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Peptidergic innervation within the prostate gland and seminal vesicle*

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Summary. An immunohistochemical study in which antisera against several neuropeptides were used demonstrated the presence of neuropeptide Y(NPY) and vasoactive intestinal polypeptide (VIP) immunoreactivity in nerve fibers in the human prostate gland and seminal vesicle, whereas no immunostaining for substance P and calcitonin gene-related peptide was observed. The peptidergic innervation was found to be generally moderate to low. NPY- and VIP-immunoreactive fibers were localized in the subepithelial connective tissue as well as the smooth muscle layers in both organs, although the peptidergic fiber networks were more prominent in the seminal vesicle. Most NPY-immunoreactive fibers were observed in the musculature of the seminal vesicle. In addition, NPY- and VIP-immunoreactive fibers were demonstrated in the walls of blood vessels. The results of our study suggest that the innervation of the prostate gland and seminal vesicle by various neuroactive peptides may be involved in the autonomic regulation of these organs in adult man, as well as sympathetic and parasympathetic nerve fibers.

Key words: Neuropeptides – Prostate gland – Seminal vesicle – Immunocytochemistry

Several studies have indicated that the cholinergic and adrenergic innervation of the prostate gland and seminal vesicle is predominantly localized in nerve fibers in the submucosal parts of these organs, whereas comparatively few fibers supply the smooth muscle cells (for review see [1]). Nerve fibers in the submucosal tissue have been demonstrated to accompany small blood vessels, which are located close to the glandular epithelium [12]. Recent studies have also demonstrated a peptidergic innervation of both prostate gland and seminal vesicle in several mammals; for example, the peptides enkephalin, neuropeptide Y (NPY), substance P (SP) and vasoactive intestinal polypeptide (VIP) have been shown to be present in the urogenital tract of the guinea pig and other mammals [23, 24, 29]. In addition, there is evidence from recent investigations that VIP and enkephalin also occur in the human urogenital tract [17, 26, 27]. Additional pharmacological studies have indicated that peptides may play a role, together with adrenergic and cholinergic innervation, in the physiological regulation of secretory activity, muscle contractility and blood supply of the urogenital tract [20, 23]. The goals of our immunohistochemical study were to expand previous investigations on the peptidergic innervation of the human prostate gland and seminal vesicle and to test the possibility that additional vasoactive peptides (i.e. calcitonin gene-related peptide; CGRP) may be present in both organs in adult man.

Materials and methods

Specimens of prostate gland and seminal vesicle were obtained soon after death (6–8 h) from two adult individuals (ages: 36 and 39 years). None of the cases had any history of urological disease. The tissue blocks were immersion-fixed in Bouin's fluid, dehydrated in ethanol, embedded in paraffin and cut on a sliding microtome at a thickness of 7 μm. Step sections at a distance of approximately 500 μm through the entire organs were processed for immunocytochemistry. Antibodies against the neuropeptides NPY, VIP, CGRP and SP were used for immunocytochemistry. The polyclonal antibodies against NPY, VIP and CGRP were produced in our laboratory by immunizing rabbits, and their specificity has been characterized previously [15, 25]. The antibody against SP was purchased from MILAB (Malmö, Sweden). After rehydration and blockage of endogenous peroxidase with 10% methanol/3% H_2O_2, the sections were incubated in the antibody dilutions for 24–48 h at 4°C. Antibodies were diluted 1:200 to 1:1000 in Tris-Triton-Carrageenan, pH 7.8 [21]. Immunohistochemical staining was carried out according to the unlabeled peroxidase-antiperoxidase method [22]. Control sections were incubated in antibody dilutions that were preabsorbed with the appropriate antigenic peptide (10 μg/ml of diluted antiserum). This procedure resulted in complete blockage of staining.

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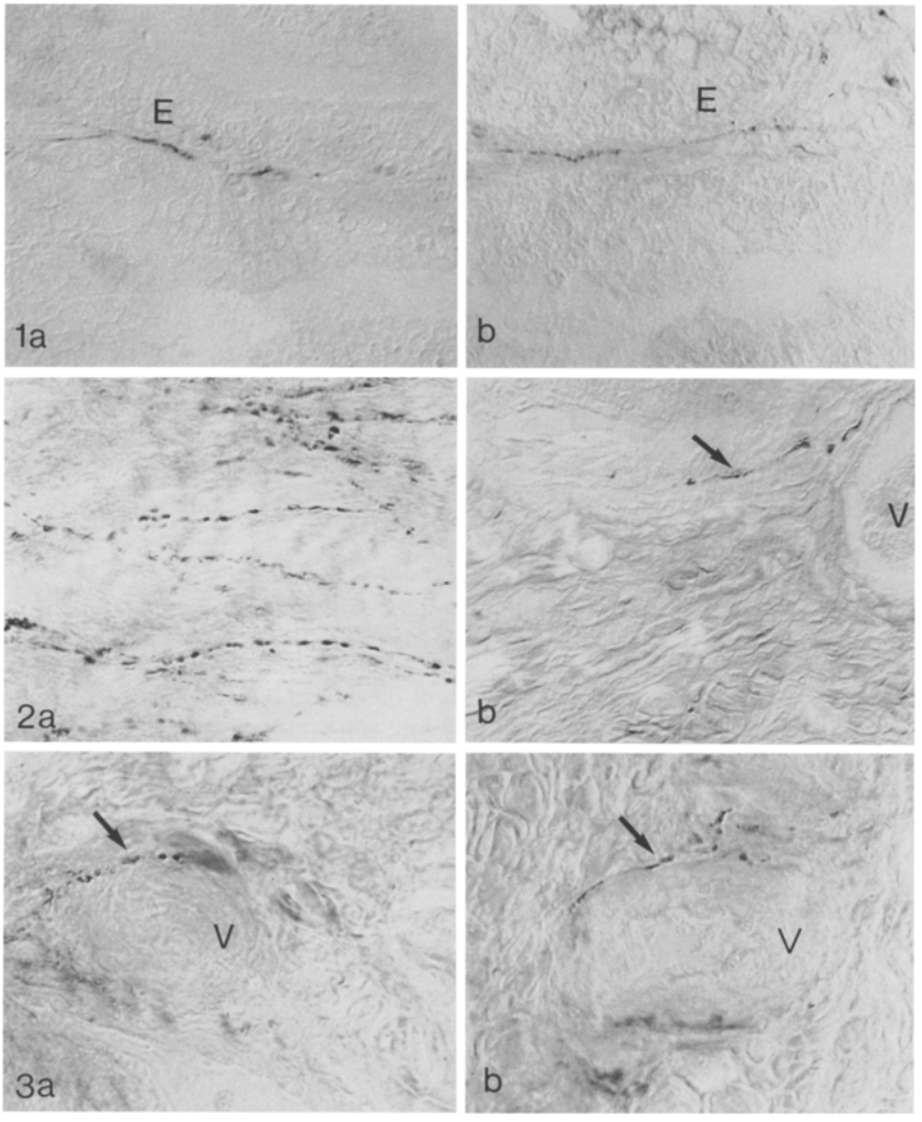

Fig. 1a, b. Section through the epithelial parts of the seminal vesicle stained for NPY (**a**) and prostate gland stained for VIP (**b**). In both organs, single NPY- and VIP-IR nerve fibers are located in the connective tissue underlying the glandular epithelium. ×800

Fig. 2a, b. NPY-IR fibers are present in the smooth musculature of the seminal vesicle (**a**) as well as of the prostate gland (**b**). Note the difference between the two organs in the number of NPY-IR varicose fibers. ×800

Fig. 3a, b. Cross sections through blood vessels within the seminal vesicle and prostate gland. **a** A VIP-IR fiber in the wall of a medium-sized artery in the seminal vesicle. **b** NPY-IR varicosities that are closely associated with a blood vessel in the prostate gland. ×800

Results

Overall, the peptidergic innervation of the prostate gland and seminal vesicle was found to be generally moderate to low. Among the four peptides investigated, only NPY- and VIP-immunoreactivity (IR) was seen in varicose fibers, whereas no immunostaining for SP and CGRP could be demonstrated, although the sensitivity and specificity of both antibodies have been demonstrated in previous immunohistochemical investigations of rat and human tissue [15]. The immunoreactive fibers were distributed at all levels throughout the glands.

Seminal vesicle

The density of NPY-IR fibers in the subepithelial connective tissue of the seminal vesicle was found to be moderate to low (Fig. 1a). However, a relatively dense network of NPY-IR fibers was located in the smooth musculature of the gland. These fibers were following the main direction of the muscle bundles (Fig. 2a). NPY-IR was also demonstrated to be closely associated with blood vessels, i. e. NPY-IR fibers were frequently observed in the adventitia and media of arteries.

Immunostaining for VIP revealed an even less dense innervation of the seminal vesicle than was demonstrated for NPY, although the general distribution followed a similar pattern. Again, VIP-IR fibers were observed in the subepithelial connective tissue as well as the walls of larger blood vessels (Fig. 3a).

Prostate gland

Single VIP-immunoreactive nerve fibers were observed in the connective tissue underlying the glandular epithelium (Fig. 1b). Occasionally, these fibers appeared to be associated with blood vessels.

Sparse NPY-immunoreactive fibers were demonstrated in the subepithelial connective tissue of the prostate as well as between strands of smooth muscle fibers (Fig. 2b).

In addition, single IR fibers, as well as small networks of NPY-IR fibers, were found surrounding larger blood vessels. A few IR fibers were seen in the media and adventitia of arteries and veins (Fig. 3b).

There was a significant difference between the seminal vesicle and the prostate gland in the number of NPY-IR fibers in the smooth musculature. The density of the NPY-IR network was found to be higher in the muscle layer of the seminal vesicle (Fig. 2a), although the musculature of the prostate gland is considerably larger.

Discussion

The innervation of the prostate gland and that of seminal vesicle have been demonstrated by several studies (for review see [1]. Both organs are innervated by the autonomic nervous system, i. e. dense cholinergic and aminergic fibers, arising from retroperitoneal ganglia [6, 7, 12] have been shown in the smooth muscle bundles [2, 9, 10, 19, 28], whereas the innervation of blood vessels in both organs was demonstrated to be less dense [3, 5]. Recent studies have expanded our knowledge on the distribution of specific neurotransmitters that may also play a role in the innervation of the urogenital tract. Sternquist et al. [24] demonstrated several neuropeptides, i. e. enkephalin, SP, VIP, NPY in the seminal vesicle of mouse, guinea pig and rabbit. The immunoreactive fibers were localized predominantly in the smooth muscle layers as well as blood vessels. In addition, a recent study demonstrated enkephalin- and VIP-IR fibers in the human genitourinary tract, including prostate gland and seminal vesicles [17, 26, 27].

Our findings in human tissue demonstrate several similarities and differences in the number and localization of peptidergic fibers between the two organs. Although our results are based on the observation in just two urologically healthy individuals, the possibility that the demonstration of a relatively sparse immunoreactive fiber network may be due to post mortem degradation of peptidergic structures can be excluded, since neuropeptides are known to be stable post mortem [4] and the tissue was fixed freshly. In both the prostate gland and the seminal vesicle solitary NPY- and VIP-IR nerve fibers were localized in the subepithelial connective tissue, as well as the wall of blood vessels, possibly innervating arteries and veins. However, a difference was noted concerning the peptidergic innervation of the smooth muscle layers of prostate and seminal vesicle. Relatively numerous NPY-IR fibers were localized between the muscle bundles of the seminal vesicles, whereas the muscle layers in the prostate gland were sparsely innervated by this neuropeptide. This finding may reflect the different ontogenetic origin of these organs. The seminal vesicle is a diverticle of the anlage of the ductus deferens which is known to be rich in autonomic innervation, whereas the prostate gland originates from the anlage of the urethra [14] and is considerably less densely innervated [1].

Our study also showed a lack of specific SP- and CGRP-IR in the human prostate gland and seminal vesicle. This finding is in contrast to a recent study that demonstrated SP-IR fibers in the seminal vesicle of mouse, guinea pig and rabbit [23]. As with some other peptides, there seem to be species-specific differences in the pattern and distribution of peptide-containing structures [16].

Our findings concerning the localization of VIP- and NPY-IR fibers in the prostate gland and seminal vesicle of man indicate that peptides may be involved in the innervation of the smooth muscle layers as well as blood vessels. Recent studies have indicated these peptides may play a role in the regulation of blood flow, muscle contractility and secretory activity of different organs [23, 24]. In the central nervous system, an interaction of neuropeptides and classic transmitters has been suggested for the regulation of synaptic activity in several brain regions [11], since several neuropeptides have been found to be co-localized with transmitters, i. e. GABA and somatostatin in thalamic nuclei [18], norepinephrine and NPY in locus coeruleus [8]. In the peripheral nervous system, NPY is co-localized with norepinephrine in nerve fibers in the urogenital tract of the guinea pig and rat [13, 24]. Although the peptidergic innervation of the prostate gland and seminal vesicle appears to be relatively low compared with the aminergic and cholinergic innervation, these results suggest that the classic concept of peripheral parasympathetic and sympathetic innervation of these organs should also include the possible modulatory role of peptidergic neurotransmission.

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References

1. Aumüller G (1979) Prostate gland and seminal vesicles. In: Oksche A, Vollrath L (eds) Handbuch der mikroskopischen Anatomie des Menschen VII/6. Springer, Berlin Heidelberg New York
2. Aumüller G, Metz W, Grube D (1976) Electron- and fluorescence-microscopical investigations of the human prostate (in German) Verh Anat Ges 70:895
3. Baumgarten HG, Falck B, Holstein A-F, Owman C, Owman T (1968) Adrenergic innervation of the human testis, epididymis, ductus deferens and prostate: a fluorescence microscopic and fluorimetric study. Z Zellforsch 90:81
4. Bird ED (1981) Problems of peptide-analysis in human post-mortem brain. In: Martin JB, Reichlin S, Blick KL (eds) Neurosecretion and brain peptides. Raven Press, New York, p 657
5. Bullon A, Lopez FL (1959) On the innervation of the human seminal vesicle and the homological organ in the dog (in German). Z Mikrosk Anat Forsch 65:133
6. Casas AP (1958) The innervation of the human prostate gland (in German). Z Mikrosk Anat Forsch 64:608
7. Denissov-Nikolskiĭ JI (1969) On the state of nerve cells of organ plexus of the prostate after castration (in Russian). Bull Eksp Biol Med [Moskva] 67:107
8. Everitt BJ, Hökfelt T, Terenius L, Tatemoto K, Mutt V, Goldstein M (1984) Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 11:443
9. Flickinger CJ (1972) The fine structure of the interstitial tissue of the rat prostate. Am J Anat 134:107
10. Hervonen A, Vaalasti A, Vaalasti T, Partanen T, Kanerva L (1976) Paraganglia in the urogenital tract of man. Histochemistry 46:307

11. Hökfelt T, Johansson O, Goldstein M (1984) Chemical anatomy of the brain. Science 225:1326
12. Jabonero V, Genis MJ, Santos L (1963) Observations on the osmium-zinciodine positive elements of the prostate gland (in German). Z Mikrosk Anat Forsch 69:167
13. Jacobowitz DM, Olschowka JA (1982) Bovine pancreatic polypeptide-like immunoreactivity in brain and peripheral nervous system: coexistence with catecholaminergic nerves. Peptides 3:569
14. Johnson FP (1920) The later development of the urethra in the male. J Urol 4:447
15. Lange W, Basting-Krieglsteiner C, Debbage P, Unger J (1986) Are motilin and CGRP cerebellar peptides? An immunohistochemical, chromatographic and radioimmunological study. Neurosci Lett [Suppl 26]:S322
16. Lange W, Unger J, Weindl A, Lang RE (1989) Demonstration of atrial natriuretic peptide/cardiodilatin (ANP/CDD) – immunoreactivity in the salt gland of the Pekin duck. Anat Embryol 179:465
17. Larsson L-I (1977) Occurrence of nerves containing vasoactive intestinal polypeptide immunoreactivity in the male genital tract. Life Sci 21:503
18. Oertel WH, Graybiel AM, Mugnaini E, Elde RP, Schmechel DE, Kopin J (1983) Coexistence of glutamic acid decarboxylase and somatostatin-like immunoreactivity in neurons of the feline nucleus reticularis thalami. J Neurosci 3:1322
19. Sjöstrand NO (1965) The adrenergic innervation of the vas deferens and the accessory male genital glands. An experimental and comparative study of its anatomical and functional organization in some mammals, including presence of adrenalin and chromaffin cells in these organs. Acta Physiol Scand 65 [Suppl 1]:1
20. Smith ER, Miller TB, Wilson MM, Appel MC (1984) Effects of vasoactive intestinal peptide on canine prostatic contraction and secretion. Am J Physiol 247:R701
21. Sofroniev MW (1983) Golgi-like immunoperoxidase staining of neurons producing specific substances or of neurons transporting exogenous tracer proteins. In: Cuello AC (ed) Immunohistochemistry. IBRO, p 431
22. Sternberger LA (1979) Immunocytochemistry, 2nd edn. Wiley, New York
23. Stjernquist M, Håkanson R, Leander S, Owman C, Sundler F, Uddman R (1983) Immunohistochemical localization of substance P, vasoactive intestinal polypeptide and gastrin-releasing peptide in vas deferens and seminal vesicle, and the effect of these and eight other neuropeptides on resting tension and neurally evoked contractile activity. Regul Pept 7:67
24. Stjernquist M, Owman C, Sjoeberg NO, Sundler F (1987) Coexistence and cooperation between neuropeptide Y and norepinephrine in nerve fibers of guinea pig vas deferens and seminal vesicle. Biol Reprod 36:149
25. Unger J (1984) Somatostatin and vasoactive intestinale polypeptide in rat and human brain and cerebrospinal fluid of patients with neurodegenerative diseases: immunohistochemical and radioimmunological investigations (in German). Inaugural dissertation, University of Munich
26. Vaalasti A, Linnoila I, Hervonen A (1980) Immunohistochemical demonstration of VIP, Met^5- and Leu^5-enkephalin immunoreactive nerve fibres in the human prostate and seminal vesicle. Histochemistry 66:89
27. Vaalasti A, Tainio H, Pelto-Huikko M, Hervonen A (1986) Light and electron microscope demonstration of VIP- and enkephalin-immunoreactive nerves in the human male genitourinary tract. Anat Rec 215:21
28. Watzka M (1928) On the occurrence of multiple nuclei in ganglionic cells of nerve fiber networks in the seminal vesicle (in German). Anat Anz 66:321

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