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

mixture was administered. $p < 0.05$ was considered significant.

Results. Figure 1 plots $\dot{V}_I$ as a function of arterial P_{CO_2} at each Tb. Significant regression slopes were obtained only at 25 and 35 °C. These slopes were not significantly different from each other. Thus, CO_2 sensitivity increased between 15 and 25 °C but not between 25 and 35 °C. The increase in $\dot{V}_I$ during CO_2 breathing at 25 and 35 °C was due to an increase in V_T as f did not change. V_T also increased during CO_2 breathing at 15 °C but this was offset by a significant decrease in f.

A marked increase in $\dot{V}_I$ above the steady-state CO_2 response level was observed during the transition back to room air breathing at 15 °C (fig. 2). This off-CO_2 transient hyperpnea was due to a dramatic increase in f and began within seconds after the first breath during CO_2 washout from the flow-through head mask. In addition, a transient increase in ventilation occurred within seconds of the start of CO_2 breathing at 15 °C. This was also seen with 7% but not 4% CO_2 at 25 °C and was absent at 35 °C. Figure 3 is the polygraph record of a control, steady-state 4% CO_2 response, off-CO_2 transient sequence obtained from a single snake at 15 °C. Note the reduced f and increased V_T during CO_2 breathing compared to control and the sharp increase in f during CO_2 washout. In this animal, approximately 20 sec elapsed between the first breath during CO_2 washout from the head mask and the onset of the off-CO_2 transient hyperpnea. In some snakes, this lag time was as short as 5 sec. The off-CO_2 transient hyperpnea was also seen at 25 and 35 °C although it was much less distinct at 25 °C and in some cases entirely absent at 35 °C.

Discussion. The increase in CO_2 sensitivity in *Coluber* between 15 and 25 °C differs from the response of *P. scripta* in which no Tb dependence of CO_2 sensitivity was observed between 10 and 30 °C[3]. The off-CO_2 transient response of *Coluber* strongly suggests the presence of an airway or intrapulmonary chemoreceptor (IPC) which has an inhibitory effect on breathing frequency. This inhibition is apparently reduced as inspired CO_2 decreases, thus allowing the excitatory effects on ventilation of blood acidosis to initiate the off-CO_2 transient response. This transient hyperpnea subsides as the respiratory acidosis is corrected. Qualitatively similar off-CO_2 transient responses have been observed in lizards[5–7], and in the aquatic snake *Acrochordus javanicus*[8]. In all cases, some degree of inhibition of breathing frequency was seen during 9–10% CO_2 inhalation. Gatz et al.[9] observed a rapid decrease in breathing effort and frequency upon decreasing airway CO_2 in halothane-anesthetized, unidirectionally ventilated lizards *(Tupinambis nigropunctatus).* This response differs from that of *Coluber* and may reflect a species difference or may be attributed to the anesthesia and surgical intervention in *Tupinambis.*

Direct evidence of IPCs in reptiles has been obtained from single unit vagal recordings in lizards[10,11]. These fibers increased their discharge frequency as CO_2 concentration decreased. Viewing both these data and the off-CO_2 transient ventilatory response of *Coluber,* it may be inferred that reptile IPCs have an excitatory influence on ventilation that is reduced as inspired CO_2 increases. The off-CO_2 transient response of *Coluber* is clearly Tb dependent, being most distinct at 15 °C, of intermediate intensity at

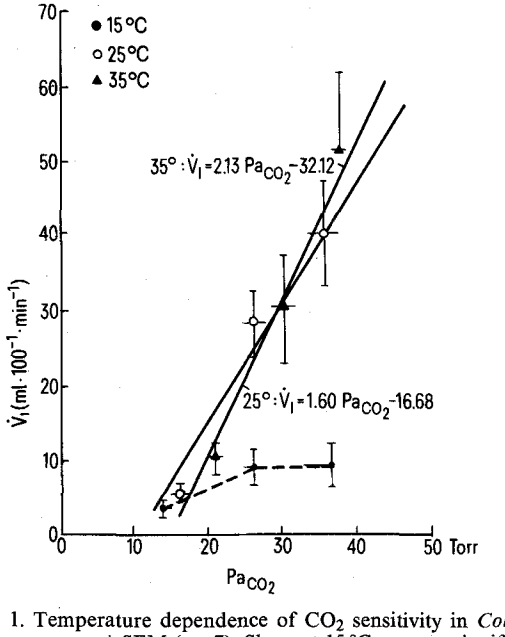

Figure 1. Temperature dependence of CO_2 sensitivity in *Coluber.* Values are mean ± SEM (n = 7). Slope at 15 °C was not significant. ($p > 0.05$). Volumes are BTPS.

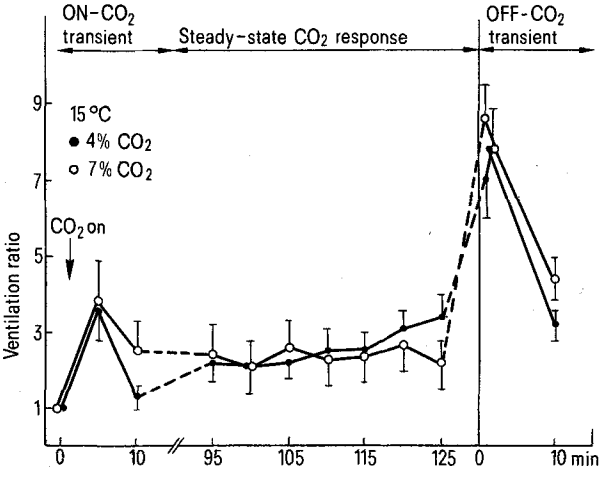

Figure 2. Ventilation ratio (VR) during the first 10 min and final 35 min of CO_2 breathing, and during the CO_2 washout period ('OFF-CO_2' transient) at 15 °C in *Coluber.* VR is the experimental minute ventilation divided by the control minute ventilation. Each point is the mean ± SEM for 14 animals.

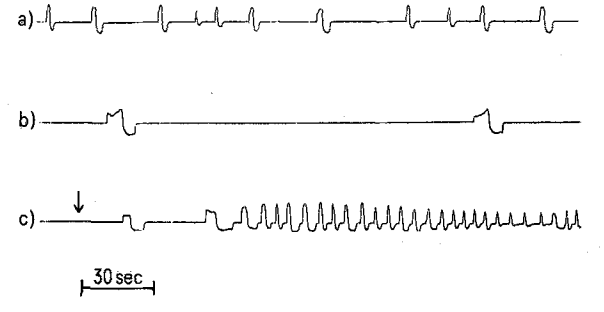

Figure 3. Polygraph record illustrating *a* control ventilation, *b* steady-state response to 4% CO_2, and *c* the off-CO_2 transient response. All traces are from the same animal at 15 °C. Upward pen deflection indicates expiration. Arrow indicates beginning of CO_2 washout from the inspired air. Control ventilation was recorded at a polygraph amplifier gain factor twice that at which the steady state and off-CO_2 transient responses were recorded.

25 °C, and minimal or absent at 35 °C. The off-CO_2 transient hyperpnea is preceeded during CO_2 inhalation by inhibition of breathing f. At 15 °C, the lower metabolic rate and lower O_2 requirements of *Coluber* may faciliate this inhibition. At the higher Tbs, a higher metabolic rate and O_2-related ventilatory drive may prevent IPC-mediated inhibition from dominating ventilation during CO_2 breathing, thus minimizing the off-CO_2 transient increase in ventilation over the steady-state response level.

P. scripta showed no sign of ventilatory inhibition during inhalation of CO_2 at concentrations up to 6% and exhibited no off-CO_2 transient hyperpnea at any Tb[3]. The difference in the Tb dependence of the steady-state CO_2 sensitivities between *Coluber* and *Pseudemys* may reside in the presence in *Coluber* but not in *Pseudemys* of IPC-mediated ventilatory inhibition which is prominent at low but not high body temperatures.

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Parasympathetically evoked parotid salivary secretion of chronically amitriptyline-treated rats

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Summary. Treatment of rats with amitriptyline for 4 weeks significantly decreased flow rate of saliva elicited from parotid glands in response to electrical stimulation of the parasympathetic innervation but did not alter calcium concentration of such saliva. The mechanism of the dissociation between flow rate and calcium concentration of parasympathetically evoked saliva induced by amitriptyline treatment remains to be explored, and may not involve an amitriptyline induced reduction in acetylcholine release.

Tricyclic antidepressant drugs such as amitriptyline have been widely used in treatment of depression. However, one of the side effects following longterm treatment with antidepressants is hyposecretion or no secretion at all from the salivary glands[2-4]. It has been suggested but not established that the reduced salivary secretion is the result of anticholinergic effects of the drug[5].

The present study was undertaken therefore to determine if salivary secretion evoked by stimulation of the parasympathetic innervation to parotid is reduced in rats chronically treated with amitriptyline. Furthermore, since calcium concentration of parasympathetically evoked saliva is related to salivary flow rate[6], the effects of amitriptyline on calcium concentration were also examined; the main objective in this case was to see if a drug-induced separation of calcium concentration from flow rate could be effected.

Materials and methods. Female Long-Evans rats, 4-6 months old, and weighing between 200 and 250 g were used in these experiments. Rats were administered daily 10 mg/kg of amitriptyline i.p. for 2 or 4 weeks. The experimental and control rats were maintained on rat lab chow and water ad libitum until 18 h before the experiments, when food but not water was removed. The rats were anesthetized by i.p. administration of sodium pentobarbital (50 mg/kg b.wt). The parasympathetic innervation to the parotid gland was stimulated using a Grass stimulator which delivered square-wave pulses of 5 msec in duration at a frequency of 16 pulses and an intensity of 4 V. Bipolar electrodes were placed around the auriculotemporal nerve as previously described[7]. Parotid saliva was collected by micropipette directly from the cut end of the duct. Saliva samples were continously collected for the period of 20 min stimula-

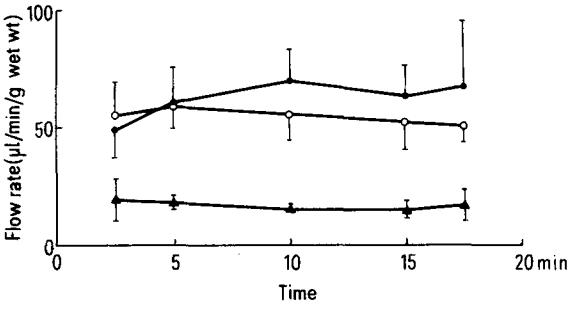

Figure 1. Change in flow rate of parotid saliva evoked by parasympathetic nerve stimulation following chronic administration of amitriptyline. ○—○, control; ●—●, amitriptyline 2 weeks; ▲—▲, amitriptyline 4 weeks.

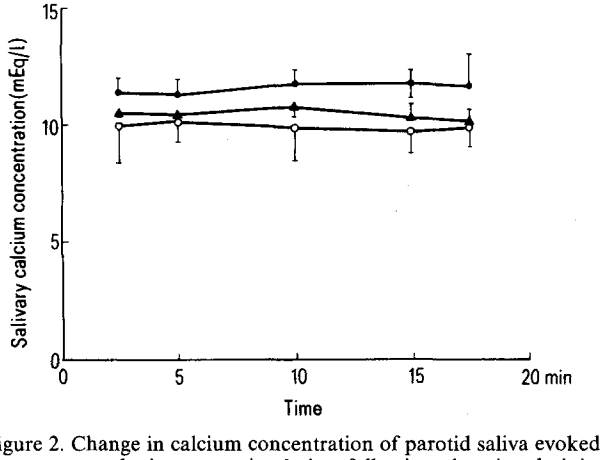

Figure 2. Change in calcium concentration of parotid saliva evoked by parasympathetic nerve stimulation following chronic administration of amitriptyline. ○—○, control; ●—●, amitriptyline 2 weeks; ▲—▲, amitriptyline 4 weeks.