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Adrenergic and peptidergic neuromuscular systems in the Fallopian tube

Sistemas neuromusculares adrenérgicos y peptidérgicos en la trompa de Fallopio

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INTRODUCTION

The smooth musculature of the Fallopian tube is crucially involved in the transport of spermatozoa and ova, in fertilization, and in transport of the blastocyst towards the uterus for implantation (Pauerstein and Eddy, 1979). Within the ampullary region, the egg is *borne* in a pattern of to and fro movements providing a net forward transport (Verdugo *et al.,* 1976). In women, egg transport is characterized by a retention in the ampulla, near the ampullary isthmic junction for 70-80 hours followed by rapid transit through the isthmus, a passage effected primarily by tubal muscular activity (Anand and Guha, 1978). In addition to this mechanism, the activity of ciliated epithelium, intraluminal secretion, and innervation of the muscular wall are of importance to ovum transport. The neuromuscular complex has attracted the greatest interest among researchers.

The advent of the fluorescence technique during the 1960s enabled the adrenergic nerve system to be studied, providing evidence that the isthmus, particularly the circular muscle layer, was well supplied with adrenergic innervation (Fig. 1). This gave reason to suppose that the isthmus acts as a sphincter (Sjöberg, 1967).

It is now well established that tubal adrenergic innervation partly derives from ganglion cells situated in the upper part of the vaginal wall and in the cervical tissue outside the uterovaginal junction (Owman *et al.,* 1966; Owman *et al.,* 1983).

In quantifying the adrenergic transmitter, noradrenaline, a liquid chromatography method has been used enabling measurements to be made in very small tissue samples. With this method it has been shown that, in all phases of the menstrual cycle, the noradrenaline concentration varies along the Fallopian tube, being higher in the isthmus than in the ampulla or in the infundibulum. The transmitter concentration thus falls successively from the uterus to the ampulla, with a more abrupt drop at the junction of the isthmus and the ampulla, the highest concentration occurring in the isthmus where earlier studies have shown the adrenergic nerves to be predominantly associated with the nonvascular smooth musculature (Fig. 2). This provided additional support for the concept that the isthmus acts as a sphincter (Helm *et al.,* 1982b).

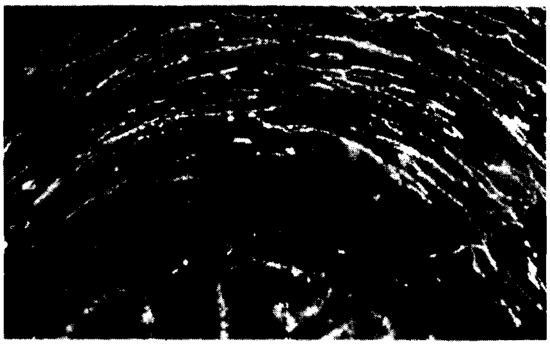

Fig. 1: Fluorescence photomicrograph of oviduct, transverse section x 100. A great number of fluorescent noradrenaline-containing nerve terminals running in the direction of the smooth muscle cells in the circular layer.

The assumption that the nerves may be able to exert motor control of this musculature presupposes a close relationship between the nerves and the muscle

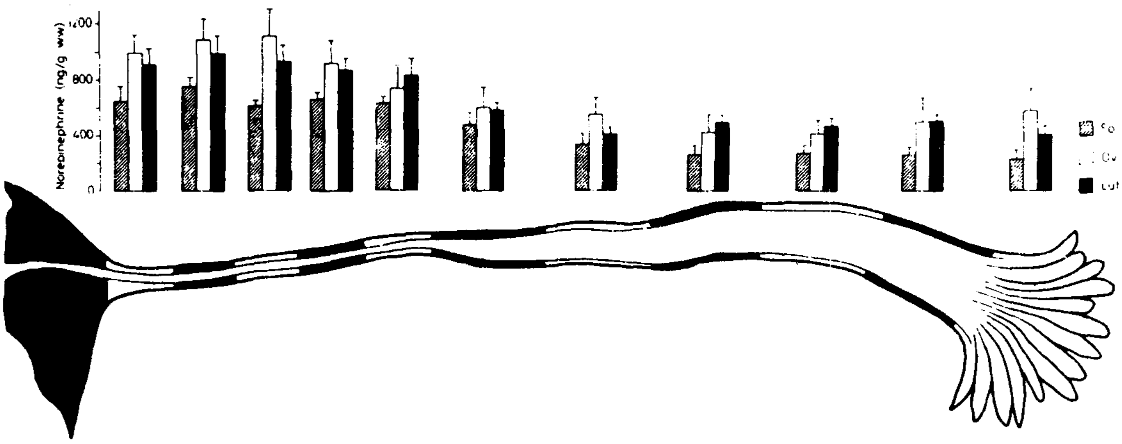

Fig. 2: Tissue concentrations of *noradrenaline (ng/g wwt)* in consecutive parts throughout the length of the human Fallopian tube (5 preparations of equal length from the isthmic and ampullary regions, respectively) during the follicular (*Fol;* n=5 for isthmus and ampulla, 14 for fimbria), ovulatory (*Ov.;* n=6 for isthmus and ampulla, 7 for fimbria) and luteal (*Lut.;* n=14 for isthmus and ampulla, 13 for fimbria) phases of the menstrual cycle.

cell. In electron microscopy the synaptic clefts have in fact been found to be narrow enough to permit the transmission of nervous excitation to the muscle cell (Fig. 3). (Sporrong *et al.*, 1982).

Several methods have been used to evaluate tubal muscular activity both *in vitro* and *in vivo*, both intratubular pressure recording system and extratubular transducer systems having been developed for this purpose. Depending on the aim of the study, essentially two types of smooth muscle preparations have been used as *in vitro* models. One consists of carefully microdissected smooth muscle elements from circular and longitudinal musculature (Lindblom, 1979). Another type of preparation uses ring-like segments

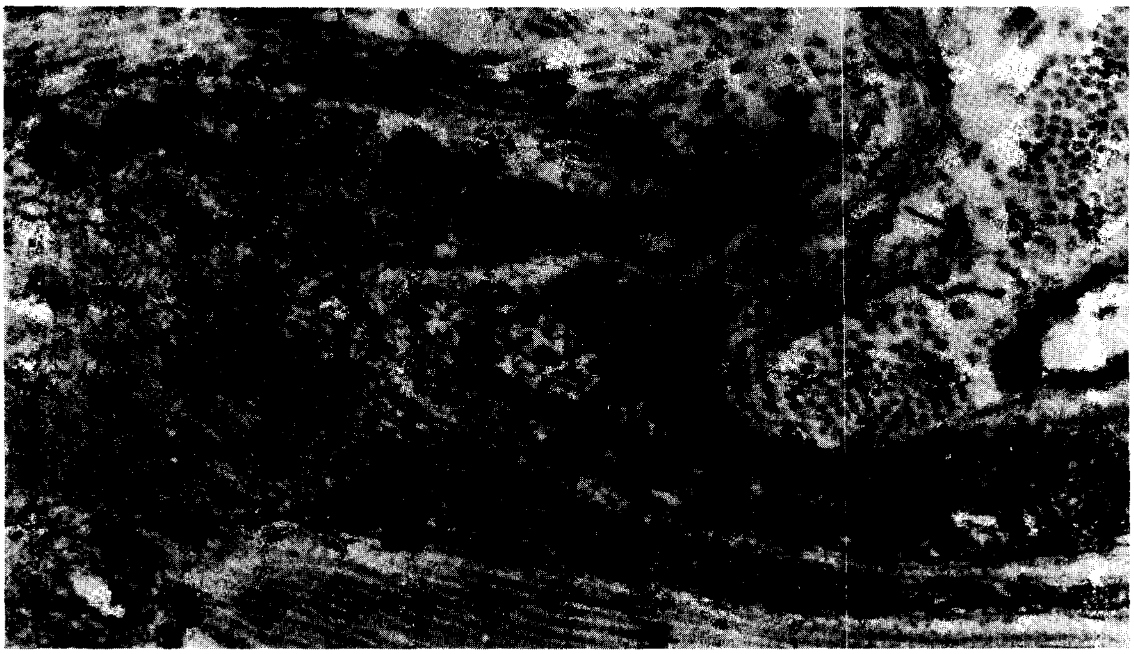

Fig. 3: Adrenergic nerve terminal located 70 nm from the membrane of a smooth-muscle cell. The apposing membrane of the terminal is not covered by the Schwann cell. The size of the contact area is considerable. (x 35,000).

of the tube to which only the external connective tissue is removed. This type of preparation was considered to provide a better representation of *in vitro* conditions (Helm *et al.*, 1982c).

In this *in vitro* system, the muscular preparations exhibit spontaneous contractions with no consistent difference in contraction frequency between isthmic and ampullary segments. The circular and longitudinal muscles differed only in the isthmus and only at the time of ovulation, when the circular musculature showed a significantly higher frequency of spontaneous contractions (Fig. 4).

Addition of noradrenaline lowered the contraction frequency of isthmic longitudinal and circular muscle in the follicular and luteal phases, but increased that of the longitudinal muscle of the ampulla in the ovulation period. The noradrenaline induced changes expressed as the area under the curve of motor activity, measured planimetrically, are given here in the form of a computer plot (Fig. 5). This

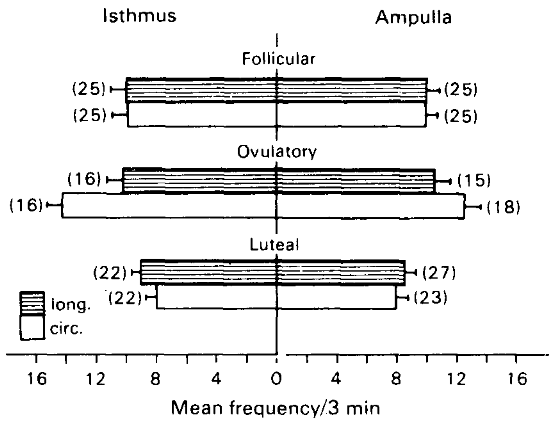

Fig. 4: Frequency of spontaneous tubal contractions (isthmus and ampullary longitudinal and circular musculature) during the follicular, ovulatory and luteal phases of the menstrual cycle. Values are means ± s.e.m. for the no. of preparations in parentheses.

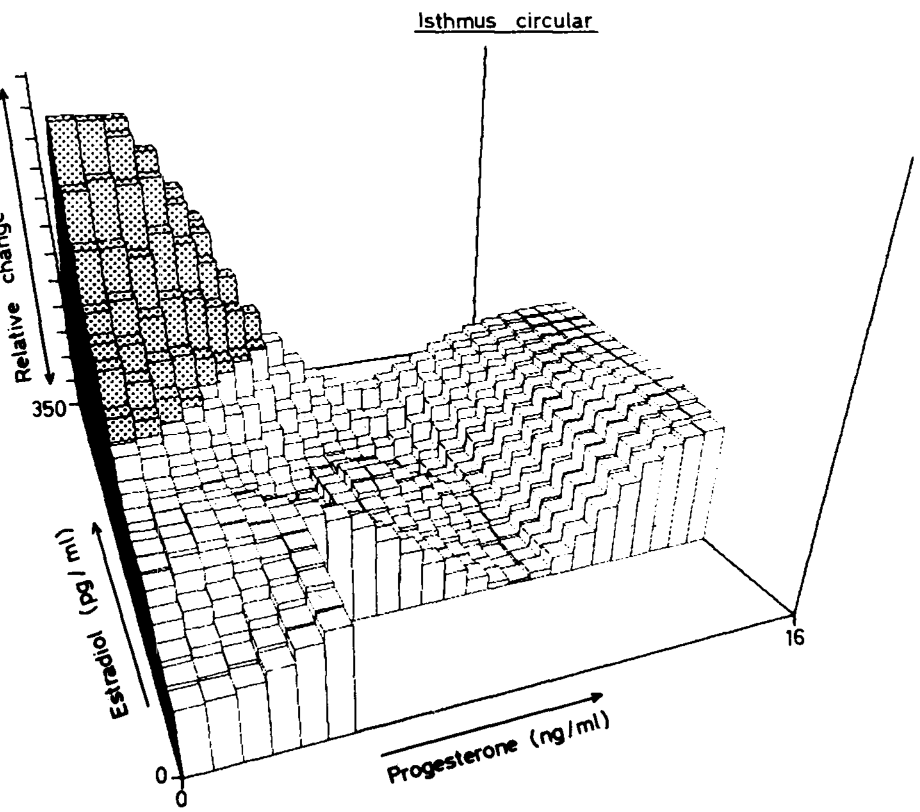

Fig. 5: Three-dimensional computer plot based on material from the circular isthmic smooth musculature (n=63), showing increase (shaded columns) or decrease (open columns) in area under curves of motor activity induced by 3 x 10^{-6} M norepinephrine, in relation to the plasma levels of estradiol and progesterone. The relative changes in area were measured planimetrically during equal time periods before and after administration of the amine.

effect of noradrenaline was studied to illustrate the net response that might be expected following sympathetic neurotransmitter release at different cyclic stages. Comparison of the areas under the curve before and after noradrenaline administration also confirmed that an α-receptor-mediated sympathomimetic increase in the motor activity prevails during ovulation, whereas the contribution by a β-receptor-mediated reduction in motor activity is more prominent during the follicular and luteal phases (Moawad *et al.*, 1976; Helm *et al.*, 1982d).

Quantitative pharmacological characterization of the two receptor types indicates that, in addition to the altered balance between α- and β-adrenoceptor function (Moawad *et al.*, 1976; Owman and Sjöberg, 1976; Helm *et al.*, 1981), there are also changes in the properties of adrenoceptors, particularly the α-receptors, which may be modified during various stages of the menstrual cycle (Helm *et al.*, 1982c).

Electrical nerve stimulation performed in an *in vitro* bath system has been shown to release sufficient noradrenaline to produce a frequency-dependent motor response in the isthmus. The amplitude of this response is about equal both in circular and in longitudinal smooth muscle preparations from the isthmus (Sporrong *et al.*, 1982). Tetrodotoxin, which blocks neural transmission, abolishes the response though, the spontaneous contractions persist. Thus together with the above-mentioned ultrastructural observations, it seems clear that the adrenergic nerves do play a functional role in modifying contractility in the human Fallopian tube.

In studying effects of estradiol and progesterone on various female reproductive tissues, there has been a general tendency to relate the results obtained to the concentrations of sex hormones in the blood. However, there is reason to suppose that the biological activity is rather governed by the tissue hormone concentrations or the steroid receptor content. Radioimmunological measurements of steroids have shown them to be much higher in tubal tissue than in plasma (Batra *et al.*, 1980), thus confirming the presence of estrogen and progesterone receptor (Robertsson *et al.*, 1975; Verhage *et al.*, 1980; Helm *et al.*, 1987a), and that there is also cycle phase-dependent variation in the concentrations of the two receptor types. This suggests that the properties of the adrenoceptors in different types of smooth musculature in different regions of the tube may be altered under the influence of sex steroids in a way that is not simply related to their plasma concentrations.

In *in vitro* studies a motor inhibition was observed to occur during transmural nerve stimulation, despite adrenergic and cholinergic blockade (Helm *et al.*, 1982d). Moreover, it was shown that addition of the nerve blocker tetrodotoxin to the *in vitro* system abolished both the α-adrenergic response and also the reduced motor activity produced by the β-receptors, showing both effects to be mediated by stimulation of the nerves in the preparations rather than a direct result of smooth muscle activation.

The presence of a nonadrenergic, noncholinergic mechanism in the human oviduct has been confirmed by Lindblom *et al.* (1979). Is has been speculated that polypeptides act as neurotransmitters, and a number of polypeptides have been shown to be widely distributed in several organs. The first peptide to be demonstrated in the female genital tract was vasoactive intestinal polypeptide (VIP), a 28 amino acid peptide (Alm *et al.*, 1977, 1980). Evidence is accumulating that this peptide represents a real neurotransmitter (Fahrenkrug, 1979).

Exposure of smooth muscle preparations from human oviduct to VIP induces a dose-dependent relaxation both in circular and longitudinal preparations (Fig. 6) (Walles *et al.*, 1980; Helm *et al.*, 1981).

In immunohistochemical studies of the distribution of VIP, VIP-containing nerve fibers have been identified in human Fallopian tubes, particularly in the smooth muscle layers, but also beneath the epithelium of the isthmic part of the tubes. In the ampullary region and in the fimbriated end, only scattered VIP terminals are observed. VIP has also been identified in nerve cell bodies occurring in clusters in

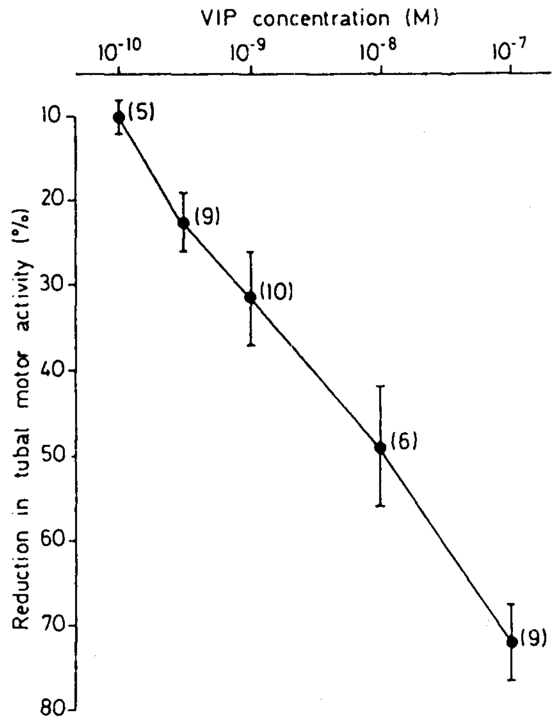

Fig. 6: Reduced contractile activity of the tubal isthmus (circular musculature) when increasing doses of VIP are added in acumulative manner. The final mean concentrations in the bath are given; vertical bars show SEMs. Number of observations within parentheses.

the paracervical tissue. This suggests that uterovaginal VIP nerves may have a local origin, analogous to the short adrenergic neurons mentioned above.

As measured by radioimmunoassay, the VIP concentration was found to be significantly higher in the isthmus of the Fallopian tube than in the rest of tubal tissue. This shows a good correlation between immunochemical and immunohistochemical findings (Helm *et al.*, 1981).

Analysis of cyclic fluctuation of VIP in the isthmic part of the Fallopian tube showed the VIP concentration to be increased in the isthmus throughout all phases of the cycle. The observed variation in VIP concentrations would seem to represent a real fluctuation at the neural level, analogous with that previously shown for the neurotransmitter noradrenaline (Helm *et al.*, 1987b).

In addition to VIP, the following peptides have hitherto been found in the Fallopian tube: substance P (SP), gastrin releasing peptide (GRP), calcitoningene-related peptide (CGRP), peptide histidine isoleucin (PHI) and neuropeptide Y (NPY) (Table 1).

NPY-immunoreactive nerve fibers are found in all tubal regions, the isthmic part being best supplied with fluorescent fibers. In the ampulla and in the infundibulum the nerve terminals were most abundant around blood vessels. In the mucosa the fibers ran close to the epithelium (Samuelsson and Dahlsgaard, 1985).

NPY inhibits in a dose-dependent manner the neurogenic contractions in the external longitudinal muscle layer over the human isthmus, and it also inhibits noradrenaline release from isthmic preparations during field stimulation, suggesting the occurrence of a prejunctional inhibitory action of the adrenergic neurotransmission (Samuelsson and Dahlsgaard, 1985).

SP enhances contractile activity in the isthmic lingitudinal muscle layer (Samuelsson, 1985). SP-immunoreactive nerve fibers were sparse in number in human oviducts. They were localized within the tubal musculature and formed networks around the tubal blood vessels. The GRP-nerve fibers were also sparse in the muscular wall of the oviduct.

CGRP-immunoreactive nerve fibers have been found in muscular layers, around blood vessels and close to the epithelium. This peptide also inhibits spontaneous

TABLE

Relative frequency of immunoreactive peptidergic nerve fibers in the oviduct of various species.
Arbitrary evaluation of the density of non-vascular nerve fibers: – no fibers; + small number of fibers; + + moderate number of fibers; + + + large number of fibers

Species	SP	GRP	VIP/PHI	NPY
Oviduct				
Rat	+ +	+	+ +	+ +
Guinea-pig	+ +	+	+	+ + +
Cow	—	—	+ +	+ +
Pig	—	—	+ +	+ + +
Human	+	+	+ +	+ + +

SP = Substance P.
GRP = Gastrin Releasing Peptide.
VIP/PHI = Vasoactive Intestinal Polypeptide / Peptide Histidine Isolcucin.
NPY = Neuropeptide Y.

contraction in a dose-dependent manner in the human oviduct, and inhibits the SP-induced muscular contraction in the oviduct (Kannisto *et al.*, 1986).

Electron microscopy of nerve terminals has shown the existence of transmitter-containing vesicles of different structure and size, a finding consistent with the evidence of coexistence of different transmitter substances (Fried *et al.*, 1985). Thus immunohistochemical investigation has shown the coexistence of VIP/PHI and NPY/NA (Lundberg and Hökfelt, 1983; Kannisto *et al.*, 1986).

Present knowledge of the autonomic nervous system in the human Fallopian tube suggests that tubal function may be subject not only to the classic autonomic transmitters, noradrenaline and acetylcholine, but also to several regulatory peptides.

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