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Functional Aspects of Adrenocholinergic and Peptidergic Innervation about Prostate and Seminal Vesicles in Mammals: A Review

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Abstract: This study was aimed to explain the functional role of the mediation in the organs of the male reproductive system (prostate and seminal vesicles of rat and hamster), representing through the visualization of adrenergic fibres (Falck method), cholinergic fibres (Martinez-Rodriguez method) and by immunohistochemical methods to detect the P substance and the CGRP (PAP) in glandular structures. The adrenocholinergic and peptidergic mediators have a growing interest for their synergic action in the control of contractility, vasodilatation and most likely, of the secretory activity of the epithelium, probably expressed by endocrine-paracrinous mechanisms.

Key words: Adrenergic-cholinergic-peptidergic innervation, P substance, CGRP, prostate, seminal vesicles

INTRODUCTION

Embryology: Prostate and Seminal vesicles are involved at the same time, throughout the development and the definitive differentiation of uro-genital portion and external genitals. We remember that the prostatic gland, in human and in other mammals, is described as an organ which arises from a sequence of endodermal invaginations placed near the primordial urethra and adjacent to the pelvic portion of uro-genital cavity, in human fetus which is 55 mm (C.R). The biggest primordial invaginations arise from differentiated mesenchyma which will originate both the muscular component and stroma.

These invaginations sink into primordial urethra opening in mesonefric duct: the future deferent duct. These primordial endodermal invaginations spread out, at this time, in 5 distinct territories:

- Frontal group
- Dorsal-cranial group
- Dorsal group
- Two Lateral groups

In this period the organ seems an infundibular gland coming from the prostatic utricle. It is the primordial male uterus; infact it rises from müllerian tubercule that is placed on the dorsal wall of the urethral-bladder junction; thus, the genital portion. The müllerian tubercule originates from mesodermal cells coming from paramesonephric and mesonephric ducts.

In this region there is a mixage of cells made up of 2 types of epithelioblasts which will be the lining of prostatic urethra, the lining of prostatic utricle and intermedium lobe.

The development of this area begins at the 5th week of intrauterine life, when the Müller duct begins to differentiate and goes on till the thirth month, when the uro-genital portion is built.

This is the caudal tract of the uro-genital duct; in this duct we can differentiate: A pelvic portion and a phallic portion, that is a part of the uro-genital cavity extending to the urethral side of the tuberculus or phallus.

The phallic portion emerges later on. In female, the uro-genital cavity will differentiate into the vestibular portion of vagina.

In male, it will differentiate into the portion of urethra placed away off ejaculatory ducts. We remember these come from Wolff's duct together to the deferent duct and epididymis; the Müller's duct will become atrophic.

In female, the Wolff's ducts will become atrophic while the Müller's will differentiate into oviducts, body and neck of uterus; these ducts develop at the 5th week;

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this time the gonad is undifferentiated. We remember that Müller's and Wolff's ducts are placed in groove of the dorsal wall: the uro-genital groove.

Therefore, the duct of epididymis, ejaculatory duct and deferent duct, which the seminal vesicles will arise from, will come from Wolff's duct.

Histochemicals and Immunohistochemical observations: The immunohistochemical evaluations about internal genital organs, in particular about their innervation, regard the anatomical organization of pelvic district.

Lewis and Sesack (1997) remember us that organization in primate nervous system shows post-ganglionic sympathethic fibres coming from cellular bodies placed at distance from effector organs, according to Owman and Sjöstrnad (1969). Thus the thoracic-lumbar district identifies its own synapsis in that sympathetic chains of paravertebral nerve centres.

The pelvic organs get, in this way, their autonomous nervous fibres both by sympathicus and para-sympathicus portion; it is necessary to regard that sympathicus post-ganglionic fibres come from peripheral nervous centres placed inside the effector organs.

For the sake of brevity, we remember by Owman and Sjöstrnad (1969) the symphathetic fibres arise from upper lumbar segments and that these come to pelvic area across ipogastric nerve, from lower mesenteric nerve centre.

The parasympathicus innervation comes from sacral territory. It is important to know that the autonomic innervation has a strong relationship among mammals.

Today, it is known that beyond adrenergic and cholinergic systems, peptidergic system (nervous mediation depended on peptide), also contributes to autonomic innervation.

The Langley and Anderson (1985a, b; 1986a, b), studies showed that the sympathicus innervation is a ganglionic peripherical power relay of internal female and male genital system placed near effector organs.

Nevertheless for a long time it has been known genital organs are provided by post-ganglionic fibres which run along ipogastric nervous centres, coming from the lower mesenteric nervous centre.

The reason of this mistake has been widely evaluated, after Sjöstrand's, by Sjöberg (1967) studies, recently the innervation of male internal genitals has been studied by particular analysis about mucous-muscle territory of deferent vessel used, as Owman and Sjöstrnad (1969) said, as a model (pattern, design) organ for pharmacological and neurophysiologycal studies regarding adrenergic mechanisms of neurotransmission.

For example, Sjöstrand shows ganglionic-inhibitor agents which are able to stop the answer of guinea pig's deferent duct when the ipogastric nerve is stimulated.

The same author shows that the chop and degeneration of ipogastric nerve doesn't decrease adrenergic content of deferent duct and seminal vesicles. Falck *et al.* (1965) shows, by histochemistry fluorescent method and use of paraphormaldehyde, that the deferent duct has a large adrenergical innervation; Falck and collaborators underlined that the adrenergic nervous centres are placed inside or near target organs.

Studies of surgical denervation, on male internal genitals of different animal species, established peripherical nature of adrenergical power relay and later these results have been confirmed by electron microscopy. These adrenergical nerve centres with their cellular bodies placed in peripherical ganglionic centres, near effector organs, have been described by Owman and Sjöstrand (1969), like short adrenergical nerve centres, in order to differentiate them from long adrenergical nerve centres which, instead, have cellular bodies placed in pre or paravertebral nerve centres.

The adrenergical short nerve centres have a selectivity of innervation towards urogenital male and female system.

These peripherical nerve centres are similar to paravertebral nerve centres, in which there are cellular bodies full of noradrenaline, acetylcholine, regulatory peptides and intrinsic and extrinsic nervous fibres. The intrinsic fibres come from small cells which are fluorescent, full of amines. These are named 'SIF cells' and have been showed by Eränkö *et al.* (1980) adrenaline is the most present amine.

Libet and Owmann (1974) showed that the SIF cells work like 'inter-nerve centres' extending collateral fibres inside the nerve centre. The short adrenergical nerve centre of this territory shows physiological and pharmacological differences from long neurons. The differences which Owman and Sjöstrnad (1969) underlines in 8 points are present in deferent duct:

- More resistence, through nervous stimulation, to the release of "quanti" of transmitters
- Lower sensitivity after loss of noradrenaline through reserpine or α-methyltyrosine
- Progressive decrease of noradrenaline's release through repeated electrical stimulation of nerve
- Resistence to neuro-toxic effects of (6-OHDA) and of 5,7-dihydroxitryptamine
- High affinity for the undue influence of indolamines
- Resistence to inhibition of smooth contraction muscle by drugs
- Increase of contractile answer
- Inhibition through exogenous noradrenaline of contraction

In seminal vesicles the muscular contraction by neurological mechanisms is not inhibited through adrenergic or cholinergic antagonists, showing the probability of other mechanisms.

It is underlined that inside of internal male genital organs of some mammals, Sjöstrand shows that nerve centres make strong clusters in terminal parts of ipogastric nerves in correspondence of vascular territories which go to prostate, coagulant glands (in particular in rodents) and deferent duct.

Most of neurons in male genital nerve centre are adrenergic. These adrenergic cells-like Sjöstrand told go inside genital organs which are dividing into granular extremities next to smooth muscle cells; thus this motor innervation, directed to prostatic gland, seminal vesicles, deferent duct and other near structures, is marked for functional homogeneity similar to several animal species, included human.

We remember that male genital system, particularly studied in Guinea pig, has been assessed by method of histoimmunofluorescence with paraphormaldehyde.

It is describe that a richness, in deferent vessel of adrenergic nervous terminations which are varicose, organized in plexus inside the circular and longitudinal muscular layers.

About functional aspects of short adrenergic neurons, how Sjöstrand told, it is believed that these are involved in carriage of spermatozoa and in the mechanism of seminal discharge. It is studied that neuropharmacology and neurohistochemical aspects belonging to these functions. This happens through an ejection of their content, which is thrown out urethra.

This ejection, according to Sjöstrand, happens through luminal reduction which should refer to third strength of smooth muscle cells.

The cholinergic mechanisms are present by vagal motor supplementar innervation (Gerebtzoff, 1959; Bell and McLean, 1967; Klinge, 1970; Bell and McLean, 1973; Baumgarten *et al*., 1975; Alm, 1982). Their presence is characterized in several species of animals, included human and it is illustrated in many researches. It is believed that cholinergic mechanisms are less than adrenergic ones and that they run adjacent to them. Sometimes, the histochemical and electromicroscopic identity of cholinergic nerves is not clear. For example, Owman and Sjöstrand (1969) told that the inhibitors of pseudo-cholinesterase (not always specific in several species) or AchE can be present in different systems of cholinergic fibres.

The so-called adrenergic nerves are able to, for example, store regulatory peptides which are able to develop collateral functions to cholinergic ones.

Certainly, the visualization of immunohistochemical systems that evidence the cholin-acetyltransferase is able to stand out cholinergic system.

On the functional view it is underlined that, however, the release of acetylcholine inside denervated deferent duct indicates how the cholinergic component has a role less important than adrenergic component.

Since 1863, Eckhard has shown that parasympathicus nervous stimulation is able to set up penis though the interest in cholinergic mechanisms of this region, today, is showed in concomitance of the discovery of important peptides.

The VIP (Vaso Intestinal Peptide), for example, often coexists with acetylcholine in nerves. It is not steady the relief of neuro-peptides present inside nervous fibres involved in mechanisms of cholinergic mediation and that as it is known for other districts, the appearance of neuropeptides during development comes out, at the same time, with presence of adrenocholinergic substances.

About peptidergic mechanisms, ultrastructural and immunohistochemical data demonstrated the presence of nerves, called purinergic, also in reproductive system (Sparrong *et al*., 1977).

One of the first neuropeptides evidenced by immunohistochemistry was VIP (Alm *et al*., 1977). This VIP is present in male genital organs and in supplementary structures.

Some nerves go inside, or next to smooth muscle cells; some nervous fibres are present in basal lamina of mucosa in relationship with blood vessels and epithelium. These VIP fibres come from same nervous centres of adrenocholinergic neurons and the nervous cells seem also to be reactive to AchE method (Lundberg *et al*., 1979). They are present in deferent vessel and have effect on contractile activity of same vessel.

In Guinea pig, further the deferent duct, also seminal vesicles have many positive VIP fibres and it is known that the peptide administered *in vitro* doesn't influence smooth muscle.

The Neuropeptide Y is evidenced in these territories (Tatemoto, 1982; Tatemoto *et al*., 1982). It has been demonstrated that the NPY is placed inside of sympathicus noradrenergic neurons. In man the immunoreactivity NPY is higher in seminal vesicles, in deferent duct, in prostate while is lower in epididymis, in cavernous and spongy body of penis, in gland and testis (Adrian *et al*., 1984).

Rush and Geffen (1980) underline the common presence of NPY and Dopamine-β-idrossilase (DβH), tipycal marker of adrenergic nerves. Another example of peptidergic innervation is the "Gastrin Releasing Peptide" (GRP) and Bombesin.

The GRP has been isolated from gastric mucosa but hasn't been isolated from antral mucosa by McDonald *et al.* (1978, 1979). The GRP has 27 aminoacids and 7 residual aminoacides-COOH terminal are homologous to Bombesin's aminoacids, a peptide of 14 aminoacids isolated from skin of amphibious (Anastasi *et al.*, 1971; Ersparmer *et al.*, 1978).

Since it has been said that the real bombesin has never been isolated in mammals, it has been suggested that the GRP is equivalent of bombesin, in these animal species, with similar functional properties, that is: contraction of smooth muscle, induction of exocrine and endocrine cells secretion. Fibres of GRP have been discovered in deferent duct and seminal vesicles in several animals.

Either GRP or bombesin determine contractions of isolated seminal vesicles. Another interesting peptide is the Galanin.

For the first time, it has been isolated in small upper intestine of pig and it is made of 29 aminoacids (Tatemoto *et al.*, 1983). Its name depends on the terminal presence of glycine and alanine. Their presence has been demonstrated immunohistochemically in central and peripherical nervous system and in male genital apparatus (Bauer *et al.*, 1986).

The fibres which have galanin are next to vascular smooth muscle, but when these fibres take a plexiform appearance, these are present also in the context of blood vessels.

It is believed that the galanin may be involved in a pre-synaptical mechanism; it is able to control the release of noradrenaline during stimulation of synaptical post-gangliar fibres.

Other neuro-peptides are represented by Enkefalins identified in female genital apparatus and little more in male genital apparatus.

The prostatic gland is full of nervous fibres which go towards connective tissue of organ branching in glandular epithelium. The same distribution is present in epididymis, deferent duct and testis.

The Substance P was one of the beginning peptides evidenced in the group of tachykinins by Von Euler and Gaddum (1931). Fibres which have Substance P were evidenced in genito-urinary apparatus (Alm *et al.*, 1978).

The distribution in male organs and particularly in testis and epididymis, assumes the form of large nervous trunks and terminations which are placed in capsule of organ and interstitial connective tissue.

The seminal vesicles also have a share of fibres with Substance P while there are not in prostate. The fibres of Substance P make perivascular nervous plexus.

The action of this peptide, like other peptides of the group of tachykinins (sisalemin, eledoisin) increases the vascular tension.

Today, it is believed that Substance P can modulate the motor effects in male genital organs, not only in loco but also in nerve centre. Infact, it is believed that Substance P is able to enlarge symphathetic motor activity.

Another peptide of primary sensitive neurons is "peptide correlated to gene of calcitonin, (CGRP)" as this peptide is codified by same genic place which is precursor of the calcitonine (Amara *et al.*, 1982; Lundberg *et al.*, 1985). It should be noticed that their effects on the contraction, induced neurologically, seem opposite at least in deferent vessels, though Substance P and CGRP are together in the same neurons.

MATERIALS AND METHODS

Our observations along the past years on cholinergic innervation of prostate through histochemicals experiments treated on acetylcholinesterasic activity in rodents (rat, hamster), during the development and during post-born life are very interesting.

Our observations made on growing old animals allow us to evaluate a composite map about specific distribution of cholinergic terminals (Gerbino, 1975; Tessitore and Gerbino, 1975) in this organs including the peptidergic component with the visualization (Peroxidase Anti-Peroxidase, PAP; our speeches at the Italian Anatomy Society and Sicilian-Calabrian Urology Society in the 90s) of CGRP. The activity AchE-ChE was evaluated by Karnovsky and Roots (1964) method modified by Martinez-Rodriguez *et al.* (1964). The selectivity of this method has been controlled using prostigmine which, as it is known, stops the activity of both cholinesterases (acetylcholinesterase and pseudo-cholinesterase). As control of Acetylcholinesterase (AchE), tetraisopropyl-pirophosphoramide (ISO-OMPA) has been used, according to Bayliss and Todrick (1956) advice, a specific inhibitor of pseudo-cholinesterases.

At the end of intrauterine life and during 1st day of life (phase of newborn) the activity of acetylcholinesterase in prostatic gland is present in isolated myocellular elements in peri-utricular seat.

It is possible, in stroma, to observe the distribution of small nervous fibres positive to AchE with lithe course, going near utricular glands. Thin reactive fibres are next to smaller vessels. In a latter phase of development the activity of AchE is showed near of muscular box coupling around utricules. The glandular epithelium shows

homogeneous reactivity placed in basal portion of cells. In periutricular stroma numerous cholinergic fibres are also present in very close relationship with vascular-glandular formation.

In animals in advanced development, the activity of AchE is more intensive, placed in muscular peri-utricular layer and at base of epithelial cells. Nervous cholinergic trunks and small nerve centres are in peri-utricular stroma.

Fibres more thin and lithe are present in peri-vascular complex. In newborn animals histochemical features similar to those shown in fetal group (at term) and newborn group are present but the intensity of histochemical reactivity seems reduced. These data show that the activity of AchE in prostate of mammals is already evidenced in fetal and newborn period.

In following periods the histoenzymatic reactivity becomes stronger in smooth muscle cells periutricular region and it is also present near trunks of nervous fibres which go in stroma and close to vessels.

In the recent past the histochemical evidence of Choline-Acetyltransferase according to Burt (1970) is shown as a weak reactivity along nervous intra-muscular fibres while a more reactivity is present in cellular bodies of neurons. These same territories seem to be immunohistochemically interested by numerous peptides like Substance P and CGRP investigated by us.

It is evidenced, moreover, that near glandular territories now characterized we describe evident zones where reactivity seems to be less marked, or absent by histochemical features.

This data in agreement with the Gerebtzoff (1959) results, suggests that the zones of reduced histochemical activity are expression of a poor function of the organ. A similar adrenergic (peri-utricular, inter-utricular) distribution is detectable in prostate, demonstrated by Falck *et al.* (1982) fluorescent histochemical technique where the fluorescent fibres make a peri-utricular or peri-vascular plexus or break up inside stroma with fibres which seem to be "grains of rosary". The classical neurofibrillar methods, according to the old observations by Frankel and Stohr and later in the 40s by Gomorasca and Kaswin and Serfaty till Stack's results at the beginnning of the 60s, contributed to studies of seminal vesicles. Other important contributes have been given by Grieten and Gerebtzoff (1957), Gerebtzoff (1959) and Falck *et al.* (1982).

Gerebtzoff (1959) described in various mammals the presence of numerous cholinergic fibres going from external connective layer to muscular layer in shape of a thick plexus. He, moreover, noticed how the presence of cholinergic terminals in smooth muscle disagreed with physiological acquisitions according which the ejeculative mechanism was characterized by ortho-sympathicus.

The adrenergic innervation of seminal vesicles was studied in different times, by Falck *et al.* (1982) and Owman and Sjöstrand (1969) in guinea pig (cavy), rat, mouse, dog and macaque, Norberg *et al.* (1967) in cat, in rabbit and in cavy (Pacini, 1969).

Our histochemical studies focus the attention to various periods of rodents' development (18, 20, 21 days of intrauterine life, newborns and adults).

The cholinesterase reactivity (AchE) of seminal vesicles in rodents appears, in our observations, more precociously: during the development in mesenchyma of tubular wall. During the first days of post-born life (about 15 days old), it is present in muscular part and in epithelial cells of mucosa. In newborns, the histoenzymatic pattern shows definitive aspects: Reactivity of external connective layer and spreading after into muscular layer and in peri-vascular territories.

In seminal vesicles of adult rat the cholinesterasic reactivity is present both at myo-cellular level as endogenous reactivity by Dumont *et al.* (1961) and at level of nervous fibres (intra-adventitial fibres, intramuscular, perivascular, intra-chorial).

The aminergic component has also been shown in early time of development and has been symbolized by specific fluorescent structures as shape of rough streaks or peri-tubular clods, expression of adrenergic mediation already in phase of organization.

In adult rats, at last, a plexus full of adrenergic varicous fibres has been shown which is developing in the context of muscular layers, but not in mucosa layers.

Adrenergic fibres also create perivascular delicate inter-laceries. About immunohistochemical view the CGRP appears on muscular component of adult hamster; as the immunohistochemical presence of choline acetyl-transferase is also documentable, in same animal species, in neonatal period.

Rodents' seminal vesicle and prostate (rat, hamster), from us studied, show a rich cholinergic varicose terminal network (Met. I.C.24), localized in the prostatic stroma and where thin AchE positive fibres are present around the utricules (Fig. 1). In the seminal vesicles a strong staining is evident in the muscular layer and around small vessels (Fig. 2). In the same way, the vesicular adrenergic network seems to be positive (Method Falck) in smooth muscular tissue forming a basket of positive fibres (Fig. 3). CGRP winding fibres are present in the smooth muscular tissue with a strong brown staining (Fig. 4).

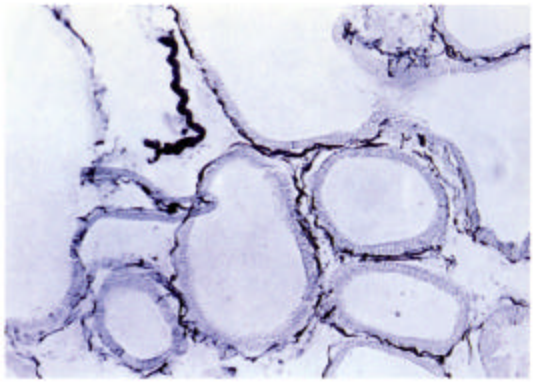

Fig. 1: Rat Prostate; AchE activity (Karnowsky-Roots mod. Martinez-Rodriguez *et al*. (1964) I.C.24); cholinergic fibres in the stroma; Oc. 10x, Ob. 16x

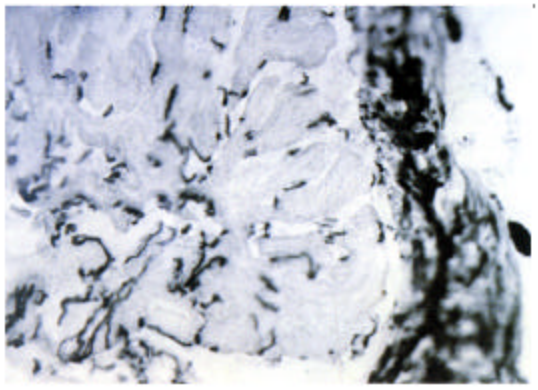

Fig. 2: Rat seminal vesicle; AchE activity (Karnowsky-Roots mod. Martinez-Rodriguez *et al*. (1964) I.C.24); very intense positive cholinergic fibres in the muscle layer, in the stroma and perivascular region; Oc. 10x, Ob. 16x

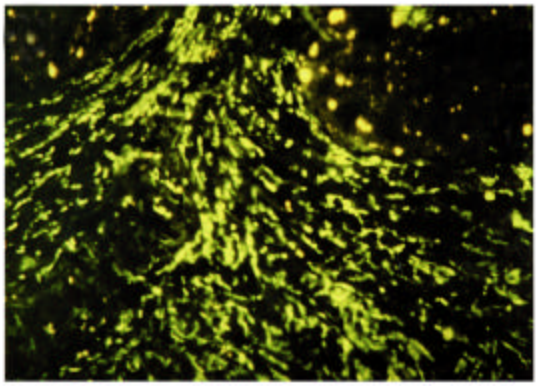

Fig. 3: Rat seminal vesicle; Adrenergic fibres in the muscular layer (Falck *et al*., 1965); Oc. 12x, Ob. 16x

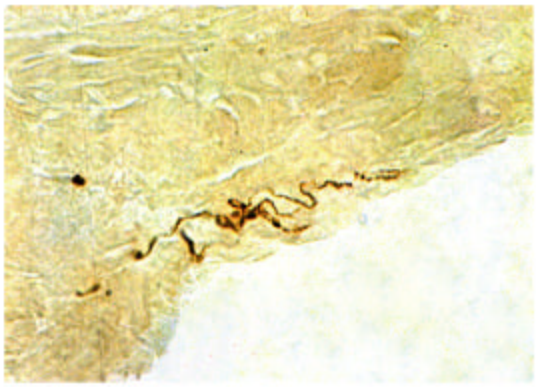

Fig. 4: Hamster seminal vescicle; CGRP fibres in the muscular region (PAP); Oc. 12x, Ob. 6.3x

CONCLUSION

The demonstration of a triple adreno-cholinergic and peptidergic activity constitutes a point of start for further studies in order to explain the functional role of adreno-cholinergic and peptidergic components.

It is believed that according to the past experiences by Picarelli and Valle (1969), Masuda (1969) and Pacini (1969) not only the epithelium of seminal vesicles and probably of prostate (like of other glands annexed to reproductive male system) are, as it is known, preferential targets of hormonal influences, but also adreno-cholinergic mediations and furthermore, peptidergic of these organs, may be influenced by hormonal condition of animal.

By histo-immunological methods, the presence of Choline-Acetyltransferase has been evidenced as a weak reactivity along the intramuscular nervous fibres, against more reactivity which is expressed in cellular bodies of intra-wall neurons. These territories, like others are interested by several peptides like Substance P and CGRP.

About the Substance P, it has been demonstrated in smooth-muscle of vascular territories and in avascular territories. Next to the more known adrenocholinergic map, a rich distributive map of peptidergic component is growing, which seems to be more involved in sensitive innervation.

The adrenocholinergic and peptidergic mediators, hence, are interested in the control of contractility, of vasodilatation and probably in secretory activity of epithelium by endocrinous-paracrinous mechanism.

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