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The development of an Usssing chamber technique for isolated human vaginal mucosa, and the viability of the in vitro system

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Abstract

An Ussing chamber technique for isolated human vagina has been developed. Human vagina is preferred as animal epithelium is keratinized to various degrees. Moreover, a slicer technique is introduced to reduce non-epithelial tissue, as only the multilayer epithelium is assumed to be the absorption barrier in vivo. The electrical properties of the tissue (short-circuit current (I_{sc}) and transepithelial potential difference (PD)) are recorded to estimate the tissue viability. The efficacy of the slicer technique is further illustrated histologically. I_{sc} and PD are relatively stable between 1 and 5 h (plateau about 23 $\mu A/cm^2$ and 12 mV). The viability of the tissue is longer than 6–8 h, where I_{sc} is greater than 80 and 50% of the I_{sc} plateau, respectively. Electrical resistance is about 500 Ω cm^2. The method is considered to be useful for in vitro studies of vaginal electrophysiology and permeability. The slicer technique may also be useful for isolation of other multilayer barriers, e.g., from the skin.

Key words: Ussing chamber; Vaginal tissue; Electrophysiology; Viability

1. Introduction

Due to increasing interest in alternative routes for application of drugs, such as peptides, and in identification of absorption promotors, there is a need for good in vitro methods as a supplement to in vivo techniques. Physiological factors and absorption properties, as well as enhancer mechanisms and toxicity, may be studied.

The Ussing chamber method is a well known in vitro system developed by Hans H. Ussing (1948). Originally, it was used to characterize the ionic transport across biological membranes. However, it has also been used to study drug absorption and tissue membrane interactions. The Ussing chamber has been used with many different tissue preparations and tissue from different species. For example, Siegel et al. (1981) and Veillard et al. (1987) used isolated oral mucosa from rabbits, hamsters and dogs to study the permeability of different compounds. Corneal drug permeability has been investigated by Schoenwald and Huang (1983), who used isolated rabbit corneas. Nasal drug absorption studies and corresponding electrophysiological measurements have been performed by Wheatley et al. (1988) and Bechgaard et al. (1992), whereas Grass and

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Sweetana (1989) and Grass et al. (1990) used the Ussing chamber to measure permeability in gastrointestinal tissue. Permeability studies and vaginal toxicity studies have been performed in isolated vaginal tissue from rats (Levin and Parker, 1987) and humans (Levin and Parker, 1988).

The Ussing chamber has also been used to study electrophysiological properties of different cell lines, e.g., MDCK (Popowicz et al., 1985) and human intestinal clonal cell line Cl.19A (Rouyer-Fessard et al., 1989).

The vagina has traditionally been used for local treatment, but systemic drug delivery is also possible. Intravaginal drug delivery has recently been reviewed by Deshpande et al. (1992). In addition to human clinical studies, various species have been used for in vivo absorption studies, e.g., rabbits (Yotsuyanagi et al., 1975), rhesus monkies (Park et al., 1979), and rats (Okada et al., 1982; Richardson et al., 1989a,b).

A common problem with the studies using vaginal tissue is the cyclical changes of the vaginal epithelium thickness. In order to standardize the thickness of the vaginal tissue, Richardson et al. (1989a) used ovariectomized rats. The vaginal epithelium of the ovariectomized rat is thin, and may, for example, be more susceptible to drug-induced damage than vaginal epithelium from a non-ovariectomized rat. Richardson et al. (1991) overcame this problem by pretreatment of the ovariectomized rat with oestradiol. In addition to the problems with varying tissue thickness, the vaginal epithelial cells in animals, but not women, are keratinized to various degrees resulting in more difficult drug penetration. To overcome these problems an in vitro method with sliced human vaginal tissue, resulting in isolation of the epithelium primarily, is introduced and the viability of the in vitro system is evaluated.

2. Materials and methods

2.1. Chemicals

The bicarbonate Ringer solution (GR) consisted of HPO_4^{2-} (1.6 mM), $H_2PO_4^-$ (0.4 mM), Mg^{2+}(1.2 mM), Cl^- (122 mM), Ca^{2+} (1.2 mM), K^+ (5 mM), HCO_3^- (25 mM), Na^+ (141 mM) and D-(+)-glucose (13 mM). These chemicals except glucose (May & Baker, Dagenham, U.K.) were of analytical grade and obtained from Merck (Darmstadt, Germany). ^{14}C-labelled polyethylene glycol 4000 (5 μCi/ml) is commercially available from New England Nuclear (Du Pont, Boston, MA, U.S.A.). Carbogen (95% O_2/5% CO_2) was obtained from Hede Nielsen A/S (Denmark). Scintillation cocktail (Pico-aqua) was purchased from Packard Instrument BV (Groningen, The Netherlands).

2.2. Apparatus

The Ussing chamber consisted of two 1 ml acrylic half-chambers as described by Bechgaard et al. (1992). The Thomas®-Stadie-Riggs tissue slicer was purchased from Thomas Scientific (Swedesboro, NJ, U.S.A.). The PL Tri-Carb scintillation counter was from Packard Instruments Co. (Downers Grove, IL, U.S.A.).

2.3. Tissue preparation

Vaginal tissue was obtained from seven non-malignant women (mean age 54, range 36–72 years) undergoing vaginal surgery performed at Bispebjerg or Hvidovre Hospital (Copenhagen, Denmark). The study was approved by the local ethical committee. Prior to vaginal operation, patients over 50 years of age were pretreated with oestrogen.

The tissue was immersed in oxygenated GR (0–4°C) until preparation. The vaginal tissue was carefully cut into shape, and the thickness was adjusted by removing underlying blood filled layers before the vaginal epithelial layer was isolated by a longitudinal slice through the whole preparation.

2.4. Ussing chamber set-up

The freshly isolated vaginal tissue was mounted between two acrylic half-chambers (0.50 cm^2 exposed surface area), which were joined to form the complete Ussing chamber. The reservoirs were equipped with a gas lift system (carbogen)

to provide both oxygenation and circulation of the chamber solutions. The total volume of each reservoir was 1.0 ml and the whole system was maintained at 37°C throughout the study.

2.5. Viability study

During a 12 h period, the electrophysiological properties of the isolated human vaginal tissue were registered to establish viability of the in vitro system. 1.0 ml GR was added to both the mucosal (M-side) and serosal side (S-side). Each experiment was terminated by control of the tissue integrity with ^{14}C-labelled polyethylene glycol 4000 as described by Bechgaard et al. (1992).

2.6. Histology

Slices of human vaginal tissue were fixed in Lilly's solution (10% buffered formalin solution), and routinely processed for paraffin embedding sections and staining with hematoxylin and eosin. A magnification of ×40 was used for microscopy.

3. Results and discussion

Fig. 1 shows that the thickness of the mucosal tissue can be reduced substantially by the slicing technique used. Vaginal epithelium can be isolated almost totally from underlying tissue as shown in Fig. 2, where the thickness of the slice is 0.44 mm, representing 0.38 mm epithelium and only 0.06 mm subepithelium. The method may also be usable for other tissues with several epithelium cell layers, e.g., skin and buccal tissue.

As shown in Tables 1 and 2 the short-circuit current (I_{sc}) and the potential difference (PD) are relatively stable between 1 and 5 h (mean about 23 μA/cm^2 and 12 mV). The viability of the tissue is longer than 6–8 h, where I_{sc} is greater than 80 and 50% of the above-mentioned mean I_{sc}, respectively. The electrical tissue resistance (TR) is about 500 Ω cm^2.

Levin and Parker (1988) have found I_{sc}, PD and TR values of about 8 μA/cm^2, 6 mV and 700 Ω cm^2, respectively, in human vaginal (ectocervix) epithelium, where the subepithelial fibrous connective tissue has been trimmed off. These I_{sc} and PD values are 2–3-times lower than ours, but the TR is in good agreement with our observation, indicating that trimming may sometimes be a good alternative to the present slicing technique.

The observed PD is about 50% lower, but still in fairly good accordance with the PD of 25 mV observed in normal women (Wagner and Levin, 1978), and they found the PD to be independent

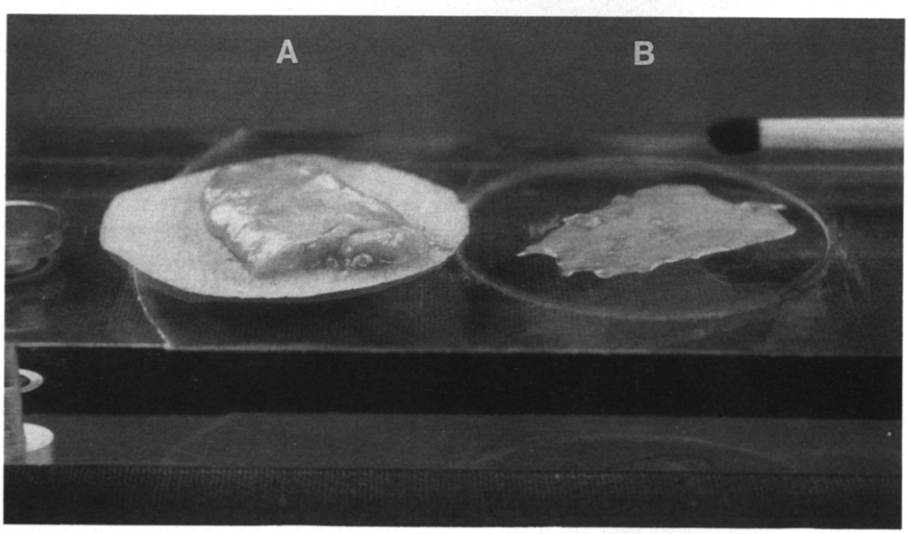

Fig. 1. Isolated human vaginal tissue prior to (A) and after slicing (B).

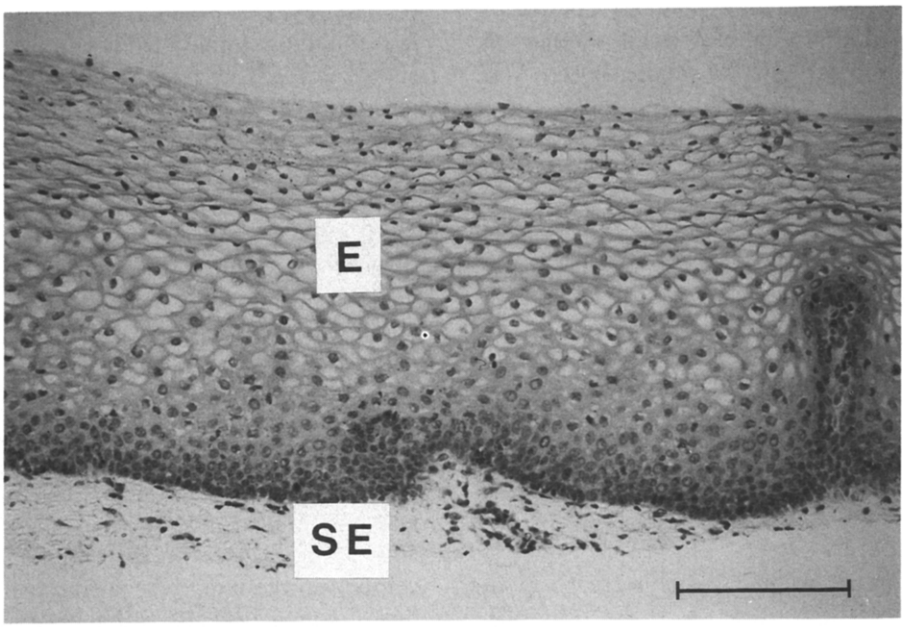

Fig. 2. Cross-section of sliced human vaginal tissue (thickness 0.44 mm including 0.38 mm epithelium). Microscophy performed with a primary magnification of ×40.

of the hormonal status. This difference between PD measured in vivo and in vitro has also been observed in rats (Edwards and Levin, 1975). They found a reduction of PD of about 60% in vitro. The electrophysiological properties of the vaginal tissue from the rat were found to be dependent on the stage of the cycle. The observed in vitro values were about 14, 4, 4, and 6 mV in oestrus, metoestrus, dioestrus, and pro-oestrus, respectively. The corresponding I_{sc} and TR values were about 10, 4, 5, and 3 $\mu A/cm^2$ and 1500, 800, 800, and 2400 Ω cm^2. One reason for the much higher TR found in rats can be due to the fact that the epithelium of the rat is keratinized.

The observed TR of about 500 Ω cm^2 may be considered as remarkably low, as the vaginal epithelial barrier consists of several cell layers. In comparison, Artursson (1990) observed a TR of

Table 1
Short-circuit current ($\mu A/cm^2$) of isolated human vaginal tissue mounted in the Ussing chamber

Experiment	Time (h)										
	0	0.5	1	2	3	4	5	6	8	10	12
I	20	18	16	12	10	10	12	8	4	0	0
II	34	30	32	32	32	28	26	22	20	10	2
III	14	8	6	6	–	4	4	4	4	2	2
IV	24	16	16	14	14	14	22	20	12	8	6
V	38	16	16	18	18	16	16	16	14	10	6
VI	46	50	60	66	68	66	60	52	32	18	12
VII	28	20	24	22	18	16	14	12	6	4	2
Mean	29	23	24	24	27	22	22	19	13	7	4
S.D.	11	14	18	20	22	21	18	16	10	6	4

–, not measured.

Table 2
Transepithelial potential difference (mV) of isolated human vaginal tissue mounted in the Ussing chamber (mucosal side is negative relative to serosal side)

Experiment	Time (h)										
	0	0.5	1	2	3	4	5	6	8	10	12
I	12.1	10.5	13.0	12.8	11.0	11.5	12.7	12.5	11.3	7.5	5.0
II	11.0	11.2	11.0	11.5	9.5	8.5	10.0	8.0	4.9	1.0	0.0
III	2.2	1.5.	2.2	2.4	2.2	2.0	1.8	1.6	1.5	0.8	0.1
IV	9.9	10.5	14.0	15.1	15.5	16.0	23.0	23.8	16.8	13.7	10.9
V	6.7	4.8	5.2	5.7	5.5	5.3	5.2	5.2	4.8	3.4	2.5
VI	12.4	12.2	17.8	26.9	30.0	30.0	30.2	33.8	30.0	24.3	20.0
VII	4.5	6.5	8.2	9.8	11.2	11.9	11.9	11.0	8.3	5.7	4.2
Mean	8.4	8.2	10.2	12.0	12.1	12.2	13.5	13.7	11.1	8.1	6.1
S.D.	4.0	4.0	5.4	7.8	9.0	9.1	9.9	11.3	9.7	8.4	7.2

about 250 Ω cm^2 in monolayers of cultured human intestinal cells (Caco-2 cells) and Bechgaard et al. (1992) reported a TR of about 70 Ω cm^2 for the leaky nasal mucosal tissue isolated from rabbits.

Based on the observed electrical properties, histology and viability of the tissue, the method is considered to be useful for in vitro studies of human vaginal electrophysiology and permeability.

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