Munsey. L.D. (1972) Salinity tolerance of African pipid frog *Xenopus laevis. Copeia* 1972, 584-586.

Padhye. A.D. and Ghate. H.V. (1988). Effect of altered pH on embryos and tadpoles of the frog *Microhyla ornata. Herpetological Journal* 1, 276-279.

Ruibal, R. (1959). The ecology of the brackish water populations of *Rana pipiens. Copeia* 1959, 315-321.

Schroeder, T.E. (1970). Neurulation in *Xenopus laevis,* model based upon light and electron microscopy. *Journal of Embryology and Experimental Morphology* 23, 427-462.

Woolf. C.M. (1968). *Principles of Biometry.* D. van Nostrand Co. Inc., New Jersey, USA.

PLASMA CONCENTRATIONS OF ALDOSTERONE AND ELECTROLYTES IN *GALLOTIA GALLOTI* (SAURIA: LACERTIDAE)

MARIO DIAZ AND ANTONIO LORENZO

Laboratorio de Fisiología Animal, Departamento de Biología Animal, Universidad de La Laguna, Canary Islands, Spain.

ABSTRACT

Plasma concentrations of aldosterone, sodium and potassium were measured in the lizard, *Gallotia galloti*. Aldosterone concentrations in control animals were 29.48 $\pm$ 8.65 ng/dl, which falls within the range reported for this hormone in mammals. Peripheral sodium and potassium concentrations were 132.81 $\pm$ 2.28 and 5.77 $\pm$ 0.32 meq/l, respectively. Plasma aldosterone and sodium were negatively correlated. A positive relationship could be established between potassium concentration and aldosterone levels. Acute or chronic administration of exogenous aldosterone increased the circulating levels of this hormone, being maximal in chronically treated animals. However, although plasma concentrations of aldosterone were augmented by acute administration, the sodium concentration in the plasma was only elevated by chronic treatment. No further changes to potassium concentration could be observed under primary hyperaldosteronism conditions. The extent to which aldosterone may be implicated in the regulation of sodium and potassium transport in reptiles and its possible action on postrenal structures of electrolyte transport are discussed.

INTRODUCTION

Adrenal corticosteroids have been implicated in the control of osmoregulation in reptiles (Bentley, 1976; Bradshaw, 1975; Callard & Callard, 1978), but their precise modes and loci of action await elucidation. Aldosterone has been isolated from reptilian adrenal tissue after *in vitro* incubation of this tissue, and it is also now known that this steroid is secreted into the plasma (Sandor, 1972; Vinson, Whitehouse, Goddard & Sibley, 1979). To date, only a few indications of the plasma level of this steroid hormone are available. Bradshaw & Grenot (1976) found that in a North African terrestrial agamid, *Uromastix acanthinurus*, and in the large omnivorous skink from Western Australia, *Tiliqua rugosa*, plasma levels were 36.04 and 31.74 ng/dl, respectively. However, Nothstine, Davis & DeRoos (1971) have reported a higher concentration of 760 ng/dl plasma in post-caval plasma from the caiman. The physiological significance of aldosterone in reptiles is not at all clear. Early studies in lizards, such as *Amphibolorus ornatus* and *Dipsosaurus dorsalis*, in which hypophysectomy and dexamethasone treatment were associated to an increased tubular reabsorption of sodium ions (Bradshaw, 1972; Bradshaw, Shoemaker and Nagy, 1972), are difficult to interpret because these treatments would also be expected to decrease plasma aldosterone (Bradshaw, 1978). However, Rice, Bradshaw and Prendergast (1982) found that in adrenalectomized *Varanus gouldii* sodium and glucose concentrations fell after the operation, and potassium concentrations rose, as has been observed in mammals. The fall in aldosterone concentrations in *Varanus gouldii* as a result of salt-loading is associated with a marked decrease in fractional reabsorption of sodium and chloride ions by the kidney, and an increase in the rate of potassium secretion, suggesting an obvious mineralocorticoid effect of aldosterone. In an effort to elucidate the physiological role of aldosterone in reptiles, peripheral plasma concentrations were measured in relation to electrolyte concentrations under several treatments in an omnivorous lacertid endemic to the Canary Islands, *Gallotia galloti*.

MATERIAL AND METHODS

ANIMALS

A total of 18 adult male and female *Gallotia galloti* lizards were trapped between May and September 1989 in the zone of Tegueste (Tenerife, Canary Islands, Spain). Lizards were transported to the laboratory and acclimatized in a large indoor terrarium. Mean body weight of experimental animals was 39.34 $\pm$ 1.23 g. Food and water were provided *ad libitum*.

TREATMENTS

Lizards were ramdomly assigned to one of three groups: (1) untreated controls (UC), (2) acutely treated (AT) which received a single intraperitonel (i.p.) injection of 100 µg/kg body weight d-aldosterone 4 hr prior to blood sampling, and (3) chronically treated (CT) which received i.p. injections 100 µg/kg body weight, at 52, 42, 28, 18 and 4 hours prior to the experiment. Aldosterone (d-aldosterone, Sigma Chemical Company) was dissolved in a 50% solution of dimethylsulfoxide (DMSO) in water and immediately administered.

ANALYSIS

Blood samples were collected, following decapitation, in heparinized tubes and centrifuged inmediately at 3000g for 10 minutes, and the plasma was stored at – 20°C until analysis. In these same animals, interrenal glands, urinary bladder and large intestine were removed and used for additional experiments regarding reptilian osmoregulation not included in the present study. Sodium and potassium concentrations were measured in 200 µl samples of plasma with an atomic absorption spectrophotometer (Perkin Elmer, Model No. 603). Plasma aldosterone concentrations were determined in 200 µl samples by a commercial radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles), using ^{125}I-labelled aldosterone and antibody-coated tube technology for the final separation of free from bound aldosterone. Since the existence of circadian oscillations of endogenous circulating aldosterone has been demonstrated (Clauss, 1984), blood samples were always collected at the same time (16.00hrs).

STATISTICS

The significance of differences between means was assessed by Student's t-Test. Treatment means were compared using Analysis of Variance coupled to Student-Newman-Keul's test. The relationship between electrolyte concentration and plasma aldosterone was assessed by means of regression analysis to a linear model followed by Analysis of Variance. Least-square linear regression equations are quoted in Table 2 with standard error of estimates of the coefficients, the correlation coefficient (r) and the significance levels of r (P). Values are quoted as means $\pm$ S.E.M. A probability value (P) of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

PLASMA ALDOSTERONE AND ELECTROLYTE CONCENTRATIONS

Results of measurements of plasma aldosterone, sodium and potassium concentrations in untreated control animals are shown in Table 1. As can be seen, both plasma concentrations of hormone and sodium fell within the range reported for mammals which, in general, for aldosterone is between 10 and 100 ng/dl depending on the sodium status of the animal. Moreover, observed concentrations in Gallotia galloti were very close to those reported for other terrestrial lizards, e.g. Tiliqua rugosa and Uromastix acanthinurus (Bradshaw & Grenot, 1976), and Varanus gouldii (Bradshaw & Rice, 1981). Reptiles, thus, in this respect

	Aldosterone (ng/dl)	Na$^+$ (meq/l)	K$^+$ (meq/l)	Na$^+$/K$^+$
UC	29.49 ± 8.66 (6)	132.81 ± 2.28 (6)	5.77 ± 0.32 (6)	23.01
AT	106.06 ± 5.97 (6) P 0.005	130.10 ± 2.37 (6) NS	5.70 ± 0.12 (6) NS	22.82
CT	190.14 ± 6.29* (6) P<0.005	145.18 ± 2.42* (6) P<0.05	6.07 ± 0.17 (6) NS	23.91

TABLE 1. Plasma aldosterone and cation concentrations in G. galloti in control (UC), single-injected (AT) and chronically treated animals (CT). Values are means SEM. Numbers in parentheses are the sample size. P values are refered to UC. *: P<0.05 between indicated value and AT animals.

appear to be closer to mammals than amphibians, the latter having very high circulating aldosterone concentrations of between 500 and 1000 ng/dl (Dupont, Leboulenger, Vaudry & Vaillant, 1976). A somewhat elevated plasma potassium was observed in relation to previous studies. This could be related to the feeding patterns of G. galloti since, although this species is considered to be omnivorous, in natural environments adults are mainly herbivorous, eating halophitic plants (Báez, 1987; Molina-Borja, in press) providing, possibly, a high concentration of dietary potassium. Related findings have been reported for Amblyrynchus cristatus, whose natural dietary items (macrophytic seaweeds) contain large quantities of this ion, with the consequence of very high concentrations of potassium in the plasma (Dunson, 1969).

Plasma aldosterone concentrations in untreated control animals appear to be a function of plasma sodium, since a significant negative correlation with r = –0.89 is evident for G. galloti (Fig. 1a, Table 2), suggesting that aldosterone is involved in the regulation of the sodium status of this species. Furthermore, a good positive relationship with r = 0.97, could also be observed for potassium (Fig. 1a). Other studies in reptiles have shown that aldosterone bears a negative correlation to plasma sodium concentration, e.g. Uromastix acanthinurus (Bradshaw & Grenot, 1976) and Varanus gouldii (Bradshaw & Rice, 1981). However, Bradshaw & Grenot (1976) have reported that such a relationship was doubtful for Tiliqua rugosa, in which it was observed that over a wide range of plasma sodium concentrations, there was very little alteration in the plasma aldosterone level.

	ion	i	s	r	SE	P
UC	Na$^+$	139.79	-0.023 ± 0.0058	-0.89	2.78	0.0157
	K$^+$	4.69	0.003 ± 0.0004	0.97	0.19	0.0009
AT	Na$^+$	102.01	0.026 ± 0.0148	0.66	4.85	0.1489
	K$^+$	6.19	-0.001 ± 0.0001	-0.22	0.32	0.6713
CT	Na$^+$	97.70	0.034 ± 0.0148	0.73	3.11	0.0172
	K$^+$	6.17	-0.000 ± 0.0014	-0.01	0.49	0.9742

TABLE 2. Single regression equations with plasma aldosterone concentrations as the independent variable. Shown are estimates for intercepts (i), slopes (s), correlation coefficients (r) standard error of estimation (SE) and probability level from the analysis of variance (P) for each experimental group and electrolyte.

Recent evidence for ACTH-like immunoreactivity and angiotensin II in the *pars distalis* and *pars intermedia* of the adenohypophysis (Bello, Tramu, Pérez-Batista, Marti & Lancha, 1988), and, furthermore, the demonstration of increased unidirectional colonic sodium absorption following exogenous aldosterone treatment (Diaz, Lorenzo, Badia & Gómez, 1988), suggest that the pituitary-adrenal axis is involved in osmoregulation in *Gallotia galloti*.

(a): UC animals

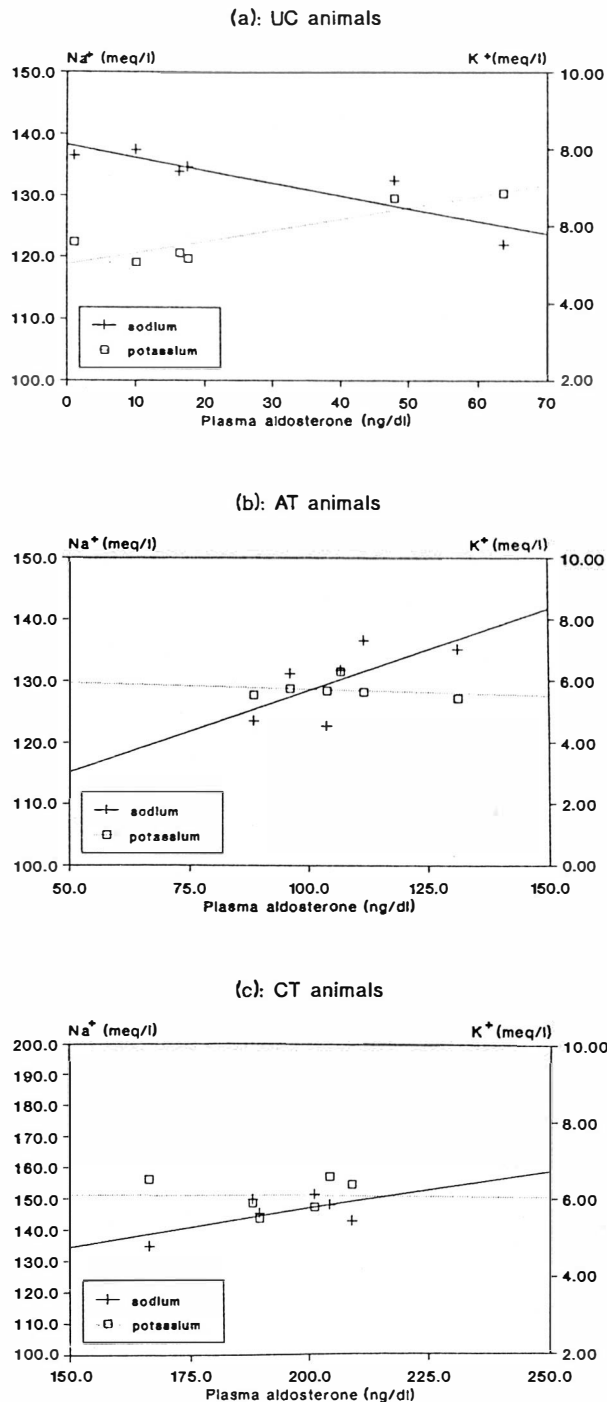(b): AT animals

(c): CT animals

Fig 1. Correlation between plasma aldosterone and sodium (crosses) and potassium (squares) concentrations in (a) control (UC), (b) single-injected (AT) and (c) chronically treated animals (CT). See Table 2 for regression equations.

EFFECTS OF EXOGENOUS ALDOSTERONE

Acute injections of exogenous aldosterone significantly increased the fractional reabsorption of sodium ions and enhanced the tubular secretion of potassium in adrenalectomized *V. gouldii* (Rice et al., 1982), suggesting that aldosterone functions physiologically in this species. More recently, Bradshaw, Tom & Bunn (1984) have reported severe hyperkalemia in *Uromastix acanthinurus* following i.p. KCl injections which were associated with increased plasma aldosterone. In contrast, mineralocorticoid concentrations were reduced when NaCl was administered. Both the above studies suggest that aldosterone regulates sodium and potassium metabolism in terrestrial reptiles. The results presented in this paper show that primary hyperaldosteronism produced changes in plasma sodium but did not affect potassium concentration in either acutely or chronically treated animals (Table 1). Moreover, no relationship could be observed between potassium and aldosterone concentrations for any treatment (Fig. 1b and 1c) in spite of the increased plasma aldosterone concentrations. These results contrast with the positive correlation observed in untreated control animals, and contrast with our previous findings that showed exogenous aldosterone to increase colonic potassium secretion by abolishing control absorptive processes (Diaz & Lorenzo, 1989). However, the fact that potassium concentrations were unaffected by aldosterone treatment suggests, and might indicate, the presence of effective additional osmoregulatory mechanisms whose loci require further investigation.

Minnich (1982) in a very complete review, has pointed out that in terrestrial reptiles, mechanisms for excreting potassium ions are usually more effective than those for excreting sodium ions. In fact, many species develop significant hypernatremia during drought. For example, *Amphibolorus* lizards (*A.ornatus, A.minor, A. minimus, A. caudicinctus* and *A.maculosus*) incur hypernatremia during the dry Australian summer (Bradshaw, 1970; Braysher, 1972). In the present paper, we could observe hypernatremia in animals in response to primary hyperaldosteronism. From Table 1, it is clear that the plasma cationic relationship (Na^+/K^+) was increased in chronically treated animals, which, additionally, indicates that elevations in plasma sodium are better tolerated than decreases in potassium concentration. In fact, our results indicated that chronic but not acute aldosterone treatment produced a significant rise in Na^+ plasma concentration. Moreover, measurements of changes in circulating concentrations of aldosterone have shown that the negative correlation observed in untreated controls was reversed to a positive relationship in chronically treated animals, which indicate the presence of a regulatory mechanism for plasma sodium by aldosterone (Fig. 1b and 1c). These results are in good agreement with our previous results in which exogenous aldosterone increased colonic unidirectional sodium absorption in this species (Diaz et al., 1988; Diaz, 1990).

The changes in aldosterone concentrations in *G. galloti* with the various treatments utilized in this study are consistent with this hormone having an active role in the control of sodium metabolism in this species.

However, it is important to emphasise that these data in no way prove the existence of such a relationship.

The situation with potassium regulation on the other hand is far less clear. Both acute and chronic treatments augmented plasma aldosterone concentrations, but the correlation with potassium concentration was so marginal and the slope of the lines so little different from zero that it is difficult to envisage this hormone being involved in the intimate regulation of potassium metabolism in this species (Fig 1b and 1c). This was despite the existence of aldosterone-induced colonic K$^+$ secretion. Nasal salt glands are absent in *G. galloti*, consequently K$^+$ concentrations could not be controlled by this means, unlike in other species where the glands are present (e.g. *Tiliqua rugosa, Amblyrhynchus cristatus*).

Comparatively, the effects of the mineralocorticoids on potassium excretion in nonmammalian vertebrates are not very clear. In birds, as in mammals, these hormones tend to increase potassium excretion (Skadhauge, 1981), whereas the effects appear to be somewhat confused in reptiles. They appear to increase sodium reabsorption in salt-loaded snakes (LeBrie & Elizondo, 1969), decrease reabsorption in turtles (Brewer & Ensor, 1980) and either have no effect, or stimulate secretion in lizards (Bradshaw & Rice, 1981, Rice et al., 1982). Further investigations concerning renal and post-renal electrolyte metabolism will be neccesary to clarify the nature of potassium balance in reptiles and in particular in *Gallotia galloti*.

ACKNOWLEDGEMENTS

REFERENCES

Báez, M. (1987). Datos sobre la reproducción de *Gallotia galloti* en Tenerife. *Rev. Esp. Herpetol.* 2, 29-32.

Bello, A.R., Tramu, G., Pérez-Batista, M.A., Marti, E. & Lancha, A. (1989). Coexistence of ACTH-Substance P and ACTH-Angiotensine II during the pituitary development in a lizard (*Gallotia galloti*). (Abstract) *Eur. J. Neurosci.* (suppl). 11.

Bentley, P.J. (1976). Osmoregulation. In: *Biology of Reptilia*. Gans C. and Dawson W.R. (Eds). New York: Academic Press, pp 365-412.

Bradshaw, S.D. (1970). Seasonal changes in the water and electrolyte metabolism of *Amphibolorus* lizards in the field. *Comp. Biochem. Physiol.* 36, 689-718.

Bradshaw, S.D. (1972). The endocrine control of water and electrolyte metabolism in desert reptiles. *Gen. Comp. Endocr. Suppl.* 3, 360-373.

Bradshaw, S. D. (1975). Osmoregulation and pituitary-adrenal function in desert reptiles. *Gen. Comp. Endocr.* 25, 230-248.

Bradshaw, S.D. (1978). Volume regulation in desert reptiles and its control by pituitary and adrenal hormones. In: *Osmotic and volume regulation*. Jorgensen, C.B. and Skadhauge, E. (Eds). Alfred Benzon Symposium XI. Munksgaard, pp 38-53.

Bradshaw, S.D. & Grenot, C.J (1976). Plasma aldosterone levels in two reptilian species, *Uromastix acanthinurus* and *Tiliqua rugosa*, and the effects of several experimental treatments. *J. Comp. Physiol.* 111, 71-76.

Bradshaw. S.D & Rice, G.E. (1981). The effects of pituitary and adrenal hormones on renal and postrenal readbsorption of water and electrolytes in the lizard *Varanus gouldii (Gray). Gen. Comp. Endocr.* 44, 82-93.

Bradshaw. S.D., Shoemaker, V. H. & Nagy, K.A. (1972). The role of adrenal corticosteroids in the regulation of kidney function in the desert lizard *Dipsosaurus dorsalis. Comp. Biochem. Physiol.* 43A, 21-635.

Bradshaw, S.D., Tom, J.A. & Bunn. S.E. (1984). Corticosteroids and control of salt gland function in the lizard *Tiliqua rugosa. Gen. Comp. Endocr.* 54, 308-313.

Braysher, M. (1972). *Water and electrolyte balance in the agamid lizard. Amphibolorus maculosus (Mitchell). and the structure and function of the nasal salt gland of the sleepy lizard. Trachydosaurus rugosus (Gray).* Ph.D. Univ. Adelaide. Australia.

Brewer, K.J. & Ensor, D.M. (1980). Hormonal control of osmoregulation in chelonia. I. The effects of prolactin and interrenal steroids in freswwater chelonians. *Gen. Comp. Endocr.* 42, 304-309.

Callard, I.P. & Callard G.V. (1978). The adrenal gland in reptilia: Physiology. In: *General, comparative and clinical endocrinology of the adrenal cortex.* Vol. 2. Chester Jones I. and Henderson I.W. (Eds). New York: Academic Press, pp 370-418.

Clauss. W. (1984). Circadian rhythms in Na transport. In: *Intestinal absorption and secretion.* Skadhauge E. and Heintze K. (Eds). Lancaster: MTP Press, pp 273-283.

Diaz, M., Lorenzo, A. Badia. A & Gómez, T. (1988). The role of aldosterone in water and electrolyte transport across the colonic epithelium of the lizard, *Gallotia galloti. Comp. Biochem. Physiol.* 91A, 71-77.

Diaz, M. & Lorenzo, A. (1989). Modulation of active potassium transport by aldosterone. *Comp. Biochem. Physiol.* 95A, 79-85.

Diaz, M. (1990). *Modulación del transporte activo de electrolitos a través de la mucosa colónica de Gallotia galloti por la d-aldosterona.* Tesis Doctoral. Universidad de La Laguna. Spain.

Dunson, W.A. (1969). Reptilian salt glands. In: *Exocrine glands.* Botelho, S.Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press, pp 83-103.

Dupont, W., Leboulenger, F., Vaudry, H. & Vaillant, R. (1976). Regulation of the aldosterone secretion in the frog *Rana catesbiana L. Gen. Comp. Endocr.* 29, 51-60.

LeBrie, S.J. & Elizondo, R.S. (1969). Saline loading and aldosterone in water snakes *Natrix cyclopion. Am. J. Physiol.* 217, 426-430.

Minnich, J. E. (1982). The use of water. In: *Biology of Reptilia. Physiological Ecology.* Gans, C. and Harvey Pough, F. (Eds). London: Academic Press, pp 325-395.

Molina-Borja, M. (1990). Alimentary habits and spatial-temporal distribution of eating behaviour patterns in a natural population of lizards (*Gallotia galloti*). *Vieraea.* (In press).

Nothstine, S.A., Davis, J.O. & DeRoos, R.M. (1971). Kidney extracts and ACTH on adrenal steroid secretion in a turtle and a crocodilian. *Amer. J. Physiol.* 221, 726-732.

Rice, G.E., Bradshaw, S.D. & Prendergast, P.J. (1982). The effects of bilateral adrenalectomy on renal function in the lizard *Varanus gouldii* (Gray). *Gen. Comp. Endocr.* 47, 182-189.

Sandor, T. (1972). Corticosteroids in Amphibia, Reptilia and Aves. In: *Steroids in non-mamalian vertebrates.* Idler, D.R. (Ed). New York: Academic Press, pp 253-327.

Skadhauge, E. (1981). *Osmoregulation in birds.* Berlin Heidelberg: Springer-Verlag.

Vinson, G.P., Whitehouse, B.J., Goddard, C. & Sibley, C.P. (1979). Comparative and evolutionary aspects of aldosterone secretion and *zona glomerulosa* function. *J. Endocrinol.* 81, 5-24.