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INFLUENCE OF ADRENALECTOMY UPON RAT ERYTHROCYTE Na^+ AND K^+ CONTENT, Na^+ EFFLUX RATE AND Mg^{2+}- AND $(Na^+ + K^+)$-Mg^{2+}-ATPase ACTIVITIES

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SUMMARY

1. Erythrocyte $[Na^+]_i$, $[K^+]_i$, $^{22}Na^+$ efflux and Mg^{2+}- and $(Na^+ + K^+)$-Mg^{2+}-ATPase (EC 3.6.1.3) activities were studied in intact and totally adrenalectomized, saline-deprived male rats.

2. Both $[Na^+]_i$ and $[K^+]_i$ were decreased post-operatively. The $[Na^+]_i$ decrement was statistically significant, and maximal 3 days post-operatively.

3. When compared with erythrocytes from intact rats, those from adrenalectomized rats showed a progressive decline in the rate constant for strophanthin-G-sensitive $^{22}Na^+$ efflux during a 5-day post-operative period.

4. Both Mg^{2+}- and $(Na^+ + K^+)$-Mg^{2+}-ATPase activities were elevated in erythrocyte membrane preparations from adrenalectomized rats. The elevation was maximal in cells taken from rats 3 days post-operatively.

INTRODUCTION

It is generally agreed that total adrenalectomy results in a decreased renal microsomal $(Na^+ + K^+)$-Mg^{2+}-ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity in the rat [1–4], and known that the decrease may be postponed and partially prevented by high dietary Na^+ intake [4]. However, few investigations have been made of the influence of adrenalectomy upon the ATPases obtained in preparations from other rat tissues. Gutman and Glushevitsky-Strachman [5] have recently shown that both Mg^{2+}- and $(Na^+ + K^+)$-Mg^{2+}-ATPase activities are elevated in the intestinal mucosa and salivary glands of adrenalectomized rats. Their result is in accord with a previous observation in rat adenohypophysis [6], but Gallagher and Glaser [7] have shown that brain ATPases remain uninfluenced. Thus, the results so far obtained in studies of extra-renal ATPases [5–7] are at variance with those established for kidney.

It is clear that the post-operative decline in renal $(Na^+ + K^+)$-Mg^{2+}-ATPase

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activity is associated with the extent of hyponatraemia [4], and it is probable that it arises from a decline in the availability of Na^+ to the active transport enzyme in situ [4, 8]. The questions therefore arise whether microsomal preparations from extrarenal cells which are known to lose $[Na^+]_i$ after adrenalectomy exhibit decreased $(Na^+ + K^+)$-Mg^{2+}-ATPase activity, and whether there is a concomitant decline in the rate of active Na^+ efflux.

Investigations of rat erythrocytes have been conducted with these questions in mind. It is known that active Na^+–K^+ exchange is closely related to $(Na^+ + K^+)$-Mg^{2+}-ATPase activity in human erythrocyte membranes [9, 10], and that a close correlation exists between the normal $[Na^+]_i$:$[K^+]_i$ ratio and the activity of this enzyme in erythrocytes from a variety of species [11–14]. Changes in these properties have been reported in hereditary spherocytosis [15], and may be induced by X-irradiation [16], storage at 4 °C [17], and photo-oxidation in the presence of rose bengal [18]. Erythrocytes have therefore been adopted as a useful model with which to approach the problem of adrenocorticosteroidal control of tissue electrolyte distribution.

MATERIALS AND METHODS

Animals and adrenalectomy

Male CFHB rats (Carworth Europe, Alconbury, Huntingdon, England) of 150–200 g body weight were totally adrenalectomized under ether vapour or sodium pentobarbitone (Boots Pure Drug Co. Ltd, Nottingham, England) anaesthesia, and maintained post-operatively on a diet consisting of Laboratory Small Animals Diet (Spillers Ltd, Oxford, England) and distilled water ad libitum.

Blood sampling and analyses

Blood was obtained by cardiac puncture in syringes containing 100 I.U. heparin (Evans Medical Ltd, Liverpool, England). Erythrocytes from duplicate aliquots were immediately separated by centrifugation at $1500 \times g$ for 10 min, and prepared for the following estimations. Erythrocyte packed cell volume was determined throughout by centrifugation at $1500 \times g$ for 30 min.

(1) *Erythrocyte* $[Na^+]_i$ *and* $[K^+]_i$. These were measured by flame emission in a Pye Unicam SP90 atomic absorption spectrophotometer, using deproteinized haemolysates prepared from erythrocytes separated at 18–21 °C.

(2) Na^+ *efflux from* $^{22}Na^+$*-loaded, intact erythrocytes.* Cells separated at 0–4 °C were twice washed in 25 mM sodium phosphate buffer (pH 7.25) containing 144 mM NaCl. After determination of their packed cell volume, they were $^{22}Na^+$-loaded at 4 ± 1 °C in saline containing 1.6 μCi ^{22}NaCl/ml (Radiochemical Centre, Amersham, Bucks, England). The cells were then washed in $^{22}Na^+$-free saline, and $^{22}Na^+$ efflux was studied in samples taken from suspensions of known packed cell volume during incubation at 37 ± 0.1 °C. Radioactivity was measured below a Mullard endwindow Geiger–Müller tube with a 2.3-mg/cm^2 mica window. Rate constants for $^{22}Na^+$ efflux were derived by the equation:

$$\text{rate constant} = \ln \frac{\text{cps/ml packed cell volume at 60 min}}{\text{cps/ml packed cell volume at 0 min}} \cdot h^{-1}.$$

Parallel experiments revealed no change in packed cell volume during this incubation period.

(3) *Mg^{2+}- and ($Na^+ + K^+$)-Mg^{2+}-ATPases in erythrocyte membrane fragments.* Erythrocytes were treated at 0–4 °C as follows. Their packed cell volume was determined after washing in 154 mM NaCl (neutralized with L-histidine), and they were then washed in saline containing 1 mM EDTA, recentrifuged and haemolysed by forced injection of 0.5 mM EDTA (neutralized with L-histidine). Haemoglobin-free ghosts were then obtained by repeated EDTA washes and centrifugations at 20 000 × *g* for 15 min. They were rapidly frozen at −86 °C and thawed in L-histidine–HCl (pH 7.2) and the membrane fragments finally suspended in 30 mM L-histidine–HCl (pH 7.2). Aliquots of these suspensions were incubated in the presence of Tris–ATP (prepared from Na_2ATP supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A.); incubations were terminated by addition of 1 ml 12% (w/v) trichloroacetic acid. The resultant supernatants were assayed for P_i [19] with reference to a phosphorus standard (Sigma Chemical Co.).

RESULTS

(1) *Erythrocyte $[Na^+]_i$ and $[K^+]_i$.* The mean $[Na^+]_i$ and $[K^+]_i$ values obtained for adrenalectomized rats sacrificed 1, 3 and 10 days post-operatively are given in Table I, in which they are compared with those obtained for control rats sampled

TABLE I

EFFECT OF ADRENALECTOMY UPON RAT ERYTHROCYTE $[Na^+]_i$ AND $[K^+]_i$

Erythrocytes were washed by suspension in 250 mM D-mannitol (neutralized with Tris) and sedimented at 1500 × *g* for 10 min. Their packed cell volume was measured in fresh D-mannitol, and they were haemolysed by forced injection of deionized water. The haemolysate was deproteinized with 1 M trichloroacetic acid and centrifuged at 15 000 × *g* for 15 min. The resultant supernatant was pooled with three subsequent washings of the precipitate and assayed for $[Na^+]$ and $[K^+]$. Values shown are mean ± S.E.; *P* values (in parentheses) calculated with reference to control. Number of animals: day 0, 44; day 1, 6; day 3, 6; day 10, 4.

Days post-adrenalectomy	[Cation] (mmoles/l packed cell volume)	
	$[Na^+]_i$	$[K^+]_i$
0 (control)	5.69 ± 0.18	95.07 ± 0.86
1	3.38 ± 0.09 (<0.001)	91.16 ± 0.60 (>0.10)
3	2.91 ± 0.08 (<0.001)	91.16 ± 0.70 (>0.10)
10	3.77 ± 0.07 (<0.01)	90.25 ± 0.64 (>0.10)

during the same experimental period. The results show that both mean $[Na^+]_i$ and mean $[K^+]_i$ decreased post-operatively, and the *P* values obtained by Student's *t* test reveal that $[Na^+]_i$ was significantly lowered during the entire period. $[K^+]_i$ values remained not significantly different from control. It is particularly noted that the post-operative changes were substantially accomplished within the first post-operative day, at which time the mean cell volume (58.3 ± 1.2 μm^3) was not significantly different from control (57.9 ± 0.6 μm^3). $[Na^+]_i$ was reduced to almost 50% of the control level at the third post-operative day.

(2) *Na^+ efflux from $^{22}Na^+$-loaded intact erythrocytes.* The results obtained in a typical series of determinations with erythrocytes from control and adrenalectomized rats are given in Fig. 1, which additionally shows the influence of $1 \cdot 10^{-4}$ M strophanthin-G (Sigma Chemical Co.) when present in the efflux medium containing cells from control rats. The influence of adrenalectomy was studied in erythrocyte samples prepared from animals sacrificed 1, 3 and 5 days post-operatively.

The $^{22}Na^+$ efflux rate constant was reduced very considerably by strophanthin-G from $-2.16\ h^{-1}$ to $-0.10\ h^{-1}$, strophanthin-G-insensitive (residual) $^{22}Na^+$ efflux representing approx. 5% of the total. The efflux rate constant showed a decline

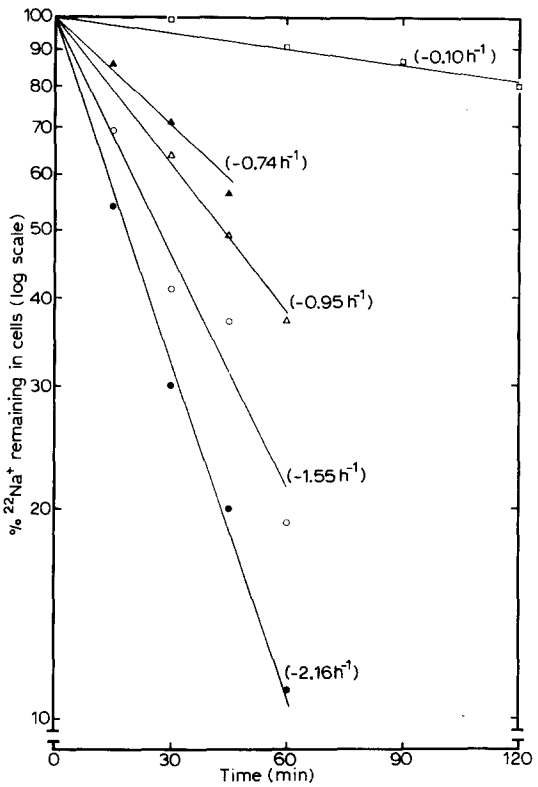

Fig. 1. Effect of adrenalectomy upon strophanthin-G-sensitive $^{22}Na^+$ efflux from rat erythrocytes. Saline-washed, $^{22}Na^+$-loaded cells were immediately suspended to three times their packed cell volume in a medium consisting of 145 mM NaCl, 6 mM KCl, 3 mM NaH_2PO_4 and 20 mM D-glucose (adjusted to pH 7.4 with Tris). Aliquots were incubated in a shaking metabolic incubator at 37±0.1 °C and sampled at convenient time intervals. Cells were quickly separated from a proportion of each sample by centrifugation at $1000 \times g$ for 3 min. Aliquots from the sample of whole suspension and its supernatant were then tested for $^{22}Na^+$ content. The difference between the respective radioactivities was taken to be due to intra-cellular $^{22}Na^+$. The value obtained immediately after $^{22}Na^+$ loading is given as 100%, and the relative subsequent values are plotted logarithmically as a function of incubation time. Lines are those of best fit, calculated by the method of least squares. Plots are shown for cells from the following rats: control, incubated alone (●); control, incubated with $1 \cdot 10^{-4}$ M strophanthin-G (□); 1 (○), 3 (△) and 5 (▲) days post-adrenalectomy. Rate constants for $^{22}Na^+$ efflux are shown in brackets.

through $-1.55\ h^{-1}$ and $-0.95\ h^{-1}$ to $-0.74\ h^{-1}$ for erythrocytes taken, respectively, from adrenalectomized rats sacrificed 1, 3 and 5 days post-operatively, even though the initial mean $^{22}Na^+$ content of the cells did not differ significantly from that of erythrocytes obtained from controls (see Table II).

(3) *Mg^{2+}- and ($Na^+ + K^+$)-Mg^{2+}-ATPases of erythrocyte membrane fragments.* Of a group of rats anaesthetized with sodium pentobarbitone, a number were totally adrenalectomized. Adrenalectomized and unoperated rats from this group were

TABLE II

EFFECT OF ADRENALECTOMY UPON $^{22}Na^+$-LOADING CAPACITY OF RAT ERYTHROCYTES

Values shown are mean±S.E. Number of animals: day 0, 5; day 1, 4; day 3, 4; day 5, 2.

Days post-adrenalectomy	$^{22}Na^+$ content after 10 h (cps/ml packed cell volume)
0 (control)	160±10.2
1	182±33.5
3	154±26.9
5	151±6.0

TABLE III

EFFECT OF SODIUM PENTOBARBITONE ADMINISTRATION AND ADRENALECTOMY UPON RAT ERYTHROCYTE Mg^{2+}- AND ($Na^+ + K^+$)-Mg^{2+}-ATPase ACTIVITIES

Aliquots of membrane suspensions were incubated in duplicate for 15–20 min at 37±0.1 °C in thermoequilibrated systems of 2 ml final volume, containing 2 mM Tris–ATP and buffered at pH 7.2 with 30 mM L-histidine–HCl. Mg^{2+}-ATPase was assayed in the presence of 4 mM $MgCl_2$; ($Na^+ + K^+$)-Mg^{2+}-ATPase in the presence of 4 mM $MgCl_2$, 100 mM NaCl and 20 mM KCl. ($Na^+ + K^+$)-Mg^{2+}-ATPase activity is given as the increment in P_i liberation obtained by inclusion of Na^+ and K^+ in the assay media. Values shown are mean±S.E.; *P* values (in parentheses) calculated with reference to control. Number of animals: day 0, 9; day 1, 3; day 3, 3; day 5, 3; day 7, 3.

Treatment of animals	Days after treatment	ATPase activity (nmoles P_i liberated/ml packed cell volume per min)	
		Mg^{2+}-ATPase	($Na^+ + K^+$)-Mg^{2+}-ATPase
None (control)	0	113.40±5.95	73.30±4.68
50 mg/kg body weight sodium pentobarbitone alone			
	1	104.17±11.94 (>0.40)	63.70±13.98 (>0.50)
	3	98.20±14.57 (>0.20)	56.83± 7.37 (>0.10)
	5	102.56± 5.97 (>0.30)	65.20± 8.03 (>0.40)
	7	112.40± 4.69 (>0.90)	73.43± 4.20 (>0.90)
50 mg/kg body weight sodium pentobarbitone and total adrenalectomy			
	1	251.17±17.18 (<0.001)	227.96± 4.38 (<0.001)
	3	413.83± 9.19 (<0.001)	292.13± 9.17 (<0.001)
	5	295.80±14.92 (<0.001)	254.10±10.66 (<0.001)
	7	224.30±11.02 (<0.001)	123.16± 6.09 (<0.001)

sacrificed 1, 3, 5 or 7 days later, and their erythrocyte ATPase activities compared with those of intact, control rats which had not received the anaesthetic. The values obtained are presented in Table III.

The data show that sodium pentobarbitone anaesthesia itself resulted in a very slight, but not statistically significant, reduction in both ATPase activities. However, these activities were markedly elevated in erythrocytes taken from adrenalectomized rats with reference to those from controls ($P < 0.001$ throughout the post-operative period). The pattern of post-operative ATPase activity change was similar for both Mg^{2+}- and $(Na^+ + K^+)$–Mg^{2+}-ATPase, respective maximal elevations of 264 and 298% being reached on the third post-operative day. Thereafter, activities decreased to yield respective 97 and 67% elevations on the seventh post-operative day.

DISCUSSION

Whilst the erythrocyte $[Na^+]_i$ loss following adrenalectomy is consistent with the change noted in rat striated muscle by Conway and Hingerty [20] and Leonard [21], it is at variance with the report of Losert et al. [22], who noted no change in muscle $[Na^+]_i$, and an increased erythrocyte $[Na^+]_i$ 3 days after adrenalectomy. This result probably arose from the use of saline-maintained rats [22]; a similar effect of saline maintenance has been noted by the present author (Radcliffe, M. A., unpublished).

It is unlikely that the decline in $^{22}Na^+$ efflux rate constant of erythrocytes from adrenalectomized rats can be solely attributed to the altered $[Na^+]_e$ and $[K^+]_e$ characteristic of adrenalectomy. Although Sachs [23] has shown that such a decline accompanies reduction in $[Na^+]_e$ for human erythrocytes in vitro, his work suggests that it is very small within the range of hyponatraemia evident in saline-deprived, adrenalectomized rats. Moreover, the degree of associated hyperkalaemia is neither concomitant with altered Na^+ efflux from human erythrocytes subjected to identical $[K^+]_e$ change in vitro [24], nor with altered Na^+ influx rate [25]. It therefore seems that altered erythrocyte membrane properties would be involved in the changes reported here.

In the light of evidence implicating $(Na^+ + K^+)$–Mg^{2+}-ATPase in the control of erythrocyte Na^+ transport [9, 10], the post-operative reduction in strophanthin-G-sensitive $^{22}Na^+$ efflux and in $[K^+]_i$ were expected to be paralleled by a selective fall in $(Na^+ + K^+)$–Mg^{2+}-ATPase activity. Whilst it is known that a non-selective elevation of both $(Na^+ + K^+)$–Mg^{2+}- and Mg^{2+}-ATPase activities accompanies reticulocytosis in rabbits [26], the reticulocyte count of adrenalectomized rats does not alter significantly during the first post-operative week [27]. The elevated ATPase activities evident in these experiments cannot, therefore, be accounted for upon this basis. Although the elevation in plasma adrenocorticotrophin hormone level occurring after adrenalectomy [28] would be a concomitant of the elevated erythrocyte ATPase activities, adrenocorticotrophin hormone is not known to influence either enzyme. Again, whilst Gutman and Glushevitsky-Strachman [5] suggest that an elevated plasma angiotensin level may enhance tissue ATPase activity in adrenalectomized rats, previous work in their laboratory [29] has shown that Mg^{2+}-ATPase remains uninfluenced by angiotensin in vitro. However, their observation that both Mg^{2+}- and $(Na^+ + K^+)$–Mg^{2+}-ATPase activities are elevated in tissues from adrenalectomized rats [5] does

remain consistent with the present findings in erythrocytes. It seems difficult to reconcile the elevated erythrocyte $(Na^+ + K^+)$-Mg^{2+}-ATPase activity and lowered $[Na^+]_i$ with the reduced strophanthin-G-sensitive $^{22}Na^+$ efflux rate which follows adrenalectomy without implicating an altered Na^+ influx rate. Since the results of preliminary experiments (Radcliffe, M. A., unpublished) suggest that $^{22}Na^+$ influx remains uninfluenced by adrenalectomy, alternative explanations are worth consideration.

In view of the fact that steroids are taken up by erythrocytes [30] and are known to influence their membrane stability [31–33] and permeability [33], it seems possible that at least one of the altered erythrocyte properties reported here could arise from the altered plasma/erythrocyte steroidal exchange which would follow adrenalectomy. For instance, the non-selective post-operative elevation in Mg^{2+}- and $(Na^+ + K^+)$-Mg^{2+}-ATPase activities in erythrocyte (and other tissue [5, 6]) membrane preparations need not, after all, reflect their functioning in intact cells in vivo; it may instead involve an increased susceptibility of the enzymatic sites to exposure by the preparative techniques employed. Such an effect has been previously noted upon treatment of intact or fragmented erythrocytes with tannic acid [34], and noted in paroxysmal nocturnal haemoglobinuria [35]. It is now being further explored in connection with adrenocorticosteroidal status.

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