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RESPONSIVENESS OF THE PAROTID SALIVARY GLAND OF RED KANGAROOS (*MACROPUS RUFUS*) TO MINERALOCORTICOIDS

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SUMMARY

During both acute and chronic mineralocorticoid administration, parotid saliva was obtained by acetylcholine stimulation at rates of 1·0–1·5 ml min^{-1} from anaesthetized red kangaroos. The Na/K ratio of saliva from chronically Na-replete kangaroos was virtually unaltered by ipsilateral intracarotid infusion of aldosterone at rates of 8, 40 or 80 $\mu g\,h^{-1}$ for 4 h, the ratio falling from 20·1 ± 1·09 to 17·5 ± 0·53 ($t_6 = 2{\cdot}07$; NS) at 80 $\mu g\,h^{-1}$. Kangaroos given intramuscular injection of the mineralocorticoid, deoxycorticosterone (DOCA), at rates of 0·25 or 0·30 mg kg^{-1} 12 h^{-1} showed a progressive fall in salivary Na/K ratio from 19·1 ± 0·47 to 1·76 ± 0·41 ($t_5 = 27{\cdot}4$; $P < 0{\cdot}001$) over the 21-day period of injection. The DOCA treatment caused hypertrophy of the ducts, particularly the intralobular ducts of the parotid gland. Aldosterone acetate given intramuscularly at 0·03 mg kg^{-1} 12 h^{-1} for 10 days also reduced the Na/K ratio of the saliva. As soon as the salivary Na/K ratio had returned to replete values, some 3–4 days after cessation of the DOCA injections, the kangaroos were given a 5-h infusion of aldosterone. Intracarotid infusion of aldosterone at 8 $\mu g\,h^{-1}$ produced a near maximal fall in salivary Na/K ratio after 3 h of infusion, and increasing the infusion to 80 $\mu g\,h^{-1}$ had little additional effect. The minimum Na/K ratio obtained at this time was 5·7 ± 1·04 ($t_5 = 14{\cdot}21$; $P < 0{\cdot}001$), which was equivalent to the ratio obtained at 3–6 days of DOCA injection. Significant regression of the intralobular ducts occurred during the 3 days following cessation of DOCA administration; 24 days after the end of DOCA treatment duct development was approaching that of Na-replete, untreated kangaroos.

The results demonstrate that the parotid glands of kangaroos from a sodium-rich environment are almost unable to respond to acute fluctuations in endogenous aldosterone levels; that chronically high levels of mineralocorticoids cause hypertrophy of the sodium-transporting ducts of the parotid gland, which results in an increasing ability to reduce the Na/K ratio of the saliva; and that responsiveness to mineralocorticoids declines rapidly in the absence of high mineralocorticoid levels due to regression of the ducts.

INTRODUCTION

In Na-replete red kangaroos, the parotid saliva has been found to have a high concentration of sodium and a low concentration of potassium (Beal, 1984). The

Key words: parotid saliva, mineralocorticoids, red kangaroo.

sodium concentration exceeds the plasma sodium concentration and the potassium concentration is less than 10 mmol l^{-1} over most of the flow range (i.e. 1 to >4 ml min^{-1}). The tendency of the concentration/flow curves for these ions to plateau at low flow rates indicates that the rate of ductal transport of these ions is very low.

The parotid gland of Na-replete ruminants also produces a saliva in which the sodium concentration exceeds the plasma concentration and the potassium concentration is very low (Coats & Wright, 1957; Bailey & Balch, 1961; Komi & Snyder, 1963; Beal, 1979) and, as a result, kangaroo and ruminant parotid salivas are quite similar with respect to the concentrations of the two ions. Sheep, both Na-replete and Na-depleted, respond to the infusion of the mineralocorticoid, aldosterone, by reducing the sodium concentration and increasing the potassium concentration of their parotid saliva, the delay in onset of this response varying between 70 and 130 min depending on the rate of steroid infusion (Blair-West *et al.* 1963). Whether elevated mineralocorticoid levels will alter the Na/K ratio of the parotid saliva of kangaroos is unknown. Grey kangaroos (*Macropus giganteus*) from mountain areas, a sodium deficient environment, have greater development of the striated or intralobular ducts in their salivary glands and higher peripheral-blood aldosterone levels than grey kangaroos from sodium-rich coastal areas (Blair-West *et al.* 1968); this may indicate that kangaroo parotid glands can respond to elevated mineralocorticoid concentrations in the blood.

This paper reports an investigation of the effect of mineralocorticoids on salivary sodium and potassium concentrations in the parotid saliva of Na-replete red kangaroos.

METHODS

Experimental procedures

Nine adult red kangaroos were used, four males weighing 37·5–46·0 kg and five non-lactating females weighing 23·5–30·0 kg. Each animal had one common carotid artery exteriorized in a skin loop, the loops having been prepared months to years previously. The kangaroos were maintained on *ad lib.* lucerne chaff, supplement cubes and dilute saline solution (25 mmol l^{-1} NaCl+25 mmol l^{-1} $NaHCO_3$).

Aldosterone infusion experiment (four males and three females)

Two days before the aldosterone infusions, the kangaroos were lightly anaesthetized with ketamine hydrochloride (Ketalar; Park Davis, Australia) given at rates of 8–18 mg kg^{-1}. This level was sufficient to tranquillize each animal so that it would lie unrestrained for about 30 min in a normal resting position on its side with its head raised and its swallowing reflex unaffected. The skin overlying one superficial lateral tail vein was infiltrated with 1% Lignocaine hydrochloride in saline (David Bull Laboratories, Victoria) to produce local anaesthesia and the vein was then cannulated with a vinyl cannula (0·86 mm i.d., 1·27 mm o.d.; Dural Plastics, N.S.W.) using the

technique of Seldinger (1953). The cannula was filled with heparinized saline (1000 i.u. ml^{-1}) and covered with a bandage. Food was removed 15–16 h before the commencement of each experiment, but the saline drinking solution was available until the experiment began.

At the beginning of each experiment the kangaroos were anaesthetized with 5% sodium pentobarbitone in saline given at rates of 25–36 mg kg^{-1} by intravenous injection through the tail vein cannula. Anaesthesia was maintained with sodium pentobarbitone throughout the experiment using the corneal reflex as a guide to the level of anaesthesia. The animals were positioned on one side (carotid loop side up) with an electrically-heated pad under the thorax to maintain normal body temperature and with an air cushion under the hind quarters to prevent pressure damage to the hip and thigh region. The trachea was intubated with a cuffed endotracheal tube which was shortened so that the dead space of the respiratory tract was not increased. A solution of NaCl:KCl (150:4 mmol l^{-1}) was infused intravenously at 1·2–2·0 ml min^{-1} for the duration of each experiment to minimize changes in body fluid composition resulting from transpiration and salivary loss. The carotid artery loop was cannulated with a polyethylene cannula (0·58 mm i.d., 0·96 mm o.d.; Dural Plastics, N.S.W.) which was inserted 10 cm in the direction of the heart using the technique of Seldinger (1953). The duct of the parotid gland ipsilateral to the carotid artery loop was catheterized with a vinyl tube (1·57 mm i.d., 2·08 mm o.d.; Dural Plastics, N.S.W.). This catheter was inserted 3 cm into the duct through its orifice in the mouth. Saliva was collected into polypropylene sample tubes which were closed except for a 20 wire gauge air-bleed. The distal end of the salivary catheter was positioned about 10 cm below the duct orifice and the dead space in the catheter was 0·4–0·5 ml.

Salivary secretion was stimulated by ipsilateral, intracarotid infusion of acetylcholine chloride (Sigma Chemical Co., U.S.A.) at rates sufficient to maintain parotid salivary flow at 1·0–1·5 ml min^{-1} depending on the size of the animal (i.e. approx. 25% of maximum sustainable flow under anaesthesia). Once the desired flow rate had been established, three or four 15-min serial samples of saliva were collected. Aldosterone (Aldocorten; Ciba Pharmaceuticals, N.S.W.; or *d*-aldosterone; Sigma Chemical Co., U.S.A.) was then infused in saline (0·1 ml min^{-1}) at rates of 8, 40 or 80 $\mu g\ h^{-1}$ (3, 2 and 7 experiments respectively) for 4 h. Serial 15-min samples of saliva were collected throughout each experiment except during the first hour of the aldosterone infusion. Blood samples (5 ml) were taken before commencement of sampling and, thereafter, at hourly intervals throughout the infusion.

The presence of mineralocorticoid activity in the aldosterone infusates used in five of the 80 $\mu g\ h^{-1}$ experiments was confirmed by measuring the urinary ratio of inert Na/K before and after injection of a volume containing the nominal equivalent of 0·1 μg aldosterone into adrenalectomized rats. The protocol used was based on that of Simpson & Tait (1952). The concentration of aldosterone in the vials of Aldocorten and *d*-aldosterone used in the same five experiments was estimated by radioimmunoassay.

Chronic deoxycorticosterone administration (four males and two females)

Each kangaroo was given deoxycorticosterone acetate (DOCA) dissolved in ethyl oleate (5 or 6 mg ml^{-1}) intramuscularly for 21 days. Husbandry of the kangaroos before this treatment, cannulation of the tail vein and carotid artery and stimulation of salivation were as described above. The cannulae were left in the artery and vein for the 21 days of this treatment and were kept patent with heparin (5000 i.u. ml^{-1}) between samplings. On the first day of the treatment (day 0) the kangaroos were anaesthetized with sodium pentobarbitone, salivation was stimulated by intracarotid acetylcholine infusion to approximately 25% of maximum sustainable flow and four serial 10- to 15-min samples of parotid saliva were collected. Immediately following this collection, three animals received a 4-h infusion of aldosterone at 80 $\mu g\,h^{-1}$ (as above) and the other three were given DOCA at the rate of 25 mg kg^{-1}. Thereafter, the kangaroos were injected with DOCA at the rate of 0·25 mg kg^{-1} 12 h^{-1} (3 animals) or 0·3 mg kg^{-1} 12 h^{-1} (3 animals) at approximately 08.00 and 20.00 h each day for 21 days (the rate was increased from 0·3 to 0·5 mg kg^{-1} 12 h^{-1} from days 15–21 in one relatively unresponsive animal). Under the conditions outlined for day 0, four serial 10- to 15-min samples of saliva were collected at the day 0 flow rate for each animal between 09.30 and 11.00 h on days 1, 3, 6, 10, 14 and 21 of DOCA administration. Blood samples (5 ml) were taken before and after salivary collection. Several procedures were used to offset the sodium-gaining and potassium-losing actions of the DOCA injections. Throughout the period of DOCA administration the kangaroos were fed lucerne chaff only (the supplement cubes had added salt), were given drinking water containing 50–100 mmol l^{-1} potassium derived equally from KCl and K_2CO_3 and, during each saliva collection, they were given an infusion containing potassium at concentrations varying between 5 and 125 mmol l^{-1}. The concentration of potassium in the drinking solution and in the infusate depended on the elapsed time through the DOCA treatment and on the changes in plasma sodium and potassium observed in each animal. The kangaroos had access to food and drinking solution except during saliva collection.

Samples of tissue were taken from the contralateral parotid glands of two of the animals before, and at 21 days of, DOCA administration. Subsequently, a second pair of kangaroos was given DOCA for 21 days at 0·25 mg kg^{-1} 12 h^{-1} with gland tissue being biopsied at 21 days DOCA, 3 days post-DOCA and 24 days post-DOCA.

Chronic aldosterone administration (one female)

Husbandry and methods for this experiment were similar to the chronic DOCA experiment except that the animal was given *d*-aldosterone acetate (Sigma Chemical Co., U.S.A.), dissolved in butyl alcohol and ethyl oleate, intramuscularly at the rate of 0·03 mg kg^{-1} 12 h^{-1} for 10 days.

Post-DOCA infusion of aldosterone (four males and two females)

After cessation of DOCA administration, the salivary Na/K ratio returned to sodium replete values within 3 days (4 days in kangaroo, LB). Over this period the

potassium was progressively reduced in the kangaroos' drinking water and, over the last 15 h (40 h for kangaroo, LB), saline and supplement cubes were made available to the animals. Once the salivary potassium concentration was below 10 mmol l^{-1} at 25% of the maximal flow rate, the kangaroos were given an aldosterone infusion. The methods used for this experiment were as described for the initial aldosterone infusion except that the aldosterone was infused at 8 μg h^{-1} for 3 h and then increased to 80 μg h^{-1} for a further 2 h.

At the end of each period of anaesthesia, the kangaroos were kept on the heated pad and air cushion until 15–30 min after the endotracheal tube had been removed. The endotracheal tube was removed when the swallowing reflex had returned and the animals were monitored over the following 15–30 min to ensure that respiration was unimpaired. Removal of cannulae and catheters was done while the kangaroos were anaesthetized. At the end of each experiment, the anaesthetized kangaroos were given a single intramuscular injection of procaine penicillin and dihydrostreptomycin (Streptopen injection; Glaxo Australia Pty Ltd; Victoria) at a rate of 1 ml 12 kg^{-1} as a safeguard against infection. Since kangaroos are essentially night-feeding animals, experiments during the daylight hours do not interfere with their food intake and thus the experimental animals gained weight during the period of these experiments.

Analytical procedures

Blood samples were taken into plastic syringes heparinized with one drop of heparin (5000 i.u. ml^{-1}) and centrifuged at 2200 ***g*** for 10 min to obtain plasma for analysis. Microhaematocrit determinations were made in triplicate on blood spun at 12 000 ***g*** for 10 min in a microhaematocrit centrifuge (Hawksley). Saliva and plasma were analysed in duplicate for sodium and potassium by atomic absorption spectroscopy using appropriate ionization suppressants.

Histological procedures

Changes in the histology of the parotid gland which occurred during and after cessation of DOCA administration were examined in four animals. Under general anaesthesia, samples of tissue were taken by excision biopsy from the dorsal cervical region of the contralateral gland before, and at 21 days of, DOCA administration from two animals (one male and one female), and at 21 days DOCA, at 3 days post-DOCA and at 24 days post-DOCA from the other two animals (one male and one female). The tissue was fixed in formol saline or buffered formalin solution, embedded in paraffin wax or methacrylate to provide two or more blocks per biopsy and, after sectioning, stained by both haematoxylin/eosin and periodic acid Schiff techniques. The mean height of the cells lining the intralobular ducts in each section was obtained by measuring 100 cells from at least 50 duct cross-sections using an eyepiece micrometer. Similarly, the mean diameter of the duct cell nuclei in each section was obtained by making two measurements of nuclear diameter at right angles to one another on the nuclei of 100 cells from at least 50 duct cross-sections. The nuclei to be measured were selected at random, with those having sharp nuclear margins being measured. Using the mean diameter, the mean nuclear volume was

estimated assuming the nuclei were spherical. The area of the sections occupied by serous cells, striated/intralobular ducts and excretory ducts was estimated by tracing the magnified image of the section projected onto a graphics tablet. The volume occupied by the intralobular ducts was expressed as a percentage of lobular volume rather than gland volume since any shrinkage of the tissue during preparation enlarges the interlobular spaces.

Statistical procedures

Responses to the various treatments were compared by *t*-test or paired *t*-test as appropriate.

RESULTS

Aldosterone infusion experiment

Aldosterone was infused at 8, 40 and 80 $\mu g\,h^{-1}$. Since the changes in salivary sodium and potassium concentrations associated with the highest rate were minimal and the effects of the lower dosages were less obvious, the data for the highest infusion rate only are presented in this paper (Fig. 1). Plasma sodium concentration rose during aldosterone infusion in all seven experiments ($P < 0{\cdot}02$), the mean increase in plasma sodium being $1{\cdot}6\,mmol\,l^{-1}$. The potassium concentration in the plasma showed a variable response, rising in some experiments and falling in others so that the mean potassium concentration fell by $0{\cdot}09\,mmol\,l^{-1}$. No change in salivary sodium concentration was associated with the aldosterone infusion, although some tendency for salivary potassium concentration to rise was observed. In three of the seven experiments, salivary potassium was unaltered or fell during the aldosterone infusion whereas, in the remainder, potassium rose by $1–2\,mmol\,l^{-1}$. As a consequence the mean sodium/potassium ratio of the saliva fell from $20{\cdot}1 \pm 1{\cdot}09$ to $17{\cdot}5 \pm 0{\cdot}53$ ($t_6 = 2{\cdot}07$; NS). When administered to adrenalectomized rats, the aldosterone infusates from five of the above experiments caused a fall in urinary sodium/potassium ratio from $1{\cdot}12 \pm 0{\cdot}336$ to $0{\cdot}206 \pm 0{\cdot}076$ ($t_4 = 3{\cdot}504$; $P < 0{\cdot}05$). Radioimmunoassay of the five stock solutions of aldosterone used in the bioassay gave an estimate of $0{\cdot}48 \pm 0{\cdot}062\,mg\,ml^{-1}$, which was not significantly different from the nominal concentration of $0{\cdot}5\,mg\,ml^{-1}$.

Chronic deoxycorticosterone administration

Changes in plasma sodium and potassium concentrations (Fig. 2) throughout the 21 days of DOCA administration were not statistically significant, with the exception of the elevated plasma potassium on day 1 of the treatment ($t_5 = 2{\cdot}581$; $P < 0{\cdot}05$). The fall in haematocrit during the first 6–10 days of this treatment was significant ($t_5 = 8{\cdot}288$; $P < 0{\cdot}001$). Throughout the period of DOCA injection, there was a progressive fall in salivary sodium concentration and an increase in salivary potassium concentration (Fig. 2), the concentrations of these ions being significantly different from the non-treated state by day 1 of the treatment ($t_5 = 5{\cdot}004$ and $6{\cdot}272$ respectively; $P < 0{\cdot}01$). As a consequence of these changes in salivary composition,

the Na/K ratio of the saliva fell from 19·1 ± 0·47 to 1·76 ± 0·41 over the period of the experiment ($t_5 = 27{\cdot}4$; $P < 0{\cdot}001$). The rate of change in salivary Na/K ratio varied greatly between animals, the values for the kangaroos giving the least and greatest responses being shown in Fig. 3. Although two levels of DOCA administration were used, the difference in response was not related to the dose rate (0·25 or 0·3 mg kg^{-1} 12 h^{-1}) and, in the case of the kangaroo showing the least response, increasing the rate of dosage to 0·5 mg kg^{-1} 12 h^{-1} for the last 7 days of treatment produced no

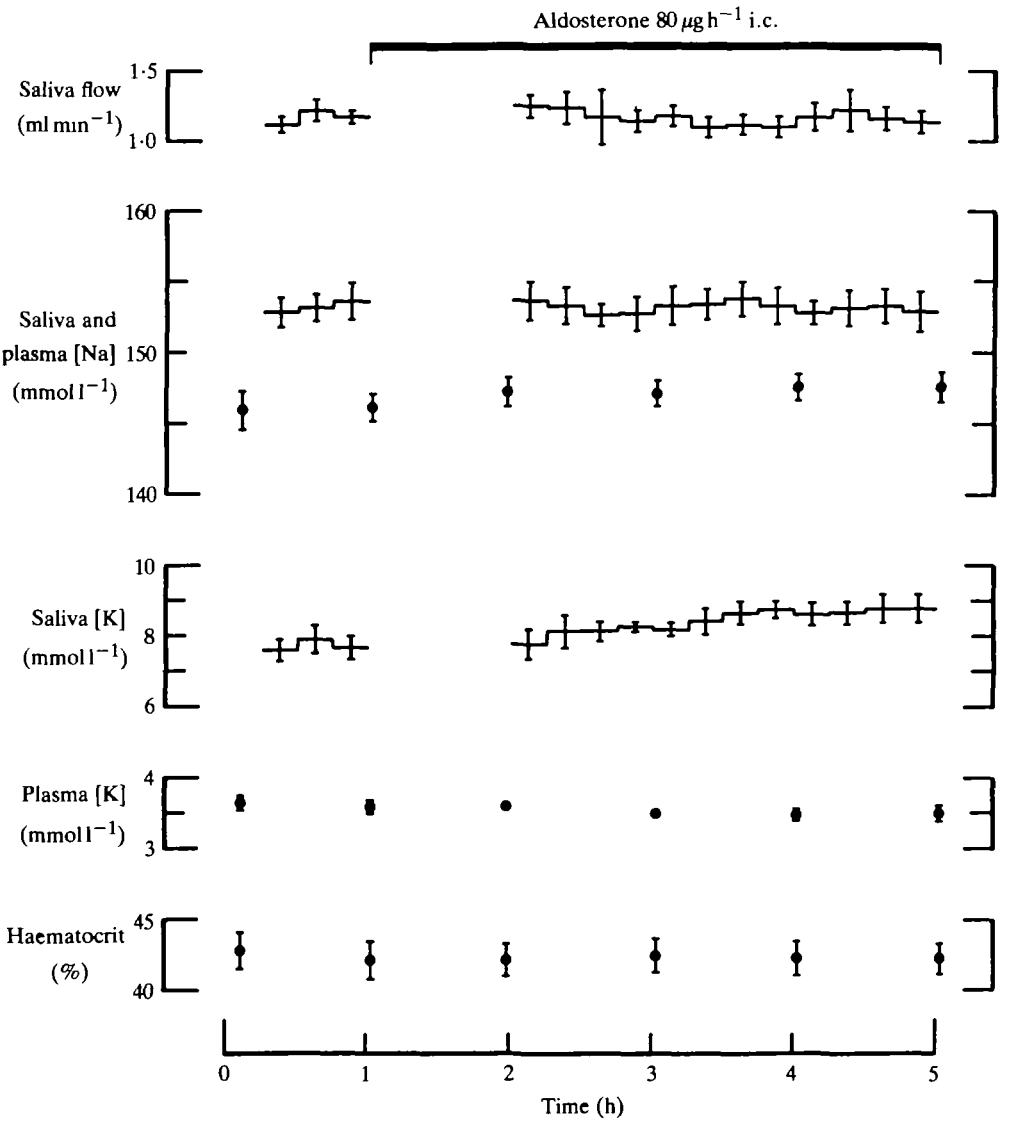

Fig. 1. Salivary flow rate and salivary sodium and potassium concentrations before and during a 4-h ipsilateral intracarotid (i.c.) infusion of aldosterone at 80 μg h^{-1}. Plasma sodium and potassium concentrations and haematocrit are given at hourly intervals ($N = 7$; means ± S.E. of mean).

obvious increase in effect. As a subjective observation, it was noted that the responsiveness to DOCA appeared to be related to whether the animal readily indulged in thermoregulatory and stress-induced body licking or not.

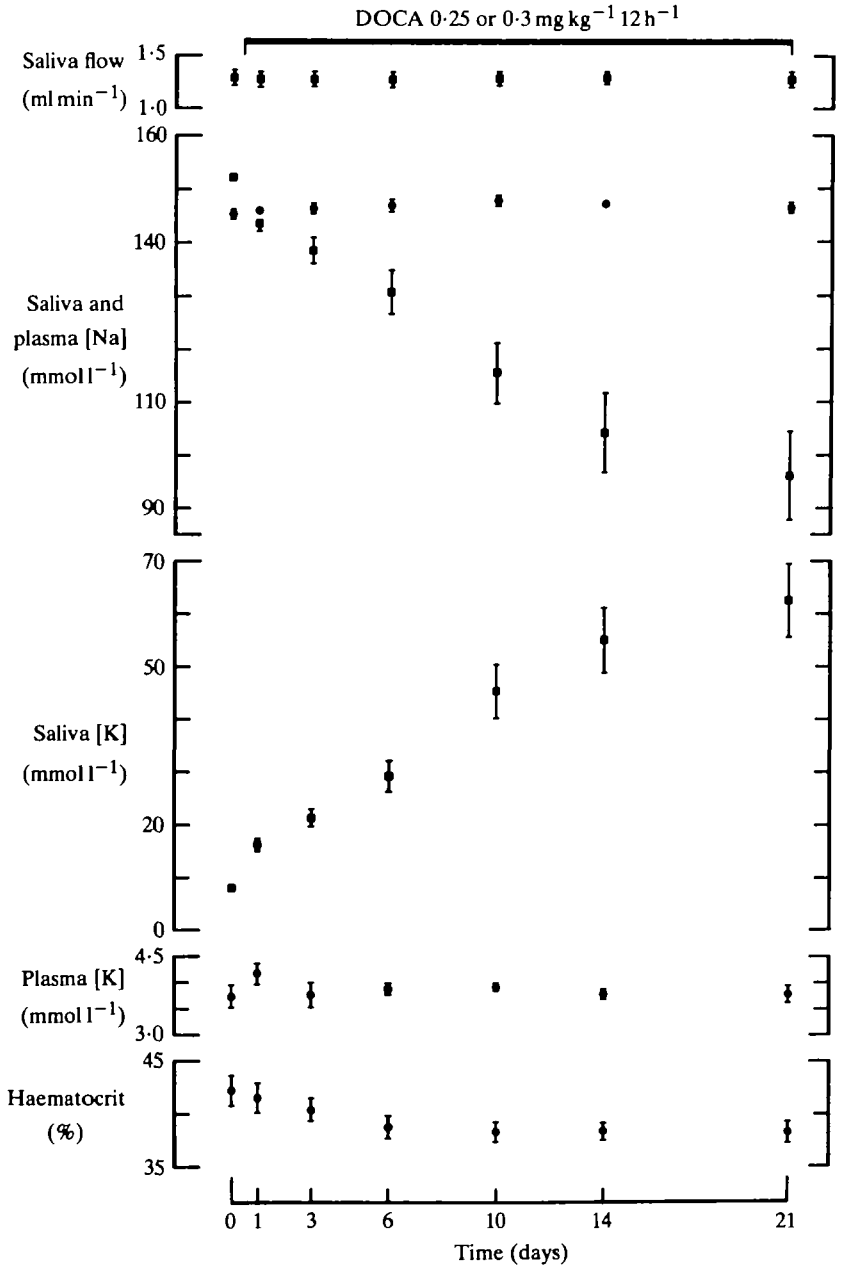

Fig. 2. Salivary flow rate, sodium concentration and potasium concentration (squares) and plasma sodium and potassium concentrations and haematocrit (circles) on days 0, 1, 3, 6, 10, 14 and 21 of intramuscular deoxycorticosterone acetate injection at 0·25 or 0·3 mg kg^{-1} 12 h^{-1} ($N = 6$; means ± S.E. of mean).

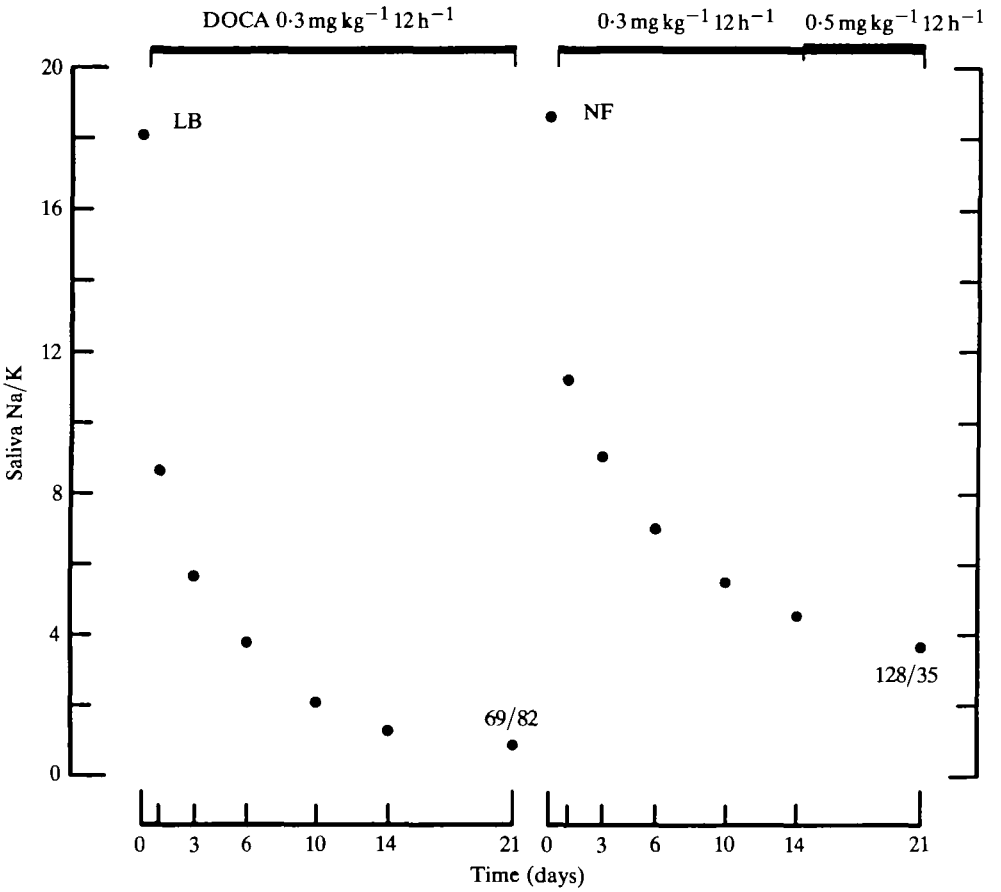

Fig. 3. Salivary Na/K ratio during 21 days of intramuscular DOCA injection into the kangaroos having the most and least responsive parotid glands. Animal LB is a male which readily indulged in stress-induced and thermoregulatory licking whereas animal NF is a female which rarely indulged in either stress-induced or thermoregulatory body licking.

Chronic aldosterone administration

As with the DOCA treatment, intramuscular injection of aldosterone acetate caused a progressive fall in salivary Na/K ratio from 19·1 to 6·69 over the 10 days of this experiment. This fall in Na/K ratio was less than that of the least responsive of the DOCA-treated kangaroos at the equivalent time in that experiment (18·6 to 5·49).

Post-DOCA infusion of aldosterone

No significant changes in haematocrit, or in plasma sodium and potassium concentrations were associated with this aldosterone infusion (Fig. 4). Salivary sodium concentration fell from a mean value of $152{\cdot}4 \pm 0{\cdot}46$ to $138{\cdot}9 \pm 3{\cdot}17$ mmol l^{-1} at 3 h of aldosterone infusion (time of change from 8 to 80 $\mu g\,h^{-1}$) and tended to plateau between 30 and 75 min (mean, 45 min) after the change to infusion at 80 $\mu g\,h^{-1}$.

Salivary potassium concentration rose from 8·55 ± 0·333 to 19·1 ± 2·64 mmol l^{-1} by 3 h of aldosterone infusion and plateaued between 15 and 75 min (mean, 45 min) after the infusion rate had been increased. Overall, the salivary Na/K ratio fell from

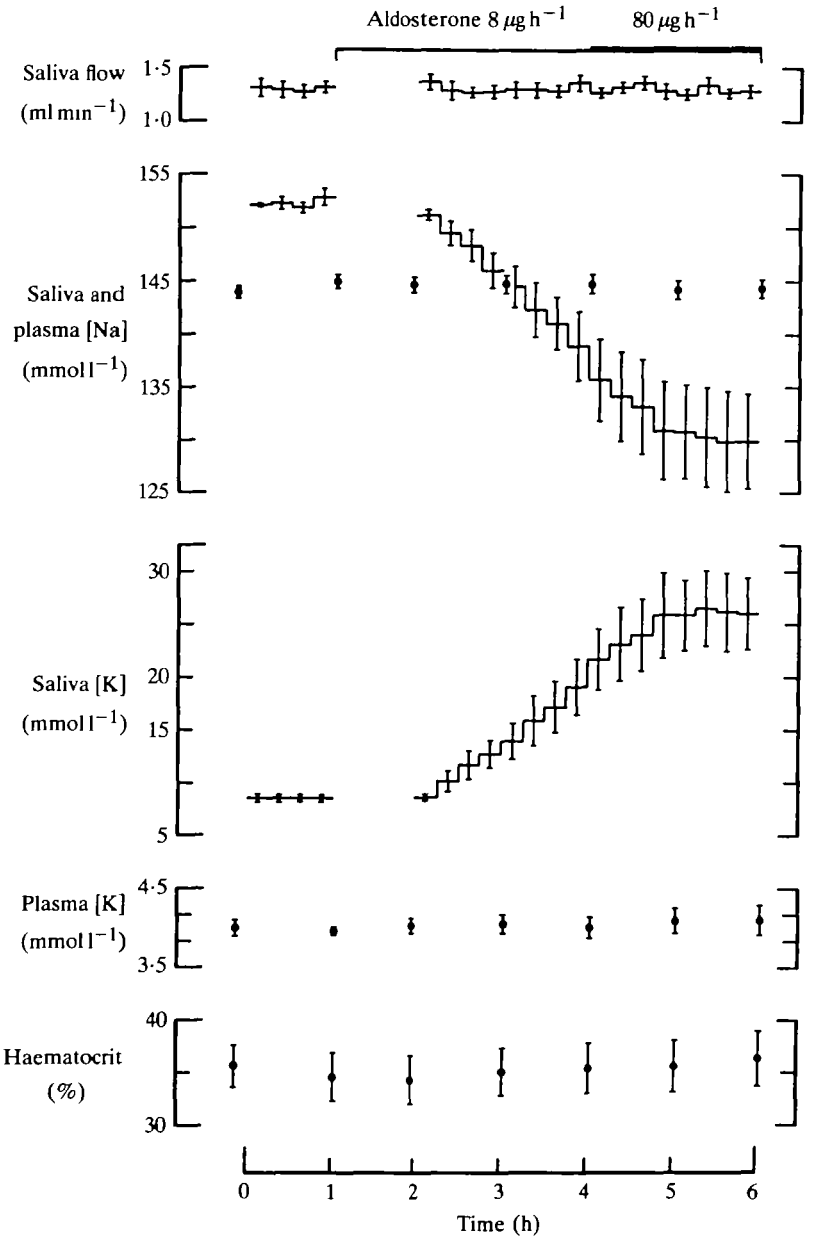

Fig. 4. Salivary flow rate and salivary sodium and potassium concentrations before and during infusion of aldosterone at 3–4 days after cessation of 21 days DOCA administration. Aldosterone was given by ipsilateral intracarotid infusion at 8 μg h^{-1} for 3 h increasing to 80 μg h^{-1} for a further 2 h. Plasma sodium and potassium concentrations and haematocrit are given at hourly intervals ($N = 6$; means ± S.E. of mean).

$18{\cdot}0 \pm 0{\cdot}72$ to $5{\cdot}7 \pm 1{\cdot}04$ ($t_5 = 14{\cdot}21$; $P < 0{\cdot}001$). The magnitude of the response by individual kangaroos to aldosterone infusion was correlated with the size of their response to chronic DOCA administration ($r_4 = 0{\cdot}909$; $P < 0{\cdot}05$). The minimum Na/K ratio achieved as a result of the aldosterone infusion was similar to the ratios obtained on days 3 and 6 of the DOCA experiment and significantly different from the ratios observed on days 1, 10 14 and 21 of DOCA administration.

Gland histology

DOCA administration for 21 days was associated with an increase in height of the intralobular duct cells ($P < 0{\cdot}001$), in volume of the nuclei in the duct cells ($P < 0{\cdot}001$) and in the lobular volume occupied by the ducts (Fig. 5; Table 1). After DOCA treatment the duct cell nuclei had a very granular appearance and prominent acidophilic nucleoli. Three days after cessation of DOCA injection, measurable regression of the duct system had occurred, the height of the duct cells having fallen by 16–17% ($P < 0{\cdot}001$), the volume of their nuclei by 21–35% ($P < 0{\cdot}001$) and the volume of ducts by 20–24%. By 24 days post-DOCA, the cell height and nuclear volume had returned to or were approaching the values found in Na-replete kangaroos prior to DOCA treatment. At this time, the duct volume was on average 5 times the duct volume found prior to DOCA administration (Table 1). The nucleoli of the intralobular duct cells remained identifiable but were now no more obvious than those of the adjacent serous cells. Periodic acid Schiff staining showed that polysaccharides were not being accumulated by the duct cells either before or during the DOCA treatment.

DISCUSSION

Throughout these experiments, the salivary flow rate was maintained at approximately 25% (i.e. 1·0–1·5 ml min^{-1}) of the maximum flow under anaesthesia. This rate was used because the kangaroo parotid gland can achieve and maintain this rate easily for long periods of time and, as a consequence of the shape of the salivary sodium and potassium concentration/flow curves (Beal, 1984), the sodium and potassium concentrations in the saliva will vary little with moderate fluctuations in flow but should show obvious changes when ductal transport of these ions is increased.

In Na-replete sheep, intravenous infusion of aldosterone at 20–30 $\mu g\, h^{-1}$ causes maximal changes in parotid Na/K ratio, the salivary potassium rising to 14–45 mmol l^{-1} and the sodium concentration falling by a similar amount (Blair-West *et al.* 1963; Kraintz *et al.* 1972). By comparison, the response of the chronically Na-replete kangaroos to acute aldosterone administration was virtually negligible. There can be little doubt that the aldosterone infusates were biologically active, for the following reasons: rat renal bioassay demonstrated the presence of mineralocorticoid activity, radioimmunoassay of the stock aldosterone solutions gave estimates approximating to their nominal concentrations, aldosterone infusion into the kangaroos was associated with increased plasma sodium concentrations, which

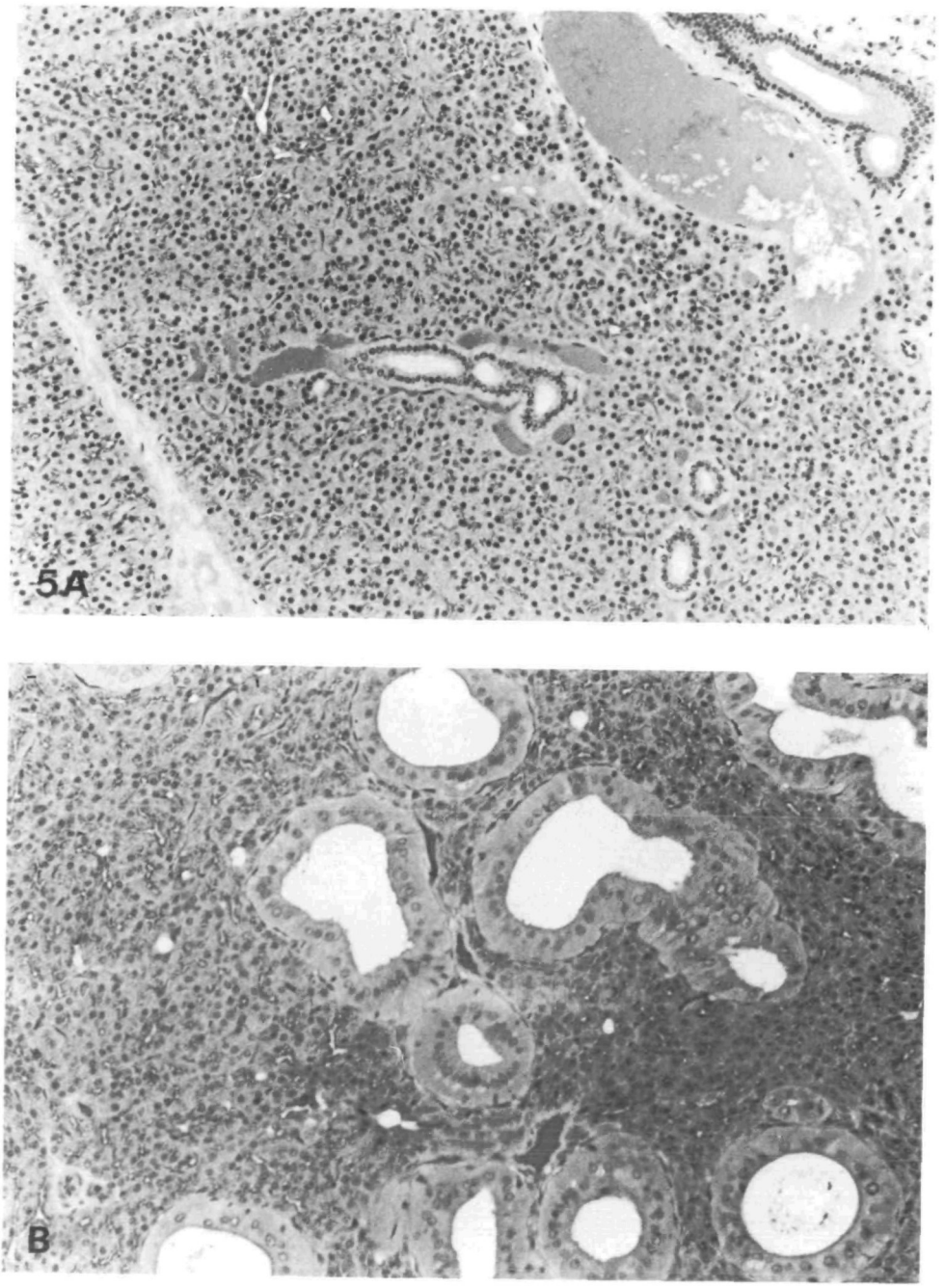

Fig. 5. Parotid gland from the kangaroo which gave the greatest physiological response to DOCA administration (animal LB). Fixed in formol saline; methacrylate sections stained with haematoxylin and eosin (×150). (A) represents the Na-replete state and (B) shows the effect of 21 days injection of DOCA at 3 mg kg^{-1} 12 h^{-1}.

may reflect extra-salivary actions of the hormone, and, when used after chronic DOCA administration, aldosterone infusion caused obvious changes in salivary sodium and potassium concentrations. Intracarotid infusion of aldosterone into the kangaroos at 80 $\mu g\,h^{-1}$ was presumably a supramaximal rate since 20–30 $\mu g\,h^{-1}$ given intravenously caused maximal salivary response in sheep (Blair-West *et al.* 1963; Kraintz *et al.* 1972) and both species have similar levels of aldosterone in their peripheral blood when in similar environmental conditions (Scoggins *et al.* 1970). Also, intracarotid infusion of aldosterone at 8 $\mu g\,h^{-1}$ after DOCA treatment was sufficient to produce maximal falls in salivary Na/K ratio since this ratio plateaued during the latent period of the change from 8 to 80 $\mu g\,h^{-1}$. Thus the parotid glands of kangaroos living in sodium-rich environments, and therefore ingesting a diet chronically high in sodium, are almost unable to respond to acute fluctuations in endogenous aldosterone levels.

For kangaroos living in sodium-deficient environments, retention of sodium would be a continual problem which results in these animals having blood aldosterone levels above those of sodium-replete animals (Scoggins *et al.* 1970). Chronic administration of mineralocorticoid to the kangaroos should produce physiological responses simulating the natural situation more closely than the alternative of sodium-depletion either by salivary loss through a duct fistula or by diuretic-induced urinary loss. The twice-daily injections of DOCA caused a slow but progressive fall in the Na/K ratio of the saliva which, at the end of 21 days, had clearly not reached the minimum possible value for the ratio. However, the rate of fall in the ratio was

Table 1. *Intralobular duct cell height (mean ± S.E.M.), estimated nuclear volume of intralobular duct cells (mean ± S.E.M.) and intralobular duct volume as a percentage of lobular volume before, during and after intramuscular administration of deoxycorticosterone acetate (DOCA) at 0·25 mg kg^{-1} 12 h^{-1} for 21 days*

Kangaroo	Sex	Before DOCA	21 days DOCA	3 days post-DOCA	24 days post-DOCA
			Intralobular duct cell height (μm)		
GT	F	14·5 ± 0·42	27·1 ± 0·48	—	—
LB	M	15·1 ± 0·36	29·9 ± 0·80	—	—
NF	F	—	30·3 ± 0·63	25·2 ± 0·52	14·9 ± 0·38
DF	M	—	40·3 ± 0·99	33·9 ± 0·62	18·4 ± 0·50
			Nuclear volume of intralobular duct cells (μm^3)		
GT	F	133·0 ± 4·56	297·5 ± 9·87	—	—
LB	M	142·8 ± 5·00	387·2 ± 10·59	—	—
NF	F	—	243·1 ± 8·52	157·0 ± 7·23	117·3 ± 5·86
DF	M	—	234·1 ± 7·06	184·0 ± 6·07	127·3 ± 3·72
			Percentage intralobular duct volume		
GT	F	0·86	16·6	—	—
LB	M	1·37	18·5	—	—
NF	F	—	13·2	10·6	4·39
DF	M	—	13·1	9·98	6·64

probably similar to the maximum rate for each animal under these conditions since there was no correlation between the dose rates of 0·25 and 0·3 mg kg^{-1} 12 h^{-1} and the rate of fall in the ratio, and increasing the dosage in one animal from 0·3 to 0·5 mg kg^{-1} 12 h^{-1} did not increase the rate of fall in the ratio. The lowest dose rate was chosen on the basis that it was 10 times the dosage necessary to maintain a 40-kg adrenalectomized sheep (i.e. 2 mg day^{-1}) and therefore might be expected to produce a near-maximal rate of fall in the salivary Na/K ratio.

Sodium deficiency in sheep, grey kangaroos and rabbits is associated with hyperplasia and hypertrophy of the striated and excretory ducts of the major salivary glands (Blair-West *et al.* 1968, 1969; Compton *et al.* 1975). The histological changes observed in the intralobular ducts of the red kangaroo parotid gland as a consequence of the DOCA injection were similar to those of the grey kangaroo although considerably more extreme. Hypertrophy of the duct cells, with histological evidence of increased nuclear activity, was clearly demonstrated, whereas duct hyperplasia could not be demonstrated beyond question. In the earlier studies it was uncertain whether these histological changes were due to the sodium deficiency itself or to concomitant changes in mineralocorticoid activity. The red kangaroos were Na-replete at the commencement of the DOCA injection and there is no reason to believe that they became Na-deficient during the course of mineralocorticoid injections. Indeed, the tendency for the plasma sodium concentration to rise during the period of injection would indicate that they remained Na-replete. Hence the duct hypertrophy seen during Na deficiency must be due to the action of endogenous mineralocorticoids on the duct cells. As a consequence of low mineralocorticoid levels, parotid glands of chronically Na-replete red kangaroos have short, relatively inactive ducts, which explains the lack of response to acute aldosterone infusion. However, with the establishment of high mineralocorticoid levels, the duct system enlarges and the duct cells become more active, which explains the slowly increasing ability of the gland to reduce the salivary Na/K ratio. The observation that animals which indulge in frequent skin licking appear to be more responsive to DOCA administration than non-licking animals may reflect a slight priming of this mechanism due to loss of sodium during this activity. During sodium deficiency in rabbits, glycogen accumulates in the duct cells of the mandibular gland (Compton *et al.* 1975; Young & van Lennep, 1978). This did not occur in the kangaroo parotid gland.

The 3–4 days required for the salivary Na/K ratio to return to Na-replete levels after cessation of DOCA administration corresponded to the time necessary for the metabolism of the residual steroid in the muscles since, in terms of sodium balance, the animal should have remained Na-replete throughout the DOCA treatment. Under these conditions, intracarotid infusion of aldosterone reduced the salivary Na/K ratio to a minimum after 3–4 h which coincided with the latent period following the increase in infusion rate from 8 to 80 $\mu g\,h^{-1}$. At the higher infusion rate, aldosterone caused no further fall in the Na/K ratio, a further indication that this infusion rate was supramaximal. The increased ability to transport Na and K was apparently very dependent on high-level mineralocorticoid support since, during the

3–4 days between the end of DOCA administration and the post-DOCA aldosterone infusion, the minimum Na/K ratios were only equivalent to those observed at 3–6 days of DOCA administration. This loss of sodium transporting ability was associated with substantial regression of the duct system, indicating that the increased activity and development of the ducts are rapidly lost when mineralocorticoid support is withdrawn. Indeed, by 24 days post-DOCA, which is equivalent to 21 days of low mineralocorticoid levels, the regression of the ducts was approaching completion. Based on these observations, one would predict that the parotid glands of kangaroos from sodium-deficient environments would lose their ability to respond to acute fluctuations in blood mineralocorticoid levels within a month of arriving in a sodium-rich environment.

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