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Spontaneous contractile pattern of isolated circular and longitudinal muscle layers from the ampullae of normal and damaged fallopian tubes

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Summary

Ampullary biopsies were obtained from twenty-three normal fertile women and from eleven women with hydrosalpinges. The contractile activity of 4-mm muscle strips was studied isometrically in organ bath. Both normal circular and longitudinal muscle strips contracted with similar frequency, duration and amplitude. Circular and longitudinal muscle strips from hydrosalpinges contracted with a lower frequency and a longer duration and the amplitude of contractions was higher than normal. The difference in frequency and duration was statistically significant. The possible explanations for these differences in rhythmicity between normal and diseased fallopian tubes are presented and the prognostic relevance discussed.

Résumé

Des biopsies ampullaires ont été prélevées à vingt-trois femmes à fécondité normale et à onze autres atteintes d'hydrosalpingite. Nous avons étudié isométriquement dans un bain, des tranches de muscles de 4 mm, afin d'en déterminer le fonctionnement contractile. Les tranches circulaires normales et celles de forme longitudinale se sont contractées avec la même fréquence, la même durée et la même amplitude. Des tranches semblables mais atteintes d'hydrosalpingite se sont contractées moins fréquemment et en une durée moins prolongée, alors que l'amplitude des contractions a été plus élevée qu'a l'ordinaire. La différence entre fréquence et durée a été très importante du point de vue statistique. Dans cette étude, nous cherchons à expliciter ces différences rythmiques entre oviductes normaux et oviductes pathologiques et nous en analysons la pertinence pronostique.

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Introduction

The mechanism of transport of ova through the ampulla and their delay in this segment is not completely understood. The relative importance of ciliary beat and of muscle contractions in ovum transport has been the reason for disagreement amongst investigators in this area. Investigators have tended to be polarized into two schools, those who believe that the cilia are more important (Halbert *et al.*, 1976; Blandau, Bourdage & Halbert, 1979), and those who favour muscular action (Anand & Guha, 1978; Jean *et al.*, 1979). Delay at the ampullary–isthmic junction (AIJ) is thought to be controlled by contractions of smooth muscle cells rather than any sphincteric action, since reversal of isthmic segments, chemical sympathectomy and autograft transplantation of the fallopian tube do not affect normal transport (Eddy, Hoffman & Pauerstein, 1976; Eddy & Black, 1974; Winston & Browne, 1974).

Muscular activity has been shown to provide the primary propulsive force in the instantaneous movement of ovum surrogates (Hodgson, Talo & Pauerstein, 1977). It, therefore, seems very likely that damage to the ampullary muscle may be an important cause of subfertility in women after tuboplasty. Little attention has been paid to the smooth muscle changes that occur in hydrosalpinges.

Ampullary dilatation alone with or without previous infection produce certain changes in the smooth muscle. These changes include, oedema, atrophy and fibrosis in humans and hypertrophy, atrophy and increased collagen deposits in animal models. (Otubu, 1984). These changes may well affect the mechanical action of the smooth muscle layers of the ampulla.

The aim of this study was to evaluate the spontaneous mechanical activity of the smooth muscle in hydrosalpinges in humans *in vitro* and to compare this activity with that in normal ampulla.

Materials and methods

Diseased fallopian tubes were obtained from eleven patients undergoing salpingostomy of the contralateral tube, salpingectomy in preparation for in-vitro fertilization or hysterectomy for benign uterine disease. Their ages ranged from 28 to 48 years.

Portions of normal fallopian tubes were obtained from twenty-three patients (aged 27–49) undergoing tubal sterilization or hysterectomy and bilateral salpingectomy for benign disease.

General anaesthesia was administered to all patients during surgery. Surgeons were most co-operative in removing fallopian tubes with as little direct handling as possible and without applying clamps. The tissues were immediately submerged in buffer. When possible venous blood was drawn before surgery. The phase of menstrual cycle was determined by histological examination of an endometrial biopsy when available and by level of serum progesterone and estradiol 17β. Regular cycles were recorded in most patients and none of the women had used hormonal contraception up to 8 weeks before surgery.

In-vitro isometric contractility

All portions of fallopian tubes taken for this study were taken from areas less than 4 cm from the fimbrial end. The number of mucosal folds and the wide diameter confirmed that the ampulla was being used. Immediately after opening the abdomen, 1–2-cm long portions of the ampullary part were excised. The tissue was immediately placed in oxygenated ice-cold Krebs–Ringer bicarbonate buffer and transported to the laboratory.

Dissection

All dissection was performed under a stereomicroscope (Olympus) at 10–16 times magnification. This aided the identification of the direction of muscle bundles. During dissection, the tissues were kept moist with ice-cold buffer.

Strips with a cross-sectional area of about 1 mm^2 were obtained. The length of the strips were kept to 4 mm. This was measured directly using a graticle attached to the eyepiece of the microscope. Strips of circular and longitudinal muscle fibres were obtained without difficulty in the normal ampullae. Diseased fallopian tubes (hydrosalpinx) were most difficult to disect. Many attempts were made on different portions of each fallopian tube specimen before fibre direction could be defined in certain areas. The time spent on dissection was slightly longer in diseased fallopian tubes compared with normal tubes.

Recording of contractile activity

A silk ligature (4.0) was tied to each end of the muscle strip and one end attached to a glass tissue holder. Muscle preparations were then transferred to a mantle organ chamber. The incubation chamber contained 50 ml of Krebs–Ringer bicarbonate buffer at 37°C. The Krebs–Ringer bicarbonate buffer had the following composition: (mmol) NaCl 122; KCl 4.7; $NaHCO_3$ 15.5; KH_2PO_4 1.19; $MgCl_2 \cdot 6H_2O$ 1.19. The chamber was continuously aerated with 95% O_2 and 5% CO_2, at a constant pH range of 7.34 ± 0.04. The ligature at the other end of the strip was connected to an isometric force transducer (Grass Model FT. 03) under a tension of 4 mN. This passive force was found to give optimal active tension in length–tension experiments. The contractile activity was recorded on a Grass Polygraph (Model 7). After the muscle strips were placed in organ baths, 60 min was allowed for equilibration.

Quantitative measurements of the frequency, duration and amplitude of contractions were then recorded. Twenty-three observations in normal tubes and eleven observations in hydrosalpinx muscle were compared in the final analysis.

Statistics

Frequency of contraction, duration and amplitude over a 10-min period in normal and hydrosalpinx tubes were compared by the Student's *t*-test.

Results

Both normal circular and longitudinal muscle strips showed spontaneous rhythmic mechanical activity. This was always the case despite the phase of the menstrual cycle. Activity commenced spontaneously within 15 min of placing the specimens in organ bath and continued for up to 3 h. The characteristic patterns of contractions of circular and longitudinal muscle strips are shown in Fig. 1.

There was no difference between the contractile patterns in the proliferative and secretory phases of the cycle. Table 1 shows a summary of the frequency of contractions recorded every 10 min and the duration and amplitude of contractions in normal circular and longitudinal layers.

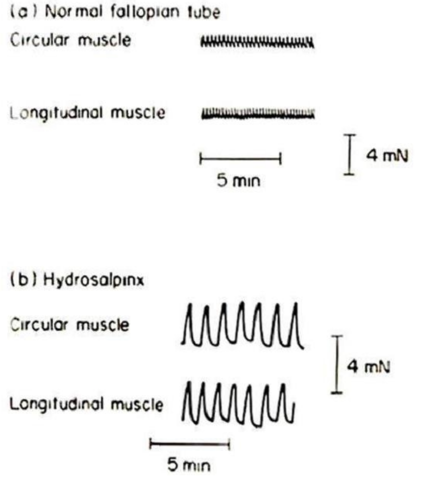

Fig. 1. Characteristic pattern of contractions in (a) normal and (b) hydrosalpinx fallopian tubes.

There was no statistically significant difference between these measurements.

Circular and longitudinal muscle strips from hydrosalpinges also showed rhythmic spontaneous activity. There was, however, no characteristic reproducible pattern. The pattern shown in Fig. 1 is one of several patterns exhibited by these muscle strips. The contractions started within 30 min of mounting the strips and continued for 2 h at the most. A few strips failed to contract spontaneously.

The summary of the frequency over a 10-min period and duration and amplitude of circular and longitudinal strips are shown in Table 1. Although muscles from hydrosalpinges contracted at a lower frequency, longer duration and higher amplitude than did muscle from normal tissue, only the differences in frequency and duration were statistically significant ($P < 0.01$).

Discussion

The advantages of dissecting circular and longitudinal muscle fibres away from each other, so that separate recordings of mechanical activity can be obtained, have been extensively discussed by Lindblom, Hamberger and Wiqvist (1978). The only report in the literature that compares the physiological activity (frequency, duration and amplitude of contractions) of normal circular and longitudinal muscles of the ampulla is that of Cascheto *et al.* (1979). The method used in that study is the same as in this study and the results are comparable. There are, however, no reported studies on the contractility of circular and longitudinal muscles from hydrosalpinx. The comparison with normal ampulla in these experiments is the first such study. These preliminary findings show that muscle taken from hydrosalpinges has a lower frequency of contraction with a longer duration and higher amplitude.

There was no significant difference between these measurements at any particular phase of the menstrual cycle. This finding is similar to Cascheto *et al.* (1979) but different from that of Helm *et al.* (1982). Differences may indeed exist in these measurements in various phases of the cycle but, since these differences were not statistically significant in this preliminary report, it cannot be said to affect the overall

Table 1. Summary of frequency of contraction in circular and longitudinal muscle layer of normal and hydrosalpinx tubes means ± s.e.m.

	Normal (n = 23)		Hydrosalpinx (n = 11)	
	Circular	Longitudinal	Circular	Longitudinal
Frequency (contraction/10 min)	41.1 ± 3.1	43.5 ± 5.8	21.8 ± 15.2	24.1 ± 14.1
Duration (sec)	14.7 ± 2.3	13.2 ± 1.4	27.9 ± 7.1	25.5 ± 13.5
Amplitude (mN)	1.1 ± 0.004	1.1 ± 0.01	1.3 ± 0.02	1.5 ± 0.06

difference between normal and damaged tubes. Although the dissection of damaged fallopian tubes was difficult in certain portions, the trauma of dissection, if any, was similar in both the normal and damaged tubes. It is, however, possible that with oedema and fibrosis in the musculature of the hydrosalpinges, one mm^2 tissue may contain fewer fibres than one mm^2 normal fallopian tube muscle. The presence of active infection might also alter contractility although infection was seldom found on histological examination at the end of the experiment. Despite these reservations, the differences in the measurements in the different tissues are real and significant.

The possible reasons for this difference in contractile pattern may include atrophy, fibrosis, hypertrophy or oedema in the damaged tube. Hypertrophy of smooth muscle has been demonstrated in mechanical hydrosalpinges in rabbits, while increased collagen content was demonstrated in muscles from hydrosalpinges (Otubu, 1984).

Caution is needed in interpreting in-vitro experiments, but one is tempted to suggest that if this altered mechanical activity shown here is confirmed *in vivo*, it may affect ovum transport through the ampulla. This is more so since longitudinal muscle fibres may be crucial for linear transport of ova, while circular fibres play a major role in ovum retention, particularly in the region of the ampullary–isthmic junction (Talo & Brundin, 1973).

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