

Model of counter-current transfer from vagina to urethra in postmenopausal women

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BACKGROUND: Low-dose vaginal oestrogens are effective in treating post-menopausal urogenital atrophy without inducing endometrial proliferation. We aimed to assess whether this dicotomic effect could be the result of a preferential vagina-to-urethra transfer via a counter-current transfer of oestrogens from vagina to the arterial blood supplying the urethra. Due to the impossibility of obtaining blood samples from urethral arteries, and since the nature of counter-current exchange of substances is similar to the transfer of heat, we investigated cold transfer throughout the anterior vaginal wall to the vesical trigone and urethra. **METHODS:** Plastic tubes filled with cold saline were inserted into the vagina of 30 menopausal women. Temperatures were recorded at the vesical trigone and at three different urethral sites. Comparisons were performed 2 and 4.5 min after starting of cooling, and 4.5 min after removal of tubes. **RESULTS:** The urethra cooled significantly more than the bladder. Urethral cooling was not homogeneous; distal sites of the urethra cooled significantly more than proximal site and bladder despite a larger distance to the vaginal cooling device. **CONCLUSIONS:** Distribution of cold from the vagina to the urethra is not the result of simple diffusion and mechanisms of preferential distribution may exist from the vagina to the middle and low part of the urethra.

Key words: counter-current transfer/preferential distribution/urethra/urinary atrophy treatment/vagina

Introduction

In western countries ~30–40% of postmenopausal women without oestrogen replacement therapy complain of symptoms related to urogenital atrophy (Stemberg *et al.*, 1996; Barlow *et al.*, 1997). In most cases, systemic oestrogen replacement therapy is effective in rapidly reversing the symptoms (Luisi *et al.*, 1980; Rud, 1980; Brandberg *et al.*, 1987; Falconer *et al.*, 1996). However, women treated several years after their menopause often accept systemic oestrogen replacement therapy poorly and in women with an intact uterus the combination with progestins is mandatory. As an alternative, oestrogens at very low dosage (only ~10–15% of that usually required to alleviate hot flushes) administered vaginally have been repeatedly reported to be effective in treating urogenital atrophy (Samsioe *et al.*, 1985; Mettler and Olsen, 1991; Raz and Stamm, 1993; Smith *et al.*, 1993; Fantl *et al.*, 1994; Barentsen *et al.*, 1997) without inducing endometrial proliferation (Vooijs and Geurts, 1995). The existence of a ‘therapeutic window’ has been proposed, where an oestrogen dose within this range should be enough to stimulate urovaginal epithelium but below the oestrogen activity required to promote endometrial growth (Samsioe, 1998). The mechanisms proposed to explain this dicotomic effect include greater sensitivity of the receptors

in the urogenital tract, or the existence of other types of oestrogen receptors or binding sites (Landers and Spelsberg, 1992). Additional mechanisms that could be advocated to explain the different effect of low-dose vaginal oestrogens on the urethra and endometrium are simple vagina-to-urethra diffusion or preferential distribution from the lower part of vagina to the urethra by means of local distribution mechanisms such as counter-current transfer. Physiologically, the counter-current exchange mechanism is known to take place between two tubes such as blood vessels that share a common (exchange) surface and have flows running in opposite directions; substances in high concentrations in venous and lymphatic vessels can diffuse to nearby arteries, where their concentrations will rise above those of other organs (Baker, 1991; Glad Sorensen *et al.*, 1991). Counter-current transfer may also play a role in case of drug administration. The effects on the uterus of progesterone administered vaginally, for instance, far exceed the expectations reasonably drawn from the relatively low blood concentrations achieved, suggesting preferential delivery of progesterone to the uterus, or first uterine pass effect (Miles *et al.*, 1995; Fanchin *et al.*, 1997). The occurrence of a counter-current transfer is strongly supported by the finding that after vaginal administration of progesterone, plasma progesterone

concentration in the uterine artery is twice as high as that from a peripheral artery (Cicinelli *et al.*, 1998). The ratio of endometrial to serum progesterone concentrations after vaginal administration at the posterior vaginal fornix was about 14 times higher than that after intramuscular administration of the same amount of progesterone; this confirms the local relationship between vagina and uterus (Cicinelli *et al.*, 2000). Accordingly, a greater endometrial efficacy of vaginal oestradiol compared with oral administration has been recently reported (Tourgerman *et al.*, 2001).

Vaginal oestrogens that are able to resolve urethral atrophy without inducing endometrial proliferation may be another example of targeted therapy based on a counter-current transfer of oestrogens from vagina to the arterial blood supplying the urethra. However, since it is impossible to obtain blood samples from the urethral arteries or collect biopsies from the urethra, and since the nature of counter-current exchange of substances is similar to the transfer of heat (Glad Sorensen *et al.*, 1991), we decided to use a heat-exchange in-vivo model. It is our experience that heat will be transferred if steroids are transferred and *vice versa*. This has been found in four different situations: between testicular vein blood and arterial blood; between ovarian vein blood and arterial blood; between vaginal-uterine vein blood and uterine arterial blood and between venous blood in the cavernous sinus and internal carotid blood (Einer-Jensen, 1988; Einer-Jensen and Khorooshi, 2000; Einer-Jensen *et al.*, 2001). In no case was transfer of one and not the other found; the reason is probably that the transfer is based on passive diffusion, no pumping mechanism has been found. In this study, we investigated cold transfer from vagina to the vesical trigone and urethra, both in close anatomical relationship with the anterior vaginal wall. Plastic tubes filled with cold saline at different temperatures were inserted into the vagina of volunteers, while the temperature was recorded at vesical trigone and at three different sites of the urethra. Any cooling of the bladder and urethra that could not be explained by a simple diffusion of cold from the vagina may therefore suggest the occurrence of preferential distribution.

Materials and methods

Thirty menopausal women were enrolled in the study after informed consent was given. The women were 54–65 years old, in good general health conditions, all complaining of dyspareunia or symptoms related to urinary atrophic condition. Exclusion criteria were any current either local or systemic hormone replacement therapy, the presence of urogenital prolapse and of urinary infection. The study protocol was approved by the local Ethical Committee.

The participants were asked to empty the urinary bladder before the trial. The women were conscious and reported only minor discomfort during the insertion of the urethral probe. No painkillers or anaesthetics were administered during the trial. Standard gynaecological procedures were used throughout; the probes were autoclaved before insertion. During the investigation, bladder and urethral temperatures were collected every 2 s with a four-canal ELLAB TM9604 electronic thermometer (WWW.ELLAB.COM). The output from the thermometer was transferred to an IBM ThinkPad, Type 2625 with ELLAB TSII software installed. The urethral probes were produced

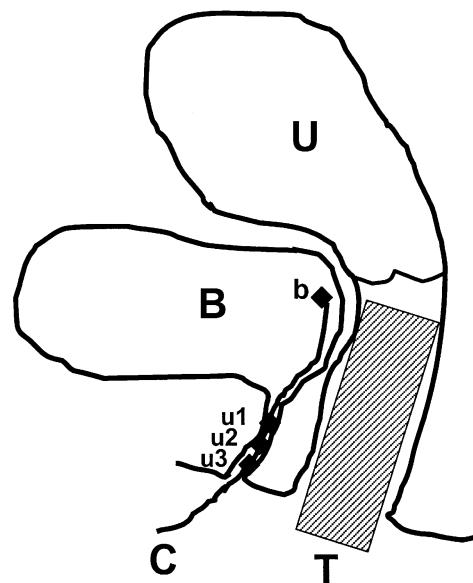


Figure 1. Scheme of the female pelvic organs (B = bladder, U = uterus). Shown are the position of the saline plastic tube (T) in the vagina as well as the four-point temperature probe (C) in the bladder (b) and in the middle and low part of the urethra (u1, u2, u3).

by ELLAB according to our specification and they were based on Cu/CuNi thermo-elements. The probes were 25 cm long, 0.8 mm in diameter, and had four points of measurements: 1 mm from the tip (bladder); 38 mm from the tip (urethra 1); 45 mm from the tip (urethra 2); and 52 mm from the tip (urethra 3). The four points were painted blue and therefore visible during the insertion. The probe was inserted into the urethra until the tip was free in the bladder and the last urethral point disappeared above the urethral external ostium; correct positioning was verified by ultrasound. The probe was taped to a leg; the clinician controlled the position throughout the trial. The probe had a 3 m long connector to the thermometer. One minute after insertion of the probe, the vagina was cooled by means of a disposable plastic tube of 3 cm diameter and 10 cm length (50 ml disposable centrifuge tubes, Corning Incorporated, NY, USA) inserted for 5 min into the vagina (Figure 1). The tubes were used in three different ways: filled with saline at room temperature (22–25°C) (14 women), at 5°C temperature (12 women) and with air at room temperature as controls (four women). Comparisons were performed 2 and 4.5 min after starting of cooling, and 4.5 min after removal of the plastic tube. All registrations were printed and the temperatures calculated manually, after which the results were transferred to a spreadsheet (MS Excel).

Results are stated as mean (\pm SEM) number. Statistical analysis was performed by one-way analysis of variance (ANOVA) for repeated measures; for the analysis temperature values at each point were expressed as percentage of start value (defined as 100). Multiple comparisons among means of temperature at different measurement points were done by Student–Neuman–Keuls test. Significance was established at the level of $P < 0.05$.

Results

The positioning of probes was easy and almost painless for all women. The temperature readings at each measurement point and at different times during vaginal cooling with tubes filled with room temperature and 5°C saline are displayed in Table I.

Table I. Temperatures (°C) in the bladder and in three different sites of the intermediate and distal portion of the urethra (distance between individual urethral measurement sites 7 mm) during vaginal cooling with a plastic tube filled with saline at 22–25°C (A), or with saline at 5°C (B). Data are mean \pm SEM

Time (min)	Bladder	Urethra 1	Urethra 2	Urethra 3
A (saline 22–25°C, 14 women)				
0	37.58 \pm 0.07	37.47 \pm 0.08	37.27 \pm 0.11	37.20 \pm 0.55
2	37.00 \pm 0.37 ^{b†,c†,d†}	35.86 \pm 0.40 ^{a†,b*}	35.38 \pm 0.40 ^{a†}	35.32 \pm 0.58 ^{a†,d§}
4.5	36.80 \pm 0.38 ^{b†,c†,d†}	35.45 \pm 0.33 ^{a†}	35.03 \pm 0.37 ^{a†}	35.08 \pm 0.50 ^{a†}
4.5 (after cooling)	37.03 \pm 0.11 ^{b†,c†,d†}	35.95 \pm 0.20 ^{a†,b*}	35.61 \pm 0.22 ^{a†}	35.14 \pm 0.50 ^{a†,d*}
B (saline 5°C, 12 women)				
0	37.39 \pm 0.06	37.08 \pm 0.13	36.81 \pm 0.18	36.73 \pm 0.11
2	37.22 \pm 0.05 ^{b§,c*,d*}	32.59 \pm 1.09 ^{a*,b§}	30.16 \pm 1.84 ^{a*}	30.57 \pm 1.38 ^{a§,d§}
4.5	36.84 \pm 0.09 ^{b†,c†,d†}	31.36 \pm 1.15 ^{a†}	30.52 \pm 1.07 ^{a†}	31.09 \pm 0.57 ^{a†}
4.5 (after cooling)	36.43 \pm 0.15 ^{b†,c†,d†}	33.35 \pm 0.47 ^{a†,b*,c*}	32.25 \pm 0.54 ^{a†,d*}	31.83 \pm 0.78 ^{a†,d*}

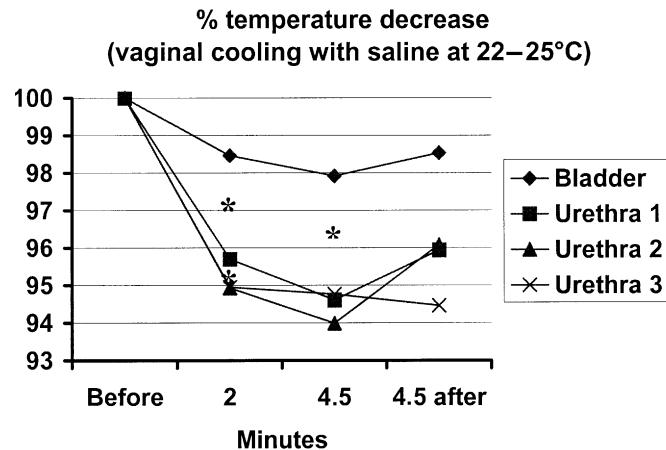
^aPercentage variation is significantly different from that of bladder.^bPercentage variation is significantly different from that of urethra 3.^cPercentage variation is significantly different from that of urethra 2.^dPercentage variation is significantly different from that of urethra 1.* $P < 0.05$.§ $P < 0.005$.† $P < 0.0005$.

Figure 2. Variation of mean temperature as percentage of start temperature at the vesical trigone and in the urethra of women after insertion into the vagina of a tube filled with saline at room temperature (22–25°C). At time 2 min, the upper asterisk indicates that reduction of temperature in the bladder is significantly different from that at all measurement sites in the urethra; the lower asterisk indicates a significant difference between urethra 1 and urethra 3. At time 4.5 min, the asterisk indicates that reduction of temperature in the bladder is significantly different from that at all measurement sites in the urethra.

When the vagina was cooled with tubes filled with saline, the temperature in the urethra decreased significantly faster and farther than at vesical trigone. In particular, maximal temperature decrease as percentage in the urethra was three and 10 times as great as that in the bladder with saline at room temperature and at 5°C respectively (Figures 2 and 3). Moreover, cooling at different sites of urethra was not homogeneous but showed significant differences at different sites. More in detail, the distal sites of the urethra (urethra 2 and 3) cooled significantly faster than the proximal one (urethra 1); in fact in both groups of women whose vagina was cooled with saline (room temperature and 5°C) at time 2 min, the temperature reduction as percentage recorded at point urethra 1

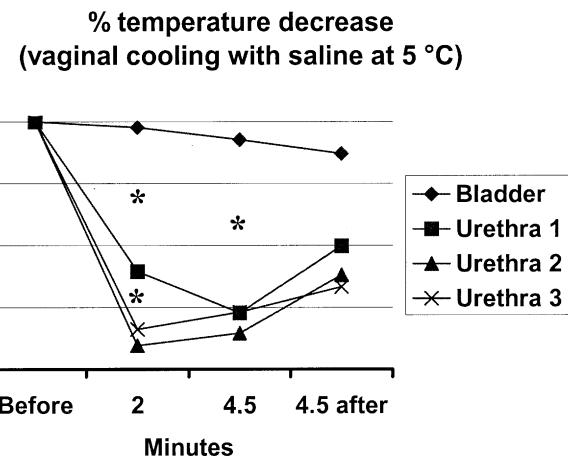


Figure 3. Variation of mean temperature as percentage of start temperature at the vesical trigone and in the urethra of women after insertion into the vagina of a tube filled with saline at 5°C of temperature. At time 2 min, the upper asterisk indicates that reduction of temperature in the bladder is significantly different from that at all measurement sites in the urethra; the lower asterisk indicates a significant difference between urethra 1 and urethra 3. At time 4.5 min, the asterisk indicates that reduction of temperature in the bladder is significantly different from that at all measurement sites in the urethra.

was significantly lower than that at urethra 3 (Figures 2 and 3). The temperature decrease was partly reversed 4.5 min after the cooling was removed.

The temperature decreased only marginally ($<0.1^\circ\text{C}$) in all positions where the temperature was followed in the control group of four women where an air-filled tube was inserted into the vagina.

Discussion

Both bladder and urethral temperatures decreased when the vagina was cooled with a tube filled with saline; however, the urethra cooled significantly faster and more than the bladder

in the area of trigone. The finding that the cooling effects were significantly different although both organs are in close anatomical contact with the anterior vaginal wall and the distance from the cooling source of the vesical trigone and urethra roughly the same, strongly suggests the occurrence of a preferential distribution of cold from the vagina to the urethra, but not to the bladder. Furthermore, percent temperature reduction had not the same velocity at the three urethral measurement sites; the distal measurement sites (urethra 2 and 3) indeed cooled significantly faster than the proximal one (urethra 1). Since the urethra has an anatomical concavity forwards, the thickness of the vagino-urethral septum is greater in the lower part than in the upper part (Krantz, 1951); therefore, simple diffusion of cold through the tissues cannot explain why the thickest part of the septum corresponding to measurement site 'urethra 3', is crossed by cold faster than the thinnest part (urethra 1). Anatomically, the lower two-thirds of the urethra is an inseparable and integral part of the anterior vaginal wall, while in the region of the upper third of the urethra the anterior vaginal wall is distinctly separate from the urethra (Krantz, 1951). The distance between the highest and the lowest urethral recording sites is 14 mm, the length of the urethra is estimated to vary between 30 and 36 mm (Krantz, 1951), and the lowest recording site was just above external urethral opening. Thus, it is clear that all the urethral recording sites were in the middle and in the low part of the urethra and that anatomical relationships between urethra and vagina were similar at all urethral measurement sites. Although it is not possible to exclude that higher bladder temperature could alter proximal urethra cooling by temperature diffusion it must be considered that, anatomically, the bladder measurement site was in correspondence of the trigone, whereas the three urethral sites were in the lower two-thirds of the urethra; the highest urethral point (urethra 1) was at ~1.5 cm from the bladder whereas the second and third urethral points were only 0.7 cm and 1.4 cm distant respectively. However, we did not observe any difference in the cooling effect between urethra 2 and 3.

Local or preferential transfer of substances is a common phenomenon in the reproductive system (Schramm *et al.*, 1986; Einer-Jensen, 1988). Among the different mechanisms advocated to explain the uterine first pass effect after vaginal administration, a counter-current exchange between adjacent veins and arteries may play a critical role (Einer-Jensen, 1988). The anatomy of the blood vessels seems to favour such a possibility, since the vagina is surrounded by rich arterial and venous meshes and the blood supply of the urethra and the anterior vaginal wall are closely related. In addition to the vaginal blood supply received from branches of the internal pudendal artery, diffuse anastomoses between them and branches of the uterine, inferior vesical, and vaginal arteries are found. The confluence of these anastomotic branches forms longitudinal azygos vaginal arteries in the midline of the anterior or posterior vaginal walls, or both (Nichols and Milley, 1995). The vaginal arteries send branches to the urethra and the bladder; the small arteries run lateral and parallel to the urethra and vagina, entering the urethro-vaginal septum where extensive perforating vessels supply the urethra; accordingly,

in the urethro-vaginal septum, extensive capillary plexuses have been demonstrated (Krantz, 1951). The upper thirds of the urethra receive branches from the inferior vesical artery, while the blood supply of the middle and lower third of the urethra derives from vaginal arteries (Krantz, 1951). The veins draining the vagina communicate with the contralateral veins around the vagina and in the paracolpium form a rich venous mesh that cranially continues in the utero-vaginal plexus. Part of the vaginal outflow reaches other larger veins via communicants with the vesical and the haemorrhoidal venous plexi (Williams *et al.*, 1989). It thus seems evident that most of the vaginal blood outlet is in close contact with arteries supplying the urethra and part of the bladder (Williams *et al.*, 1989).

The anatomy provides a possible interpretation of results. The vaginal wall, and therefore the venous blood, is cooled by the saline. The close contact between the veins and arteries means that the colder vein blood cools the vaginal arterial blood. The non-vaginal tissues, including the urethra, supplied with the cooler arterial blood will, therefore, decrease their temperature. The mechanism is similar to the heat transfer that takes place between the pampiniform venous plexus and the testicular artery in the ram (Waites and Moule, 1961). Temperature gradients between Graafian follicles and ovarian stroma also indicate heat transfer in the ovarian adnexa or between the intra-ovarian blood vessels (Hunter *et al.*, 2000). The vascular distribution may thus explain why larger and more rapid changes in temperature were found in the middle and in the lower third of the urethra than in the upper site. We postulate that such changes happen because the lower part of the urethra is supplied by arteries that run close to the vagina; the upper half of the urethra, on the other hand, is mainly supplied with arterial blood from the bladder. It is possible to conceive that vaginal arterial blood flowing from the upper part to the lower part of the vagina is exposed for longer to cold venous blood, so that blood to the lower part of the urethra could be colder than that to the upper part of the urethra and bladder. An additional hypothesis is that part of cold blood from venous vaginal plexus drains in the clitoral and bulbo-cavernosus venous plexus cooling vessels of the ischiocavernosus, bulbo-cavernosus and clitoral arteries that supply the lowest part of the urethra. The small changes in bladder temperature we have observed are in agreement with the hypothesis indicating that vesical vessels are not included in the transfer system; a simple 'temperature buffer' effect of urine is excluded as patients voided their bladder before the trial.

The present data support the hypothesis of a preferential distribution to the urethra of vaginally administered oestrogens. However, according to that previously demonstrated for progesterone (Miles *et al.*, 1994; Cicinelli *et al.*, 2000), a greater endometrial effect of oestradiol administered vaginally compared with that administered by systemic routes has been recently reported (Tourgeman *et al.*, 2001). This suggests that also in case of vaginal administration of oestradiol, direct vagina to uterus transport takes place and therefore, while some vaginal weak/low dose oestrogens are proven safe (in

the absence of progestin), the risk of administration of any oestrogen preparation should be always considered.

In conclusion, our results indicate that the distribution of cold from vagina to the urethra is not the result of simple diffusion and that mechanisms of preferential distribution may exist from vagina to the urethra. The preferential distribution may contribute to explaining the efficacy of low-dose oestrogen therapy by the vaginal route for treating low urinary atrophy without inducing endometrial proliferation. On the basis of arterial distribution to the pelvic organs, a therapeutic scenario could be conceivable that introduces a new concept of targeted therapy via vaginal administration. In fact, by applying oestrogen in the lower part of the vagina, preferential distribution mainly from the vagina to the urethra could take place; on the other hand, by applying oestrogens or progesterone in the upper part of the vagina, preferential distribution to the uterus via the uterine artery could occur (Cicinelli and De Ziegler, 1999; Tourgeman *et al.*, 2001).

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Received on 2 April, 2001; accepted on 28 August, 2001