URINARY CHARACTERISTICS OF THE CAPE PORCUPINE *HYSTRIX AFRICAEAUSTRALIS*: EFFECTS OF PHOTOPERIOD AND TEMPERATURE

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ABSTRACT

The Cape porcupine *Hystrix africaeaustralis* is a large (11-18 kg), nocturnal, burrowing and group-living rodent. It experiences a metabolic response to seasonal acclimatization and is a hind gut fermenter. Changes in the urinary electrolyte and free urinary catecholamine concentrations of Cape porcupines were related to combined changes of ambient temperature and photoperiod regime. Three groups, A) $T_a = 25°C$, 12L:12D; B) $T_a = 32°C$, 16L:8D; C) $T_a = 10°C$, 8L:16D, were studied to assess the influence of seasonal acclimatization on urinary bicarbonate and catecholamine concentrations.

Urine volume was significantly ($p < 0.001$) higher in group C than in the other two groups. In groups B and C urinary pH was above 7 and this was associated with high concentrations of HCO_3^-. The total amount of catecholamines was higher in groups B and C than in group A. It is apparent that seasonal acclimatization of the Cape porcupine is also reflected by these parameters.

KEY WORDS

photoperiod, seasonal acclimatization, catecholamine, rodents, bicarbonate, urinary pH

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Accepted 1 July 1992

INTRODUCTION

The Cape porcupine *Hystrix africaeaustralis* is a large nocturnal, burrowing rodent and is widely distributed over the southern African subregion /1, 2/. They are monogamous, attain sexual maturity when 10-14 months of age, and breed seasonally in the summer rainfall areas of southern Africa /1/. Cape porcupines are territorial /3/, live in extended family groups /1/, and are considered food generalists. They feed on the bulbs, tubers, roots, bark, twigs and leaves of a variety of plants, but food preferences could not be explained in terms of protein or mineral contents /4/. Caecal fermentation enables the efficient utilization of food with a high fibre content /5/. Metabolic rates and thermoregulation respond to seasonal climatic change, but the former is up to 35% lower than that expected for a mammal with such a body mass /6, 7/. However, acclimation to cold and a long scotophase (10°C; 8L:16D) resulted in increased metabolic rate and food consumption. Increased heat production partly resulted from an increase in digestible matter intake which caused a decrease in digestible efficiency /6/. Therefore, it seems of great interest to determine whether such metabolic changes are also reflected by changes in urine composition.

The mammalian kidney is a major osmo-iono regulatory organ which maintains the internal environment of the individual at a constant level /8/. It is assumed that urine composition differs according to the environment. The urine of subterranean mammals contains higher levels of free calcium, magnesium carbonate and bicarbonates than that of surface living mammals /9-11/. However, the urine of some herbivorous mammals, such as the rock hyrax *Procavia capensis* /12, 13/, the pack rat *Neotoma albigula*, the hamster *Mesocricetus auratus* /14/ and the domestic horse *Equus cabbalus* /15/, also contains high levels of carbonates and bicarbonates. Subterranean mammals apparently use calcium, magnesium, carbonate and bicarbonate excretion through the kidneys to off-load carbon dioxide, thereby reducing the hypercapnic atmosphere within their burrows /10/. Since the Cape porcupine is a relatively large (11-18 kg), semi-fossorial, herbivorous mammal with caecal fermentation, spending 12-16 hours daily with family members in subterranean burrows /3/, a study of urine composition with emphasis on carbonates and bicarbonates is of great relevance. However, the significant metabolic changes in seasonal acclimatization /6/ would presumably reflect the activity of the sympatho-adrenal system. Such changes in activity can be detected by changes in the urine catecholamine concentrations.

Environmental effects on thermogenesis are principally controlled by the sympathetic nervous system /16-18/. In mammals, the turnover of circulating catecholamines is very high, but the response of the sympatho-adrenal system to cold can be assessed by measuring changes in free urinary catecholamine concentrations /19/. Cold, as well as long scotophase acclimation, caused an increase in free urinary catecholamines in the rat /20, 21/.

The aim of the present study was to compare the urine composition of Cape porcupines exposed experimentally to different ambient temperatures and photoperiod regimes, thus assessing the effects of seasonal acclimatization on urinary bicarbonate and catecholamine concentrations.

MATERIALS AND METHODS

Six captive bred adult Cape porcupines (three males, three females) were kept in a climatic chamber (Spech Scientific) with an average relative humidity of 60%. They were fed on rat pellets (Epol Pty. Ltd.) and given water *ad libitum*. Porcupines were acclimated to three different ambient temperatures (T_a) and at each of these to a different photoperiod regime. Acclimation of the groups for at least three weeks to the following conditions was as follows:

Group A: T_a = 25°C; 12L:12D
Group B: T_a = 32°C; 16L:08D
Group C: T_a = 10°C; 08L:16D

Urine collection

Each porcupine was kept in a customized metal cage (101 x 75 x 65 cm) with a stainless steel tray below it, in which urine accumulated and was collected through a funnel into a jar with paraffin oil. The jars with urine were removed at 24 h intervals and replaced with clean jars containing paraffin oil. Each porcupine was sampled for a minimum period of five days. Samples collected for measuring catecholamine content were kept at pH 3 by adding 0.1 N HCl to the jar before collecting urine. Urine volume was measured to the nearest 0.5 ml.

Urine analysis

The concentrations of sodium, potassium, chloride and urea (after treatment with urease) were determined using iono-specific electrodes in an Astra-8 instrument (Beckman). Calcium and magnesium concentrations were determined by atomic absorption spectrometry using lithium nitrate dilution. Bicarbonate was measured by adding 0.1 ml urine to 0.5 ml 0.1 N HCl and titration with 0.1 N NaOH, using phenol red as an indicator. Phosphate was determined by colour development as described by Taussky and Shorre /22/ and monitored on a fleacigen (ENI). Urine osmolarity was measured using a 5130C vapour pressure osmometer (Wescor Inc.) while pH was recorded with a Labotec pH meter.

Urinary free catecholamine content was determined using the method of Pisano /23/ for analysing normetanephrine (m-O-methylnorepinephrine) and metanephrine (m-O-methylepinephrine) (NMA) and the method of Pisano *et al.* /24/ for analysing vanilmandelic acid (VMA). In both methods, catecholamines were transformed to vanillin which was assayed spectrophotometrically at 350-360 nm for VMA and 360 nm for NMA.

All results are given as means followed by one standard deviation (±S.D.) of the means. Significance of differences is based on Student's t-values ($n = 24$ for all variables but catecholamines, for which $n = 6$).

RESULTS

The mean volume of urine voided by group C (10°C; 08L:16D) was significantly ($p < 0.001$) higher than that of the other two groups, while urine osmolarity was significantly ($p < 0.001$) higher in group B (32°C; 16L:08D) than in the other two groups. The urinary pH of group A (25°C; 12L:12D) was in the acidic range (6.0±0.4) while those of the two other groups were in the basic range (7.7±0.3 for group C and 7.8±0.5 for group B; Table 1).

HCO_3^- concentration was significantly ($p < 0.001$) lower in samples of porcupines from group A than those of the other two groups.

TABLE 1
The comparison of the urine content of several variables (mean±SD) in the Cape porcupine *Hystrix africaeaustralis* acclimated to different ambient temperatures and photoperiod regimes (n = 24)

Group	pH	Na^+ mmol/l	K^+ mmol/l	Cl^- mmol/l	HCO_3^- mmol/l	PO_4^{--} mmol/l	Ca^{++} mmol/l	Mg^{++} mmol/l	Urea mmol/l	Osmol mmol/kg	Volume ml/24 h
A 25°C 12L:12D	6±0.4	54.6±7.6*	39.8±4.7	67.5±8.2	20.3±3.2*	6.7±1.2	11.4±2.3	4.3±0.6	259.6±30.7	586±61.3	216.3±57.4
B 32°C 16L:8D	7.8±0.5	79.0±8.7	43.9±4.2	63.3±7.1	55.4±6.2	2.8±0.7	16.5±2.7*	4.2±0.8	281.6±29.1	751±67.9*	232.7±69.7
C 10°C 8L:16D	7.7±0.3	87.2±16.2	72.4±9.7*	103.3±18.6*	54.2±9.5	0.3±0.1*	10.9±2.1	4.6±0.3	220.3±22.7	591.2±87.3	467.8±86.7*

*P<0.001

This correlates with the difference in pH and the observation that urine samples from these groups contained noticeable sediments of carbonate. Cl^- was significantly ($p < 0.001$) higher in the urine of group C than in the urine of the other two groups, but in this group PO_4^{--} was significantly ($p < 0.001$) lower than in groups A and B (Table 1).

Ca^{++} concentration was significantly ($p < 0.001$) higher in group B than in the two other groups. No differences were noted in the concentration of Mg^{++}. K^+ was significantly ($p < 0.001$) higher in porcupines of group C than in the other two groups, while Na^+ was significantly ($p < 0.001$) lower in group A than in the other two groups (Table 1).

Urea concentration was significantly higher in porcupines from group B than in those from groups A ($p < 0.05$) and C ($p < 0.001$) (Table 1).

The highest concentrations of catecholamines (VMA and NMA) were recorded in group B. These values were significantly ($p < 0.05$) higher than those of group A but the total amount in group B was similar to that of porcupines from group C (Table 2).

TABLE 2

The mean (±S.D.) concentrations of urinary free catecholamine (VMA - vanilmandelic acid and NMA - normetanephrine and metanephrine) and urine volume in three groups of Cape porcupines, *Hystrix africaeaustralis* (n = 6)

Group	VMA mol/d	NMA mmol/d	Volume ml in 24 h
A 25°C 12L:12D	5.5±3.2	0.20±0.05	277±37.6
B 32°C 16L:8D	10.7±2.3	0.36±0.15	253±33.2
C 10°C 8L:16D	7.4±2.1	0.18±0.07	405±40.8*

*P<0.001

DISCUSSION

As in other rodents /25-28/, the Cape porcupine can respond to seasonal climatic changes by modifying its metabolic rates as well as its thermal conductance /6/. In porcupines acclimated to T_a = 32°C 16L: 8D, the lower critical point was at T_a = 32°C with Resting Metabolic Rate (RMR) values 50% less than that expected for body mass, while in those kept at T_a = 10°C, 8L;16D, the lower critical point was at T_a = 24°C with RMR values only 19% less than expected. The present study illustrates that urine volume is significantly higher in Cape porcupines maintained at a low temperature and long scotophase (group C) than in those kept at higher temperatures and longer photophases (groups A and B). Observed increases in metabolic rates and food intake were accompanied by an increase of water intake /6/ which may explain the increase in the urine volume of porcupines from group C. Contrary to expectation, the urine of this group was not more dilute than in the other groups, and has a osmolarity similar to that of group A. The urinary osmolarity of group B was high, with high concentrations of HCO_3^-, Ca^{++} and Mg^{++}, due to low water intake /6/.

Urinary concentrations of Ca^{++} and Mg^{++} are higher in porcupines than in the laboratory rat *Rattus norvegicus* /11/ and the ground squirrels *Xerus inauris* and *X. princeps* /29/, but similar to those recorded in the rabbit *Oryctolagus cuniculus* /30, 31/, the pack rat *Neotoma olbigula* and hamster *Mesocricetus auratus* /14/ and the rock hyrax *Procavia capensis* /12, 13/. Free calcium and magnesium carbonate and bicarbonate are also excreted in the urine of the domestic horse *Equus cabbalus* /15/, the subterranean mole rat *Spalax ehrenbergi* /9/ and *Cryptomys hottentotus* /10/. Like Cape porcupines /5/, all these mammals are herbivorous, caecal fermenters with alkaline urine. Such an adaptation in fermenters could presumably develop as a mechanism for releasing components produced during fermentation.

In the present study urinary bicarbonate concentrations were affected by photoperiod and ambient temperature, clearly illustrating the porcupines' ability to excrete bicarbonate through the kidney, this being supported by the high pH of the urine (see Table 1). The subterranean environment of burrow-dwelling mammals is characterized by hypoxic and hypercapnic conditions, with extreme values (10.0% O_2 and 10.8% CO_2) having been recorded in the burrows of euthermic hamsters /32/. Haim *et al.* /9-11/ have hypothesised that the high values of bicarbonate and carbonate in the urine of subterranean mammals is an adaptation to off-load CO_2 without increasing its concentration in the hypercapnic atmosphere of their burrow environment. The hypercapnic atmosphere is accompanied by relatively low concentrations of O_2, and the release of HCO_3^- through the urinary pathway may reduce CO_2 concentrations in the air inhaled while in the burrow. From the "Bohr shift", it may be assumed that at lower CO_2 concentrations, more O_2 will bind to the haemoglobin of a mammal exposed to a hypoxic environment. Under these conditions, with CO_2 being excreted through the kidneys, venous blood entering the lung conceivably contains lowered concentrations of HCO_3^-. The significant increase of HCO_3^- in the urine of group C suggests the existence of such a mechanism, but needs further investigation. Increased O_2 consumption and CO_2 production resulting from increased metabolism as recorded in the cold and long scotophase acclimated porcupines - group C /7/ - may increase the hypercapnic and hypoxic conditions in the burrow. Therefore, it may be assumed that an off-load of CO_2 through the kidney may be of significance for survival in a hypercapnic, hypoxic environment such as that of group-living porcupines.

Since the turnover of catecholamines released into the blood stream is very high, the response of the sympatho-adrenal system to cold stimulus could be assessed by measuring the increase in free urinary catecholamine content. A six-fold increase in the noradrenaline content of the rat's urine during the first day of exposure to cold was reported by LeBlanc and Nadeau /20/. Leduc /33/ showed that adrenaline content in the urine reaches its maximal value on the sixth day of exposure to cold. However, during the development of non-shivering thermogenesis (NST), both LeBlanc and Nadeau /20/ and Leduc /33/ reported a decrease of noradrenaline values.

The present study illustrates that acclimation both to high temperature and long photophase, and to cold and long scotophase, have an effect on VMA concentrations, which increased relative to group A. However, the low concentration found in group C is a result of the high urine volume. In group B the total amount (concentration x volume) is significantly higher when compared with that of the two other groups (Table 2). In the rat, acclimation to a long scotophase (8L:16D at 25°C) caused an increase in urinary catecholamines /21/. It may be assumed that, as in the rat, urinary catecholamines increase in the porcupine only as a response of acclimation to long scotophase. The increase in the total amount of urinary catecholamine reflects the higher activity of the sympato-adrenal system in group C which showed an increase in metabolic rates /6/. However, norepinephrine turnover rates in brown adipose tissue and hearts of hamsters were not affected by short photoperiod (8L:16D) acclimation /18/. These discrepancies cannot be explained with the limited information at our disposal and require further investigation.

ACKNOWLEDGEMENTS

Financial support was provided by the Foundation for Research Development. Urinary analyses were performed at the Faculty of Medicine of the University of Pretoria. Martin Haupt, Babsie Potgieter and Andre van Rooyen assisted technically.

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