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The Effect of Urine on Ureteral Motility*

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Summary. Ureteral rings were used to study in vitro spontaneous phasic contractions, similar to the peristaltic waves in vivo. Addition of small amounts of sheep or human urine inhibited or totally blocked rhythmic contractions, and induced tonic contracture. Changes in osmolality induced by the addition of urine were analysed and electrolyte and protein catabolites determined. Similar changes in osmolality which were induced by the addition of urine were elecited by adding sucrose to the organ bath. This had the opposite effect, it increased both the frequency and the amplitude of rhythmic contractions. Therefore, an increase in osmolality per se cannot be responsible for the observed changes of motility. A reduction of pH, resulted in similar changes of motility. In an in-vivo situation with a damaged urothelial barrier there is reason to believe that entrance of urine to the lamina propria and smooth muscle cells will induce profound changes of motility.

Key words: Ureter, Urine, Osmolality, Smooth muscle contraction.

To study the effect of urine on the contractile function of the ureter may seem redundant in view of the protective capacity of the mammalian urinary tract epithelium. There are, however, at least two conditions under which the barrier function is challenged and urine may become accessible to the contractile elements: renal calculus and urinary tract infections. Impactation of a renal calculus may give rise to local trauma and an inflammatory reaction thus damaging the inner coat of the protective transitional epithelium which covers the lamina propria and the underlying smooth muscle fibers of the ureter. This can result in entry of urine and exposure of the contractile elements to the non-physiological environment of urine, which inter alia is characterised by hyperosmolality. A similar situation may occur as the result of urinary tract infection when virulent bacterial toxins initiate structural changes within and beyond the protective transitional epithelium. This has recently been shown by Fussel and Roberts [4].

Hyperosmolar solutions have pronounced and diverse effects on smooth muscle contraction [13] but studies on the effect of urine on ureteral peristalsis have to our knowledge not been reported. The aim of the present study was to elucidate the influence of urine and osmolality on rhythmic contractions the of the sheep ureter.

Material and Methods

Sheep kidneys with attached ureters were obtained early in the morning from the local abbatoire. The specimens were put into chilled Krebs-Henseleit solution and transported in thermos flasks to the laboratory. The ureters were dissected free of and 3 mm rings were cut from the proximal part. These preparations were vertically suspended in 10 ml organ baths filled with Krebs-Henseleit solution, at 37 °C with 95% oxygen and 5% carbon dioxide. The rings were attached to the bottom of the organ bath and the upper end connected with a Bioscience UFI force transducer. Isometric tension was continuously recorded on a Lectromed MC216 2 channel recorder. After suspension of the rings a starting pretension of 2 g was obtained by adjustment with a micrometer screw. When the preparations were allowed to equilibrate for 30 min, usually phasic rhythmic contractions started spontaneously. After a stable pattern of contractions was established usually within 30–50 min, 0.5–4 ml of sheep or human urine or sucrose solution was added to the bath and the changes of frequency and amplitude observed and measured after 10 min. Sheep urine was obtained from the same animal from which the ureters were harvested and collected by needle aspiration of the bladder. Human urine was taken from healthy male volunteers. A summary of the experimental set-up is shown in Fig. 1.

The Krebs-Henseleit solution contained (mM): NaCl, 115.3; KCl, 4.6; $CaCl_2$, 2.3; $MgSO_4$, 1.2; $NaHCO_3$, 22.1; KH_2PO_4, 1.1 and glucose 7.8; pH 7.4.

Osmolality of the samples added to the organ bath and the resultant final osmolality after addition was determined with a Microosmometer Model 3MD of Advanced Instruments Inc., Needham Heights, Mass. USA, after calibration with a 290 m OSM/kg

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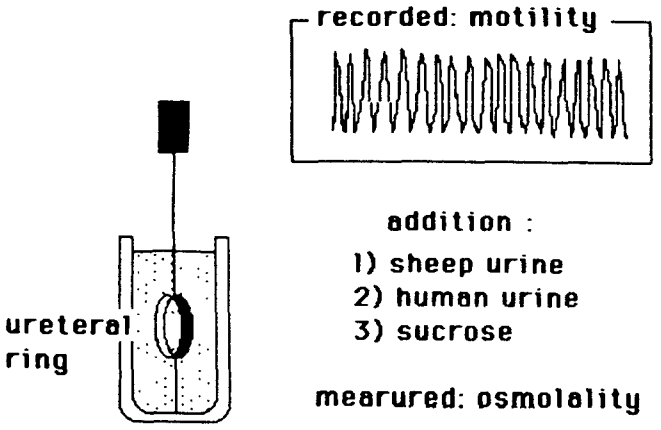

Fig. 1. Experimental set-up: organ bath with ureteral ring. *Inset:* record of spontaneous ureteral motility

reference solution. Urine electrolytes NaK and Cl were determined using the Astra ion selective electrode system, Beckman Instruments, Inc. Calcium was measured on the Technicon Autoanalyzer II using a method modified after [6, 10]. Creatinine and urea nitrogen was also determined on the Technicon Autoanalyzer II using a method, based on the reaction of saturated picric acid with creatinine with presence of alkali. Urea nitrogen was measured using a method modified after [12].

Results

The normal pattern of contraction of ureteral preparations is shown on the left side of panels A–D of Fig. 2. This illustration displays the rhythmic phasic contractions of isolated sheep ureteral ring preparations which are characterised by a fast rise in tension from the baseline, reaching a peak level, followed by a quick relaxation. The contractions come in succession as a continuous rhythmic pattern, mostly with equal intervals or sometimes arranged in trains of intermittent activity. The ureteral contraction observed in vitro are very similar to the normal peristalsis seen in vivo [3]. The contractile waves can be characterised by their frequency (contractions per minute) amplitude (mg) and basal tension. Usually the pattern of contraction in a particular preparation is quite stable over long periods (hours) but frequency and amplitude usually decline with time and eventually cease [14].

The remarkable stability of this preparation depends on a constant stable environment provided by the composition of bathing medium and by a temperature. The effect of adding urine to the organ bath is shown in Fig. 2. From these original recordings of ureteral contractions it can be seen that addition of small amounts of urine profoundly affect motility: rhythmicity is suppressed or stopped and basal tone is enhanced. This pattern is characteristic of a spastic contraction or contracture. Figure 2 further displays the effect of hypertonicity and acidity on peristalsis. Addition of sucrose caused increased frequency and amplitude of contractions and addition of a drop of hydrochloric acid

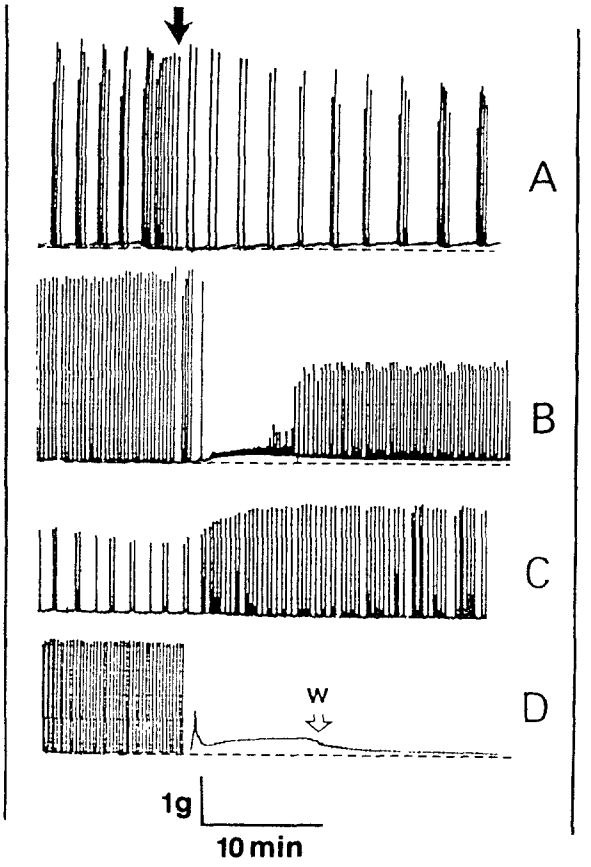

Fig. 2. Original records of sheep ureteral motility from isolated rings. *Panel A* and *B* 1 ml of sheep urine added at *arrow*. Resulting osmolality: 311 in A and 369 mOsm/l in B. *Panel C* 3 ml of 30% sucrose solution added, resulting osmolality: 389 mOsm/l. *Panel D:* HCl added at arrow (pH reduced to 4.8). At *open arrow:* wash (*w*). Note changes in baseline tone in *A*, *B* and *D*

immediately stopped the rhythmic process of phasic contractions and induces an increase in basal tone. Figure 3 shows a plot of the change in frequency of contractions 10 min after addition of urine. In 10 out of the 19 experiments ureteral motility stopped altogether, in the rest frequency was reduced. The result of all experiments is given in Table 1 which also lists the effect of human urine. From this it can be seen that sheep urine was more potent in reducing rhythmic peristalsis than was human urine, despite a slightly higher mean osmolality in the latter.

In order to find out if the changes in motility were due to alterad osmolality we also studied the effect of increasing concentrations of sucrose added to the organ bath. This is shown in Fig. 3 and Table 2. The results were quite clear cut: hyperosmololity per se cannot be made responsible for the profound motility changes observed with addition of sheep urine. On the contrary, an increase of osmolality alone achieved with sucrose enhances frequency and amplitude of ureteral contractions and does not have any ureteroplegic actions.

Table 3 lists some important osmotically active urinary constituents, electrolytes and protein catabolites in sheep

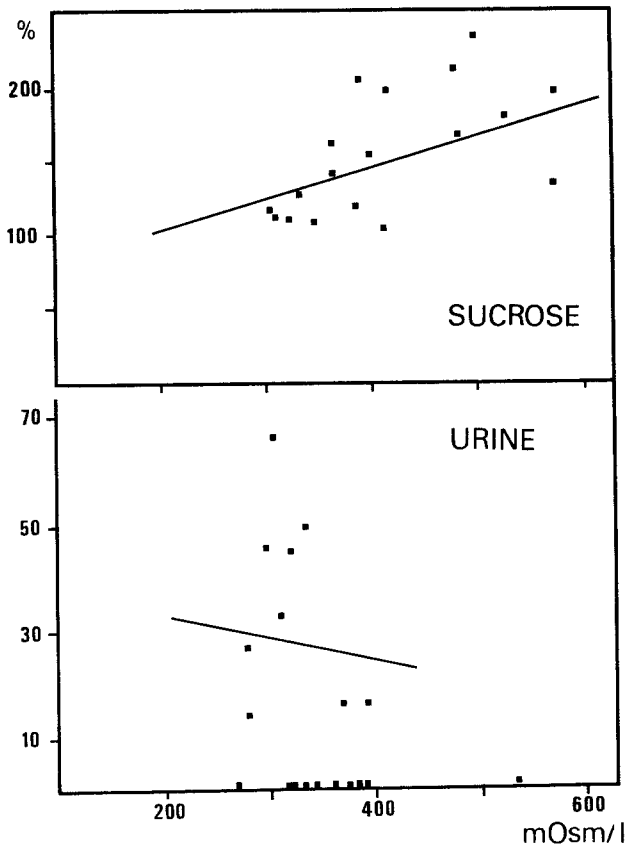

Fig. 3. Change of frequency of ureteral contractions 10 min after addition of sucrose (18 experiments) and sheep urine (19 experiments). *Note:* increase of frequency with sucrose and decrease with urine

and human urine used in our experiments. As seen from these data both electrolytes and protein catabolites are very similar in both series. If anything, there is a slightly higher concentration of electrolytes and osmolality in the human urine, compared to the values obtained from sheep. No detailed correlation analysis of the motility and urine data were made at this stage except for total osmolality. Even if there seemed to be a weak correlation this was not proof of a causal relationship since it could only imply that larger urine admixture meant more profound contractile disturbances. This, however, did not identify the crucial component. The data in Table 3 also show large standard deviations, which implies a great variation in urinary constituents, a natural consequence of difference in diet and hydration.

Table 2. Change of frequency and amplitude (measured over a 10 min, interval) of spontaneous ovine ureteral contractions after treatment with 400 m Osm/l of sheep urine, human urine, and sucrose. Values are extrapolated from regression equations from diagram in Fig. 3 and represent percent of control

Parameter	Sheep urine	Human urine	Sucrose
Frequency	8.4 (n = 19)	69.5 (n = 18)	146.3 (n = 18)
Amplitude	25.2 (n = 19)	84.7 (n = 18)	111.4 (n = 18)

Table 3. Electrolytes and protein catabolities in sheep and human urine. (Values are arithmetic means +/− SD. n = number of samples)

	Sheep urine	Human urine
Na (mmol/l)	120.5 ± 93.3 (n = 10)	126.8 ± 96.6 (n = 9)
K (mmol/l)	157.9 ± 89.9 (n = 10)	171.8 ± 84.8 (n = 9)
Ca (mmol/l)	16.8 ± 42.1 (n = 10)	18.2 ± 44.0 (n = 9)
Cl (mmol/l)	201.0 ± 116.6 (n = 10)	211.4 ± 117.9 (n = 9)
Creatinine (mmol/l)	18.3 ± 11.8 (n = 10)	20.4 ± 10.6 (n = 9)
Urea (mmol/l)	228.9 ± 181.5 (n = 10)	230.7 ± 189.4 (n = 9)
pH	6.4 ± 1.6 (n = 10)	5.8 ± 1.1 (n = 10)
Osmolality (mOsm/kg)	956.2 ± 447.2 (n = 9)	975.9 ± 423.8 (n = 9)

Table 1. Frequency and amplitude ratio of spontaneous ureteral contractions under control conditions (Krebs-Henseleit solution) and after addition of 1 ml of sheep or human urine. Osmolality data refer to values obtained after addition of urine (Arithmetic means ± SEM)

	Frequency	Amplitude	Osmolality	P value	
				freq	ampl
Krebs-Henseleit	0.92 ± 0.05	0.94 ± 0.02	295.96 ± 0.97	–	–
Sheep	0.16 ± 0.4	0.43 ± 0.11	345.63 ± 13.65	***	*
Human	0.67 ± 0.07	0.90 ± 0.02	365.22 ± 4.71	<0.1 NS	<0.2 NS

*** = <0.0005; * = <0.05

Discussion

To our knolwedge the effect of urine on ureteral motility has not been studied before although the possible interaction of this usually hyperosmolar acidic and electrolyte concentrated fluid could be of importance for the understanding of certain pathological mechanisms in which the smooth muscle of the ureter is exposed to its content. Such a situation should occur whenever the protective barrier of the urothelium is damaged. In our in vitro model with cut rings of the ureter immersed in a physiological bathing fluid, conditions are present which are comparable to the pathologic state since the cut edges of the preparation expose the muscle layers. Therefore, this experimental model lends itself very well to studies of the effect of a "*leaky urothelial barrier*".

In order to evaluate the effect of increased osmolality most studies on smooth muscle have been performed with addition of sucrose, which, contrary to urea, does not readily penetrate cell membranes. This leads to osmotic exchange of water molecules from the inside of the cell, resulting in shrinkage of cell volume. In rhythmically active smooth muscle like the portal vein of the rat hypertonicity induced by sucrose abolished phasic contractions but caused a tonic increase in tension [2]. In nonrhythmic smooth muscle preparations of the saphenous vein and aorta sucrose again induced tonic contraction, whereas urea was without effect [9, 11]. In electrophysiological studies on the guinea pig ureter sucrose causes depolarisation of smooth muscle cells and is associated with spike potential development [1]. The stimulatory mechanism of hyperosmolality seems to be related to Ca^{++} release from intracellular stores. Hyperosmolar changes of cell volume such as shrinkage probably induces conformational changes of calcium storage sites, thus triggering their release [11]. Smooth muscle controlled by adrenergic mechanisms may respond with a reduction in tone; the relationship between hyperosmolality and the contractive state therefore is based on a complex interactions which have not been properly explained [7]. A reduction in pH associated with the addition of urine seems to be an important parameter in determining ureteral contractility as shown in our study. A decrease in external pH reduced intracellular free Ca and the opposite holds for an increase in pH (cf. [8]).

Contrary to blood and other body fluids which make up the environment of the cells, urine being a waste product has a much more variable composition, depending on diet and the metabolic state of the oragnism. It is, however, fair to conclude that urine usually is hypertonic, acidic and has a higher ionic strength. All of these factors affect eucaryotic cellular function including smooth muscle. The present study only centers on contractile properties of ureteric smooth muscle but it is fair to assume that a diversity of other cellular systems are equally affected including nerves and connective tissue. In this context it seems very likely that a *leaky urothelial barrier* is a potent source of pain fiber stimulation, since it is known that hypertonic solutions as well as acids, have potent algesic properties (cf. Vyklicky [16]). Therefore urine seeping into the lamina propria of the ureter might be a contributing factor in the pain of renal colic, pyelonephritis and related disorders.

Another possiblity for the disturbance of ureteral motility as the result of the irritating effect of urine may be related to activation of mast cells in the wall of the ureter and a consequent spastic contraction. This has become a possibility with the recent demonstration of an abundance of mast cells in the human [15] ureter. Moreover histamine stimulation is associated with tonic, spastic contractions [4, 15]. Fussel and Roberts [5] in their extensive ultrastructural studies in experimental urinary tract infections in the nonhuman primate found significant early changes in the epithelial layer of the ureter. These changes which include damage of the luminal intercellular junctions suggest a breakdown of the urinary barrier which would permit penetration of urine into the interior. The authors speculate that this mechanism can be responsible for an increase in connective tissue growth from fibroblasts. Our findings with the profound effect of small amounts of urine added to the organ bath support the importance of the integrity of an intact epithelial barrier which, if damaged, can result in an immediate disruption of ureteral peristalsis. This means that infection and the ureteroplegic action of bacterial toxins can be considered as an additional factor for the disruption of ureteral transport function. Mechanical injury to the protective transitional epithelium would be enough to set forth a chain of events that induce secondary damage e.g. through entry of urine, invasion of bacteria and release of toxins etc.

Our study has shown that sheep and human urine, when added to isolated sheep ureteral ring preparations suppress spontaneous rhythmic contractions and induce spastic tonic contraction. This effect is not due simply to a change in osmolality but seems to be related to other as yet undefined constituents. Further studies are needed to clarify if these are ionic in nature or related to other factors.

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