C57BL/6. **a, b** Sparse noradrenergic axons in detrusor (**a**) and trigone (**b**) muscle. **c** Trigone muscle (mucosal surface to the top of micrograph), with many axons containing both peptides (*arrows*) but also some axons containing only NPY (*arrowheads*). **d** Trigone mucosa, with subepithelial axons containing both peptides (*arrows*) or, rarely, axons containing only NPY (*arrowheads*). **e** Detrusor muscle (mucosal surface to *top*) showing that the majority of axons have only NPY, although a minority have both peptides (*arrows*) in this strain (C57BL/6). **f** Detrusor muscle (QS strain), where most muscle axons had both peptides (*arrows*) and a small minority only VIP (*arrowheads*). *Bar in* **a** represents 8 μm (**d**), 20 μm (**a, b, f**), 30 μm (**e**) or 40 μm (**c**)

ply to blood vessels in each organ. Many vessels also had a sparse supply of VIP axons, some of which also contained NPY.

Vas deferens and seminal vesicles

The pattern of innervation and relative prevalence of each axon type were similar in the two organs; however, the overall density of innervation of both the muscle and mucosa was substantially higher in the vas deferens (Fig. 5a, b). Noradrenergic NPY axons were prevalent in the smooth muscle and formed a dense network throughout its thickness (Fig. 5c–e). A sparse population of VIP axons existed in the muscle and provided a slightly higher density of innervation to the most inner muscle layers (Fig. 5c–e). All of these VIP axons also contained NPY. In the mucosa a delicate plexus of VIP axons was found with small blood vessels and beneath the epithelium (Fig. 5c–e). In C57BL/6 mice most of these axons contained VIP alone, whereas in QS mice they also contained NPY. Noradrenergic axons were very rarely seen in the mucosa.

Prostate gland

Noradrenergic NPY axons formed a dense plexus around each secretory acinus (Fig. 5f–h). The muscle layers associated with secretory ducts were very thin and it was not possible to determine which axons targeted smooth muscle, secretory epithelia or both. A smaller supply of VIP axons was present around the secretory acini of the prostate gland and some (C57BL/6) or all (QS) of these contained NPY (Fig. 5g, h).

Urinary bladder

Noradrenergic NPY axons comprised only a minor component of the nerve supply of the bladder muscle and mucosa, although their innervation of blood vessels supplying the bladder was as pronounced as for the other pelvic organs. Noradrenergic axons formed a sparse supply in the trigone muscle and were quite rare in the detrusor muscle (Fig. 6a, b). They were absent from the mucosa. Cholinergic axons (as marked by peptides and absence of TH immunoreactivity) were prevalent in muscle (Fig. 6c, e, f) and mucosa layers (Fig. 6d). The same chemical classes of cholinergic axons were found in the mucosa as in the muscle and the relative prevalence of each type was the same (e.g. the most prevalent chemical class in the muscle was also the most prevalent chemical class in the mucosa). In the trigone muscle of both strains, the most common class of axons was those containing both VIP and NPY; some axons with NPY alone were also present (Fig. 6c). In C57BL/6 there were also rare axons with VIP alone in the trigone muscle. In the detrusor muscle the most common axon type varied between strains. In C57BL/6 mice, axons with only NPY were the most common class (Fig. 6e), whereas in QS mice the most common axon type contained both VIP and NPY (Fig. 6f). In both strains axons with only VIP were rare in the muscle of both bladder regions.

Discussion

This study provides the first description of the structural and histochemical features of the male mouse major pelvic ganglion, which provides all of the parasympathetic and much of the sympathetic nerve supply to the lower urinary tract, lower bowel, internal reproductive organs and penis. In comparison with rats, relatively few studies have been performed on the physiology of these nerve pathways in mice. However, the development of numerous types of knockout and transgenic mice provides a trigger to initiate their basic physiological characterisation in genetically unmodified (wild-type) animals.

Our study identified numerous similarities to rat pelvic ganglia (Keast 1999) and a smaller number of differences. Both mouse and rat major pelvic ganglia have comparable structural features, including basic macroscopic organisation (location of pelvic, penile and hypogastric nerves) and cell structure (virtually all neurons are monopolar). The mixed sympathetic-parasympathetic nature of the pelvic ganglia has also been demonstrated physiologically in rats in vitro, by activation of synaptic and action potentials from stimulation of the hypogastric and pelvic nerves (Tabatabai et al. 1986). While the basic membrane and firing properties of mouse pelvic ganglia have been described (Rogers and Henderson 1990; Rogers et al. 1990), the origin of their synaptic inputs has not been determined. From the present study we would predict that there would be slightly more parasympathetic than sympathetic neurons, assuming all noradrenergic neurons are sympathetic and all cholinergic neurons parasympathetic. However, in male rat pelvic ganglia there are also some sympathetic cholinergic neurons (Keast 1995a), and lesion studies or electrophysiological experiments would need to be performed to determine if this was also the case in the mouse.

Further similarities between rat and mouse pelvic autonomic innervation lie in their histochemical properties. In both species, all pelvic ganglion cells are either noradrenergic or cholinergic (but not both) (rat: Keast et al. 1995; mouse: this study). These have distinct primary targets, with noradrenergic axons targeting the smooth muscle of reproductive organs and the cholinergic axons supplying bladder smooth muscle or glandular mucosa of reproductive organs (Keast et al. 1989; Keast 1999). Similarly to the rat, these can be distinguished by their neuropeptide content, with the mucosal axons containing VIP and many of the bladder muscle axons (especially in the detrusor) being VIP negative. In this study we did not include analysis of pelvic ganglion projections to the lower bowel, as this requires lesion or anterograde tracing studies to distinguish these from axons arising from enteric neurons. However, in the rat pelvic ganglion, bowel-projecting cholinergic neurons contain NPY but not VIP. It is unlikely that this is also the case in mice as this chemical class of neurons comprises less than 20% of pelvic ganglion cells and many of these innervate the urinary bladder. Therefore at least some bowel-projecting cholinergic neurons are likely to contain both peptides. Another target of VIP-containing pelvic ganglion cells is the cavernosal tissue of the penis (mouse: Laurikainen et al. 2000; rat: Dail et al. 1983, 1985; Keast and de Groat 1989). Finally, it should be considered that some of the axons we stained in the pelvic organs were of sensory origin. This may account for a small proportion of the immunostained fibres, but is unlikely to represent the majority of axons stained for TH, VIP or NPY, as these substances are absent or found in only a very small proportion of mouse dorsal root ganglion neurons (Green and Dockray 1988; Corness et al. 1996; Holmberg et al. 2001).

The male mouse pelvic ganglion differs in a number of ways from that of the rat. Most obviously, the mouse ganglion has fewer cellular layers. Being much thinner, this makes it ideal for study as a whole-mount (unsectioned) preparation. This will be particularly advantageous for studies where responses in the whole population of ganglion cells need to be monitored. The major chemical difference from rats is that in mice a higher proportion of pelvic ganglion cells contain the neuropeptide NPY. This is particularly marked in the QS strain, where virtually all neurons express this peptide. This raises the question of its function in pelvic viscera. Various roles have been proposed for NPY in the peripheral autonomic system, particularly in the vasculature, where it can have vasoconstrictive actions as well as modulation of noradrenergic vasoconstriction by pre- or postsynaptic actions (Morris 1995). In the vas deferens and urinary bladder, NPY can also modulate excitatory transmission to the smooth muscle (Lundberg et al. 1990;

Iravani and Zar 1994; Smith-White et al. 2001). However, these studies do not show strong effects of NPY and all nerve-mediated postsynaptic events can be blocked by noradrenergic, cholinergic or purinergic blockers. Therefore it is possible that NPY is a genuine cotransmitter with noradrenaline or acetylcholine in all or most mouse pelvic organs, but if so its relative importance compared with these two substances (or with ATP that is also released from the same nerves) is likely to be minor.

In male mouse pelvic ganglia many or all of the parasympathetic neurons appear to contain VIP. This is a common feature of parasympathetic ganglion cells, and it is thought to have actions alone or in conjunction with acetylcholine on blood vessels (dilatation) or epithelia (secretion) (Morris and Gibbins 1992). We found that the level of coexpression of VIP and NPY in cholinergic mouse pelvic ganglion is quite high, and in the QS strain all VIP neurons contained NPY. This is unusual in the autonomic system and it would be of interest to examine the presence and impact of corelease of both peptides in the pelvic viscera. Of particular interest would be the smooth muscle of the bladder detrusor, where the prevalence of cholinergic axons with both peptides differs between strains.

Our studies add to the growing body of data on the autonomic ganglia in mice. Previous studies have shown that many but not all sympathetic noradrenergic neurons contain NPY (Gibbins 1991; Jobling and Gibbins 1999; Guidry and Landis 2000), as our studies have also shown to be true for the pelvic ganglia. This has been observed primarily for noradrenergic axons supplying blood vessels. We also saw noradrenergic NPY neurons associated with vasculature of pelvic organs, but many axons of the same chemical type supplied non-vascular targets, particularly the smooth muscle of reproductive organs and prostate secretory acini. Pelvic sympathetic neurons have a very simple structure almost all of them being monopolar, and this is very different to other sympathetic ganglion neurons in mice (Jobling and Gibbins 1999), which typically have dendrites.

In comparison with rats, very few of the pelvic viscera have been studied in depth in mice. Nevertheless the potential for detailed investigation of the development, normal function and plasticity of pelvic ganglion pathways is now expanded greatly by the use of genetically modified animals (e.g. Mulryan et al. 2000; Trendelenburg et al. 2001; Vlaskovska et al. 2001). Information on normal physiology and structure of these nerve circuits forms a critical basis for this next major step forward. Our observations on strain differences in some chemical aspects of autonomic innervation indicate the importance of maintaining consistency in the types of mice used in these experiments and warn against broad extrapolations between strains.

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