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THE INFLUENCE OF ISOTONIC SALINE ADMINISTRATION ON THE URINARY EXCRETION OF KALLIKREIN IN RATS

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Abstract—Acute saline loading is known to increase kallikrein excretion. To clarify whether this is a specific stimulatory effect or rather a non-specific wash-out, pentobarbital anesthetized rats were loaded thrice (5 min infusions) at 40 min intervals with a volume of 150 mM NaCl equal to 5% of their body wt. The effect of such a load on the central venous pressure was studied in a separate group of rats. Control animals did not receive the infusions. Kallikrein excretion (amidolytic assay) increased with the first, but decreased with the subsequent saline administrations. The rise observed after the first load lost significance when kallikrein excretion was related to that of creatinine. The reduction observed after the second and third infusions remained significant even when expressed per mg of creatinine. Thus, saline load induced kallikrein "stimulation" is due to a non-specific wash-out. Similar transient enhancements of central venous pressure were observed after each of the three loads. This, together with the unchanged creatinine excretion (except for the rise seen after the first load) indicate that the lack of kallikrein stimulation after the second and third loads was not due to the appearance of heart failure. Saline loaded rats had a renal kallikrein activity at the end of the experiment which did not differ from that of controls. Plasma aldosterone concentration was reduced in saline infused rats, and it correlated with the kallikrein excretion when both, NaCl loaded and control rats, were taken into account.

It has been repeatedly shown that an acute load of isotonic saline increases the urinary kallikrein excretion in the rat, dog and sodium depleted man [1–5]. This finding led to the suggestion that increased activity of the renal kallikrein–kinin system could be responsible for the natriuresis and diuresis that follows an expansion of the extracellular fluid volume [3, 6].

However, it has also been reported that an infusion of isotonic saline given after the urine flow had been enhanced by a previous administration of isotonic dextrose, in spite of increasing the excretion of sodium, did not affect that of kallikrein [7]. It is possible that the higher excretion of kallikrein found after an acute sodium loading, instead of reflecting a real stimulation of its renal activity, represents a urine flow dependent wash-out of the enzyme from the renal tubular system.

That kallikrein could be washed-out into the urine by an increased diuresis has been suggested by the transient elevation in kallikrein excretion observed after acute administration of furosemide [8, 9], bumetanide [10], mannitol [11], hypertonic dextrose [12] and isotonic saline [13]. To distinguish between a physiological stimulatory effect of acute sodium chloride administration on renal kallikrein and a non-specific wash-out caused by a brisk diuresis, kallikrein excretion and renal kallikrein activity were measured in rats repeatedly loaded with isotonic saline. If saline loading were an appropriate stimulus, kallikrein excretion should increase repeatedly. Contrariwise, if the effect is non-specific, the response should subside.

MATERIALS AND METHODS

Male Wistar rats (Dr. Karl Thomae GmbH, 7950 Biberach, Federal Republic of Germany), weighing 300–400 g had free access to food and water while kept in individual cages for at least 3 days prior to the experiment. Under sodium pentobarbital anesthesia (40 mg/kg body wt, i.p.) a polyethylene catheter (inner diameter, 0.6 mm; outer diameter, 1 mm) was introduced into the left carotid artery for blood pressure recordings and blood sampling. Isotonic saline (150 mM NaCl) was given to the rats of the experimental group (IIA) through a catheter (i.d.: 0.5 mm, o.d.: 0.7 mm) placed into the right jugular vein. Urine was collected through a catheter placed into the bladder through a small suprapubic incision. The urethra was ligated.

I. *Control rats* (N = 8). These animals did not receive i.v. isotonic saline. Urine collection was started immediately after surgery and continued for 160 min. Each collection period lasted 20 min. Blood was collected at the end of the experiment for the measurement of plasma aldosterone and hematocrit, then the left kidney was excised for the measurement of renal kallikrein.

IIA. *Sodium chloride loaded rats* (N = 8). Urine was collected for two control periods of 20 min each. Subsequently, a volume of isotonic saline equal to 5% of the body wt was infused i.v. within 4–6 min at an approximate rate of 4 ml·min^{-1}. Urine was then collected for periods of 5 min during 130 min.

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The infusion of saline was repeated twice, at 40 min intervals. At the end of the experiment blood was collected for the measurement of plasma aldosterone (one sample was lost) and hematocrit, and the left kidney was excised to measure the renal kallikrein activity.

IIB. *Sodium chloride loaded rats* (N = 6). The same infusion protocol described for group IIA was used, with the exception that the saline infusions were given through the femoral vein. A catheter was advanced through the jugular vein into the vena cava to measure central venous pressure by means of a strain-gauge.

Analytical methods. Urine volume was determined gravimetrically and urine creatinine was measured by the method of Popper *et al.* [14]. Sodium and potassium concentrations in the urine were measured by internal standard flame photometry. Kallikrein was estimated by incubating appropriately diluted urine (1:50–1:100) with the synthetic amide substrate D-valine-leucine-arginine-paranitroanilide in Tris–HCl buffer (0.1 M, pH 8.2) at 37° for 30 min. The enzyme–substrate reaction was stopped by adding 50% acetic acid. Extinction was measured at 405 nm against a blank, prepared for each urine sample in the same manner except that in addition it contained 100 KIU/ml of aprotinin. Results are expressed in units. One unit being the amount of enzyme capable of hydrolysing 1 μM of substrate per min. Urinary kallikrein activity assayed by this method correlates linearly with the kininogenase activity ($r = 0.96$, $P < 0.001$; [15]). Electrolyte and kallikrein excretions (concentration or activity times the urine volume) were calculated per min.

The kallikrein activity of the kidneys was measured by incubating homogenates of renal cortex with the synthetic substrate D-valine-leucine arginine-paranitroanilide as described elsewhere [16]. The protein concentration of the renal cortex homogenates was measured by the method of Lowry *et al.* [17]. Plasma aldosterone concentration was measured by radioimmunoassay [18]. Results are expressed as means ± standard error of the mean ($\bar{x} \pm S.E.M.$). The Wilcoxon test was used to evaluate the differences in the kallikrein/creatinin ratio between saline loaded and control animals. All other parameters were analysed by the Student's t test. The coefficient of correlation between kallikrein and aldosterone was calculated by regression analysis, and its significance tested against zero.

RESULTS

Repeated saline administration to the rats of group IIA produced consistent enhancements of urine flow, sodium excretion and potassium excretion ($P < 0.001$ at the crest for all three parameters, Fig. 1). By contrast only the first saline administration produce a significant increase of kallikrein excretion ($P < 0.02$). The successive saline loads induced a progressive decrease of kallikrein excretion ($P < 0.02$ and $P < 0.001$ at the second and third valleys respectively, Fig. 2, upper panel). Except for a small but significant ($P < 0.05$) enhancement occurring after the first loading creatinine excretion remained unchanged and stable (middle panel of Fig. 2). As a consequence, when the kallikrein excretion was expressed per mg of excreted creatinine, no significant rise was observed after the first load ($P > 0.5$). Successive loads brought about a reduction of the kallikrein excreted per mg of creatinin ($P < 0.01$) at the second and third valleys of the lower panel in Fig. 2). No change in arterial blood pressure was observed (group I: initial BP 110 ± 6 mm Hg, final BP 116 ± 5 mm Hg; group IIA: initial 116 ± 3 mm Hg, final 115 ± 7 mm Hg). Central venous blood pressure reached a maximum of 70 ± 9, 68 ± 9 and 70 ± 7 mm H_2O shortly after the first, second and third saline infusions (group IIB), returning in about 10 min towards, but not quite reaching control values. A typical tracing is shown in Fig. 3.

The kallikrein activity of the renal cortex of rats that were loaded with saline did not differ from the activity of controls at the end of the experiment (group I: 0.36 ± 0.02 mU/mg protein, group IIA: 0.41 ± 0.04 mU/mg protein). Plasma aldosterone was lower in saline loaded than in control rats (group I: 0.38 ± 0.05 ng/ml plasma; group IIA 0.17 ± 0.03 ng/ml plasma, $P < 0.01$). When data from both groups (I and IIA) were taken into account, aldosterone correlated with the kallikrein excreted per mg of creatinine during the last period of urine collection (Fig. 4).

Saline loaded rats (group IIA) had a weight gain of 27 ± 2 g ($P < 0.001$) and controls a weight loss of 4.13 g ($P < 0.001$) by the end of the experiment. The hematocrit, which was similar in both groups at the beginning of the experiment (I: 45 ± 1%, IIA: 43 ± 1%, $P > 0.3$), increased significantly in control rats (+2.6 ± 0.7%, $P < 0.01$) and did not change in rats which received saline (+0.5 ± 1.0%, $P > 0.6$).

DISCUSSION

The role played by the renal kallikrein-kinin system in the regulation of electrolyte and water excretion still awaits clarification. Urinary kallikrein activity has been reported to rise during long term sodium depletion but also during short term saline loading [1–5, 19–23]. It has been suggested that the stimulatory effect of sodium depletion could be due to the increased secretion rate of aldosterone [20, 21]. This idea is supported by the finding that a prolonged administration of mineralocorticoids rises the urinary kallikrein excretion [20, 24–26]. The stimulatory effect of a short-term sodium loading cannot be produced by the same mechanism, since saline reduces rather than increases plasma aldosterone concentration (group IIA vs I).

The rise of kallikrein excretion after an acute saline load was attributed to the expansion of the extracellular fluid volume [3] or some "critical fluid volume" [4] and thought to play a role in the ensuing diuretic and natriuretic response. The fact that the kallikrein excretion remains unaffected in man with an expanded central volume (and increased sodium excretion) provoked by a head-out water immersion shed doubt on the validity of this interpretation [27]. However, head-out water immersion promotes a redistribution of blood rather than an increase of the extracellular fluid volume. It may be also argued that

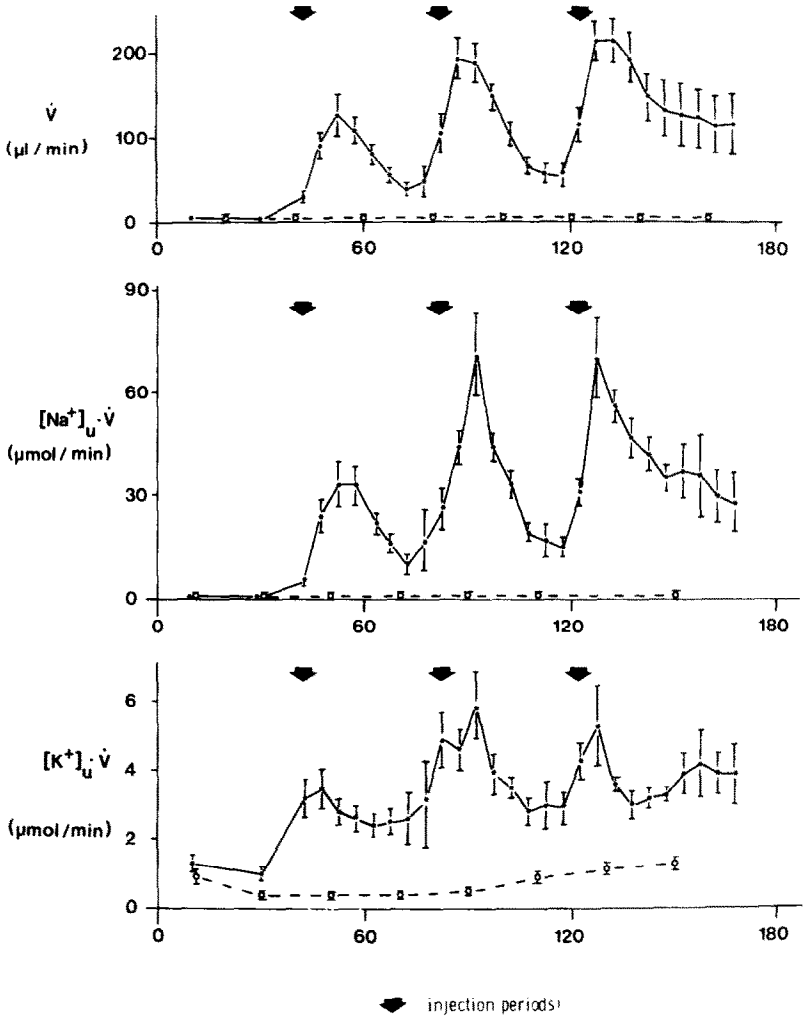

Fig. 1. Urine volume ($\dot{V}$), sodium ($[Na^+]_u$ $\dot{V}$) and potassium ($[K^+]_u$ $\dot{V}$) excretion in control rats (I: - - -○- - -) and in rats repeatedly infused with a volume of isotonic saline equal to 5% of their body wt (IIA: ——*——). The arrows indicate the 4–5 min infusion period.

a lower plasma aldosterone concentration and/or unaffected renal plasma flow, known to occur in this situation, may have blunted the kallikrein response, if an increase on central volume rather than an enhanced ECFV were the appropriate stimulus for kallikrein activation [28, 29].

A hint that the effect of short-term sodium loading on kallikrein excretion represents a non-specific wash-out secondary to an increase in urine flow has been given by experiments with diuretics. It has been shown that both acute and chronic administration of diuretics increase kallikrein excretion [8–11, 19]. The latter could be due to the secondary rise of endogenous aldosterone. Several studies on the acute effect of furosemide, bumetanide and mannitol have revealed that the kallikrein excretion increases sharply with the appearance of diuresis, and that it rapidly returns to normal or even subnormal levels while urine flow remains elevated [8–11, 19]. One dose of furosemide increases urinary kallikrein excretion only transiently even when fluid losses are replaced [8]. When the injections of a diuretic are repeated at relatively short intervals, a progressive decline in the kallikrein response is observed [11]. This probably explains why kallikrein excretion does not increase when the diuresis is stimulated by other means prior to the administration of furosemide [7].

We have found that the acute administration of isotonic saline increases the kallikrein excretion when it induces a rapid diuretic response (group IIA vs I). However, this rise is not significant when it is related to the simultaneous change of creatinine, thus indicating that kallikrein activity is not stimulated, but the enzyme displaced at an accelerated rate from the urinary tract into the urine collection tube (wash-out or dead-volume effect). Repetition of the saline injections had no stimulatory effect on the kallikrein excretion, although they effectively expanded the volume of the extracellular space as indicated by the weight gain, the unchanged hematocrit (as opposed to controls who showed an increase) and the diuretic peaks. It could be argued that the incapacity of the successive injections of saline to stimulate kallikrein activity reflects a col-

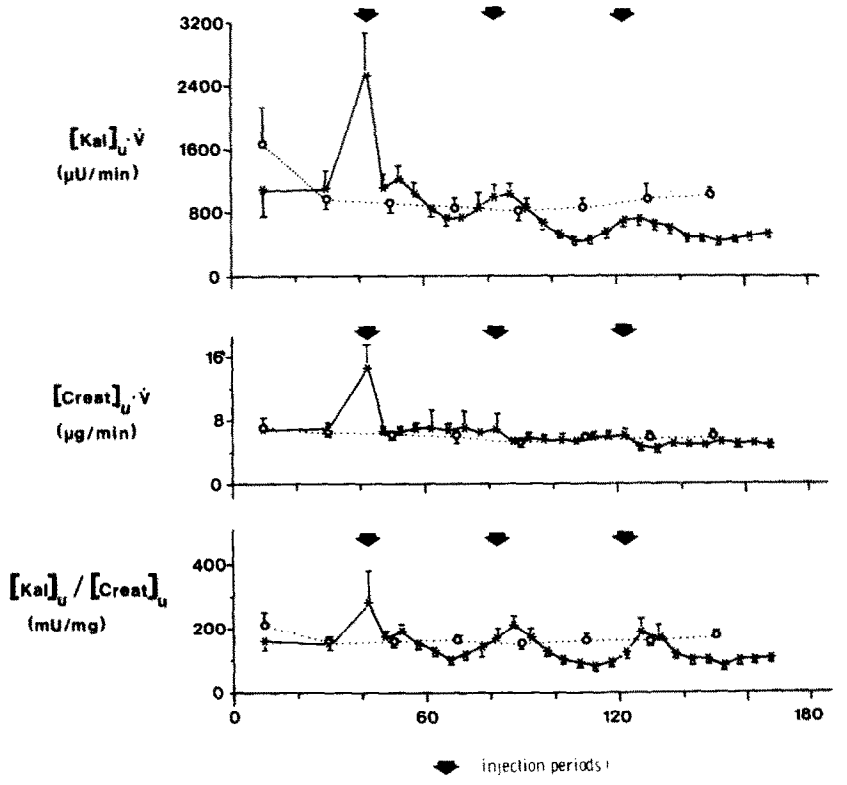

Fig. 2. Kallikrein excretion ($[Kal]_u \dot{V}$), creatinine excretion ($[creat]_u \dot{V}$) and kallikrein/creatinine ratio ($[Kal]_u/[creat]_u$) in control rats (– – –○– – –) and in rats repeatedly infused with a volume of isotonic saline equal to 5% of their body wt (IIA: ——*——). The arrows indicate the 4–5 min infusion period.

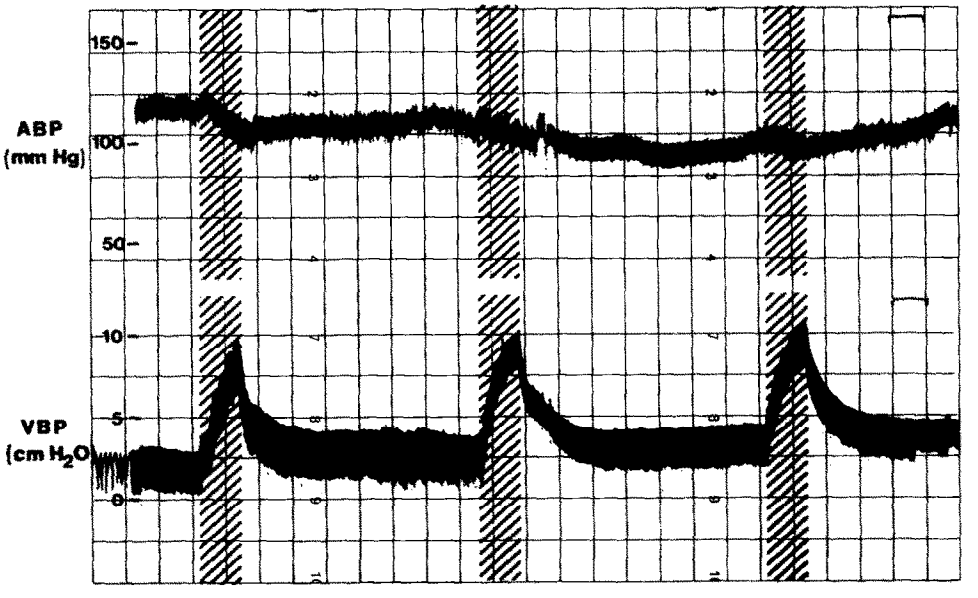

Fig. 3. Arterial blood pressure (ABP) and central venous pressure (VBP) of a rat who received thrice 5 min infusions of isotonic saline corresponding to 5% of his body wt (shadowed area). The horizontal line at the upper right angle represents 5 min.

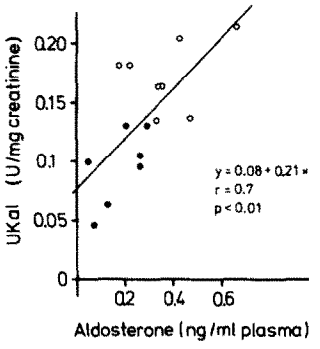

Fig. 4. Correlation between plasma aldosterone concentration (abscissa) and kallikrein (ordinate) excreted in the urine of control rats (I: ○) and in the urine of rats who had received three infusions of isotonic saline solution corresponding to 5% of the body wt each (IIA: ●).

lapse of the renal circulation due to an acute heart failure. This does not seem to be the case, since the transient rise of the central venous pressure following the second and third injections did not differ from the increase which appeared after the first one. In addition, the arterial blood pressure and the creatinine excretion remained within the normal range, and the diuretic response after the second and third injections were slightly higher than those following the first injection. Thus, it can be concluded that the lack of kallikrein stimulation after successive saline injections is due to the wash-out of the enzyme by the first rise of urine flow.

The different kallikrein responses to the same load refutes the hypothesis that an elevation of the ECFV or a certain critical fluid volume are the cause for higher kallikrein excretion after an acute saline load. The involvement of a rate of change rather than of a volume can be also disregarded. The data from our saline infusion experiment are consistent with the interpretation that an acute enhancement of urine volume increases kallikrein in a non-specific manner (wash-out or dead-volume effect). The reduced kallikrein excretion observed after subsequent saline loadings is probably due to the lowering of the aldosterone secretion rate which follows the ECFV expansion. This is suggested by the significant correlation found between kallikrein excretion and plasma aldosterone concentration.

Mills and Ward proposed that the renal kallikrein-kinin system plays a role in the regulation of water excretion which is opposed to the action of antidiuretic hormone [30]. They derived their proposal from the observation that kallikrein correlates positively with urine volume and negatively with urine osmolality. However, if acute changes of urine volume would wash-out kallikrein from the kidney, the finding of such correlations would be of no physiological significance.

The results reported here also show that a marked acute expansion of the ECFV actually reduces the kallikrein excretion. This suggest that not only chronic but also acute changes of the aldosterone concentration may affect the kallikrein excretion.

It is unclear why saline infusion increases kallikrein excretion in sodium depleted but not in sodium repleted man [2, 5]. Perhaps sodium depleted humans have increased renal kallikrein activity (like rats, 16). Then, a greater amount of kallikrein could be removed from the kidney when urine flow increases.

Although the results reported here show that renal kallikrein release is not specifically stimulated by a saline load, they do not exclude the possibility that kinins (perhaps of extrarenal origin) may provoke natriuresis through renal vasodilatation. Such a role has been suggested by the blunting of a saline diuresis after passive immunization against kinins and by the stimulation of natriuresis with the administration of kininase inhibitors [31–33].

It may be concluded that the acute increase in kallikrein excretion which occurs simultaneously with a diuretic response to isotonic saline infusion is transient, abolished by a previous enhancement of diuresis and as judged by its relation to creatinin excretion, non-specific. Perhaps most, if not all the substances, which in acute experiments were shown to stimulate kallikrein excretion (diuretics, vasoconstrictors, vasodilators [34] induce a urine flow mediated wash-out or void-volume effect.

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