Invited Review

Neuropeptides in the seminal vesicles: locations, binding sites and functional implications

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Summary. The importance of neuronal factors in the normal physiology of the seminal vesicles has been traditionally underestimated when compared to the trophic role of androgens. Immunohistochemical, autoradiographical and pharmacological experiments have, however, raised the possibility that neuropeptides, such as vasoactive intestinal polypeptide (VIP), neuropeptide tyrosine (NPY) and calcitonin gene-related peptide (CGRP), are necessary for full seminal vesicle function and development. These neuropeptides may be involved in the regulation of secretion, smooth muscle tone and blood flow. Furthermore, neuropeptides may have functional interactions with androgens affecting, probably, androgen receptor-dependent gene expression in these glands. It is now timely to focus attention on the biological relevance of neuropeptides in the seminal vesicles.

Key words: Male accessory sex glands, Nerves, Vasoactive intestinal peptide, Neuropeptide Y, Calcitonin gene-related peptide

Introduction

The seminal vesicles are accessory glands of the male genital tract that contribute their secretions to the seminal plasma. They are paired, elongated, sac- or tubule-like structures comprised of a secretory mucosa surrounded by a smooth muscle coat and an external connective tissue sheath. Like other male accessory sex tissues, the seminal vesicles are highly hormonesensitive organs, dependent primarily on a continuous supply of androgens to maintain their structural and functional integrity (see Luke and Coffey, 1994). Data accumulated over the last years have, however, suggested that transmitter substances released from nerve endings supplying the male accessory sex glands are also involved in the regulation of specialized functions of these tissues (Guthrie et al., 1990; Wang et al., 1991a,b; Martínez-Piñeiro et al., 1993).

The seminal vesicles receive a dense autonomic innervation arising primarily from the pelvic ganglia (plexus) located close to many of the pelvic organs. In most species, neurons of the pelvic ganglia are a mixture of sympathetic and parasympathetic types, receiving synapses from preganglionic fibres travelling within the hypogastric nerves or pelvic nerves, respectively (see Dail, 1993). The nerves supplying the male sex accessory tissues contain not only the «classical» neurotransmitters acetylcholine and noradrenaline, but also an array of different peptidergic transmitter candidates. Of the peptides identified to date in seminal vesicle nerves the most widely distributed are neuropeptide tyrosine (NPY), vasoactive intestinal polypeptide (VIP), and calcitonin gene-related peptide (CGRP). However, the functional significance of the neuropeptides found in the seminal vesicles is still obscure.

In this paper, the immunocytochemical and autoradiographical evidence suggesting that several neuropeptides may be transmitter and/or modulator substances in the innervation supplying the seminal vesicles is described. Then, the possible functional roles of these neuropeptides are focused upon.

Vasoactive intestinal peptide (VIP)

Location and binding sites

In the seminal vesicles of several mammalian species, including humans, VIP-immunoreactive nerve fibres have been mainly found associated with the basis of the glandular epithelium and, in a smaller proportion, with the smooth muscle coat and blood vessels (Fig. 1A) (Vaalasti et al., 1980, 1986; Stjernquist et al., 1983; Moss et al., 1987; Lange and Unger, 1990; Yuri, 1990; Abou-Elmagd et al., 1992; Rodrigues et al., 1992; Pinho et al., 1994). As in other exocrine glands, VIP appears to be localized in cholinergic nerve fibres. Support for this hypothesis comes from studies showing that VIP immunoreactivity (i) is present in acetylcholinesterase (AChE)-positive nerve fibres supplying the hamster

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seminal vesicles (Pinho et al., 1994) as well as in tyrosine hydroxylase-negative (putative cholinergic) neuron somata of the rat major pelvic ganglion (Keast, 1995) and (ii) is localized to large, dense-cored vesicles in nerve varicosities which also contain small agranular vesicles similar to those found in the classical cholinergic terminals (Fig. 1B) (Gu et al., 1983; Vaalasti et al., 1986; Pinho et al., 1994).

Similarly to what has been demonstrated in other

peripheral tissues (see Lincoln et al., 1992), VIP may also coexist with nitric oxide synthase (NOS), the enzyme which catalyzes nitric oxide production, in cholinergic nerve fibres supplying the seminal vesicles. In the human seminal vesicles, NOS activity was found to be very high (Ehrén et al., 1994) and NOS-immunoreactive nerve fibres were shown to be numerous in both the muscular coat and the base of the epithelium (Jen et al., 1996). Since many of the subepithelial nerves



Fig. 1. VIP-immunoreactivity and VIP binding sites in the guinea-pig (A, B) and hamster (C, D) seminal vesicles. A. Cryostat section of guinea-pig seminal vesicle immunostained for VIP. Numerous varicose nerve fibres are observed near the epithelium (ep) and in the muscle coat (m). x 206. B. Ultrastructural localization of VIP-immunoreactivity in an axon profile of the guinea pig seminal vesicle. VIP immunogold labeling (15 nm gold particles, thick arrows) is localized over large granular vesicular profiles in varicosities also containing numerous unlabeled small agranular vesicular profiles (thin arrows). x 42,000. C and D. Light microscope autoradiographs showing the total binding of [1²⁵I] VIP to the hamster seminal vesicle tissues. Dark-field (C) and bright-field (D) photographs of the same field are shown. Note the higher grain density over the muscular coat (m) and blood vessels (bv). Iu, gland lumen. x 126

in the human seminal vesicles are known to contain VIP (Gosling and Dixon, 1994; Tainio, 1995), it seems likely that NOS and VIP also coexist in subepithelial nerves of these glands.

Binding sites for VIP have been identified, so far, in the seminal vesicles of rodents by autoradiography on tissue sections (Inyama et al., 1987; Power et al., 1988; Rodrigues et al., 1992; Pinho et al., 1994) and binding assays using isolated membrane preparations (Guijarro et al., 1991; Rodriguez-Pena et al., 1991a, 1994). In contrast to the distribution of VIP-immunoreactive nerve fibres, which is similar in the glands of all mammalian species studied, species variations in the localization of VIP binding sites have been reported. While in the rat (Power et al., 1988) and guinea-pig (Inyama et al., 1987; Power et al., 1988; Rodrigues et al., 1992) VIP binding sites appear to be associated almost exclusively with the secretory epithelium, in the hamster they are also found in the smooth muscle coat and in the wall of small-sized blood vessels (Fig. 1 C,D) (Pinho et al., 1994).

Functional implications

There is good evidence that VIP receptors in the seminal vesicles, as in most tissues, are coupled to cyclic AMP stimulation (Guijarro et al., 1991; Rodriguez-Pena et al., 1991a,b, 1994). Even though direct evidence for transmitter roles of VIP in the male accessory sex glands is still lacking, this neuropeptide has been receiving increasing attention as a putative endogenous modulator of secretory activity in these glands. While VIP acts typically as a secretagogue in exocrine glands (Lundberg et al., 1980; Peatfield et al., 1983; Holst et al., 1987; McMillian and Talamo, 1989; Tobin and Ekström, 1992), this effect has not been definitely proven in the male accessory sex glands. For instance, in the dog (Smith et al., 1984) and rat (Jacobs and Story, 1989) prostate, as well as in the guinea-pig seminal vesicles (Sjöstrand and Hammarström, 1995), VIP by itself has no effect on secretion, but in the dog prostate, this neuropeptide potentiates the secretory response to the cholinergic agonist pilocarpine whereas in the guineapig seminal vesicles it significantly reduces fructose secretion in response to the cholinergic agonist carbachol. The apparent confusion in the interpretation of the current literature on the effects of VIP on the secretion of male accessory sex glands may arise from species and/or organ differences and from the use of distinct experimental procedures.

Recent work from our laboratory showed that VIP may interfere with the secretory activity, as well as with the contractility of the hamster seminal vesicles (Pinho et al., 1994). It was shown that, in vitro, exogenous VIP stimulated per se secretory protein release, increasing exocytosis efficiency, and inhibited the contractile response to carbachol, without affecting the resting tension of the glands. On the other hand, in the smooth muscle of the guinea-pig seminal vesicles, where VIP binding sites are not found (Inyama et al., 1987; Power et al., 1988; Rodrigues et al., 1992), the neuropeptide appears to have no effect (Stjernquist et al., 1983). It is therefore conceivable that the conjugation of the VIP secretagogue and relaxant effects on the hamster seminal vesicles might lead to the accumulation of secretion in the gland lumen and help to avoid fluid expulsion from the organ in between ejaculations.

In addition, since VIP is a potent vasodilator in most vascular beds (see Fahrenkrug, 1993), it may also enhance seminal vesicle secretion by increasing the blood supply to the glandular elements. The presence of VIP binding sites in small arteries and arterioles of the hamster seminal vesicles further supports this hypothesis (Pinho et al., 1994). There are already precedents in other exocrine glands for secretion flow being positively correlated with blood flow (Buckle et al., 1995) and for VIP-induced secretory effects being mediated through local vasodilation (Lundberg et al., 1980). Any proposal that VIP acts physiologically to stimulate secretion in the seminal vesicles requires the characterization of the effects of activation of VIPergic nerves which has not been done yet.

Peptide histidine isoleucine

Peptide histidine isoleucine (PHI) derives from the same precursor molecule as VIP and in fact it colocalizes with VIP in many peripheral autonomic neurons (Bishop et al., 1984; Fahrenkrug et al., 1985; Holst et al., 1987). Immunoreactivity to PHI has been observed in human (Tainio, 1995), rat and guinea-pig seminal vesicles (Lamano Carvalho et al., 1986). In the guinea-pig and rat seminal vesicles, nerve fibres immunoreactive for PHI, although less numerous, exhibit a distribution similar to that of VIP-containing nerves, suggesting co-localization. Double-labeling studies using antisera with proven specificity are, however, required to confirm this co-existence. Future characterization of the effects of activation of VIPergic nerves in the seminal vesicles may have to take into consideration the simultaneous release of PHI and the possible effects of the two neuropeptides together, rather than each of them separately.

Neuropeptide tyrosine (NPY)

Location

High levels of NPY-immunoreactivity have been found in the reproductive organs which receive a dense sympathetic nerve supply, such as the vas deferens and the male accessory sex glands. In the seminal vesicles of man (Adrian et al., 1984; Lange and Unger, 1990; Tainio, 1995) and rodents (Lamano Carvalho et al., 1986; Moss et al., 1987; Stjernquist et al., 1987; Schindelmeiser et al., 1989; Yuri, 1990; Properzi et al., 1992; Iravani and Zar, 1994) NPY-immunoreactive nerves appear to be the major peptide-containing neuronal component. These nerves are predominantly

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found in the smooth muscle layer of the glands. However, some NPY-immunoreactive nerve fibres are also detected in the subepithelial connective tissue and associated to blood vessels (Fig. 2A). As observed in other peripheral tissues (see Sundler et al., 1986), NPYimmunoreactive nerve fibres in the seminal vesicles have a similar distribution pattern to that of nerves containing the cathecolamine synthesising enzymes tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH), thus suggesting that they represent noradrenergic neurons (Lamano Carvalho et al., 1986; Stjernquist et al., 1987; Yuri, 1990; Tainio, 1995). However, nonnoradrenergic populations of NPY-immunoreactive nerve fibres may also occur in the seminal vesicles (Fig. 2). This is suggested by studies showing that (i) extrinsic denervation or chemical sympathectomy leads to a large reduction, but not abolition, of the NPY-immunoreactivity in the rat seminal vesicles (Lamano Carvalho et al., 1986; Iravani and Zar, 1994), and (ii) NPYcontaining nerves are observed beneath the epithelium of the prepubertal human seminal vesicles and neither THnor DBH-containing nerves are detected in this location (Gosling and Dixon, 1994). NPY-immunoreactive nerves, lacking noradrenaline and/or containing other peptides (e.g. VIP), have already been demonstrated in several urogenital organs of animals and humans (Schmalbruch and Wagner, 1989; Keast, 1992; Edyvane et al., 1995). Data concerning the coexistence of NPY with other substances in nerves supplying the seminal vesicles are not yet available.

Functional implications

The predominant distribution of NPY in the muscle

layer of the seminal vesicles suggest a physiological role for the peptide in the regulation of the glands' smooth muscle tone. However, the identity of the substance(s) responsible for the motor transmission in the seminal vesicles is still unclear. While some authors suggest a role for noradrenaline (Iravani and Zar, 1994) or both noradrenaline and acetylcholine (Hib et al., 1984; Terasaki, 1989; Sadraei et al., 1995), others report the existence of non-cholinergic, non-adrenergic mechanisms (Stjernquist et al., 1983). Apart from differences in experimental setups, these discrepancies may also reflect species variations since male accessory sex tissues are known to exhibit marked anatomical and biochemical diversity (see Luke and Coffey, 1994). Despite the controversy, it is generally agreed that noradrenaline exerts a major tonic influence on the seminal vesicles musculature, which does not preclude the possible actions of other neurotransmitters or neuromodulators in these glands. Results from pharmacological studies in both the guinea-pig and rat seminal vesicle indicate that NPY has no direct motor effects on these glands since their resting tone is unaffected by the peptide (Stjernquist et al. 1987; Iravani and Zar, 1994). However, NPY inhibits electricallyinduced, neurally mediated contractions of the isolated rat seminal vesicles, but not contractile responses elicited by noradrenaline applied exogenously (Iravani and Zar, 1994). This effect of NPY is adrenergically mediated and a presynaptic mode of action (e.g. inhibition of noradrenaline release) is suggested (Iravani and Zar, 1994). A similar interpretation has long been given for the NPY-inhibition of sympathetic nerveevoked responses in the rodent vas deferens (see Lundberg, 1996).



Fig. 2. Cryostat sections of guinea-pig seminal vesicles immunostained for Neuropeptide Y (A, NPY) and tyrosine hydroxylase (B, TH). In the connective tissue beneath the epithelium (ep), NPY-immunoreactive nerve fibres are more numerous than TH-containing nerves. m: muscle coat. x 206

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Apart from a presumable role in the regulation of smooth muscle tone, NPY may also participate in the regulation of epithelial secretory function in the male accessory sex glands. This is supported by the presence of NPY-immunoreactive nerve fibres in association with the basal glandular epithelium (Fig. 2A) and by recent data suggesting the existence of NPY receptors in epithelial cells of the rat ventral prostate (Solano et al., 1994).

Calcitonin gene-related peptide (CGRP)

Location and binding sites

The presence of CGRP in nerve fibres of the guineapig (Lamano Carvalho et al., 1986; Rodrigues et al., 1992), rat (Lamano Carvalho et al., 1986), hamster (Afonso et al., 1996) and human (Tainio, 1995) seminal vesicles has been documented by immunohistochemical and/or radioimmunological analysis. In general, CGRP immunoreactivity is localized in nerve bundles running along the periphery of the muscle coat and in single nerve fibres occurring within the muscle coat, subepithelial connective tissue and around blood vessels. In many other tissues, CGRP has been shown to coexist with substance P in afferent nerves (see Maggi, 1995). In the seminal vesicles, the two peptides also appear to coexist, although as a general trend the number of CGRP-immunoreactive nerves outscores that of substance P (personal communication). The sensory origin of these nerve fibres in the seminal vesicles has not been demonstrated yet, but it has been determined in other parts of the genitourinary system from various species by using the sensory neurotoxin capsaicin (see Maggi, 1995). Recently, we have reported, for the first time, the presence of CGRP binding sites in the muscle coat of the hamster seminal vesicle and prostate anterior lobe (Fig. 3) (Afonso et al., 1996).

Functional implications

A neuroeffector role for CGRP in the seminal vesicles has not been demonstrated yet. In other reproductive organs, such as the rat (Goto et al., 1987; Tan et al., 1994) and mouse (Al-Kazwini et al., 1986) vas deferens, CGRP has been shown to exert a relaxant action on the smooth muscle. Recent pharmacological studies have also revealed a relaxant action of CGRP in both noradrenaline and carbachol-contracted preparations from the hamster seminal vesicles (Afonso et al., 1996). These results point towards a physiological role for CGRP as an inhibitory modulator of the autonomic control of contractility in the seminal vesicles.

The presence of specific binding sites for CGRP in the secretory epithelium of the male accessory sex glands of the hamster could not be demonstrated (Afonso et al., 1996). However, the presence of CGRPcontaining nerves close to the seminal vesicle epithelium in all the species studied suggests that the neuropeptide may also affect secretory processes in these glands, as reported for other exocrine tissues (Kuo et al., 1990; Salo et al., 1995). This hypothesis is supported by recent results showing that CGRP is able to activate adenylyl cyclase in cultured normal human prostate epithelial cells as well as in several prostate cancer cell lines



Fig. 3. Light microscope autoradiographs showing the total binding of [¹²⁵I] CGRP to the hamster seminal vesicle tissues. Dark-field (A) and bright-field (B) photographs of the same field are shown. Note that silver grains are preferentially accumulated over the muscular coat (m). ep, epithelium; lu, gland lumen. x 202

(Gkonos et al., 1995).

Other neuropeptides

Other peptides have been identified in seminal vesicle nerves by immunohistochemical and radioimmunological studies in addition to those mentioned above. Among them are substance P (Rama Sastry et al., 1982; Gu et al., 1983; Stjernquist et al., 1983; Lamano Carvalho et al., 1986; Yuri, 1990), enkephalins (Vaalasti et al., 1980, 1986; Rama Sastry et al., 1982; Tainio, 1995), somatostatin (Gu et al., 1983; Tainio, 1995) and gastrin-releasing peptide (Stjernquist et al., 1983). Substance P-immunoreactive nerve fibres occur in different amounts in the smooth muscle and mucosa of the seminal vesicles from different species. Nerve fibres with gastrin-releasing peptide immunoreactivity are found in a small number, in the smooth muscle layer. Controversy exists concerning the existence of enkephalin-immunoreactive nerve fibres within the human seminal vesicles. While Gu et al. (1983) were unable to demonstrate immunoreactivity for enkephalins within the seminal vesicles, Vaalasti et al. (1980) and Tainio (1995) reported occasional fibres in the muscle coat. As with VIP or NPY, these neuropeptides may also be colocalized in some of the cholinergic or noradrenergic nerves in the seminal vesicles or, alternatively, may form separate non-cholinergic, nonadrenergic nerve populations. The effects of these neuropeptides on the seminal vesicles have so far received very little attention. One in vitro study on the guinea-pig seminal vesicles reported that substance P and gastrin-releasing peptide had contracting effects on the smooth muscle and also potentiated the response to electrical stimulation (Stjernquist et al., 1983). In the same study, Leu-enkephalin had no effect on either the resting tension or the response to transmural electrical stimulation.

Interaction of neuropeptides with androgens

Much of the effort devoted to the understanding of how androgens interact with neurotransmitters at the level of the male accessory sex glands has focused on the classical transmitters acetylcholine and noradrenaline. For instance, Kinghorn et al. (1987) demonstrated that only in the presence of these neurotransmitters were androgens able to induce secretory protein synthesis in cultured rat seminal vesicle epithelial cells. Using denervated prostatic renal grafts, Guthrie et al. (1990) showed that the restoration of the expression of a prostatic secretory protein by isoprenaline, a α -adrenergic agonist, required the presence of androgens.

Studies in other steroid-responsive tissues support a model by which neurotransmitters, through the generation of second-messengers, phosphorilate and activate steroid receptors in a ligand-independent manner, thereby modulating steroid receptor-dependent gene transcription (Mani et al., 1994; Katzenellenbogen, 1996). The observation that a typical neuropeptide second messenger such as cyclic AMP is able to produce androgen-like induction of several enzymes in the seminal vesicle is consistent with that model (Singhal et al., 1970).

Effects of castration on neuropeptides and their receptors

Studies in many neuronal systems have revealed that the levels of neuropeptides and their receptors are also under the influence of the endocrine environment (De Kloet et al., 1985; Rostene et al., 1992; Usdin et al., 1994). In the case of pelvic autonomic ganglia supplying the male sex tissues, a considerable variability in the sensitivity of peptidergic nerve types to sex steroids appears to exist. Also the sensitivity of a given neuropeptide to the hormonal environment varies in relation to the species and, within the same species, to the organ and region of the organ. For example, castration decreases the NPY content in both the epididymal and the prostatic segments of the rat vas deferens, but affects differently the response of the two segments of the ductus to NPY (Bitran et al., 1991). Likewise, short photoperiods, with low androgen levels comparable to those observed after castration, decrease the density of NPY-containing nerves in the smooth muscle layer of the djungarian hamster vas deferens but have no effect on the NPY nerve density in the seminal vesicles (Schindelmeiser et al., 1989).

Possible interactions between neuropeptides, e.g. VIP, and androgens have been recently studied by our group in the seminal vesicles of medium- (15 days) and long (2 months)-term castrated adult golden hamsters (Pinho et al., 1996). This study provided evidence that (i) castration-induced changes in autonomic nerve fibres supplying the hamster seminal vesicle are not generalized, affecting some groups of nerves, while leaving others, such as those containing VIP, relatively unscathed; (ii) VIP binding sites in the muscular coat of the hamster seminal vesicle are sensitive, but not to a high degree, to androgens since they display a delayed response to castration. Our studies confirm previous work by other investigators indicating that the static levels of VIP in the seminal vesicles do not seem to be affected by androgens (Rotsztejn et al., 1980), but that these hormones induce or maintain VIP receptors in the male sex accessory glands (Carmena et al., 1986, 1988; Juarranz et al., 1994; Gkonos et al., 1995).

It has been shown that the sensitivity of VIP binding sites to castration and subsequent androgen replacement depends on their anatomical location in the male accessory sex glands. For example, in the rat prostatic epithelial cells, the number of VIP binding sites is dramatically decreased 2 days after castration and returns to normal levels when subsequently treated with testosterone for 4 days (Carmena et al., 1986). In the muscle coat of the hamster seminal vesicle, VIP binding sites are long-lived in the face of androgen deprivation since a 15-day period of castration has no effect on their density (Pinho et al., 1996). Interestingly, in the hamster seminal vesicles too, the same castration period is able to suppress VIP binding sites in the vascular smooth muscle (Barroso et al., 1995). The differential vulnerability of VIP binding sites to hormonal manipulation in tissues from the male sex glands may reflect, in part, the difference in the degree of androgendependency of the specific tissues, which is known to vary between species, and within the same species, between different lobes of the same gland (Higgins et al., 1976; Prins and Birch, 1993; Mata, 1995). On the other hand, it is still unknown whether the two separate forms of VIP receptor identified so far, the VIP1 and VIP2 receptors, are asymmetrically distributed in these glands, and if so, whether they show a different sensitivity to androgens. In rat organs such as the brain, pituitary, testes, ovary and uterus they have distinct, sometimes overlapping, patterns of expression and may subserve different functions (Usdin et al., 1994; Sheward et al., 1995). Considering the general mechanism of action of steroid hormones, it is possible that changes in VIP binding site density following androgen manipulation are due to changes in the transcription rate and/or stability of the VIP receptor mRNA.

The influence of androgens on neuropeptide receptors in the male accessory sex glands may not only be manifested as changes in binding site density but also as alterations in receptor coupling to signal transduction systems. Androgen-related alterations in coupling efficiency between adenylyl cyclase and the VIPreceptor complex have been reported in rat prostatic epithelial cells during sexual maturation (Carmena et al., 1986) and after castration (Carmena et al., 1988). These changes have been related to modifications within the plasma membrane fluidity which could influence the coupling between the subunits that integrate this system of signal transduction (Carmena et al., 1988, 1991, 1992a). This adds another level at which the VIP receptor/effector system may be modulated by androgens in the male accessory sex glands. Important changes have also been described in the VIP receptor/ adenylyl cyclase system in both the seminal vesicles and prostate of rats with experimentally-induced diabetes mellitus suggesting a physiopathological role for this neuropeptide in the diabetic neuropathy of the genitourinary tract (Carmena et al., 1992b; Rodríguez-Pena et al., 1994). In contrast to VIP receptors, androgens may not be required for the expression of CGRP receptors in the male accessory sex glands. This idea is suggested by a recent study showing that CGRP activation of adenylyl cyclase in cultured normal human prostate epithelial cells as well as in several prostate cancer cell lines occurs in both androgen-responsive (LNCaP, normal prostate) and non-responsive (PPC-1) cells (Gkonos et al., 1995).

Further investigations in the male sex glands to determine androgen-related changes on neuropeptide

receptor coupling to second messenger systems or on expression of mRNA for particular neuropeptide receptors would be of great interest.

Neuropeptides and development

From the analysis of the very few developmental studies on neuropeptides and male accessory sex glands that are on record, one can postulate a role for neuropeptides in gland growth and functional development during the early stages of life. For instance, the peak of the VIP stimulatory effect on adenylyl cyclase activity in the rat ventral prostate is found between 1 and 2 months of age (Juarranz et al., 1994), a period of extensive growth and cytodifferentiation of these glands (Flickinger, 1974), and coincides with an increase in the density of nerve fibres immunoreactive for VIP and other peptides in the male genital system at this age (Properzi et al., 1992). Interestingly, in the human seminal vesicles, except for the subepithelial VIPergic innervation, the pattern and density of the peptidergic innervation seems to be already established in infancy (3 years), suggesting that, contrary to the rat, it is not dependent upon pubertal-induced hormonal changes (Gosling and Dixon, 1994). The absence of subepithelial VIP-containing nerves in human prepubertal glands, which is in marked contrast to the adult glands, was interpreted as a consequence of a difference in secretory activity between immature and the mature glands. This observation argues for a role for VIP in the functional differentiation of the secretory cells in the seminal vesicles.

Concluding remarks

It is apparent from the above outline that many of the neuropeptides present in autonomic nerves supplying the seminal vesicles are likely to coexist with other peptides and/or with «classical» neurotransmitters such as noradrenaline and acetylcholine. The densities of peptide-containing nerves and specific receptors for neuropeptides in the seminal vesicles may vary in different species, and within the same species, in different tissues of the glands as well as with age and under different hormonal environments. However, current knowledge of the involvement of neuropeptides in nerve-stimulated responses in the seminal vesicles is still very limited, and this would seem to be an important issue for future research in this field. For instance, demonstration of neuropeptide release after nerve stimulation and inhibition of nerve- induced responses with specific blockers for neuropeptides are obligatory events in determining whether these substances participate as neurotransmitters in this gland. Furthermore, detailed studies on the characterization and localization of neuropeptide receptors and on their pharmacological actions within the seminal vesicles would certainly shed additional light on the issue. Finally, understanding the functional interactions

between androgens and neurotransmitter substances in the seminal vesicles will improve our ability to perceive the mechanisms underlying the regulation of the male accessory sex gland function.

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