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Protein gene product 9.5 (PGP 9.5)

A new neuronal marker visualizing the whole uterine innervation and pregnancy-induced and developmental changes in the guinea pig

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Summary. The guinea pig uterus is supplied by different populations of nerves which can be demonstrated by specific immunocytochemical and histochemical techniques. So far, there has been no single marker displaying entire peripheral innervation patterns. Recently, protein gene product (PGP) 9.5, a cytoplasmic protein in neurons and neuroendocrine cells, was found to visualize both different populations and subtypes of nerves. This prompted the present study of using PGP 9.5 for visualization of the whole uterine innervation. This was performed by the indirect immunofluorescence method using antiserum to PGP 9.5 raised in rabbits.

PGP-immunoreactivity was present in all neuronal parts of the extrinsic and intrinsic uterine innervation, including different subpopulations of nerves. This was verified by chemical sympathectomy and sensory denervation with 6-hydroxydopamine and capsaicin-treatment respectively, and double immunostaining.

By term a disappearance of uterine PGP-nerve-immunoreactivity was observed which was almost complete in fetus-bearing uterine tissue and further strengthens previous assumptions of a general, pregnancy-induced uterine neuronal degeneration.

The developmental time-course and morphology of PGP-immunoreactive nerve structures was similar to that for other neuronal markers and support the suggestion of PGP-immunoreactivity as a general marker for the entire uterine innervation, and suggests that the presence of PGP 9.5-immunoreactivity may coincide with functional maturation of uterine innervation.

Introduction

The innervation of the guinea pig uterus is predominantly adrenergic (Thorbert et al. 1977; Alm and Lundberg 1988). There is a minor contribution of peptide-containing nerves, which are separated into three different populations comprising adrenergic nerves containing neuropeptide Y (NPY), substance P (SP)-/calcitonin gene related peptide (CGRP)-/neurokinin A (NKA)-IR nerves – all three immunoreactivities occurring in the same nerves, and a third consisting of peptide histidine isoleucine (PHI)-IR nerves (Alm and Lundberg 1988). There are no or very few acetylcholinesterase-positive (presumably cholinergic) nerves in the guinea-pig uterus (Thorbert et al. 1977; Hammarström and Sjöstrand 1979).

In comparison to other organs the uterine innervation is unique in that it undergoes pronounced degenerative changes during pregnancy and at full term no adrenergic or peptide-containing nerves can be histochemically visualized (Thorbert et al. 1978; Fried et al. 1985; Alm and Lundberg 1988; Alm et al. 1988a). This phenomenon probably reflects a pregnancy-induced structural degeneration of the whole uterine innervation as supported by the simultaneous disappearance of S-100 protein, which is a well-known marker for Schwann cells, neurofibrillary protein (NF) and neuron specific enolase (NSE), which are widely used general neuronal markers (Alm et al. 1988a; Lundberg et al. 1987). However, NF and NSE do not seem to be well represented in all populations of nerves and in all neuronal regions (Bishop et al. 1985; Hacker et al. 1985). These drawbacks in the demonstration of complete tissue innervation patterns might be overcome by the use of protein gene product 9.5 (PGP 9.5) as a marker. In a recent study on cardiovascular innervation in the guinea pig PGP 9.5 seemed to be a general cytoplasmic marker demonstrating all types of efferent and afferent nerve fibres, unlike NSE and NF (Gulbenkian et al. 1987). PGP 9.5 is a neuronal cytoplasmic protein unrelated to NSE, with unknown function, and having a molecular weight of about 27000 and a mobility of 9.5 cm in one dimension polyacrylamide gel electrophoresis (Jackson and Thompson 1981). It has been demonstrated in neurons of the guinea pig and other species (Doran et al. 1983; Thompson et al. 1983; Jackson et al. 1985; Rode et al. 1985).

The present study was therefore undertaken with the aim of visualizing the whole uterine innervation and its pregnancy-induced changes, using PGP 9.5 as a presumed more sensitive neuronal marker.

Materials and methods

Twenty virgin sexually mature guinea pigs, (400–600 g body weight) with free access to water and standard pellets, were used.

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Four animals underwent chemical sympathectomy by 6-hydroxydopamine (6-OHDA; for details see Alm et al. 1988a). Four animals were sensory denervated by capsaicin (8-methyl-*N*-vanillyl-6-nonenamide, Fluka) with a total dose of 100 mg/kg, following the scheme for administration and anaesthesia according to Gulbenkian et al. (1987). Four pregnant (primiparous) animals carrying fetus(es) in only one of the uterine horns were killed at full term pregnancy (65–70 days of pregnancy, which was verified by measuring crown-rump lengths and weights of the fetus(es); (see Draper 1920; Kaufmann 1969). The study also included four female fetuses from four different pregnant animals at a gestational age of about 65 days, and female animals – newborn, and at the age of 1, 2 and 4 weeks (four animals in each group).

Under narcosis of Ketalar (Parke-Davies; 2.25 ml/kg) and Rompun (Bayer; 0.20 ml/kg) or ether, the inferior mesenteric ganglia (IMG), aortico-renal ganglia (ARG), the coeliac-superior mesenteric ganglia (C-SMG), the suspensory ligaments and/or the uteri with adjacent parametrial and paracervical tissue, were rapidly dissected out. The uteri were divided into parametrial tissue, uterine horns and cervix (including the surrounding paracervical tissue). All tissue specimens were fixed and processed for the indirect immunofluorescence method of Coons (Coons et al. 1955) previously described with minor modifications (Alm and Lundberg 1988; Alm et al. 1988a, b, c). Cryostat sections were cut at a thickness of 15 µm and air-dried for about 30 min. After incubation for about 2–4 h in 0.3% Triton X-100 followed by the impregnation for 1 h in the dye Pontamine sky blue (PoSB; to reduce unspecific background, see Cowen et al. 1985; 0.05% PoSB in PBS containing 1% dimethylsulfoxime (DMSO)) the sections were given a 5 min rinse in PBS, and then incubated in the presence of PGP 9.5 antiserum, raised in rabbits (see below). After this the sections were rinsed for 10 min (three rinses) in PBS, incubated in fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit immunoglobulins (Dakopatts, Sweden; diluted 1:20 in PBS) at room temperature for 1 h, rinsed in PBS and mounted in buffered glycerol containing *p*-phenylenediamine to prevent fluorescence fading (Johnson and Araujo 1981). For the simultaneous localization of PGP- and substance P (SP)-immunoreactivities double immunostaining was performed by incubating sections in the presence of both PGP and SP antibodies (the SP antibody being a rat monoclonal, see below). After three rinses in PBS, the sections were incubated for 1 h with rhodamine (TRITC)-conjugated swine anti-rabbit immunoglobulins (Dakopatts, Sweden; diluted 1:40 in PBS), rinsed in PBS and then exposed to FITC-conjugated goat anti-rat immunoglobulins (Sigma, USA; diluted 1:20 in PBS) for 1 h. After rinsing the sections were mounted as previously described.

Antiserum to PGP 9.5, raised in rabbits and used in a dilution of 1:2500 in PBS, was obtained from UltraClone, Cambridge, England; (for further information see Gulbenkian et al. (1987)). SP rat monoclonal antiserum (Sera Lab, code no MAS 035) was used in a dilution of 1:100 in PBS. The specificity of the immunohistochemical reactions was checked by omitting the primary antibody step, by substituting the primary antisera with serum from non-immunized rabbits, by omitting the FITC incubation-steps or when using antisera absorbed with excess of the respective antigens. As cross-reactions with other proteins with amino-acid sequences similar to the respective antibodies cannot be excluded the immunohistochemical products demonstrated are referred to as PGP- and SP-immunoreactive. The double immunostaining procedure was tested according to Wessendorf and Elde (1985) to exclude different possibilities of artifacts of co-existence.

The sections were examined in a Zeiss fluorescence microscope, equipped with epi-illumination and with filter settings described elsewhere (Alm and Lundberg 1988).

In the sections the immunoreactive nerves found were evaluated with respect to subtype (nerve trunks, non-varicose and varicose nerves), relative frequency (arbitrarily graded as very few = (+), few = +, moderate number = ++, rich = +++, or in the developmental studies as much less (ML) and less (L) than at complete development, and number at complete development (CD)), distribution pattern and structure (presence of signs of degeneration, and morphologic appearances compared to the situation at complete development) (see Tables 1 and 2).

Results

Virgin, untreated animals (see Table 1)

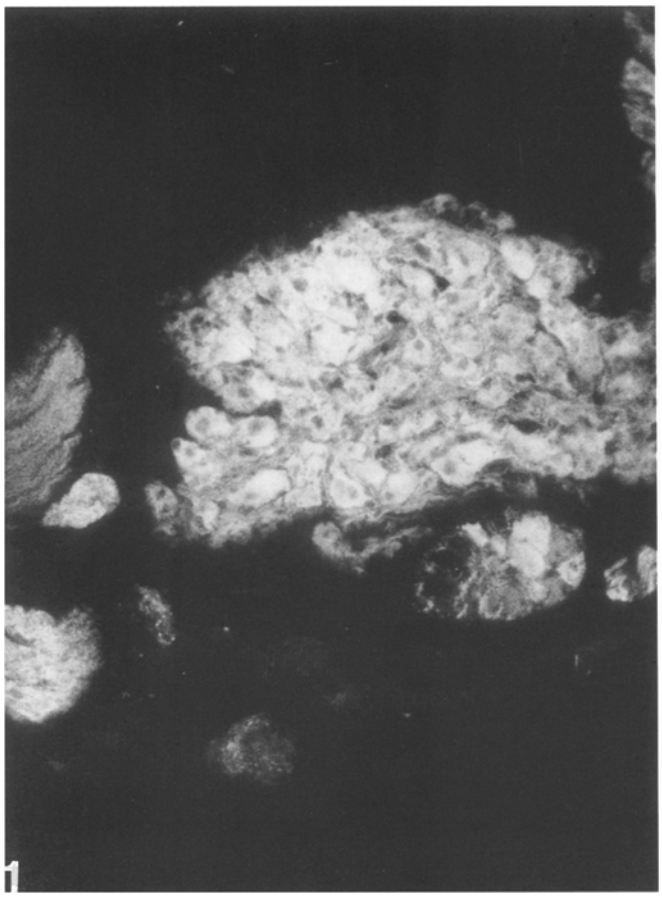
In the prevertebral ganglia (IMG, ARG and C-SMG) the cytoplasm of all neuronal cell bodies showed PGP-immunoreactivity, with immunofluorescent staining surrounding unreactive nuclei. The nerve bundles leaving the ganglia also displayed PGP-immunoreactivity (Fig. 1).

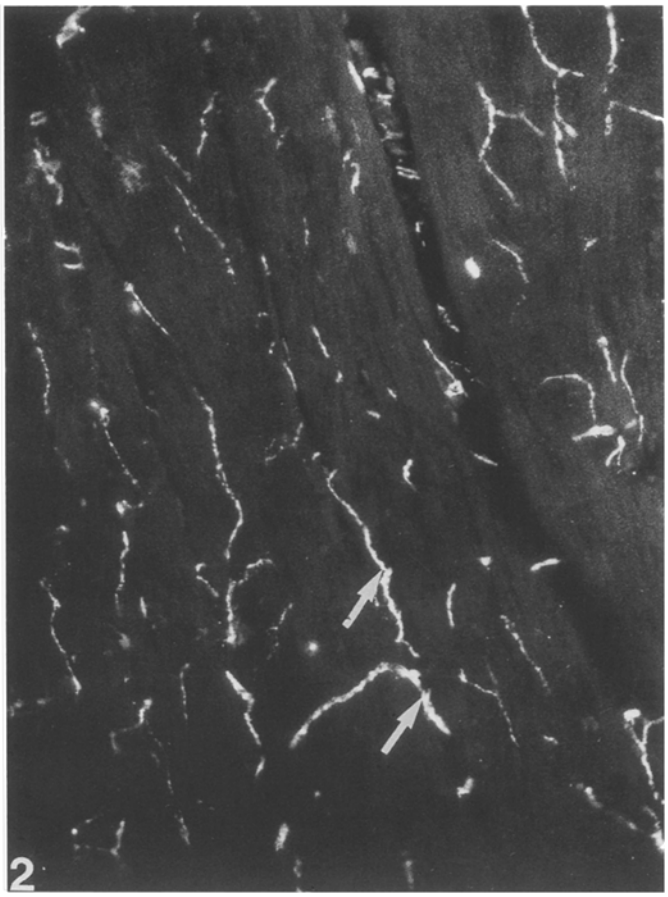
In the suspensory ligaments there were few PGP-IR nerve trunks whereas PGP-IR non-varicose and varicose nerves were more frequent (Fig. 2). All PGP-IR nerve structures ran along bundles of fibrous and smooth muscle tissue directed to the tubal end of the uterine horn.

In the fibrous and fat-containing paracervical tissue there were freely running nerve trunks and non-varicose nerves with PGP-immunoreactivity whereas in the thin parametrial tissue (Fig. 3) freely running nerve fibres were only of the non-varicose category. In the parametrial and paracervical tissues there were also paravascular non-varicose

Table 1. Relative frequency of PGP-immunoreactive (*IR*) nerve structures (arbitrarily graded) in the uterine horn in virgin animals, untreated or following chemical sympathectomy with 6-hydroxydopamine (6-OHDA) or sensory denervation with capsaicin. 0 = absence of IR nerve structures, (+) = very few, + = few, ++ = moderate number, +++ = rich number. *NT* = nerve trunk, *NVNF* = non-varicose nerve fibre, *VNF* = varicose nerve fibre. FBH = fetus-bearing uterine horn, *NFBH* = non-fetus-bearing uterine horn

	PGP-IR		
	NT	NVNF	VNF
Uterine horns			
Outer myometrium			
Virgin	+	+	+→++
6-OHDA treatment	+	(+)	0
Capsaicin treatment	+	+	+→++
FBH-full term pregnancy	0	0	0
NFBH-full term pregnancy	+	+→(+)	(+)
Intravascular space			
Virgin	+	+	+++
6-OHDA treatment	+	(+)	+
Capsaicin treatment	+	+	+++
FBH-full term pregnancy	0	0	0
NFBH-full term pregnancy	+	+	++(+)
Inner myometrium			
Virgin	0	+	++
6-OHDA treatment	0	(+)	(+)
Capsaicin treatment	0	+	++
FBH-full term pregnancy	0	0	0
NFBH-full term pregnancy	0	(+)	(+)
Endometrium (stroma)			
Virgin	0	(+)	++
6-OHDA treatment	0	(+)	+
Capsaicin treatment	0	(+)	++
FHB-full term pregnancy	0	0	0
NFBH-full term pregnancy	0	(+)→0	(+)
Suspensory ligament			
Virgin	+	++	+++





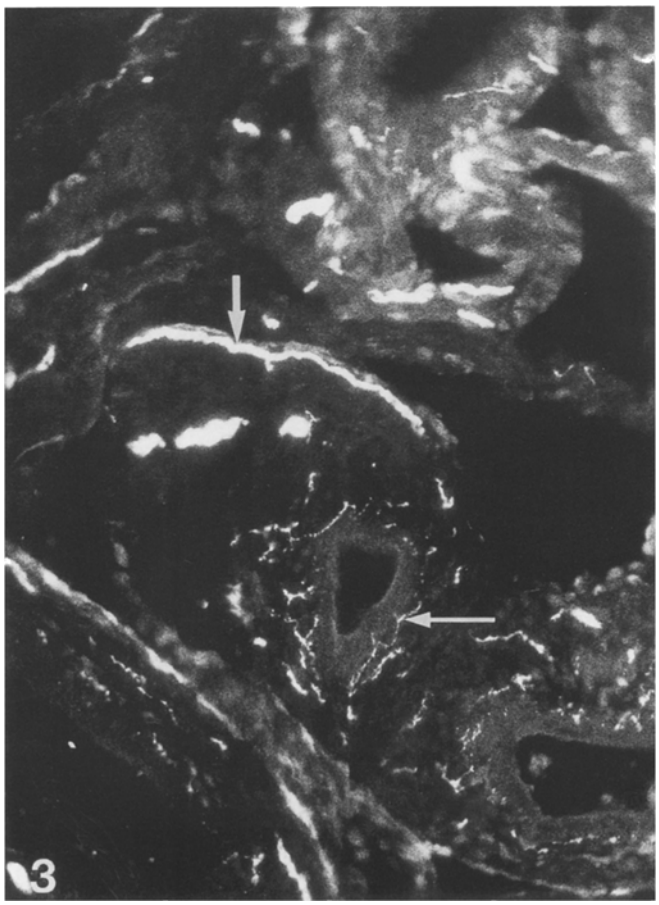
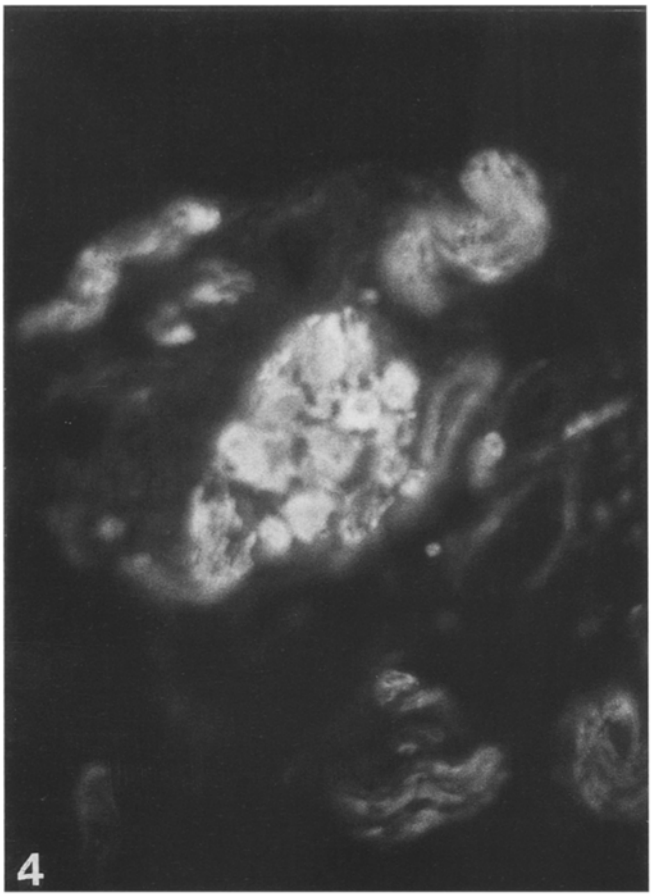


Fig. 1. Inferior mesenteric ganglion. Neuronal cell bodies showing strong cytoplasmic PGP-immunoreactivity. FITC-fluorescence micrograph, ×130

Fig. 2. Suspensory ligament. Rich amounts of PGP-IR varicose nerves. Some non-varicose nerves (*arrows*) are also present. FITC-fluorescence micrograph, ×130

Fig. 3. Parametrial tissue. Thin sheaths of mesouterine tissue with freely running PGP-IR non-varicose nerves (*thick arrow*), and vessels surrounded by plexus of fine varicose PGP-IR nerves (*thin arrow*). FITC-fluorescence micrograph, ×130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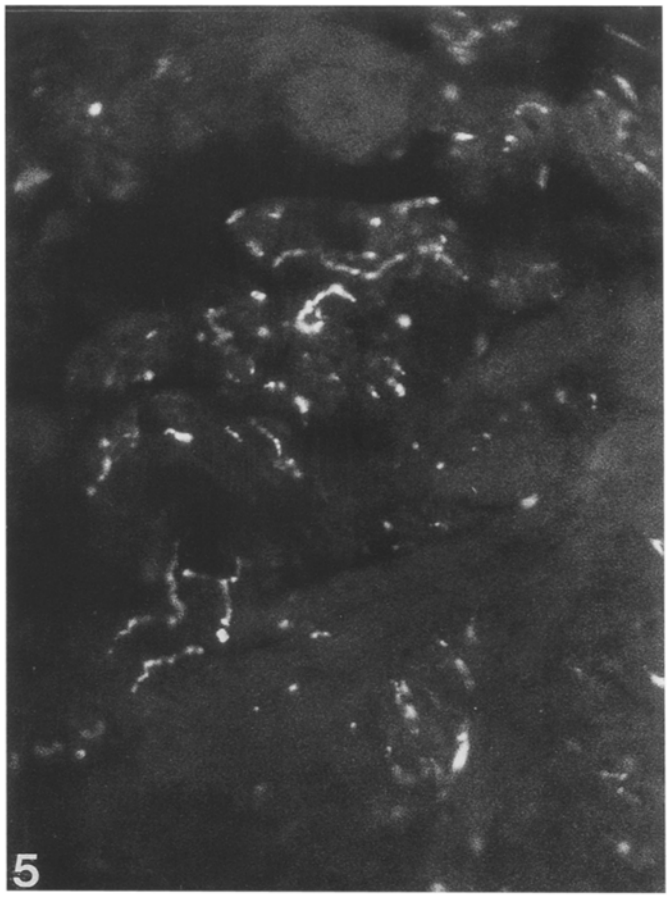
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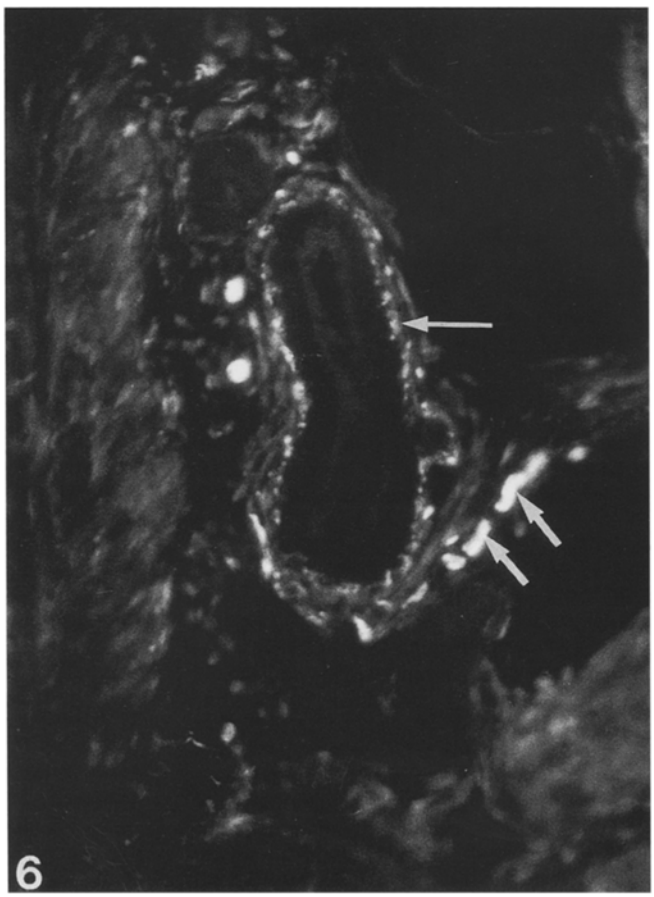
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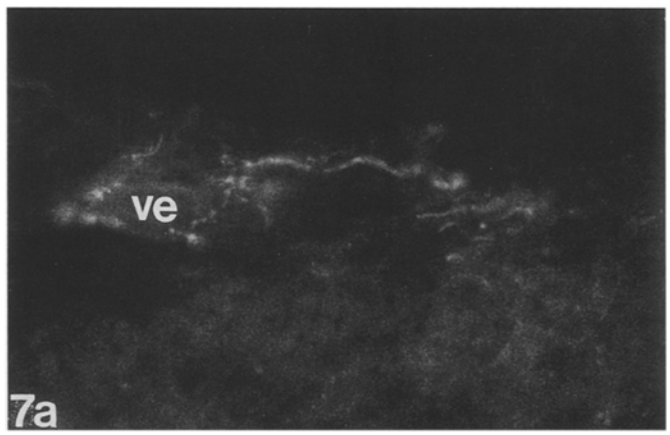
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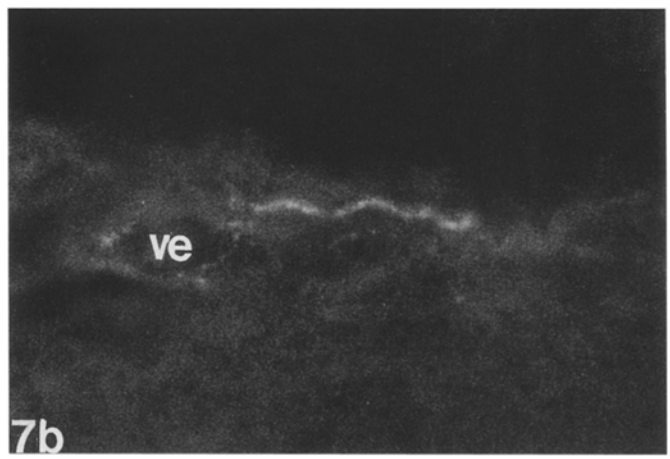
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nerves, which gave off varicose fibres forming perivascular plexiform networks that were more dense around arteries than around veins (Fig. 3). In the paracervical tissue there were also single or clusters of neuronal cell bodies, which formed more or less well-demarcated ganglia that gave off coarse nerve trunks (Fig. 4).

The outer myometrial smooth muscle layer was penetrated by few PGP-IR nerve trunks which ran to the intramyometrial vascular space where they were divided into non-varicose nerves. Branches of the latter passed into the outer and inner myometrial layers where they were further ramified into smooth muscle-related varicose nerves (Fig. 5), appreciably more frequent in the inner than in the outer myometrial layer. Moreover, in the intramyometrial vascular space branches of non-varicose nerve fibres also accompanied vessels and gave off varicose nerves which formed dense vessel-surrounding plexus (Fig. 6). The plexus continued around branches of vessels which penetrated the inner myometrial layer and extended into the endometrial stroma.

The number and distribution patterns of PGP-IR nerve structures were essentially similar in the cervix and in the uterine horns except for the subepithelially located endometrial nerves which were more frequent (few to moderate) in the cervix.

Co-existence of PGP- and SP-immunoreactivities within the same nerve fibre structure was also found in some vessel-related varicose nerves (Fig. 7a and b). In the endometrium there were non-varicose nerves with PGP-immunoreactivity, which were localized subepithelially (Fig. 8) and in which PGP- and SP-immunoreactivities also co-existed.

Chemical sympathectomy with 6-OHDA (see Table 1) produced a very clear reduction of all myometrial and vessel-related PGP-IR varicose nerves. The number of PGP-IR non-varicose nerves was slightly reduced with the nerve fibres showing overt signs of degeneration (variations in calibre, uneven contours and irregularly distributed PGP-immunoreactivity). The number and structure of nerve trunks was unchanged. In the endometrium, the number and structure of subepithelially located non-varicose PGP-IR nerves was not appreciably changed whereas the number of the stromal nerves was diminished.

Sensory denervation with capsaicin (see Table 1) caused no evident change in the number of the PGP-IR nerves, and the structure of these were mostly unchanged except the subepithelially located nerves (Fig. 8) which could show signs of degeneration. In comparison, no SP-nerve-immunoreactivity could be detected.

Effects of full term pregnancy (see Table 1)

In the fetus-bearing uterine horn practically no PGP-IR nerve structures could be found (Fig. 9).

In the non-fetus-bearing horn the number of myometrial and endometrial stromal varicose nerves and endometrial subepithelially located non-varicose nerves was reduced. In contrast, the number and structure of vessel-related varicose nerves in the intramyometrial vascular space was not significantly changed, and further, the number of PGP-IR nerve trunks and non-varicose nerves (except in the endometrium), was not or only slightly reduced.

In the cervix only scattered remnants of PGP-IR varicose nerves in relation to myometrial smooth musculature remained. There was also reduced number of PGP-IR nerve trunks and non-varicose nerves and vessel-related varicose nerves (the latter being reduced from rich to few/moderate), all the related nerve structures displaying clear signs of degeneration. In the endometrium no stroma-related varicose nerves could be detected and very few subepithelially located non-varicose nerves with clear signs of degeneration were observed (Fig. 10).

In parametrium adjacent to the fetus-bearing uterine horn the number of freely running non-varicose nerves and the densities of the vessel-surrounding plexus were significantly reduced, with a few remaining remnants displaying overt signs of degeneration (Fig. 11). In contrast, in parametrium adjacent to the non-fetus-bearing horn there were no apparent changes in number and morphology of PGP-IR nerve structures (Fig. 12).

Pre- and postnatal development of PGP-IR nerve structures (see Table 2)

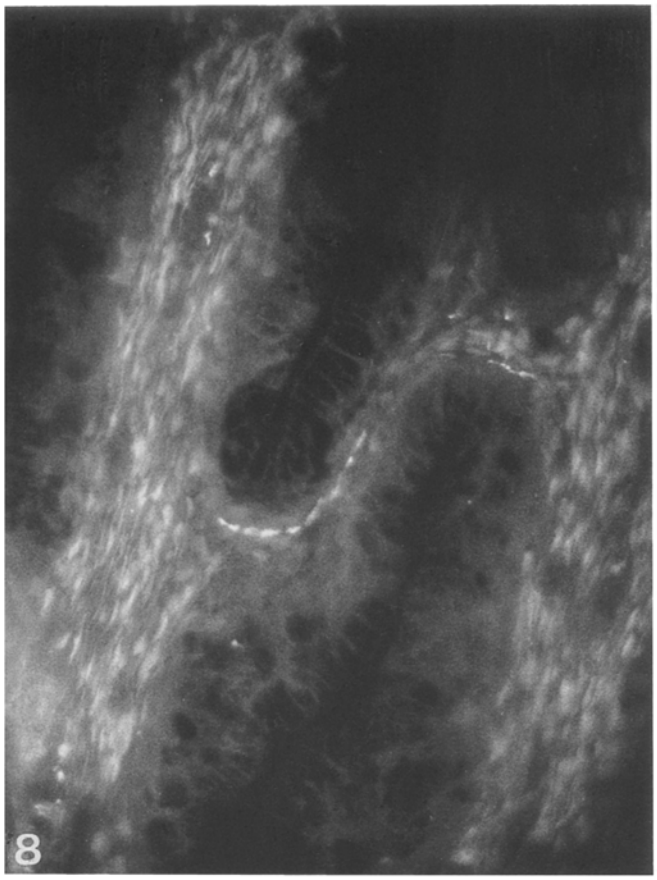
At a gestational age of about 65 days few PGP-IR nerve fibre structures resembling PGP-IR nerve trunks and non-varicose nerves at complete development were found in the outer myometrial layer and in the intramyometrial vascular space (Fig. 13). At one week of age the PGP-IR nerve fibre structures had extended into the inner myometrial layer. Some of the PGP-IR nerves branched into fibres with varicosities of irregular shapes and sizes, and either followed the vessels in the intramyometrial vascular space or ran into the inner myometrial layer (Fig. 14). Single PGP-IR nerve fibre structures resembling non-varicose nerves at complete development could also be found subepithelially in the endometrium. At 2 weeks of age the number, distribution pattern and morphologic appearance of the vessel-related PGP-IR varicose nerves were as at complete development. In comparison, this was not the case for the myometrial smooth muscle related varicose nerves until 4 weeks

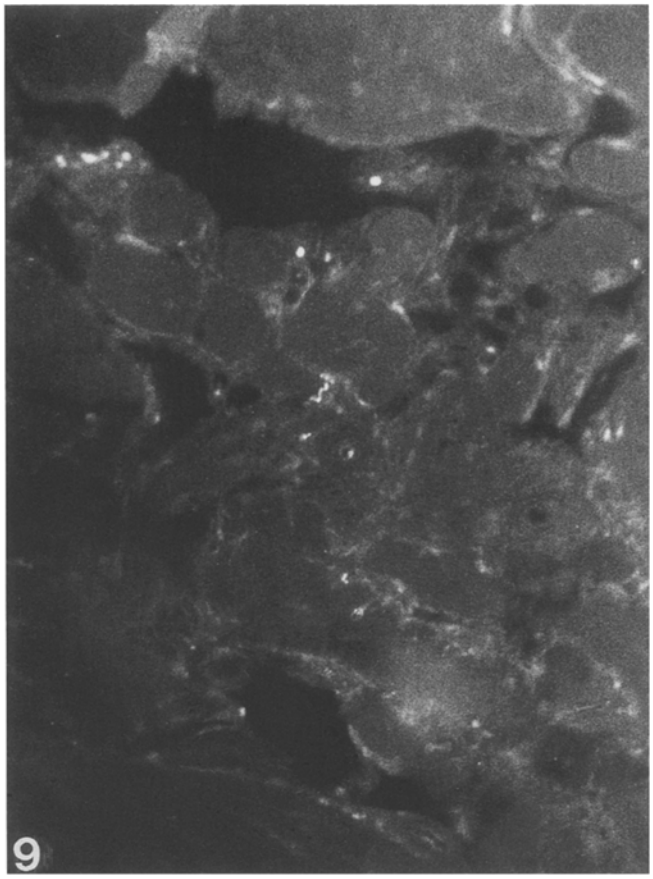
Fig. 4. Paracervical ganglion consisting of clusters of neuronal cell bodies with strong cytoplasmic PGP-immunoreactivity. A very coarse nerve trunk is leaving the ganglion. FITC-fluorescence micrograph, × 200

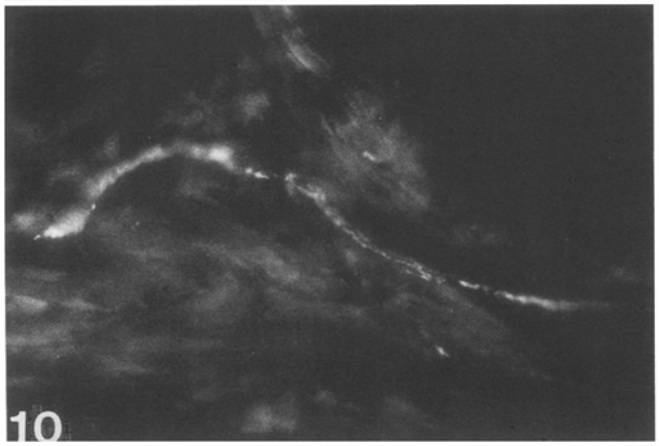
Fig. 5. Uterine horn, inner myometrial layer. Moderate numbers of PGP-IR varicose nerves are running along smooth muscle bundles. FITC-fluorescence micrograph, × 130

Fig. 6. Uterine horn, intramyometrial vascular space. Artery surrounded by plexus of PGP-IR varicose nerves (*thin arrow*). Paravascular PGP-IR non-varicose nerves (*thick arrow*) are seen. FITC-fluorescence micrograph, × 130

Fig. 7. a Uterine horn. Intramyometrial vascular space with a small vessel (*ve*) accompanied by PGP-IR nerves. TRITC-fluorescense micrograph, × 200. **b** Same sections as in **a**. SP-immunoreactivity co-existing with PGP-immunoreactivity within the corresponding nerve fibre structure shown in **a**. FITC-fluorescence micrograph, × 200







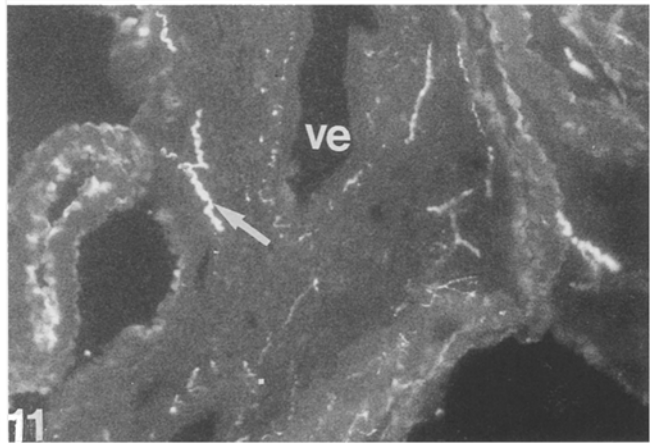


Fig. 8. Cervix, endometrium, capsaicin treatment. Subepithelially located PGP-IR nerve. FITC-fluorescence micrograph, ×200

Fig. 9. Fetus-bearing uterine horn, full term pregnancy. There is an almost total absence of PGP-IR nerve structures. FITC-fluorescence micrograph, ×130

Fig. 10. Cervix, full term pregnancy. Endometrial, subepithelially located PGP-IR nerves with signs of degeneration – uneven contours and irregularly distributed immunofluorescence in granular and globular accumulations of varying sizes. FITC-fluorescence micrograph, ×200

Fig. 11. Parametrium adjacent to fetus-bearing uterine horn. In the mesouterine tissue there is a single remnant of a degenerating PGP-IR non-varicose nerve fibre (*arrow*) and a vessel (*ve*) surrounded by few PGP-IR varicose nerves. FITC-fluorescence micrograph, ×100

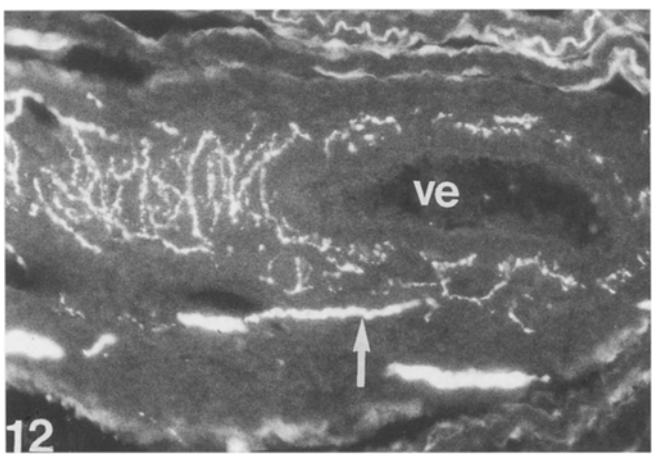
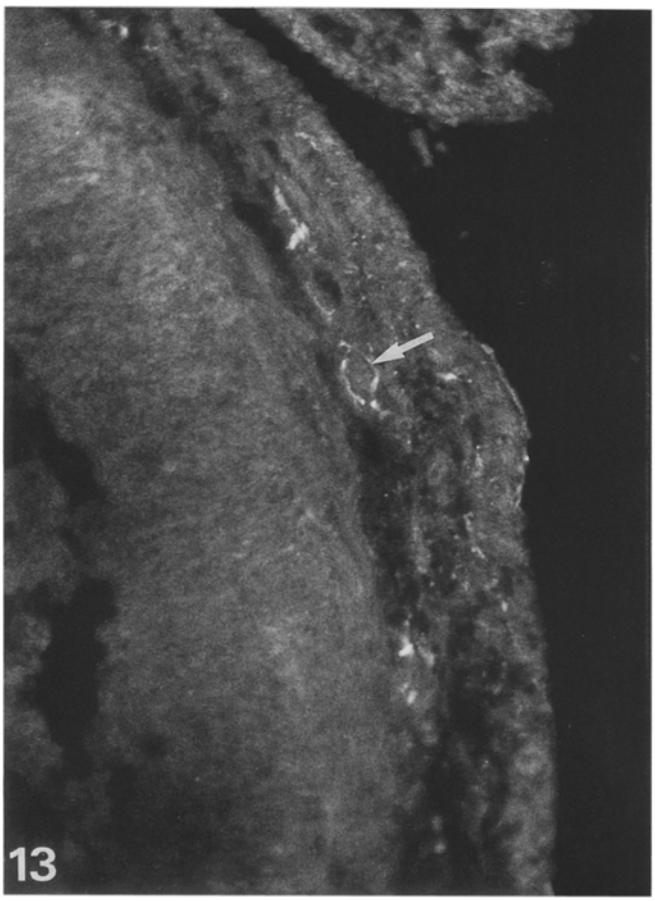


Fig. 12. Parametrium adjacent to non-fetus-bearing uterine horn. In the mesouterine tissue there is a vessel (*ve*) surrounded by a dense plexus of PGP-IR varicose nerves. Paravascularly there are PGP-IR non-varicose nerves (*arrow*). FITC-fluorescence micrograph, ×130

Table 2. Appearance of PGP-IR nerve structures in the uterine horn. 65 days = gestational age of about 65 days. 1, 2 and 4 weeks refer to postnatal age. *CD* = number of nerve structures at complete development, *L* = nerve structures in less number than at complete development, *ML* = nerve structures in much less number than at complete development. *NT* = nerve trunk, *NVNF* = non-varicose nerve fibre, *VNF* = varicose nerve fibre

	65 Days			1 Week			2 Weeks			4 Weeks		
	NT	NVNF	VNF	NT	NVNF	VNF	NT	NVNF	VNF	NT	NVNF	VNF
Outer myometrium	ML	ML	–	L	L	ML	CD	CD	L	CD	CD	CD
Intramyometrial – vascular space	ML	ML	–	L	L	L	CD	CD	CD	CD	CD	CD
Inner myometrium	–	–	–	–	L	ML	–	CD	L	–	CD	CD
Endometrium – vascular related	–	–	–	–	–	ML	–	–	L	–	–	CD
Endometrium – subepithelially located	–	–	–	–	ML	–	–	L	–	–	CD	–



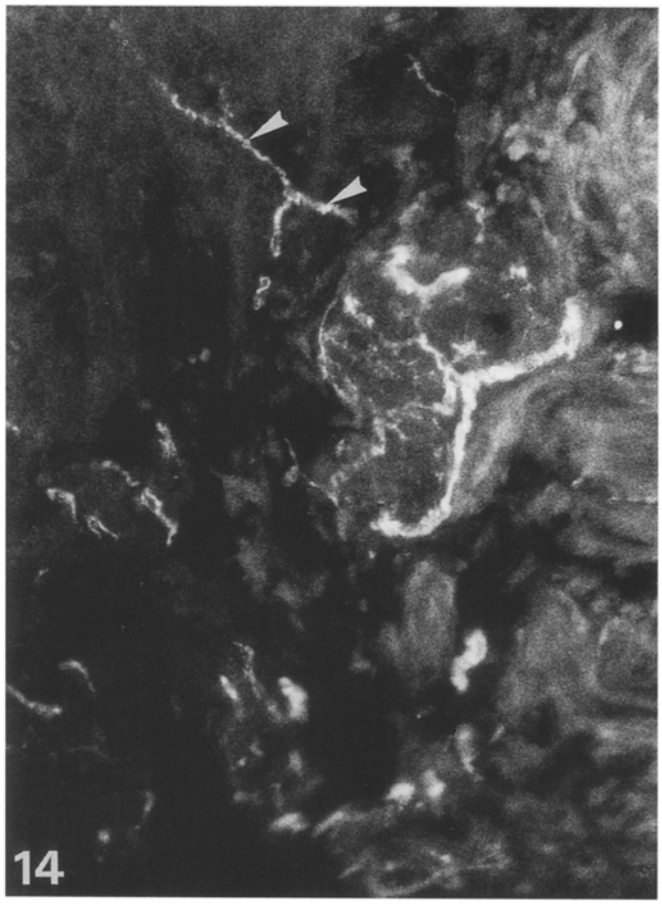


Fig. 13. Uterine horn, gestational age 65 days. Few PGP-IR nerve structures are seen in the outer myometrial layer and in the intramyometrial vascular space (*arrow*). FITC-fluorescence micrograph, × 130

Fig. 14. Uterine horn, 1 week of age, intramyometrial vascular space. Nerve trunks and non-varicose nerve fibres running paravascularly with branches of nerves with irregularly shaped varicosities (*arrowheads*) extending up into the inner myometrial layer. FITC-fluorescence micrograph, × 240

of age, when the development of the whole uterine supply of PGP-IR nerve structures was completed. The time-course for the development of PGP-IR nerve structures in the cervix was similar to that in the uterine horns.

Discussion

The present use of PGP 9.5 as a general neuronal marker demonstrated nerve trunks and non-varicose nerves with PGP-immunoreactivity. The amount and distribution pattern of the PGP 9.5-immunoreactive innervation of the guinea pig uterus was similar to that of NF- and NSE-IR nerve structures (Alm et al. 1988a, b). However, PGP 9.5 also disclosed large amounts of varicose nerves which could not be detected by NF- and NSE-immunostaining. This discrepancy in immunostaining between NSE and PGP is noteworthy as both are cytoplasmic proteins (Schmechel et al. 1978; Doran et al. 1983). One explanation could be differences in intraneuronal distribution and/or function. The lack of NF-immunoreactivity in terminal varicose

nerve fibres is in accordance with earlier findings showing a correlation between neurofilament content and axonal calibre, there being more neurofilaments in larger axons, and less in smaller ones (Peters and Vaughn 1967; Friede and Samorajski 1970; Hoffman et al. 1984). Further, intraneuronal differences in antigen subunit structures or the masking of specific epitopes by neuronal structures have also been suggested for the differences in the demonstration of NF-immunoreactivity between various neuronal regions (Dahl et al. 1984; Lawson et al. 1984; Trojanowski et al. 1985; Bignami et al. 1986). PGP 9.5 seems to be a more sensitive general neuronal marker than NF and NSE because of its discrimination of more terminal axonal structures.

The adrenergic/NPY-IR innervation of the guinea pig uterus is derived from nerve trunks and non-varicose nerve fibres travelling in the suspensory ligament, and from non-varicose nerve fibres and vascular-related varicose nerves running in the mesouterus (Thorbert et al. 1977; Alm et al. 1988b). The number and distribution pattern of PGP-IR nerves in both the extrinsic and intrinsic uterine innervation corresponds with that of adrenergic/NPY-IR nerves (Thorbert et al. 1977). Moreover, the effect of chemical sympathectomy with 6-OHDA on PGP-IR nerve structures was similar to that on adrenergic/NPY-IR nerves, there being a very pronounced reduction of PGP-IR varicose nerves and only a few remaining vascular-related varicose nerves. In contrast, the number and structure of the subepithelially located PGP-IR nerves appeared to be unchanged. The distribution pattern of the 6-OHDA resistant PGP-IR nerves corresponded to that of SP-/CGRP-/NKA-IR nerves (Alm and Lundberg 1988). It seems reasonable to assume that PGP 9.5 is a marker for all nerve populations and neuronal regional parts of the uterine extrinsic and intrinsic innervation. Furthermore, PGP-immunoreactivity has also been demonstrated in prevertebral and paracervical ganglia from which the uterine adrenergic/NPY-IR innervation originates (Alm and Lundberg 1988).

Previous catecholamine histofluorescence and physiological findings suggested a pregnancy-induced degeneration of the uterine adrenergic nerves, which is almost complete in the fetus-bearing uterine regions and less extensive in non-fetus-bearing uterine horns and in the cervix (Thorbert 1979). The similar disappearance of peptide-containing and NF-, NSE-, and S-100-IR nerves prompted the assumption of a pregnancy-induced degeneration of the whole intrinsic uterine innervation, in the fetus-bearing uterine horn extending to the adjacent parametrian extrinsic nerves (Alm and Lundberg 1988; Alm et al. 1988a, b; Lundberg et al. 1987). This was further strenghtened by the loss of the more sensitive and comprehensive neuronal marker PGP 9.5.

PGP 9.5 is a cytoplasmic protein which is unrelated to previously used neuronal markers employed in studies of uterine innervation, these including NF, NSE, neuropeptides, and tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH). It is interesting that the present developmental time-course and morphology of PGP-IR nerve structures is similar to that for NF- and NSE-IR nerves, adrenergic/NPY-IR nerves, and also subepithelially located SP-IR nerves, which are presumably sensory in function (Alm et al. 1988c). Hypothetically, it could be assumed that there is a coincidence in the maturation of structure and function of the uterine innervation.

Capsaicin is a neurotoxin, which selectively interferes with sensory function, and depletes SP-, CGRP- and NKA-immunoreactivities from sensory nerves (Gamse et al. 1979; Burks et al. 1985; Lundberg et al. 1985a, b; Theodorsson-Norheim et al. 1985). In the present study capsaicin produced a depletion of SP-nerve-immunoreactivity, but had no significant effect on the number of PGP-IR nerve structures. One explanation to this could be that the SP-IR nerves form a minor population of the PGP-IR nerves and therefore a reduction of the latter might not be detectable. This seems further likely as the PGP-IR nerves predominantly represent the adrenergic/NPY-IR nerves (see above), which are more abundant that the SP-IR nerves (Alm and Lundberg 1988). However, it should be observed that there were signs of degeneration in the subepithelially located endometrial PGP-IR nerves. This might be in accordance with earlier findings that capsaicin can induce changes in guinea pig neuronal structure (Papka et al. 1984).

Previous investigations have shown a rich supply of adrenergic/NPY-IR varicose nerves in the suspensory ligaments, and in relation to vessels and in myometrial smooth musculature of the guinea pig uterus (Thorbert et al. 1977; Alm and Lundberg 1988; Alm et al. 1988a). The present study displayed similar amounts of PGP-IR varicose nerves in the suspensory ligaments and in relation to vessels but comparatively less amounts in the outer myometrial layer. The reason for this discrepancy is unknown. One explanation could be the occurrence of less amounts of PGP 9.5 in these varicose nerves which hypothetically might have a functional background.

In conclusion, the use of PGP 9.5 visualizes the entire uterine innervation of the guinea pig as well with respect to the different nerve populations as all the neuronal parts as the developmental changes. It also presents the occurrence of a pregnancy-induced general neuronal degeneration of the guinea-pig uterine innervation. Thus, PGP 9.5 seems to be a sensitive general neuronal marker for peripheral innervation patterns and related changes of neuronal plasticity.

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