man OM-14). This method has proved to be the most reliable method for measuring $\dot{V}O_2max$[4]. Maximal heart rates were determined as previously described[5] essentially by electronic recording or manual palpatation immediately after exercise. Excessive electrical noise from skeletal muscle activity forced the use of manual palpatation periodically. No difference in heart rate was noted in animals measured by both methods. All determinations were made at 20 °C. DL-propranolol (Sigma) treatment groups were 0.1, 0.2, 0.4, 1, 10 µg/toad. Toads were run through a sequence of control then 2 propranolol treatment in 3 successive days. The doses of propranolol were sequentially increased in concentration. Fresh propranolol solutions were made before the experiments in distilled water and injection volume was 0.1 ml into the dorsal lymph sac. Metabolic and heart rate measurements were made an hour post injection.

The relation between post exercise heart rate and $\dot{V}O_2max$ is shown in figure 2. There was significant linear correlation ($r=0.87$) between maximal heart rate and $\dot{V}O_2max$. These data are consistent with the hypothesis that blood oxygen transport is the limiting process for $\dot{V}O_2max$ in anuran amphibians. Previous studies dealing with both intraspecific variability in hemoglobin concentration[2] and interspecific variability in cardiovascular parameters[5] have both implicated a cardiovascular limit to $\dot{V}O_2max$ in anuran amphibians. These data are not consistent with the hypothesis that respiratory surface area represents the limit to $\dot{V}O_2max$ in anuran amphibians[6]. A similar decrease in $\dot{V}O_2max$ following β-adrenergic blockade in humans has previously been reported[7].

Propranolol aside from reducing heart rate is also known to decrease hemoglobin-oxygen affinity in mammals[8,9]. Decreased hemoglobin affinity for oxygen increases oxygen delivery to tissues. Therefore the observed decrease in $\dot{V}O_2max$ as blood oxygen transport decreases is at worst an underestimate, since right-shifted hemoglobin would tend to compensate for diminished blood oxygen transport capacity[10].

Since amphibian hearts have a poorly developed sarcoplasmic reticulum[11,12] and Ca^{2+} for excitation-contraction coupling enters from the extracellular space, rather than being released from internal Ca^{2+} stores[13], the kinetics of a contraction-relaxation cycle are slower in relation to the mammalian cardiac muscle cycle. Consequently maximal heart rates of similar sized mammals are much greater. Therefore from an evolutionary perspective amphibian maximal metabolic capacity may be ultimately limited by the kinetics of cardiac muscle Ca^{2+} exchange, if blood oxygen transport does limit $\dot{V}O_2max$ in amphibians as these data suggest.

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Relationship between the renal kallikrein activity and the urinary excretion of kallikrein in rats[1]

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Summary. Adrenalectomy reduces, and sodium depletion increases, both the daily urinary excretion of kallikrein and the kallikrein activity in the renal cortex. These 2 variables were found to correlate significantly in normal, sodium depleted and adrenalectomized rats, thus supporting the view that kallikrein excretion reflects the activity of the enzyme in the kidney.

It is known that glandular kallikrein is synthetized, among other organs, in the kidney[2], probably in the distal tubular cells[3]. Although it has been suggested that part of the kallikrein excreted in the urine might be of extrarenal origin[4], most of the urinary kallikrein appears to be secreted in the distal segments of the nephron[5]. Several investigations have been performed in the last 10 years in an attempt to elucidate the physiological role of the renal kallikrein-kinin system or its probable involvement in hypertension (for references see Levinsky[6]). Most of this work relied on the estimation of the urinary kallikrein excretion, assumed to reflect the activity of the enzyme in the kidney. However, until now there has been no proof for this assumption. Since in acute experiments we found an inverse relationship between the excretion of kallikrein and its activity in the kidney[7], we investigated whether this was also the case under 'steady state' conditions.

Methods. Rats from different experimental groups were placed in stainless steel metabolic cages for the collection of urine for 24 h. Apart from normal rats (200 g b.wt) fed a normal rat chow or a sodium deficient diet for 14 days (2 mmoles/kg dry food), adrenalectomized rats (19 days after operation) given 1% NaCl to drink were also used. The rats were placed in the metabolic cages at least 3 days prior to the urine collection. At the end of the experimental period the animals were anesthetized with pentobarbital (40 mg/kg b.wt, i.p.) and the kidneys were excised after rinsing them with an intraarterial perfusion of 150 mM NaCl until they were macroscopically free of blood. Kallikrein was brought into solution by mechanical homogenization and

addition of desoxycholate[8]. The homogenates were then centrifuged at 50,000×g and 4 °C for 30 min and the supernatants were used to measure kallikrein.

The kallikrein activity was estimated in appropriately diluted homogenates or in urine (1:50–1:100) either by the kininogenase method, i.e.: incubation with dog kininogen and measurement of the kinins released with an specific radio-immunoassay using synthetic bradykinin as standard[9,10] or by the amidolytic method, i.e.: incubation with D-val-leu-arg-paranitroanilide (S2266, Kabi Diagnostica, Stockholm, Sweden) and photometric determination of the paranitroaniline released[11]. The kininogenase activity was calculated as kinins released per min of incubation and g of cortex (wet wt) or ml of urine. The amidolytic activity was expressed in units or mU, 1 unit beeing the amount of kallikrein capable of hydrolysing 1 µmole of amide substrate per min of incubation.

Results. The amidolytic activity of the urine and of the kidney homogenates increased after sodium depletion and decreased after adrenalectomy (table 1).

The activity of kallikrein in the kidney of sodium depleted, adrenalectomized and control rats correlated significantly with the activity of the enzyme excreted in urine during the preceding 24 h (table 2). The regression line calculated with the renal and urinary amidolytic activities had an intercept with the y axis (fig. 1) which within 95% confidence limits did not differ from zero (-0.20 ± 0.25). The kininogenase activity of the renal cortex of normal rats was 0.91 ± 0.11 µg bradykinin $\cdot$ min$^{-1} \cdot$ g^{-1} and that of the urine was 20.88 ± 2.23 µg bradykinin $\cdot$ min$^{-1} \cdot$ day^{-1}. The regression line calculated with these parameters (fig. 2) had an ordinate intercept that, within a 95% confidence limit, differed from zero ($+4.91 \pm 2.32$).

Discussion. The urinary kallikrein excretion and the activity of this enzyme in the kidney may be inversely correlated under certain conditions. Kidney kallikrein activity increases shortly after its excretion has been reduced by a renal ischaemia[7,12] and it decreases after a transient rise of the kallikrein excretion has been elicited by an osmotic diuresis[8]. This, along with the ephemeral enhancement of kallikrein excretion found after the acute administration of diuretics[13,14], suggests that rapid changes of the urine flow may influence the amount of kallikrein excreted; i.e. a wash-out by an abrupt enhancement of diuresis and an accumulation by a reduction of urine flow.

However, the results reported here indicate that under stable conditions the renal and the urinary kallikrein activities change in the same direction. In normal rats, a significant correlation was found between the urinary and the renal kininogenase activities. The intercept of the regression line with the ordinate at a point different from zero suggest that some kallikrein of extrarenal origin appears in the urine. The urinary and renal amidolytic activities of rats from different experimental groups were also correlated. But their regression line has an intercept that does not significantly differ from zero, indicating that the bulk of the enzyme is synthetized in the kidney. This is supported by the finding that kidney slices synthesize kallikrein in vitro[2], and that the isolated perfused rat kidney releases kallikrein both to the perfusate and the urine[15]. The possibility that

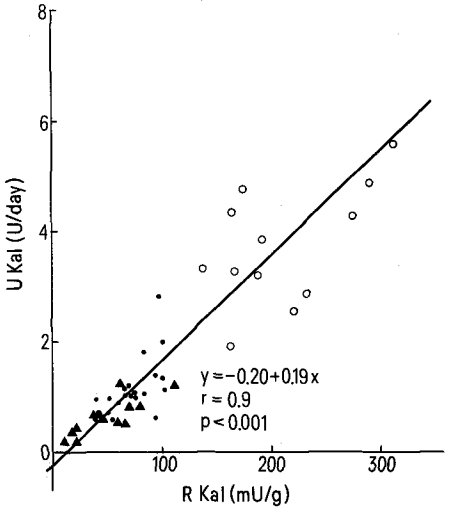

Figure 1. Correlation between the kallikrein activity in the renal cortex (RKal) and the kallikrein excreted in the urine (UKal) in normal (●), sodium depleted (○) and adrenalectomized (▲) rats. The enzyme was estimated with an amidolytic assay using D-val-leu-arg-paranitroanilide as substrate.

Table 1. Kallikrein excretion and renal kallikrein activity measured with an amidolytic assay in rats

Experimental group	No. of rats	Kallikrein excretion (U/day)	Renal kallikrein activity (mU/g)
Control	22	1.14 ± 0.11	71 ± 5
Adrenalectomy	12	0.60 ± 0.11[b]	51 ± 9[c]
Sodium depletion	14	4.08 ± 0.37[a]	216 ± 15[a]

Values are $\bar{x} \pm$ SEM. [a] Differs from control with a $p < 0.001$; [b] differs from control with a $p < 0.005$; [c] differs from control with a $p < 0.05$.

Table 2. Correlation between the renal kallikrein activity and the urinary kallikrein excretion in rats

Experimental group	No. of pairs	Coefficient of correlation	Significance	Method
Control	30	0.83	$p < 0.001$	K
Control	22	0.61	$p < 0.01$	A
Adrenalectomy	12	0.83	$p < 0.001$	A
Sodium depletion	14	0.56	$p < 0.05$	A

A, amidase activity measured with D-val-leu-arg-paranitroanilide; K, kininogenase activity measured with dog kininogen and radioimmunoassay of the kinins released.

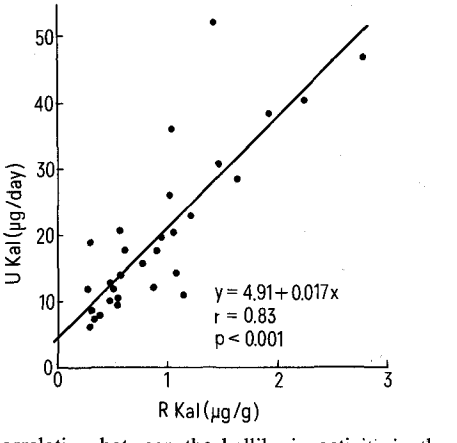

Figure 2. Correlation between the kallikrein activity in the renal cortex (RKal) and in the urine (UKal) of normal rats. The enzyme, estimated by incubation with dog kininogen and radioimmunoassay of the kinins released, was expressed in µg of bradykinin released per min of incubation and g of wet weight (RKal) or 24-h urine volume (UKal).

extrarenal kallikrein could accumulate in the distal segments of the nephron prior to its excretion cannot be disregarded. The distinction between a renal or an extrarenal origin of the urinary kallikrein is perhaps immaterial: if the activity in the kidney is high (or low), its excretion will be high (or low), irrespective of its origin.

The stimulatory effect of sodium depletion and the inhibitory effect of adrenalectomy on kallikrein excretion were confirmed[16]. Additionally, we have found that these experimental maneuvers induce similar changes in the kallikrein activity of the kidney.

Since p-toluenesulfonyl-L-arginine methyl esther (TAME) esterases other than kallikrein have been found in the urine and in the kidney[17,18], it is likely that the kininogenase[9] and the amidolytic[11] assays used in this study are more specific than the more commonly used TAME esterase assay[19].

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Ventilatory responses to CO_2 at different body temperatures in the snake, *Coluber constrictor*

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Summary. Ventilatory responses to CO_2 were examined at different temperatures in the snake, *Coluber constrictor*. CO_2 sensitivity increased between 15 and 25 °C but not between 25 and 35 °C. A rapidly occurring off-CO_2 transient hyperpnea suggested the presence of an intrapulmonary chemoreceptor.

Ventilatory responses to inspired CO_2 have been examined in a number of reptile species[2-5]. Because of the potential for large alveolar-arterial P_{CO2} gradients in reptiles[6], studies in which arterial P_{CO2} measurements are not made provide only limited information regarding CO_2 sensitivity. To date, the work of Jackson et al.[3] on the turtle *Pseudemys scripta* is the only study examining ventilation as a function of blood acid-base status during CO_2 breathing in a reptile. Furthermore, few investigators have addressed the question of the temperature dependence of the CO_2 response[3,5]. The present study examines the ventilatory response to changes in arterial CO_2 tension at different body temperatures in the black racer snake, *Coluber constrictor*.

Materials and methods. Ventilation was measured during 0, 4 and 7% CO_2, 21% O_2, balance N_2 breathing at body temperatures (Tb) of 15, 25 and 35 °C in 14 unanesthetized black racer snakes (b.wt of 125–400 g, average 260 g). Inspiratory flow was determined with a pneumotachograph in the line downstream from a flow-through head mask. Gas was supplied through the head mask at 350 ml/min. Deviations below this control flow occurred during inspiration. These were integrated electronically to provide tidal volume (V_T). Breathing frequency (f) was obtained from a polygraph record and minute ventilation ($\dot{V}_I$) was calculated. 7 snakes under cold-anesthesia had catheters placed in the posterior-most segment of the dorsal aorta for removal of blood sample. Tb was monitored with a thermistor probe inserted 8 cm into the cloaca. Each snake was kept at the experimental Tb for 18 h prior to testing. Tests at the 3 Tb were run on consecutive days. Arterial P_{CO2} was measured in 140–160 µl blood samples using a Radiometer BMS 3 Mk 2 Blood Micro System calibrated at the Tb of the animal. During the experiments, each snake was loosely restrained in a muslin sleeve and kept in a darkened constant temperature chamber. The arterial catheter was brought out through a hole in the chamber wall to enable blood sampling without disturbing the subject. Gas mixtures passing through the head mask were vented to the outside of the chamber so that the rest of the body was exposed to normal room air. Experimental protocol was as follows. Restrained and instrumented animals were allowed 1.5–2 h to adjust to experimental conditions. Control ventilation (room air breathing) was then measured for 30 min and a blood sample drawn. Either the 4% or the 7% CO_2 mixture (determined at random) was then administered for 2 h. The steady-state ventilatory response was measured and a blood sample taken during the final 30 min of CO_2 breathing. The snake was then returned to CO_2-free air and the off-CO_2 transient ventilatory response measured. After a 75-min 'rest' period, the alternate CO_2