Urol Res (1987) 15:273–276

Urological Research


# The Effect of Uropathogenic Bacteria on Ureteral Motility*

O. Thulesius[1] and G. Araj[2]

[1] Departments of Pharmacology-Toxicology and
[2] Microbiology, Faculty of Medicine, Kuwait University, Safat, Kuwait

**Summary.** The effect of bacterial products of various strains obtained from patients with urinary tract infections was tested on peristalsis of sheep ureteral rings. In 55–70% spontaneous rhythmic contractions were inhibited by addition of small amounts of growth supernatants from *Escherichia coli, Pseudomonas aeroginosa* and *Klebsiella pneumoniae.* The isolates were also tested on mesenteric artery ring preparations. In these vessels the isolates induced tonic contractions, particularly when the vessel had been depolarised and precontracted with a 40 mM KCl solution. This response is characteristic of a calcium ionophore, known to occur in some bacterial toxins. The active principle of the *bacterial ureteroplegic factor* (BUF) is heat sensitive and distinct from endotoxins which speed up peristalsis. It is suggested that the ureteroplegic action depends on an exotoxin with ionophoric properties.

**Key words:** Ureter, Pyelonephritis, Smooth muscle, Bacterial toxins, Calcium ionophore.

## Introduction

Upper urinary tract infections are the cause of serious morbidity and may provoke chronic renal failure, hypertension and urosepsis. The mechanism of ascending upper urinary tract infections is multifactorial and involves bacterial virulence factors and host defense. Bacterial attachement to urinary tract epithelium as determined by epithelial cell-receptors is important [10]. In addition several predisposing factors in the host including urinary obstruction, bladder atonia, vesico-ureteric reflux, ureteral dilatation such as in pregnancy and trauma also contribute to urinary tract infections. Normal ureteral peristalsis may be analogous to emptying mechanisms of the bladder in the pathophysiology of upper urinary tract infections [5].

A number of experimental studies have attempted to clarify the role of bacterial infections on ureteral motility in vivo and in vitro [9, 12, 17, 18]. The result of these investigations has been variable, but in the majority an inhibitory action was found although the mechanism of action has not been clarified. The aim of the present study was to test uropathogenic strains of gram-negative bacteria with regards to their effect on ureteral peristalsis and to assess if any ureteroplegic action is related to intracellular changes of calcium transport affecting the contractile process of smooth muscle.

## Materials and Method

*Ureters.* Sheep kidneys with attached ureters were obtained early in the morning from the local abbatoire. The specimens were transported to the laboratory in chilled Krebs-Henseleit solution. The ureters were dissected free of connective tissue and fat and 4 mm rings were cut from the upper part, close to the pelvis. The preparations were suspended vertically in 10 ml organ baths filled with Krebs-Henseleit solution, maintained at 37 °C and gassed with 95% oxygen and 5% carbon dioxide. The rings were attached to the bottom of the organ bath and the upper end connected to a Bioscience UFI force transducer. Isometric tension was continuously recorded on a Lectromed MX 216 two channel recorder. After a starting pretension of 2 g was obtained by adjustment with a micrometer screw, the preparation was allowed to equilibrate for 30 min after which time usually a stable pattern of rhythmic contractions was established. At this time supernatants of bacterial cultures or lipopolysaccharide endotoxin (LPS) were added with a syringe and the effect on frequency and amplitude observed. Changes in frequency were assessed by comparing the number of contractions during a 10 min period prior and after administration of the supernatant or control broth. In addition, if possible, stop times were measured i.e. the time in minutes after adding the bacterial preparation until stoppage. Further details about the method cf. Thulesius and Angelo-Khattar [19].

*Mesenteric arteries* were obtained from male Merino sheep which were slaughtered by exsanguination. The arteries were dissected free of adherent fat and ring preparations were cut for testing isometric tension with the same techniques as used for the ureters. The effect

* Supported by Grant Nr. MR 019, Kuwait University

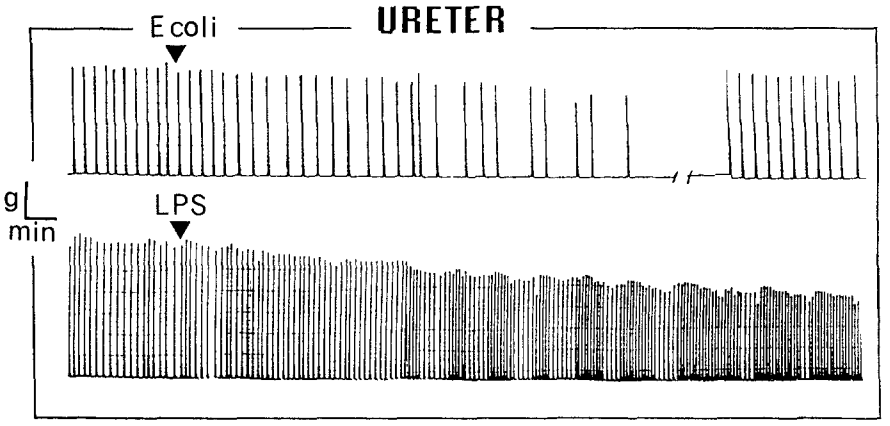


Fig. 1. Sheep ureter, isolated ring preparations with spontaneous rhythmic contractions. *Upper panel* at arrow administration of 1 ml of bacterial supernatant to 10 ml organ bath (*E. coli*). *Lower panel:* at arrow administration of 200 µl of LPS

**Table 1.** Frequency and amplitude of spontaneous ureteral contractions under control conditions and 10 min after addition of 1 ml of Krebs Solution (= control), nutrient broth, isolates of *E. coli, Pseudomonas aeroginosa, Klebsiella pneumoniae* and 200 µl of *E. coli* endotoxin (LPS). Arithmetic means ± / − SE. Comparisons to control conditions in Krebs-Henseleit buffer expressed as ratio before/after Number of experiments = *n*

| | | | *P*-value | |
|---|---|---|---|---|
| | Frequency | Amplitude | Freq | Ampl |
| Control | 0.93 ± 0.04 ($n$ = 13) | 0.97 ± 0.01 ($n$ = 13) | – | – |
| Broth | 0.66 ± 0.05 ($n$ = 9) | 0.94 ± 0.02 ($n$ = 9) | < 0.0005 | < 0.15 |
| *E. coli* | 0.49 ± 0.09 ($n$ = 16) | 0.73 ± 0.1 ($n$ = 16) | < 0.0005 | < 0.05 |
| *Pseudomonas* | 0.34 ± 0.12 ($n$ = 6) | 0.78 ± 0.16 ($n$ = 6) | < 0.0005 | < 0.10 |
| *Klebsiella* | 0.58 ± 0.20 ($n$ = 2) | 0.92 ± 0.02 ($n$ = 2) | < 0.005 | < 0.0005 |
| LPS | 1.04 ± 0.04 ($n$ = 12) | 0.97 ± 0.01 ($n$ = 12) | < 0.05 | < 0.40 |

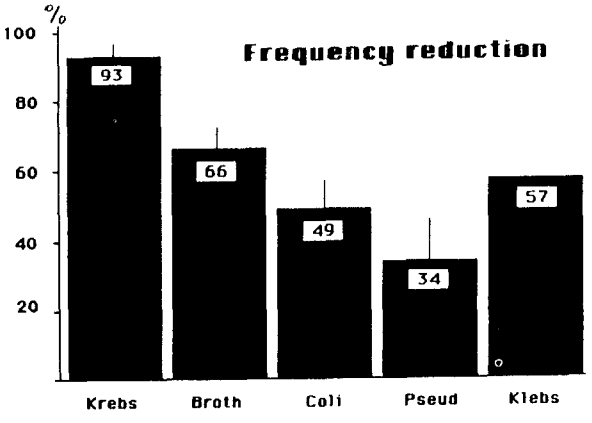


Fig. 2. Frequency of spontaneous rhythmic contractions 10 min after administration of control solutions expressed as per cent control (Krebs-Henseleit solution, culture broth) or 1 ml of supernatant of various bacterial strains (*E. coli, Pseudomonas aeroginosa, Klebsiella pneumoniae*)

of bacterial suspensions and control broth was tested on basal tone and after depolarisation with 40 mM KCl solution. Changes in isometric tension were recorded. 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 at pH 7.4. The calcium channel activator BAY K 8644 was kindly supplied by Prof. K. Triggle, Buffalo, N. Y., *Bacterial Isolates:* Bacteria were isolated from urine specimens of patients with urinary tract infections and identified according to established procedures. The strains consisted of *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Briefly, each isolate was inoculated into several tubes containing 10 ml of nutrient broth (Oxoid Ltd., London, UK) and incubated at 37 °C for 48, and 72 h. The nutrient broth contained: Hydrolyzed protein, 8.6 g/l, Na, 89 mM/l, Ca, 1.2 mM/l, Mg 0.08 mM/l. At the end of the incubation period, growth was adjusted equivalent to McFarland No. 3 density tubes, (9 x 10 bact/ml), and divided into four equal parts as follows:

- whole suspension
- whole suspension – heated (100 °C for 30 min)
- supernatant (after centrifugation at 200 rpm for 20 min)
- supernatant – heated (as above).

As a control nutrient broth alone was used. In addition to the bacterial isolates also lipopolysaccharide (LPS) endotoxins of *E. coli* 0111:B4 and 055:B5 obtained as the lyophilized product by List Biological Laboratories, Campbell, Calif. USA were used.

*Statistics.* Mean value + S. E. of all data are shown. Standard statistical methods were used for calculations of paired t-tests and regression analysis.

## Results

The effect of adding 1 ml of bacterial isolates of various strains of bacteria and 200 µl of LPS on spontaneously contracting ureteral preparations is shown in Fig. 1 and Table 1. Figure 1 shows a marked inhibition of phasic contractions which abolished peristalsis within 10 min. Washing with Krebs-Henseleit buffer leads to reestablishment of concentrations. The LPS treated preparation does not show any inhibition, on the contrary, there was an increase in frequency of contractions with a concomitant reduction in amplitude. Table 1 gives a summary of all data. From this it can be seen that mainly frequency of phasic contractions was affected, this is further demonstrated in Fig. 2. There was no statistical difference in the motility response of

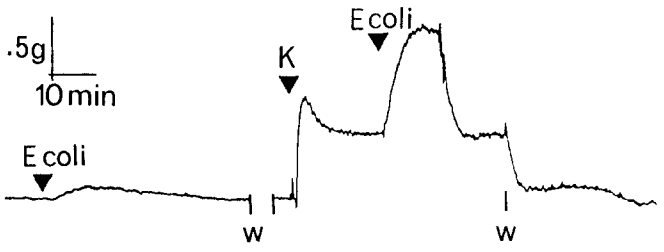


**Fig. 3.** Sheep mesenteric artery, isolated ring preparation. At arrows administration of 1 ml of bacterial supernatant (*E. coli*). Second half of recording: depolarisation with Krebs solution containing 40/mM KCl solution and administration of *E. coli*. *W*, wash

**Table 2.** Effect of bacterial isolates on sheep mesenteric artery tone in the basal state and after depolarisation with 40 mM KCl. (Depolarized II = heat treated material). Data are arithmetic means of mg tension +/− SEM, *n* = number of experiments

| | Basal tone | Depolarised I | Depolarised II |
|---|---|---|---|
| Broth | 57.5 ± 23.0 ($n$ = 10) | 142.5 ± 52.5 ($n$ = 10) | – |
| *E. coli* | 78.5 ± 39.1 ($n$ = 7) | 896.4 ± 137.8 ($n$ = 14) | 516.7 ± 174.6 ($n$ = 6) |
| *Pseudomonas* | 412.5 ± 208.5 ($n$ = 4) | 1015.0 ± 205.3 ($n$ = 5) | 591.7 ± 205.3 ($n$ = 3) |
| *Klebsiella* | 100.0 ± 50.0 ($n$ = 3) | 250.0 ± 150.0 ($n$ = 2) | – |
| LPS | 0 ± 0 ($n$ = 4) | 0 ± 0 ($n$ = 4) | |

whole bacterial suspensions or supernatants. In 4 out of 26 experiments with different strains spontaneous activity ceased within 10 min whereas this did not occur in any of the control experiments with Krebs solution or nutrient broth. If a frequency reduction below 60% is taken as a positive response, this occured in 55% of the *E. coli* strains and in 70% of Pseudomonas aeroginosa. When the calcium channel activator BAY K8644 at $10^{-6}$M was administered this also resulted in stoppage or ureteral contractions. This however, was preceded by a temporary increase in frequency and increased basal tone.

Figure 3 is the record of an experiment performed on an isolated mesenteric artery preparation. Here it can be noticed that addition of 1 ml of bacterial isolate elicited a weak contraction in the basal state but a much more pronounced increase of tone in the KCl depolarised and precontracted state. From Table 2 it can be seen that both isolates from *E. coli, Pseudomonas aeroginosa* and *Klebsiella pneumoniae* induced tonic contractions of the mesenteric artery but LPS alone failed to do so. Heat treatment of bacterial isolates reduced but did not eliminate a contractile response.

## Discussion

Conflicting results have been obtained by in-vitro [9], and in-vivo [17] studies about the action of bacteria on ureteral motility. The ureteral inhibition being shown in the former but not in the latter may have to do with the fact that the putative smooth muscle inhibitory principle of bacterial isolates has easy access to the smooth muscle layer in the in-vitro situation but not in vivo. Experiments with isolated ureteral strip preparations involve cutting of the ureter, thus exposing the contractile elements directly to any toxic material that is introduced into the organ bath, whereas short periods of ureteral perfusion may leave the protective transitional epithelium intact. The role played by bacteria adhering to the ureteral surface has been nicely demonstrated by Fussel and Roberts [6] who described a breakdown of intercellular junctions of the urothelium after chronic infections of 1–3 months. This, however, does not eliminate the possibility that certain toxins, depending on their molecular weight and structure, could get access to the contractile elements before that period, but clearly exposed muscle fibers should be more easily affected. In the present series of experiments in most instances we noted a prompt effect which probably was facilitated by the in-vitro technique.

The question of the type of bacterial toxin or product involved in the ureteroplegic action remains controversial. King and Cox [9] noted inhibitory effects by *E. coli* endotoxin whereas Struthers [17] reported the opposite effect. In the present study we observed either no effect with *E. coli* endotoxin (LPS) or a slight increase in frequency of contractions, confirming Struthers [17] work in this respect. This could be explained on the basis of the well known effect of stimulation of prostaglandin synthesis by endotoxins [2]. We have previously shown that prostaglandins are motility promoting factors in sheep and human ureters [3].

When considering the possible pharmacodynamic principle of the ureteroplegic action of bacterial extracts we did not immediately consider agonists or antagonists of the autonomic nervous system since we have shown that ureteral motility is not influenced by hyoscine, phenoxybenzamine, propranolol, mepyramine and methysergide [19]. Therefore we rather looked for a mechanism involving control of excitability or contractility exerted by a calcium mediated process. It is already known that the calcium channel blocker nifedipine dose-dependently blocks spontaneous phasic rhythmic activity of the human [8] and dog ureters [15]. Therefore, in order to test any potential calcium channel blocking effect we chose a mesenteric artery ring preparation in which administration of a calcium blocker gives rise to a relaxation, particularly after depolarisation with KCl [7].

To our surprise, however, bacterial isolates did not inhibit vascular smooth muscle tone, but on the contrary markedly enhanced contraction, particularly during partial depolarization induced by 40 mM KCl. Such a response is characteristic of a calcium agonist or calcium inophore. The recent-

ly introduced calcium agonist BAY K 8644 is a dihydropyridine with a nifedipine like structure. The effects of BAY K 8644 are voltage dependent; in the rat aorta or tail artery it has no effect on basal tone but induces contraction after partial potassium depolarisation. Under these conditions it can, however, also act like a calcium antagonist, particularly at higher concentrations (cf. [16, 17]). This was also observed in the present investigation in which we saw an inhibition of ureteral motility after an initial increase in basal tone and frequency of phasic contractions. Such an effect was, however, not noticed with bacterial isolates, inhibition of ureteral peristalsis was gradual without change of basal tone. The possibility therefore exists that material from bacterial isolates have the function of an organic calcium inophore. The best known inophores A 23187 and X-537 A both are extracted from bacteria [4]. A 23187 has been shown to possess a sealing effect on gap junctions [14], structures that are of critical importance for the maintenance of phasic rhythmic contractions in ureteral ring preparations as recently shown by us [20]. Further evidence for the assumption that a calcium inophore mechanism may be involved comes from the work of Aikin et al. [1] who showed that calcium inflow into guinea-pig ureters elicited by 30 mM KCl solution led to a cessation of action potential and contracture. The calcium channel activator BAY K 8644, however, has a mechanism of action which is distinct from the bacterial calcium ionophores in that it acts on specific sites in cellular calcium channels [21].

In conclusion our results suggests that the *bacterial ureteroplegic factor* (BUF) seems to be a heat labile extracellular product which has some properties of a calcium ionophore. The heat lability of toxic material from bacterial isolates was also previously described [9] and is a typical feature of some *E. coli* enterotoxins [13]. Further work is needed to clarify the cellular mechanism.

## References


1. Aickin CC, Brading AF, Burdyga TV (1984) Evidence for sodium exchange in the guinea-pig ureter. J Physiol 347:411–430
2. Anderson FL, Jubitz W, Fralios AC, Tsagaris TJ, Kuida H (1972) Plasma prostaglandin levels during endotoxin shock in dogs. Circulation 45:124–130
3. Angelo-Khattar M, Thulesius O, Nilsson T, Cherian T, Leon J (1985) Motility of the human ureter, with special reference to the effect of indomethacin. Scand J Urol Nephrol 19:261–265
4. Campbell AK (1985) Intracellular calcium, its universal role as regulator. Wiley Chichester, pp 34–35
5. Chisholm GD (1980) Pathophysiology of obstructive uropathy. In: Chisholm GD, Williams DJ (eds) Scientific foundations of urology, 2nd edn. Heinemann, London, pp 98–105
6. Fussell EN, Roberts JA (1978) Chronic pyelonephritis. Electron microscopic study. III. The ureter. Invest Urol 17:108–119
7. Hogestatt E (1984) Characterization of two different calcium entry pathways in small mesenteric arteries from rat. Acta Physiol Scand 122:483–495
8. Hertle L, Nawrath H (1984) Calcium channel blockade in smooth muscle of the human upper urinary tract. I. Effects on depolarization-induced activation. J Urol 132:1265–1269
9. King WW, Cox CE (1972) Bacterial inhibition of ureteral smooth muscle contractility. I. The effect of common urinary pathogenes and endotoxin in an in-vitro system. J Urol 108: 700–705
10. Lomberg H, Hanson LA, Jacobsson B, Jodal U, Leffler H, Svanborg Eden C (1983) Correlation of P blood group, vesicoureteral reflux, and bacterial attachment in patients with recurrent pyelonephritis. New Engl J Med 308:1189–1192
11. Mikkelsen E (1985) Comparison of effects of a new dihydropyridine, Bay K 8644, and nifedipine on spontaneous mechanical activity in rat portal vein. Br J Pharmacol 85:383–385
12. Roberts JA (1975) Experimental pyelonephritis in the monkey. III. Pathophysiology of ureteral malfunction induced by bacteria. Invest Urol 13.117–120
13. Robertson DC, McDonel JL, Dorner F (1985) *E. coli* heat-labile enterotoxin. Pharmacol Ther 28:303–399
14. Rose B, Loewenstein WR (1976) Permeability of cell junction and the local cytoplasmic calcium activity. J Membrane Biol 28:87–91
15. Sakanashi M, Kato T, Migamoto Y, Takeo S, Noguchi K, Ito J, Nakasone J, Aniya Y (1985) Comparison of the effects of nifedipine on ureter and coronary artery isolated from the dog. Drug Res 35.584–587
16. Schramm M, Towart R (1985) Modulation of calcium channel function by drugs. Life Sci 37:1843
17. Struthers NW (1976) The effect of *E. coli* and *E. coli* endotoxin on peristalsis in the canine ureter. Urol Res 4:107–109
18. Teague N, Boyarsky S (1968) The effect of coliform bacilli upon ureteral peristalsis. Invest Urol 5:423–429
19. Thulesius O, Angelo-Khattar M (1985) The effect of indomethacin on the motility of isolated sheep ureters. Acta Pharmacol Toxicol 56:298–301
20. Thulesius O, Ugaily-Thulesius L, Angelo-Khattar M (1986) Generation and transmission of ovine ureteral contractions with special reference to prostaglandins. Acta Physiol Scand 127: 485–490
21. Triggle DJ, Janis RA (1984) The 1.4-dihydropyridine receptor: a regulatory component of the $Ca^{+2}$ channel. J Cardiovasc Pharmac 6:S949–S955



Prof. O. Thulesius
Department of
Pharmacology-Toxicology
Faculty of Medicine
Kuwait University
P.O. Box 24923, Safat
Kuwait