J. Anat. (1990), **171**, pp. 169–178
With 4 figures
Printed in Great Britain

Serotonin-, somatostatin- and chromogranin A-containing cells of the urethro-prostatic complex in the sheep. An immunocytochemical and immunofluorescent study

A. VITTORIA, E. LA MURA, T. COCCA AND A. CECIO

Department of Biological Structures, Functions and Technology, University of Naples "Federico II", Via F. Delpino 1, 80137 *Naples, Italy*

(*Accepted* 23 *January* 1990)

INTRODUCTION

Neuroendocrine (NE) cells (Pearse, 1977) or paraneurons (Fujita, 1976, 1980) produce bio-amines and/or hormonal peptides and are diffused throughout the body of many animal species. They produce also three chromogranins, acidic proteins recently assumed to be their 'markers', the most widespread of which is chromogranin A (Rindi *et al.* 1986).

Since Pretl (1944) and Feyrter (1951) first showed histochemically the presence of such cells in the human urethral and prostatic epithelia, they have been described in the urethro-prostatic complex of various mammals. Histochemical (Koch & Engelhardt, 1959; Kazzaz, 1974; Vittoria, Paino, Cocca & Cecio, 1983; Paino *et al.* 1985), fluorescent (Hakanson, Larsson, Sjoberg & Sundler, 1974) and ultrastructural (di Sant'Agnese & de Mesy Jensen, 1984) studies showed that urogenital NE cells are a cellular population consisting of different morphological cytotypes, one of which produces the bio-amine 5-hydroxytryptamine (5-HT) or serotonin. Recently, immunohistochemical studies showed the widespread distribution of the serotonin cytotype (Fetissof *et al.* 1983; di Sant'Agnese, de Mesy Jensen, Churukian & Agarwal, 1985; Abrahamsson *et al.* 1986; Iwanaga, Hanyu & Fujita, 1987) and the presence of somatostatin- (di Sant'Agnese & de Mesy Jensen, 1984), calcitonin- (Fetissof *et al.* 1986; di Sant'Agnese, 1986), bombesin- (di Sant'Agnese, 1986), α-HCG- (Fetissof, Arbeille, Guilloteau & Lanson, 1987) and TSH- (Abrahamsson *et al.* 1986) containing NE cells in the normal prostatic and urethral epithelia of man. In the urogenital tract of non-human species, only serotonin-producing paraneurons have been found by means of immunohistochemical techniques. They have been described in the urethro-prostatic complex of the buffalo (Vittoria *et al.* 1986*a*) and dog (Hanyu, Iwanaga, Kano & Fujita, 1987).

The localisation and distribution of serotonin-, chromogranin A- and somatostatin-containing NE cells in the normal prostate and pelvic urethra of the sheep are immunocytochemically described here, as well as the co-localisation of the amine and chromogranin A in the same cell.

MATERIALS AND METHODS

The prostate of the ram is dispersed along the mucosa of the pelvic urethra from its collicular segment to the caudal one third. Samples of urethra and prostate were simultaneously collected from the proximal, middle and distal thirds of the pelvic

Table 1. *Details of the antisera used*

Antigen	Source	Code no.	Raised in	Dilution	Incubation time (hours)	T °C
Chromogranin A	Immunonuclear Corp. (Stillwater, MN)	20086	rabbit	1:100–1:500	1·30	37
5-HT	Immunonuclear Corp.	20080	rabbit	1:100–1:500	1·30	37
5-HT	Immunonuclear Corp.	20079	goat	1:100	1·30	37
Somatostatin 1–14	Dako/Accurate (Westbury, NY)	A 556	rabbit	1:300	16	4
Bombesin	Immunonuclear Corp.	30H2T	rabbit	1:100	24	4
ACTH	Immunonuclear Corp.	24H2T	rabbit	1:100	24	4
Calcitonin	Immunonuclear Corp.	25H2T	rabbit	1:100	24–48	4
Gastrin 1–17	Immunonuclear Corp.	05H2T	rabbit	1:100	24	4
Substance P	Immunonuclear Corp.	20064	rabbit	1:100	24	4
VIP	Immunonuclear Corp.	20077	rabbit	1:100	24	4

urethra of six rams, after death, by means of coronal sections. The animals were killed by barbiturate overdose. The material was fixed in Bouin's fluid or in 4% paraformaldehyde in phosphate buffer (pH 7·3; 0·1 M) or in 10% formaldehyde, dehydrated and embedded in Paraplast. Histological sections (4–5 μm) were stained by the argentaffin technique of Masson–Hamperl modified by Singh (1964) and by the argyrophil methods of Grimelius (1968), Bodian modified by Singh (1962) and Linder (1978). The last-named technique has been recently applied to the study of NE cells (Cecio, Vittoria & Budetta, 1985; Cecio, Vittoria, Budetta & Corona, 1988*a*). Sections from formaldehyde-fixed material were hydrated, immersed in the same fixative for 15 minutes to 1 hour, mounted and observed by fluorescence microscopy. Such a treatment is found to enhance the formaldehyde-induced fluorescence (FIF) of tissue serotonin. Other sections were stained by means of the peroxidase-antiperoxidase (PAP) technique of Sternberger (1979) using the primary antibodies listed in Table 1. The site of the antigen-antibody reaction was revealed by diaminobenzidine (DAB) or aminoethylcarbazole (AEC). To obtain negative controls the antisera were pre-absorbed with an excess of the respective antigens and the immunological complexes were separately incubated on sections. The anti-5-HT reactions were controlled by absorbing the two primary antibodies, from the rabbit or goat (see below), with the complex 5-HT-aldehyde-bovine serum albumin.

The co-localisation of 5-HT and chromogranin A in the same NE cell was studied by a double-labelling immunofluorescent technique (Wessendorf & Elde, 1985), a brief description of which follows. The primary antibodies, anti-5-HT, raised in the goat and anti-chromogranin A, raised in the rabbit, were simultaneously incubated on sections at a dilution of 1:100. For reaction time and temperature see Table 1. In the second immunological step, a lissamine-rhodamine isothiocyanate (LRITC)-labelled swine antibody to goat IgG (SwaG; Tago, Burlingame, 6301) and a fluorescein isothiocyanate (FITC)-labelled swine antibody to rabbit IgG (SwaR; Dako, F205) were applied to the sections in a mixture in which each antibody had a final dilution of 1:8, for one hour at room temperature. Controls were performed as described by Wessendorf & Elde (1985) so as to exclude inappropriate reactions among the primary and/or secondary antibodies used. Such artefacts could simulate co-existence of 5-HT and chromogranin A in the same cell. The technique was performed on sets of serial sections randomly collected from the urethro-prostatic complex. Only paraneurons that were clearly observed were considered for the co-localisation counting. The

sections were examined with a Leitz microscope equipped with a reflecting illumination system for fluorescence, using a filter set for FITC (450–490 nm bandpass excitation, and 525/20 nm bandpass barrier-filter) and another to LRITC (546/14 nm bandpass excitation, and 580 nm longpass barrier-filter). Using such filter sets, no red emission was ever visible during blue excitation and conversely no yellow-green emission was visible during green excitation.

The double immunofluorescent staining as well as the staining for anti-5-HT, PAP,DAB (or AEC) were performed only on sections from paraformaldehyde-fixed material. Some sections were previously incubated at 37 °C with 0·06% trypsin in TRIS buffer (pH 7·6, 0·05 M) for 20 minutes.

The co-localisation of serotonin and chromogranin A was also studied by means of serial sections (3 μm) alternately immuno-stained for the amine and the protein by means of the PAP method.

RESULTS

Both argyrophil (Fig. 1) and argentaffin (Fig. 2*a*,*b*) paraneurons were contained in the prostatic and urethral epithelia of the ovine pelvic urethra. They were very numerous in the proximal third of the urethra and decreased in number cranio-caudally along its middle and distal segments. Their highest density was formed in the collicular segment.

Prostatic NE cells were present in both the ductal and acinar epithelia in which they were generally interposed between the basal membrane and the exocrine cells. They were rounded cells that rarely showed cytoplasmic dendrite-like processes directed towards the basal membrane. Acinar NE cells were isolated or grouped in small clusters whose elements were contained in the epithelium of adjacent glands.

Urethral paraneurons were round, triangular or elongated in shape. The round paraneurons were generally small and widely spaced while the elongated cells had dendrite-like processes directed towards the neighbouring cells, subepithelial spaces or luminal surface. Often a single paraneuron showed two or three cytoplasmic processes variously directed.

Linder- and Grimelius-positive cells were much more numerous than Bodian-positive cells, both in prostatic and urethral epithelia. The argyrophilia of such cells was generally, but not always, diffused throughout the whole cytoplasm. On the contrary, the argentaffinity of Masson–Hamperl-positive cells was restricted to clusters of granular material contained in the cellular body or in dendritic processes.

Formaldehyde re-fixed sections from each segment of the pelvic urethra showed the presence of prostatic and urethral yellow-green fluorescent paraneurons (Fig. 2*c*). Such cells were mainly polymorphic in shape.

The anti-substance P, -calcitonin, -bombesin, -gastrin, -ACTH and -VIP reactions never showed positive results.

The localisation and distribution of chromogranin A- (Fig. 3*a*,*b*) and serotonin-positive (Fig. 3*c*,*d*) NE cells were very similar, as was expected, to those just described histochemically as argyrophil paraneurons. It must be emphasised that such cells were widely distributed in both the prostatic and urethral epithelia along the collicular segment.

The double labelling immunofluorescent staining of sections from the proximal, middle and distal third of the urethra did not show a high percentage of NE cells containing both serotonin and chromogranin A. In particular, 200 paraneurons were studied; 25% of them were found to contain both the substances, 25% serotonin

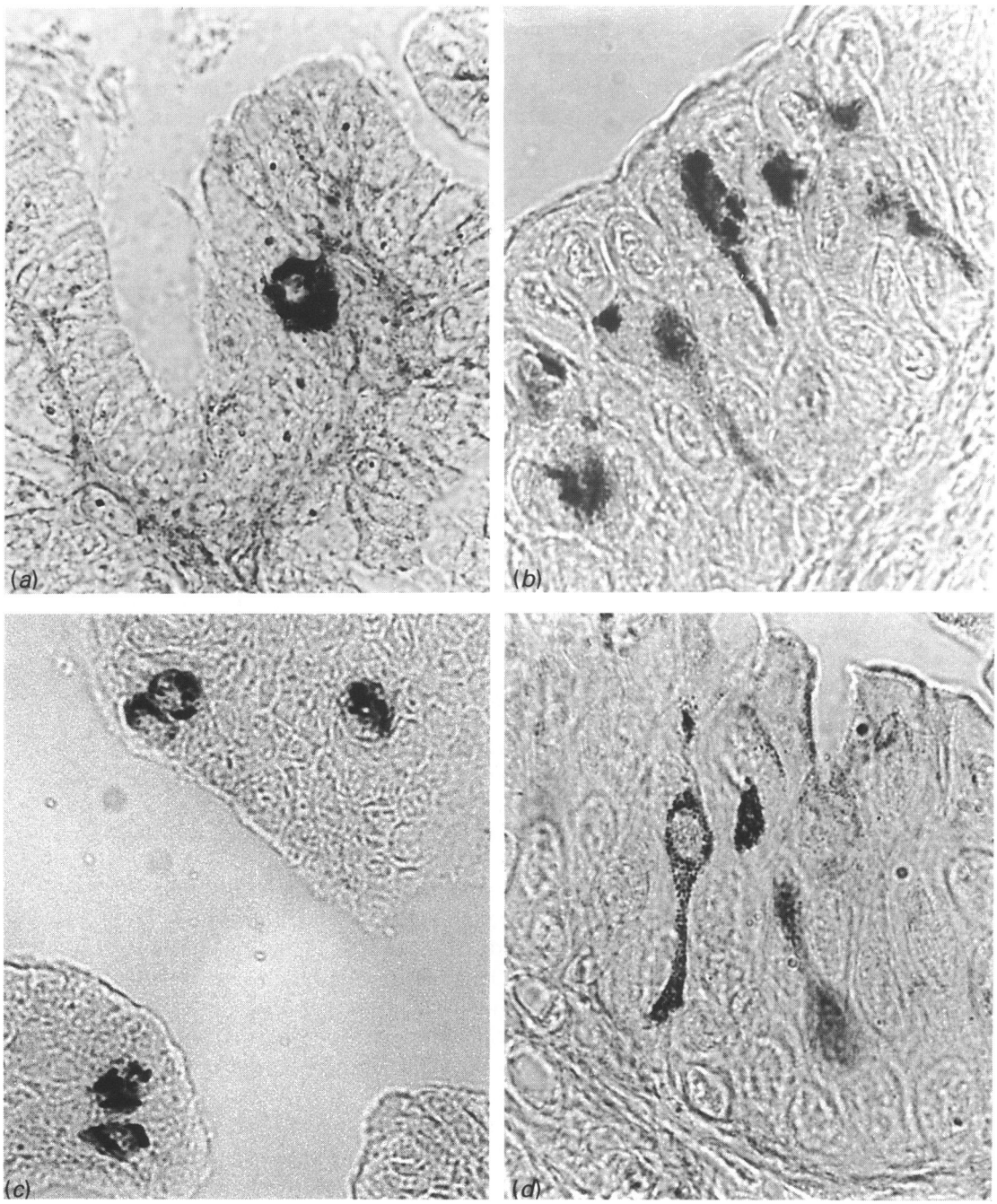

Fig. 1 (*a–d*). Linder- (*a, b*) and Grimelius- (*c, d*) positive neuroendocrine cells in the prostatic (*a, c*) and urethral (*b, d*) epithelia. × 1280.

alone and 50% of them chromogranin A (Fig. 4*a,b*). The careful observation of 130 paraneurons, serially sectioned and immuno-stained by the PAP method as described above showed the percentage of cells containing both substances to be slightly higher (30%).

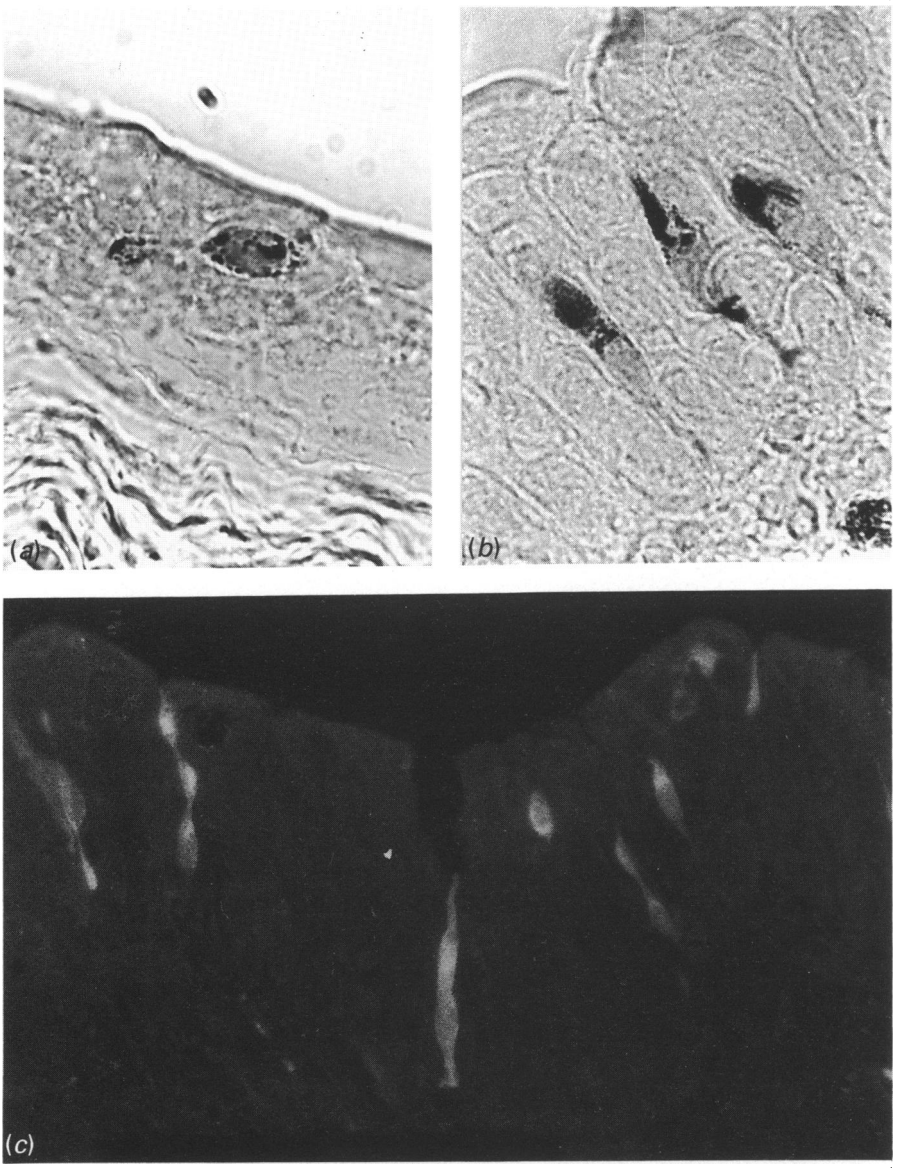

Fig. 2(*a–c*). Prostatic (*a*) and urethral (*b, c*) paraneurons visualised by the argentaffin technique of Masson–Hamperl (*a, b*) and by formaldehyde-induced fluorescence (*c*). (*a, b*) ×1280; (*c*) ×640.

Such cells were more numerous in urethral than in prostatic epithelium and normally occurred in every section observed. They were generally elongated in shape with cytoplasmic processes often extending from the luminal surface to the basement membrane. The number of such cells seen was not modified by trypsinisation.

Rare somatostatin-containing NE cells were found in the collicular prostate and in the epithelia of the proximal and middle thirds of the urethra (Fig. 4*c,d*). They never showed an intense staining reaction and often presented a unique cytoplasmic extension directed towards the basal membrane.

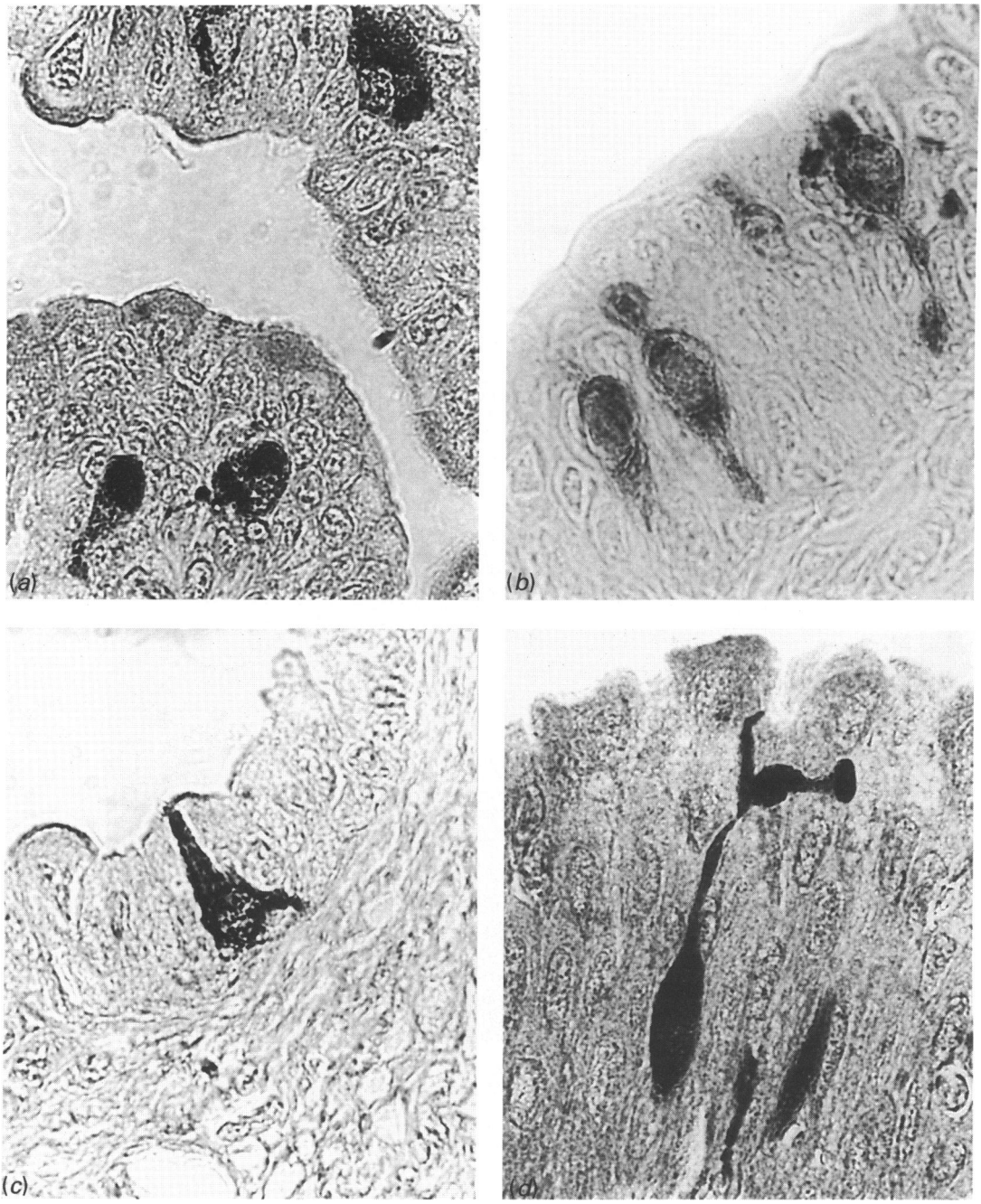

Fig. 3 (*a–d*). Chromogranin A- (*a, b*) and 5-HT- (*c, d*) containing neuroendocrine cells in the prostatic (*a, c*) and urethral (*b, d*) epithelia. Both 'closed' and 'open' cellular types are evident. × 1280.

DISCUSSION

The collicular segment of the human (di Sant'Agnese *et al.* 1985), canine (Hanyu *et al.* 1987) and ovine urethra contains a high number of NE cells both in the prostatic and urethral epithelia. This segment probably plays a critical role in the emission of urogenital fluids, at least in the species reported above.

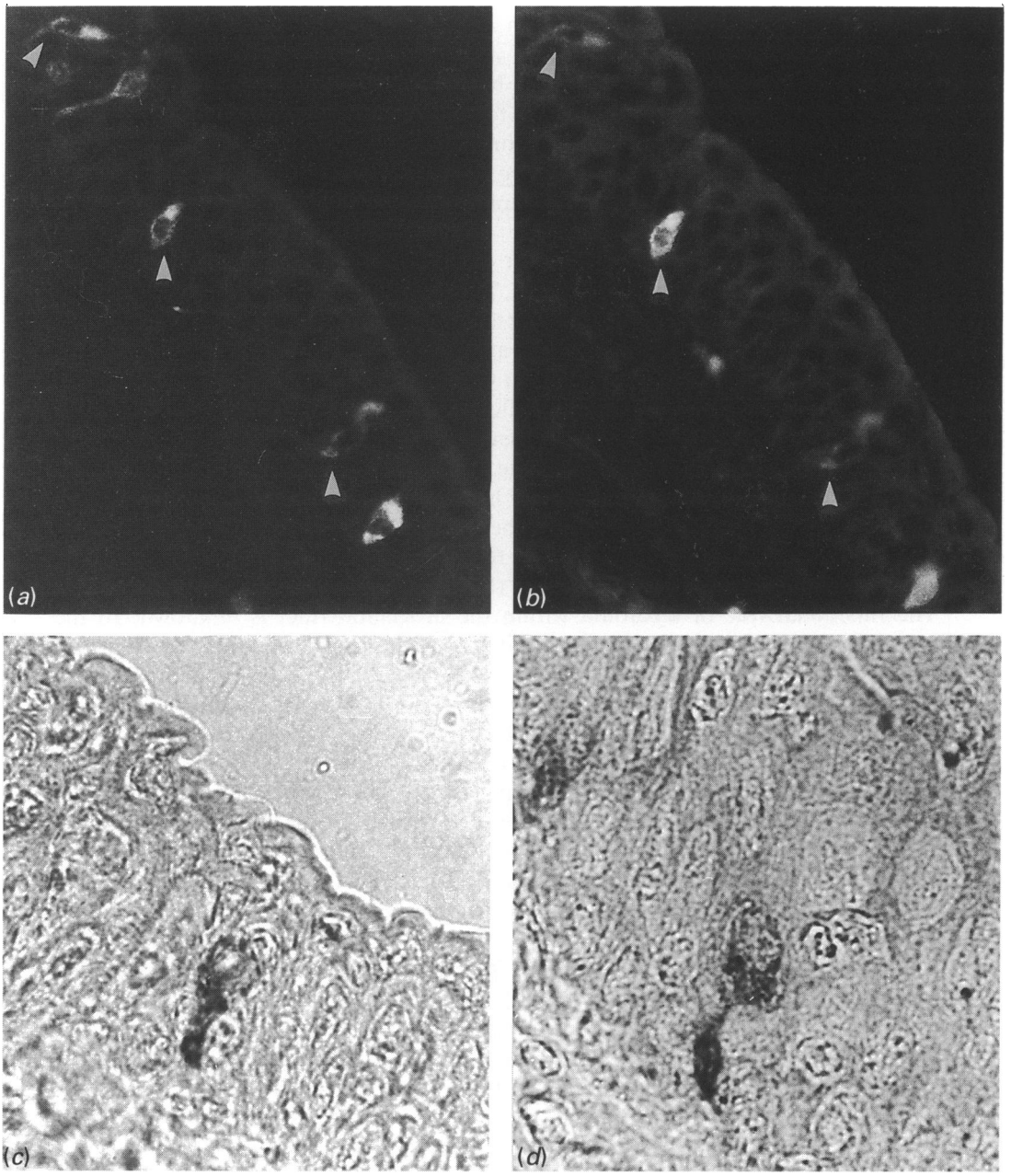

Fig. 4(*a–d*). Urethral (*a*, *b*, *d*) and prostatic (*c*) paraneurons stained by a double labelling fluorescent technique (*a*, *b*) and by the PAP method (*c*, *d*). In (*a*, *b*), a single section shows three neuroendocrine cells (arrowheads) containing both chromogranin A (*a*) (green-yellow FITC fluorescence) and serotonin (*b*) (red LRITC fluorescence). In (*c*, *d*) somatostatin-containing paraneurons. (*a*, *b*) ×450; (*c*, *d*) ×1280.

Urogenital NE cells are not only endodermal in origin, as generally described, but also mesodermal. In fact they are present both in tissues derived from the urogenital sinus and in the endometrial glands of the pig, horse and mouse (Vittoria *et al.* 1986*b*; Paino, La Mura, Budetta & Vittoria, 1987; Cecio *et al.* 1988*b*; Cecio & Vittoria, 1988; Vittoria *et al.* 1989).

Almost all the human urethro-prostatic NE cells contain both the amine 5-HT and

the 'marker' protein neuron specific enolase (di Sant'Agnese *et al.* 1985). Moreover, the amine coexists with chromogranin A in the vast majority of the analogous cells of the dog (Hanyu *et al.* 1987). Our findings are only partially in agreement with those of the cited authors. Ovine urogenital NE cells probably constitute a cellular population more heterogeneous than that found in man and in the dog as far as the serotonin content and distribution of chromogranin A are concerned. It is possible that ovine urogenital paraneurons lacking chromogranin A may contain chromogranin B and/ or C, as happens, for example, in the gastro-intestinal tract of several animal species (Rindi *et al.* 1986).

On the basis of our results, at least four different NE cell types are present in the prostatic and urethral epithelia of the ram. The first cytotype contains serotonin, the second chromogranin A, the third both these substances and the fourth somatostatin. Further morphological and comparative as well as experimental studies are to be made to clarify the peptidergic production of the urogenital paraneurons.

Urogenital serotonin-producing cells often show a narrow cytoplasmic extension whose tapered end reaches the organic lumen (Iwanaga *et al.* 1987). Such cells are said to be 'open' in type and are termed 'taste cells' (Fujita, 1976) on account of their capability to receive 'signals' from the luminal surface. It is not clear if such paraneurons release the amine in subepithelial spaces only or in the lumen as well. Such luminal release was hypothesised for analogous cells of the cat intestine (Gronstad *et al.* 1985) and rat duodenum (Nilsson *et al.* 1987).

The functional role of serotonin within the urogenital tract is unknown. In the gastro-entero-pancreatic system serotonin stimulates the secretion of exocrine epithelia, particularly of mucous cells (Furness & Costa, 1982). This function has been related, in the urogenital apparatus, to the composition of semen and to its fertilising capability (di Sant'Agnese, Davis, Chen & de Mesy Jensen, 1987).

Serotonin induces contraction of smooth muscle (Furness & Costa, 1982) and stimulates peristalsis (Gonella, 1981) of guinea-pig ileum when applied to its luminal surface. The serotonin-induced contraction of urogenital organs could regulate the emission of urine and/or semen (di Sant'Agnese *et al.* 1985; Hanyu *et al.* 1987). This hypothesis is supported by preliminary observations in the dog, whose urinary bladder and urethra are caused to contract by the injection of the amine into the iliac artery (Hanyu *et al.* 1987).

The ability of somatostatin to inhibit gastro-intestinal endocrine and exocrine secretions is well-known (Reichlin, 1983*a*). The hormone is generally released from cytoplasmic dendrite-like processes into interstitial spaces, acting on the neighbouring cells (Reichlin, 1983*b*). This modality of action is called 'paracrinia'.

Multiple molecular forms of somatostatin have been isolated from the brain and intestine of some mammals (Bohlen *et al.* 1981). Biochemical data on a genital form are not available for any animal species. Up to now urogenital NE cells containing such a hormone have been described in human subjects only (di Sant'Agnese & de Mesy Jensen, 1984). Their functional role is said to be inhibitory with regard to local exocrine and endocrine secretions.

Urethral somatostatin-containing paraneurons of the sheep probably have an endocrine modality of action often showing a unique cytoplasmic extension directed towards the subepithelial spaces.

SUMMARY

The urethral and prostatic epithelia of the sheep contain a large number of amine- and/or peptide-producing neuroendocrine cells (NE), also called paraneurons. Four

different cell types have been immunohistochemically recognised among them. The first contains the amine serotonin, the second the protein chromogranin A, the third the amine and the protein together and the fourth the hormone somatostatin. Serotonin-producing cells are elongated in shape and often show cytoplasmic dendrite-like processes directed towards the basal membrane and/or the lumen. Chromogranin A-containing cells are polymorphic and constitute the more numerous NE subpopulation. Cells containing both the bioactive substances seem to be less numerous than the chromogranin A cells and slightly more frequent than the serotonin cells. All these cell types are diffused along the whole urethro-prostatic complex and show their highest density in the collicular zone. Somatostatin-containing cells often show a unique cytoplasmic extension directed towards the basal membrane and are rare.

It is supposed that the presence of serotonin in the urogenital tract is functionally correlated with the emission of urine and/or semen, while somatostatin is associated with the inhibition of local exocrine and/or endocrine secretions.

Some of the findings reported in this paper were presented at the 17th Congress of the European Association of Veterinary Anatomists, Regensburg, West Germany, August 1988, and at the International Symposium on Neurons and Paraneurons, Niigata, Japan, October 1988.

The authors wish to thank Mr Antonio Calamo for his excellent help with the photographic prints. This work was supported by a grant from the Italian Ministry of Education.

REFERENCES

ABRAHAMSSON, P. A., WADSTROM, L. B., ALUMETS, J., FALKMER, S. & GRIMELIUS, L. (1986). Peptide-hormone and serotonin immunoreactive cells in normal and hyperplastic prostate glands. *Pathology, Research and Practice* **181**, 675–683.

BOHLEN, P., BENOIT, R., LING, N., GUILLEMIN, R. & BRAZEAU, P. (1981). Isolation and characterization of rat hypothalamic somatostatin-14. *Endocrinology* **108**, 2008–2010.

CECIO, A., VITTORIA, A. & BUDETTA, G. (1985). Applicazione del metodo argirofilo di Linder allo studio del sistema neuroendocrino. Atti del 20° Congresso della Società Italiana di Istochimica, Milano, 30–31 May.

CECIO, A. & VITTORIA, A. (1989). Urogenital paraneurons in several mammals. *Archives of Histology and Cytology* **52**, *Suppl.*, 403–413.

CECIO, A., VITTORIA, A., BUDETTA, G. & CORONA, M. (1988*a*). Staining of neuroendocrine cells by Linder's argyrophil method. *Journal of Anatomy* **157**, 197–201.

CECIO, A., VITTORIA, A., LA MURA, E., PAINO, G., BUDETTA, G. & COCCA, T. (1988*b*). Neuroendocrine cells within urogenital organs of several mammals. 17th Congress of the European Association of Veterinary Anatomists, Regensburg, 28 Aug.–1 Sept.

DI SANT'AGNESE, P. A. (1986). Calcitonin-like immunoreactive and bombesin-like immunoreactive endocrine-paracrine cells of the human prostate. *Archives of Pathology and Laboratory Medicine* **110**, 412–415.

DI SANT'AGNESE, P. A., DAVIS, N. S., CHEN, M. & DE MESY JENSEN, K. L. (1987). Age-related changes in the neuroendocrine (endocrine-paracrine) cell population and the serotonin content of the guinea pig prostate. *Laboratory Investigation* **57**, 729–736.

DI SANT'AGNESE, P. A. & DE MESY JENSEN, K. L. (1984). Endocrine-paracrine cells of the prostate and prostatic urethra: an ultrastructural study. *Human Pathology* **15**, 1034–1041.

DI SANT'AGNESE, P. A., DE MESY JENSEN, K. L., CHURUKIAN, C. J. & AGARWAL, M. M. (1985). Human prostatic endocrine-paracrine (APUD) cells. Distributional analysis with a comparison of serotonin and neuron-specific enolase immunoreactivity and silver stains. *Archives of Pathology and Laboratory Medicine* **109**, 607–612.

FETISSOF, F., ARBEILLE, B., GUILLOTEAU, D. & LANSON, Y. (1987). Glycoprotein hormone α-chain-immunoreactive endocrine cells in prostate and cloacal-derived tissues. *Archives of Pathology and Laboratory Medicine* **111**, 836–840.

FETISSOF, F., BERTRAND, G., GUILLOTEAU, D., DUBOIS, M. P., LANSON, Y. & ARBEILLE, B. (1986). Calcitonin immunoreactive cells in prostate gland and cloacal derived tissues. *Virchows Archiv A* **409**, 523–534.

FETISSOF, F., DUBOIS, M. P., ARBEILLE-BRASSART, B., LANSON, Y., BOIVIN, F. & JOBARD, P. (1983). Endocrine cells in the prostate gland, urothelium and Brenner tumours. Immunohistological and ultrastructural studies. *Virchows Archiv B* **42**, 53–64.

FEYRTER, F. (1951). Ueber des urogenitale Helle-Zellen System des Menschen. *Zeitschrift für mikroskopisch-anatomische Forschung* **57**, 324–344.

FUJITA, T. (1976). The gastro-enteric endocrine cell and its paraneuronic nature. In *Chromaffin, Enterochromaffin and Related Cells* (ed. R. E. Coupland & T. Fujita). Amsterdam: Elsevier Scientific Publishing Co.

FUJITA, T. (1980). Paraneuron, its current implications. *Biomedical Research* **1** *Suppl.*, 3–9.

FURNESS, J. B. & COSTA, M. (1982). Identification of gastrointestinal neurotransmitters. In *Handbook of Experimental Pharmacology*, **59/1**, 383–462 (ed. G. Bertaccini). Berlin: Springer.

GONELLA, J. (1981). The physiological role of peripheral serotoninergic neurons. A review. *Journal of Physiology* **77**, 515–519.

GRIMELIUS, L. (1968). A silver nitrate stain for α_2-cells in human pancreatic islets. *Acta Societatis medicorum upsaliensis* **73**, 243–270.

GRONSTAD, K. O., DE MAGISTRIS, L., DAHLSTRÖM, A., NILSSON, O., PRICE, B., ZINNER, M. J., JAFFE, B. M. & AHLMAN, H. (1985). The effect of vagal nerve stimulation on endoluminal release of serotonin and substance P into the feline small intestine. *Scandinavian Journal of Gastroenterology* **20**, 163–169.

HAKANSON, R., LARSSON, L. I., SJOBERG, N. O. & SUNDLER, F. (1974). Amine-producing endocrine-like cells in the epithelium of urethra and prostate of the guinea pig. A chemical, fluorescence histochemical and electron microscopic study. *Histochemistry* **38**, 259–270.

HANYU, S., IWANAGA, T., KANO, K. & FUJITA, T. (1987). Distribution of serotonin-immunoreactive cells in the lower urinary tract of dogs. *American Journal of Anatomy* **180**, 349–356.

IWANAGA, T., HANYU, S. & FUJITA, T. (1987). Serotonin-immunoreactive cells of peculiar shape in the urethral epithelium of the human penis. *Cell and Tissue Research* **249**, 51–56.

KAZZAZ, B. A. (1974). Argentaffin and argyrophil cells in the prostate. *Journal of Pathology* **112**, 189–193.

KOCH, G. & ENGELHARDT, G. (1959). Ueber das argyrophile Helle-Zellen-Organ in der Harnröhren Schleimhaut des Säugetieres. *Naunyn-Schmiedeberg's Archiv für experimentelle Pathologie und Pharmakologie* **236**, 463–473.

LINDER, J. E. (1978). A simple and reliable method for the silver impregnation of nerves in paraffin sections of soft and mineralized tissues. *Journal of Anatomy* **127**, 543–551.

NILSSON, O., AHLMAN, H., GEFFARD, M., DAHLSTRÖM, A. & ERICSON, L. E. (1987). Bipolarity of duodenal enterochromaffin cells in the rat. *Cell and Tissue Research* **248**, 49–54.

PAINO, G., VITTORIA, A., COCCA, T., BUDETTA, G. & CECIO, A. (1985). Osservazioni citomorfologiche ed istochimiche sui paraneuroni uretro-prostatici in bufalo, coniglio e uomo. *Atti della Società italiana di scienze veterinarie* **39**, 87–89.

PAINO, G., LA MURA, E., BUDETTA, G. & VITTORIA, A. (1987). Istochimica ed immunoistochimica di cellule neuroendocrine uterine in un caso di pseudoermafroditismo suino. *Atti della Società italiana di scienze veterinarie* **41**, 179–181.

PEARSE, A. G. E. (1977). The diffuse neuroendocrine system and the APUD concept: related 'endocrine' peptides in brain, intestine, pituitary, placenta and anuran cutaneous glands. *Medical Biology* **55**, 115–125.

PRETL, K. (1944). Zur Frage der Endokrinie der menschlichen Vorsteherdrüse. *Virchows Archiv B* **312**, 392–404.

REICHLIN, S. (1983*a*). Somatostatin: I. *New England Journal of Medicine* **309**, 1495–1501.

REICHLIN, S. (1983*b*). Somatostatin: II. *New England Journal of Medicine* **309**, 1556–1563.

RINDI, G., BUFFA, R., SESSA, F., TORTORA, O. & SOLCIA, E. (1986). Chromogranin A, B and C immunoreactivities of mammalian endocrine cells. *Histochemistry* **85**, 19–28.

SINGH, I. (1962). The staining of enterochromaffin cells by the Bodian silver impregnation method. *Acta anatomica* **51**, 292–297.

SINGH, I. (1964). A modification of the Masson–Hamperl method for staining of argentaffin cells. *Anatomischer Anzeiger* **115**, 81–82.

STERNBERGER, L. A. (1979). *Immunocytochemistry*, 2nd ed. New York: John Wiley & Sons.

VITTORIA, A., PAINO, G., COCCA, T. & CECIO, A. (1983). Presenza di paraneuroni nella prostata di coniglio e di bufalo. Atti del XIX Congresso della Società Italiana di Istochimica, Perugia 13–15 Oct.

VITTORIA, A., LA MURA, E., BUDETTA, G., PAINO, G. & CECIO, A. (1986*a*). Immunocitochimica delle cellule a 5-HT nel complesso uretro-prostatico di Bubalus buffalus. Atti del 41° Convegno della Società Italiana di Anatomia, Torino 28 Sept.–1 Oct.

VITTORIA, A., LA MURA, E., BUDETTA, G., PAINO, G., COSTAGLIOLA, A. & CECIO, A. (1986*b*). Prime osservazioni di cellule neuro-endocrine nell'utero di suino. Studio istochimico ed immunocitochimico. Atti della Società Italiana di Scienze Veterinarie **40**, 174–175.

VITTORIA, A., PAINO, G., LA MURA, E., BUDETTA, G. & CECIO, A. (1989). Chromogranin- and somatostatin-containing neuroendocrine cells in the porcine uterus. An immunocytochemical study. *Anatomia, Histologia, Embryologia* **18**, 136–142.

WESSENDORF, M. W. & ELDE, R. P. (1985). Characterization of an immunofluorescence technique for the demonstration of coexisting neurotransmitters within nerve fibers and terminals. *Journal of Histochemistry and Cytochemistry* **33**, 984–994.