20–30 min extraction at 1–4 °C, the washing solution (150 m*M* KCl, 10 m*M* $MgCl_2$, 5 m*M* EGTA, 10 m*M* *Tris* buffer, pH 8.0) was introduced at room temperature. Then, one of two types of the reactivating solution was applied at the room temperature; one was beat-reactivating solution (the standard composition; 150 m*M* KCl, 10 m*M* $MgCl_2$, 1 m*M* ATP, 5 m*M* EGTA, 10 m*M* *Tris* buffer, pH 8.0) and the other was the arrest-reactivating solution (the standard composition; 150 m*M* KCl, 10 m*M* $MgCl_2$, 0.05 m*M* $CaCl_2$, 1 m*M* ATP, 10 m*M* *Tris* buffer, pH 8.0). The Ca ion concentrations higher than 10^{-6} *M* in the arrest-reactivating solution were obtained by a simple addition of $CaCl_2$ to the solution without EGTA as shown in the standard composition. However, when Ca ion concentration of the arrest-reactivating solution was lowered than 3×10^{-7} *M*, 'Ca-buffer' was used; the solution contained 5 m*M* EGTA and appropriate concentration of $CaCl_2$ (below 5 m*M*).

Results. The lateral cilia almost immediately began to beat vigorously when the washing solution was replaced by the beat-reactivating solution with the beat frequency, reaching a steady value within a few minutes; full reactivation was obtained in the solution containing 2–6 m*M* ATP, 2–6 m*M* $MgCl_2$, pH 8.0. The frequency was dependent on ATP and Mg ion concentrations, as has been shown in other cilia and flagella by many authors.

When the arrest-reactivating solution was applied after the washing solution, it was observed that in Ca ion concentrations lower than 10^{-7} *M*, the lateral cilia continued to beat vigorously at high frequencies as in the beat-reactivating solution. In concentrations higher than 10^{-7}–10^{-6} *M*, however, the lateral cilia did not beat but exhibited the arrest response; the cilia inclined in the direction of the recovery stroke just as do the live cilia in response to an electrical stimulation. The amplitude of the arrest response could be estimated by measuring the change in the angle of inclination of the cilia, which was observed when the arrest-reactivating solution was introduced (Figure). The amplitude of the response became larger as the Ca ion concentration was increased. When the Ca ion concentration was again decreased to less than 10^{-7} *M*, the cilia which had exhibited an arrest response resumed beating. When the reactivating solution contained Ca ions and ATP without Mg ion, neither the beating nor the arrest response was observed. Similar results were obtained when the reactivating solution contained Ca and Mg ions without ATP. It may be concluded that Mg ions are indispensable not only for the ciliary beating but also for the arrest response, and that the arrest response cannot be induced by ATP and Ca ions only. The optimal pH for the initiation of the arrest response was 8.0, the same value as that for the beating. An appreciable arrest response was also induced by ADP but not by ITP, GTP, CTP, UTP and c-AMP.

Concerning the ciliary activity in *Paramecium*, it has been suggested that there are two separate motile systems: one which produces the cyclic beating, requiring ATP and Mg ions for activation, and the other which determines the orientation of ciliary movement, requiring ATP and Ca ions. A similar mechanism is also supposed to exist in *Crithidia*[7,8]. However, the present results, particularly the finding that the arrest response requires Mg ions for activation as does the beating, which is inhibited by Ca ions, may indicate that here the two types of ciliary activity depend on a single ATP-utilizing system. Detailed results of this work will be given elsewhere[10].

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Release of Renal Kallikrein to the Perfusate by Isolated Rat Kidney

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Summary. The addition of furosemide to the fluid used to perfuse isolated rat kidney increases the kallikrein activity found in the perfusion fluid. The experiments favour the concept that furosemide activates a kallikrein precursor or/and the synthesis and release of kallikrein in the kidneys.

Renal kallikrein seems to play an important role in the kidney as antagonist of the renin-angiotensin system[1,2]. Experiments from our laboratory[3] suggest that renal kallikrein is released into the blood circulation, and therefore may also exert a systemic action. In the present study, this possibility was explored in the isolated, perfused kidney using kallikrein-free perfusion medium and furosemide, a well-known diuretic and natriuretic agent, which produces a significant increase in the total amount of urinary kallikrein in the rat[4].

Methods. 15 male rats (Sprague-Dawley, 220–300 g) were anesthetized i.p. with Dial-Urethane (10 g of diallylbarbituric acid, 40 g of urethane and 40 g of monoethylurea in 100 ml of water), 0.12 ml/100 g body wt. 9 rats were used for control experiments and 6 for Furosemide experiments. The renal circulation was isolated by exposing the inferior vena cava, abdominal aorta and superior mesenteric artery; all other small blood vessels of the kidney area were tied off. The right renal artery was cannulated without ischemia by placing a PE-50 tubing initially in the superior mesenteric artery. Both kidneys were perfused with perfusion medium (after tying off the abdominal aorta) for 10 min to wash out all the renal blood. Then, the cannula was advanced into the right renal artery. Immediately, the left kidney was removed and frozen for subsequent assay. At the end of the experiment, the right kidney was also excized, frozen and assayed. The inferior vena cava was cannulated with PE-240 tubing so that the tip of the cannula was placed at the right renal vein in order to collect the renal venous outflow. The isolated right kidney was perfused and controlled by pulsatil perfusion pump (Sigmamotor T-8). Systolic perfusion pressure was held constant at 120 mm Hg and mean perfusion pressure varied between 90 and 115 mm Hg. Under these experimental conditions, renal flow was 8–10 ml/min. Perfusion pressure was measured by stain-gauge manometer (Statham P23). Renal outflow

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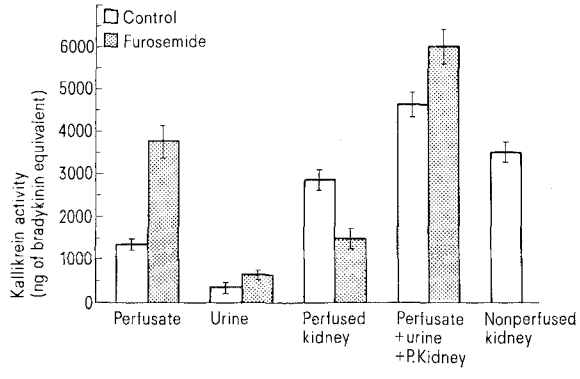
was continually monitored by a photoelectric drop counter (Grass Model PTTI). The measurements were recorded on a Grass polygraph. A single-pass perfusion system was used and was set up in a similar way as have been described previously[5].

The perfusion medium was bicarbonate Krebs-Henseleit solution (Na^+, 126 mM; K^+, 6.0 mM; Ca^{++}, 3.3 mM; Mg^{++}, 1.1 mM) modified by addition of 15 g/l bovine albumin (Calbiochem); 35 g/l Dextran 70,000 MW (Sigma Chemical Co.); 2 mM Na-pyruvate, 6 mM glucose, 6 mM urea and 10 mg/l creatinine. The solution was constantly equilibrated with a mixture of O_2/CO_2 (95/5, respectively)[5]. After equilibration, the pH ranged from 7.40 to 7.45.

To investigate the effects of furosemide, 20 mg/l of Laxur® (Hoechst AG) were added to the modified Krebs-Henseleit solution after the removal of the blood from the kidneys. Glomerular filtration rate was determined by the creatinine clearance using the method described by Folin and Wu[6].

To determine kallikrein activity, samples of renal perfusion outflow were taken every 15 min for 60 min, while urine samples were collected every 30 min for 60 min. Kallikrein activity was measured by methods previously described in perfusion outflow, urine samples[5] and renal tissue[7].

Results and discussion. Mean creatinine clearance in control kidneys was 0.57 ± 0.02 (SD) ml/min and 0.27 ± 0.01 in furosemide-treated kidneys. These values are in agreement with previous reports of others[8] and indicate adequate functioning of the kidneys. No significant changes in the vascular resistance were observed either in the control or in the furosemide perfused kidneys. Control and furosemide data are presented in the Figure.



Control and furosemide experiments. The height of the column represents the mean value for each group of samples; the bars indicate SD.

Kallikrein activity of the furosemide perfusate was significantly ($p < 0.001$) greater than the activity of the control perfusate. A significant increase is already observable even in the samples collected at the end of the first 15 min period. The highest concentration was obtained in the second sample and from then on it keeps constant. The difference between the urine samples were not statistically significant ($p < 0.01$). Kallikrein activity of the furosemide-treated kidney was significantly ($p < 0.001$) lower than of the control perfused kidney. The sum of kallikrein activity found in perfusate, urine and kidney of the furosemide experiments was significantly greater ($p < 0.01$) than the sum of the activities in control experiments.

The kallikrein activity of non-perfused kidneys was significantly lower ($p < 0.01$) than the total kallikrein activity (i.e. perfusate + urine + kidney) found in control perfused kidneys.

The significantly higher total kallikrein activity found in control perfused kidneys, compared to non-perfused kidneys indicates that perfusion of the kidneys with kallikrein-free medium activates some kallikrein precursor or/and stimulate synthesis and release of renal kallikrein. Furosemide intensifies further these effects, as shown by the significant difference between furosemide-treated and control perfused kidneys. Importantly, the effects of both perfusion and furosemide result mainly in a large increase of kallikrein activity in the perfusate. These findings strongly support the notion that renal kallikrein can be released to the blood stream in the physiological condition, and they therefore strengthen the possibility of a systemic role played by renal kallikrein.

Experiments on the effect of several factors including furosemide which increase diuresis-natriuresis upon renal and urinary kallikreins in normal rats[9] and the ability of rat kidneys slices to synthetize kallikrein[10], give support to the concept that furosemide many stimulate the synthesis of the renal enzymes. The amount of kallikrein excreted in the urine, under furosemide was slightly higher than the control, but statistically not significant.

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Changes in Heart Rate Levels During Avoidance Conditioning in the Rabbit

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Summary. During avoidance conditioning heart rate levels tend to increase or to decrease according to their initial values and these changes are not related to learning or performance of the task.

The general level of activation of an animal is usually indicated by several physiological measures such as the heart rate (HR) and the electroencephalographical activity (EEG)[2]. For instance, in the rat, it has been reported that HR levels are monotonically related to hours of water deprivation[3], while performance and deprivation

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