ing the Mo-protein binding in nitrogenase and nitrate reductase.

Earlier biochemical studies have clearly shown that V influences the rate of N_2 fixation in *Azotobacter* species by stabilizing Mo-protein binding in nitrogenase[24]. The present study, therefore, strongly indicates that V at a lower concentration has a role in stabilizing the Mo-protein binding of nitrogenase and nitrate reductase in *Nostoc muscorum*. Although there have been earlier reports on the occurrence of a V-nitrogenase[25] for N_2 fixation in *A. vinelandii* and *A. chrococcum*[26], and *Clostridium butyricum*[27], these unusual results need confirmation, because others are of the opinion that these organisms still contain traces of Mo which are responsible for nitrogenase activity[24]. The present results too emphasize that V is incapable of functionally replacing Mo, though it can help Mo-protein binding in nitrogenase for an increased N_2 fixation. Further in vitro studies on Mo-V interactions at the level of Fe-Mo- and Mo-containing co-factors of nitrogenase and nitrate reductase, as well as in vivo effects of graded V concentrations on these enzyme activities, may lead to the use of V as a trace element to increase N_2-fixing potential in this group of organisms.

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Inhibition of the ureteral contractions induced by rat urine with kallikrein antibodies[1]

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Summary. Purified urinary kallikrein induces contractions of the rat ureter in vitro. Antibodies against kallikrein block the contractile response of the isolated ureter to rat urine.

We have previously shown that rat urine induces contractions of the isolated rat ureter[2]. This effect was attributed to urinary kallikrein, since it was abolished by the serine protease inhibitor aprotinin and also by acid and heat treatment of the urine, but not by dialysis. To test the validity of this assumption, we investigated the effect of purified kallikrein on the muscular tone of the isolated ureter, as well as the capacity of kallikrein antiserum to block the urine - induced ureteral contractions.

Methods. Wistar rats (200-300 g b.wt) were placed in stainless steel metabolic cages, fed a normal rat chow and given free access to drinking water. Urine was collected at room temperature in plastic containers for 24-h periods and stored frozen. To purify urinary kallikrein, the urine was concentrated by ultrafiltration (100:1) and dialysed against 6.7 mM citrate/10 mM phosphate/11.4 mM borate buffer pH 9.5[3]. The concentrate was applied to a chromatography column (1 × 10 cm) containing aprotinin bound to CNBrSepharose[4]. Kallikrein elution, which was achieved with a pH gradient occurred between pH 3.6 and 4.5. The fractions containing the enzyme were pooled and applied to a Sephadex G-100 column (5 × 100 cm) which was developed with phosphate buffer (0.1 M, pH 5.5). The fractions which contained the kallikrein activity were pooled, concentrated by ultrafiltration, dialysed against distilled water and freezedried. Purified kallikrein (15 µg/cm^2) was found to run as a single band on SDS electrophoresis.

Kallikrein (100 µg) emulsified in complete Freund adjuvant was injected intradermally (i.d.) in multiple sites of the back of a New Zealand rabbit. The injections were applied once a week for 5 weeks. A month later the same amount of kallikrein emulsified in imcomplete Freund adjuvant was injected i.d. Blood was drawn before immunization and 1 week after after each injection. The antiserum was heated at 56 °C for 30 min and stored frozen[4].

The kallikrein binding capacity of the antiserum was tested by immunoelectrophoresis. The electrophoresis was performed with purified urinary kallikrein, concentrated urine and an homogenate of rat salivary gland on agarose gel in the presence of barbital buffer (0.04 M, pH 8.2) at a current of 5.6 mA and 250 V for 60 min. The antigen was then precipitated by incubating with kallikrein antiserum for 24 h. After washing in 150 mM NaCl for 24 h the agarose gel was stained with 0.5% amido black and unstained with methanol-acetic acid (9:1) as described by Scheidegger[5].

The capacity of the antiserum to block the biological effects of kallikrein was investigated by testing its influence on the blood pressure reduction induced by the purified enzyme. Rat urinary kallikrein and antiserum were incubated at 4 °C for 5–10 min prior to i.a. injection into anesthetized rats (pentobarbital 60 mg/kg b.wt i.p.). Samples were injected through the left carotid artery, while blood pressure was continuously monitored through a catheter introduced into the femoral artery[6]. Kallikrein, kallikrein-antiserum incubates and synthetic bradykinin were injected in a volume of 100 µl 150 mM NaCl followed by an additional 100 µl NaCl bolus to rinse the catheter.

The influence of kallikrein antiserum on ureteral contractions induced by rat urine was investigated in ureters obtained from rats anesthetized with diethylether. Both ureteral ends were ligated and the organs immersed, with 0.1 g tension, in 10 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 an organ bath and constantly flushed with 5% CO_2 and 95% O_2. Pooled rat urine was thawed and dialysed at 4 °C for 24 h against Tyrode's solution prior to addition to the organ bath. The isometric contractions obtained by addition of rat urine (25 µl/ml) or purified urinary kallikrein (0.2 µg/ml) were amplified and registered by means of a Heathkit EU20B recorder. The influence of kallikrein antiserum was investigated by incubating the dialysed urine with 20 µl antiserum for 60–120 min prior to its addition to the organ bath. After 2–4 min contact time the added solutions were rinsed with fresh Tyrode's solution.

Results. A single precipitation line was obtained when antibodies raised against purified urinary kallikrein were used for the immunoelectrophoresis of concentrated urine and an of homogenate of rat salivary gland. These precipitation lines were symmetrical to that obtained by immunoelectrophoresis of urinary kallikrein. The blood pressure lowering effect of purified kallikrein was blocked by incubation of the enzyme with antiserum prior to the intraarterial injection.

Purified kallikrein induced contractions of the isolated rat

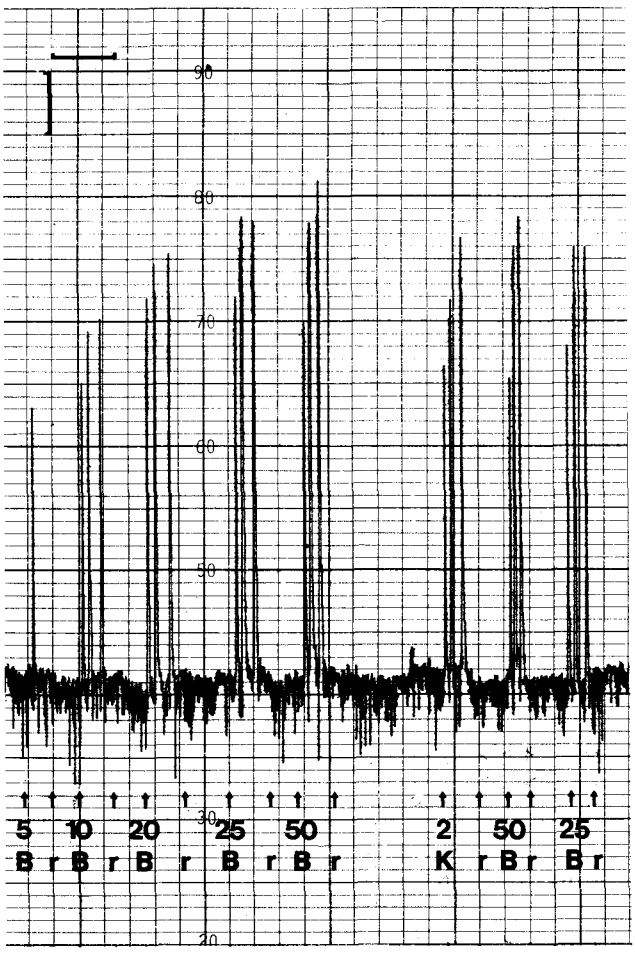

Figure 1. Influence of bradykinin (B) in doses of 5–50 ng (as indicated by numbers above B) upon the isolated rat ureter. The Tyrode solution was replaced (r) after each dose. Purified kallikrein was added at K (2 µg). The vertical line in the upper left corner represents 20 mg tension, and the horizontal one 5 min.

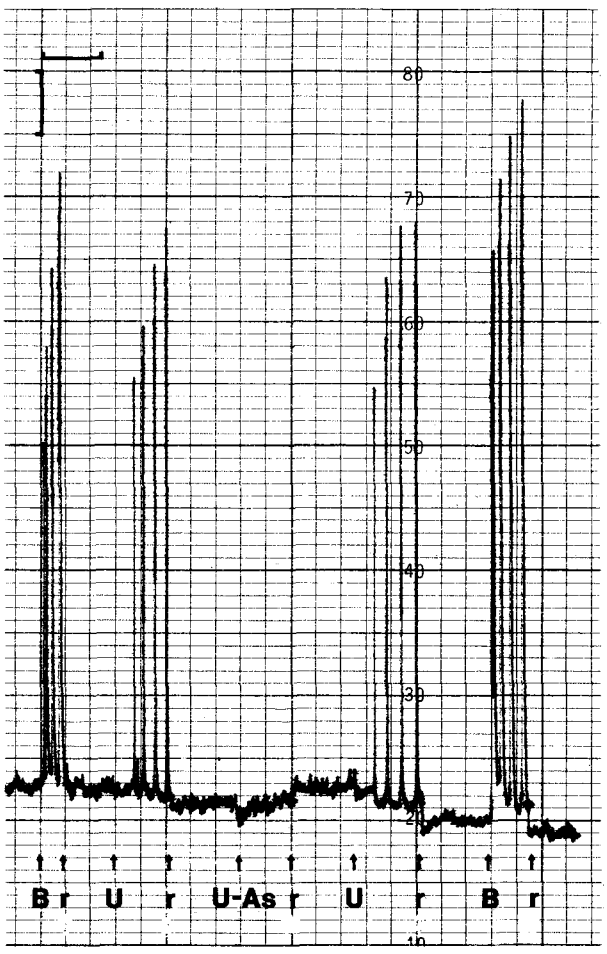

Figure 2. Influence of bradykinin (B = 250 ng), dialysed rat urine (U: 250 µl) and dialysed rat urine preincubated with kallikrein antiserum (U-As: 250 µl urine + 20 µl antiserum, 4 °C, 2 h) on the isolated rat ureter. At r the Tyrode solution was replaced by a fresh one. Calibration lines in upper left corner as in fig. 1.

ureter (fig. 1). The ureteral contractions induced by rat urine were abolished by pre-incubation of the enzyme with kallikrein antiserum (fig. 2).

Discussion. The antibodies obtained against purified rat urinary kallikrein proved to be homogenous when analyzed by immunoelectrophoresis. Since the antibodies blocked the blood pressure lowering effect of kallikrein it may be suggested that they bound to the active site of the enzyme, although it is also possible that they prevented its binding with kininogen.

Rat urine induces contractions of the isolated rat ureter[2]. The same effect can be evoked by purified urinary kallikrein. This, together with the inhibition of the contractions to rat urine obtained with kallikrein antiserum supports our previous suggestion that the stimulatory effect of urine is caused by its kallikrein content. Kininogen is also present in urine[7]. Thus, locally released kinins could facilitate urine flow through the ureters. The stimulatory effect of kinins upon the ureteral musculature in vivo was described several years ago[8].

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cGMP stimulates active K^+ uptake in rat submandibular slices

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Summary. The 8-bromo derivative of cGMP was found to stimulate the ouabain-sensitive uptake of K^+ and to reduce the net release of K^+ induced by acetylcholine in rat submandibular gland slices incubated in vitro.

Parasympathomimetic stimulation of the rat submandibular gland causes both the release of K^+ in vitro[1-4] and the formation of cyclic GMP[5,6]. Both responses are dependent on the availability of external Ca^{++} and appear to occur in essentially similar time courses[5,6]. The net release of K^+ is the result of 2 opposing and almost simultaneous mechanisms, a passive efflux and an active reuptake. The latter seemingly depends on the activation of an ouabain-sensitive Na^+, K^+, ATPase[1,3,4]. The role of cyclic GMP in K^+ release from salivary glands is, however, still unclear. One study on the parotid gland suggested a role for the cyclic nucleotide in K^+ release[7] while others have questioned the involvement of cGMP in this process[6,8-10]. The latter view is based upon experimental observations showing that 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, enhanced the effect of agonists on cGMP but not on the release of K^+. However, these experiments were routinely carried out in the presence of ouabain, which inhibits the Na^+, K^+ ATPase presumably responsible for active K^+ uptake. More recent observations have shown, on the other hand, that cyclic GMP stimulates the hydrolysis of p-nitrophenyl-phosphate (pNPP), an alternate substrate for the Na^+, K^+ ATPase[11], in both the salt gland of the duck[12] and in slices of the rat submandibular gland[13]. The present study was carried out, therefore, to assess a possible function of cyclic GMP on the active uptake component of the K^+ release mechanism of the rat submandibular gland.

Methods. Adult, male rats of the Sprague Dawley strain were used in all the experiments. The animals weighed between 200 and 280 g and were fed a standard pelleted diet and water ad libitum. Submandibular glands were excised under pentobarbital anesthesia, separated from the adjoining sublingual gland and rapidly cut into slices of approximately 1 mm^3 in a small quantity of incubation medium which had been bubbled with a 95% O_2-5% CO_2 mixture and warmed to 37 °C for at least 30 min prior to use. Slices from the glands of 4-6 rats were pooled, thoroughly mixed and then divided into approximately equal portions containing 150-200 mg tissue. Each portion was rapidly placed in a nitrocellulose tube containing 2 ml of the incubation medium for a 10-min pre-incubation. Each slice system was then washed with oxygenated, warm medium and then placed in 2 ml of fresh medium for the final incubation. The medium used throughout this procedure was a Krebs-Ringer bicarbonate solution (KRB) enriched with betahydroxybutyrate, adenine, inosine and glucose as previously described[3]. The time when the slices were placed in the final incubation medium was considered as zero time. Secretagogues or other agents were added, alone or in combination, to the slice systems. Aliquots of the medium were subsequently taken at timed intervals

Net K^+ release from submandibular gland slices of control rats

Stimulant	Net K^+ release percent of total (10 min)	N
Acetylcholine	21.0 ± 1.2	6
Acetylcholine + cGMP	6.2 ± 0.2	6
Acetylcholine + MIX	12.9 ± 0.9	6

Experimental details are described in the text. Acetylcholine was added at zero time to the final incubation medium in a dose of 2×10^{-5} M; cGMP was added to a final concentration of 10^{-5} M; 3-isobutyl-1-methylxanthine (MIX) was used in a final concentration of 10^{-5} M. These 2 agents were added 10 min before acetylcholine. Release of K^+ is expressed as the percent of the K^+ content of the slices released after 10 min of incubation. Results are means ± SD.