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Ureteral contractions induced by rat urine in vitro: probable involvement of renal kallikrein

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Summary. Rat urine, even at a 1:10 final dilution in Tyrode's solution, stimulates contraction of the ureteral musculature in vitro. This effect can be ascribed to the presence of kallikrein or a kallikrein-like enzyme in urine. Isometric contractions of ureters were prevented by previous addition of aprotinin to the organ bath. Urine also lost its activity after inactivation of enzymes by heat or acid treatment.

Renal kallikrein, an enzyme clearly different from plasma kallikrein, has been localized in the distal nephron[2,3]. At this site it is probably released into the tubular fluid, and then excreted in the urine[4,5]. The physiological role of this enzyme is still unknown, although it has been suggested that it may be involved in the control of sodium excretion[6,7], in the renal response to mineralocorticoid hormones[8,9] or in the control of renal blood flow[10]. Renal kallikrein could also have a function beyond this organ. Kallikrein stimulates contraction of the isolated dog intestine and of the isolated rat uterus in oestrous[11-14]. This activity has been used to measure kallikrein biologically[5,15]. The urinary tract has a common embryologic origin with the distal nephron, therefore, it appeared to be of interest to investigate whether rat urine, through its content of kallikrein, could also stimulate the ureteral smooth muscle in vitro.

Methods. Ureters were obtained from rats sacrificed by a blow on the head. Both ureteral ends were ligated and the organs immersed, with 0.1 g tension, in 12–15 ml Tyrode's solution (NaCl 128 mM, KCl 4.7 mM, $NaHCO_3$ 11.9 mM, NaH_2PO_4 1.2 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 2.5 mM, glucose 10 mM) kept at 37 C in a glass bath and constantly flushed with 5% CO_2 and 95% O_2. Isometric contractions were amplified and registered by means of a Heathkit EU20B recorder.

24-h-urine samples were collected from rats (n = 10) placed in metabolic cages and fed normal rat chow and with free access to drinking water. Urine was kept frozen until used. Urine was added to the isolated muscle bath immediately after thawing or after dialysis against 2 changes of isotonic saline chloride or Tyrode's solution (volume ratio 1:200) at 4 C for 48 h. Urinary enzymes were inactivated by immersing the dialyzed aliquots in a boiling water bath for 60 min or by the addition of one volume of 10% trichloroacetic acid (TCA). TCA-precipitated urine samples were neutralized with NaOH prior to addition to the organ bath. After 2–4-min contact time the preparation was rinsed with fresh Tyrode's solution.

Results. Rat urine induced an ureteral contraction of a moderate tonic character with superimposed clonic episodes (figure 1). The contractions of the isolated ureters continued until rinsing (figure 3). In some preparations repetitive addition of urine resulted in a disappearance of effect. The type of contraction elicited was identical to that evoked by bradykinin. Prolonged dialysis of urine aliquots did not affect contractile activity which, however, disappeared after enzyme inactivation by boiling (figure 1) or acid treatment of urine (figure 2). The stimulating activity of rat urine could be totally suppressed by a serine protease inhibitor (aprotinin, 1000 kIU/ml) added to the organ bath (figure 3).

Discussion. The effect of bradykinin on the ureteral musculature of dogs and rats in vivo was described several years ago[16]. The influence of bradykinin on ureteral musculature in vitro, has not yet been reported , to our knowledge.

The present study indicates that rat urine at a 1:10 final dilution induces contractions of isolated ureters. The active principle is not dialysable and can be inhibited by heat or acid denaturation, and blocked by the previous addition of aprotinin to the organ bath. The response could be markedly reduced, albeit not inhibited, by the addition of bradykinin-binding antibodies to the bath. This could be due to a greater association constant for bradykinin with receptors than with antibodies, or to an incapacity of the antibodies to compete against bradykinin to reach the site at which kallikrein releases the biologically active kinins. It may be concluded that the observed ureteral stimulation by urine depends on its kallikrein content or on a kallikrein-like enzyme, which would release kinins from kininogen present in the ureters. The amount of kininogen available in the isolated ureters (weighing 5–10 mg) is undoubtedly very small. If kallikrein stimulation of the ureter is mediated through kinin release from kininogen, the disappearance of response in some ureters could be due to consumption of the available kininogen. Since urine was added to the serosal side of the ureters, the present experiments suggest but do not prove that the kallikrein-kinin system modulates ureteral peristalsis in vivo.

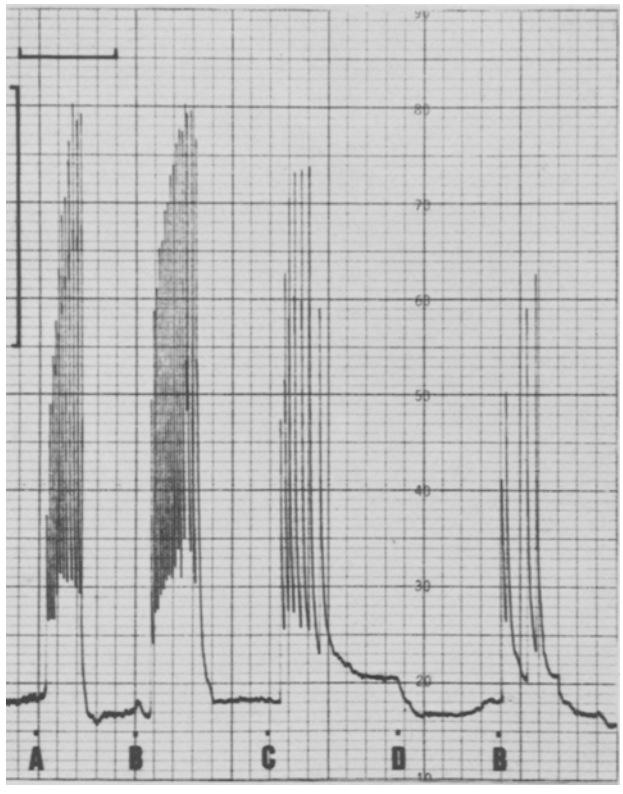

Fig. 1. Isometric contraction of a rat ureter immersed in 12 ml Tyrode's solution, after addition of *A* 0.25 µg synthetic bradykinin, *B* 1 ml rat urine, *C* 1 ml dialyzed rat urine and *D* 1 ml dialyzed rat urine which was boiled for 60 min. The horizontal line, in the upper left corner, corresponds to 5 min and the vertical one corresponds to 50 mg tension.

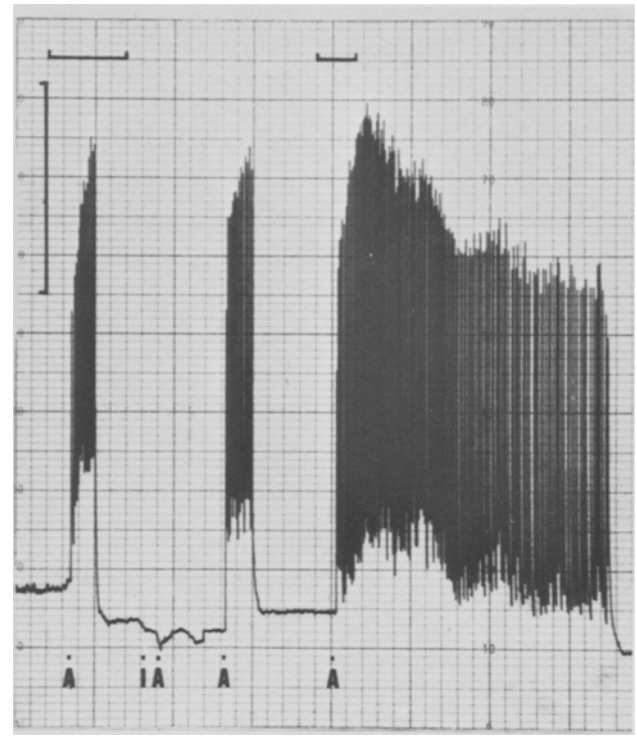

Fig. 3. Isometric contraction of a rat ureter immersed in 12 ml Tyrode's solution, after addition of *A* 1 ml rat urine, *I* 1 ml aprotinin (1000 kIU/ml). The horizontal line in the upper left corner corresponds to 5 min and the vertical line corresponds to 50 mg tension. After the 3rd addition of rat urine, the recorder speed was changed (horizontal line in the middle represents 5 min) and the urine was left in contact with the ureter for more than 30 min, instead of being washed away after 1-2 min contact time.

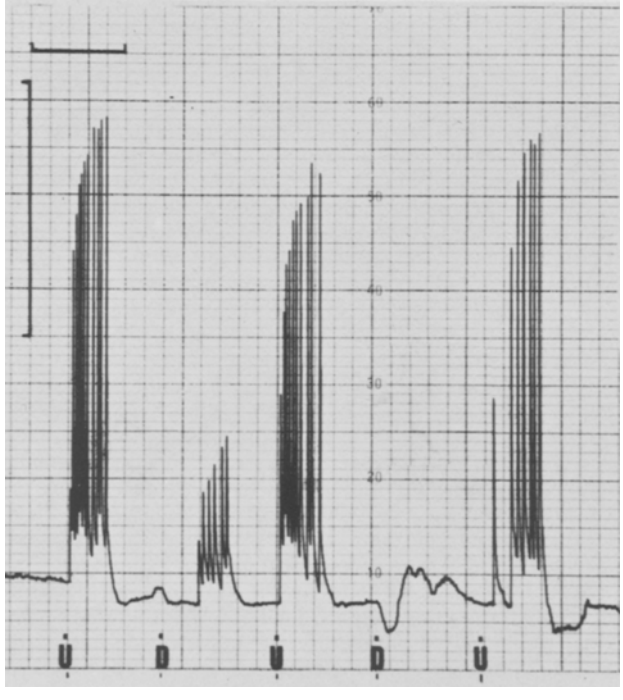

Fig. 2. Isometric contraction of a rat ureter immersed in 12 ml Tyrode's solution after addition of *U* 1 ml rat urine, *D* 1 ml rat urine after acid denaturation The horizontal line, in the upper left corner, corresponds to 5 min and the vertical line corresponds to 50 mg tension.

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