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STUDIES ON (Na^+-K^+)-ACTIVATED ATPase

XXI. CHANGES IN (Na^+-K^+)-ACTIVATED ATPase ACTIVITY AND OUABAIN-SENSITIVE $^{86}Rb^+$ UPTAKE RATE IN REGENERATING RAT LIVER

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SUMMARY

1. In view of the reported ability of the regenerating rat liver after partial hepatectomy to maintain a higher potassium and a lower sodium concentration than normal liver the activity of the cation pump in the regenerating rat liver was studied.

2. The activity of the (Na^+-K^+)-activated ATPase system was increased. A maximal increase of about 57 % was observed after 3 to 6 days of regeneration.

3. The ouabain-sensitive $^{86}Rb^+$ uptake rate was increased from 2 to 6 days after regeneration by about 54 % maximally.

4. The passive efflux of $^{86}Rb^+$ was decreased by about 41 % from 1 to 6 days after partial hepatectomy. The passive $^{22}Na^+$ influx was not significantly changed.

5. The increase in active cation transport, assisted by the decreased passive cation efflux, was held responsible for the simultaneous increase in potassium level and decrease in sodium level in regenerating rat liver.

INTRODUCTION

In recent years the cation levels in regenerating rat liver after partial hepatectomy have received some attention. UYEKI[1] reported a large increase in potassium concentration and a small increase in sodium concentration 2–4 days after hepatectomy, but MYERS[2] could not demonstrate statistically significant changes in cation levels under similar conditions. Subsequently HUMPHREY[3] established that slices of regenerating liver could maintain a higher potassium concentration and a lower sodium concentration than those of normal liver. The effect began after 12 h and was maximal between 2 and 5 days. He suggested that this effect was caused by a decrease in the membrane permeabilities for sodium and potassium.

It occurred to us that the findings of HUMPHREY could also be explained by assuming an increased activity of the cation pump in liver cells after partial hepatectomy. It seemed to be more likely that a single change, *viz.* increased cation pump activity, rather than two simultaneous changes, *viz.* decreased passive permeabilities for both sodium and potassium, would cause the increased potassium concentration and lowered sodium concentration in regenerating liver.

In recent years the active cation transport in many tissues has been found to be ouabain-sensitive and to be closely related or even identical to an ouabain-sen-

sitive (Na^+-K^+)-activated ATPase system (SKOU[4], POST *et al.*[5], DUNHAM AND GLYNN[6], BONTING AND CARAVAGGIO[7]). The presence of this enzyme system in liver has been demonstrated by BONTING, CARAVAGGIO AND HAWKINS[8], EMMELOT *et al.*[9] and AHMED AND JUDAH[10] and the properties of the enzyme in rat liver have recently been determined in our laboratory[11]. The present paper reports studies of the activity of this enzyme system and the active uptake and passive efflux of $^{86}Rb^+$ in regenerating rat liver after partial hepatectomy.

METHODS

Partial hepatectomy was performed on adult male Wister rats of about 250 g, 3–3.5 months of age, according to the method of HIGGINS AND ANDERSON[12], the left lateral and medium lobes being excised under ether anesthesia. All operations were carried out at the same time of day, at 11.00 a.m. At 1, 2, 3, 4, 5, 6 and 7 days, respectively, after hepatectomy the animals were sacrificed by stunning and decapitation and the livers removed. Tissue preparation and assay of (Na^+-K^+)-activated ATPase activity were carried out as previously described (BONTING, CARAVAGGIO AND HAWKINS[13]) with the minor modification that all volumes used were 50-fold magnified. The pretreatment with 1.5 M urea[11] was not applied. The part of the liver, which was removed at operation and immediately lyophilized, was used as control for the liver taken from the same animal after regeneration.

For the determination of $^{86}Rb^+$ uptake rate regenerating and normal livers were sliced with a Stadie–Riggs microtome. Two to three slices of 0.5 mm thickness (total wet weight 50–100 mg) were preincubated in 3 ml of a potassium-free balanced salt solution for 1 h at 0° in air. The balanced salt solution had the following composition (mmoles per l): Na^+ 149, Ca^{2+} 1, Mg^{2+} 1, Cl^- 144, HCO_3^- 6, HPO_4^{2-} 1.5, $H_2PO_4^-$ 0.15, glucose 5.6 at a pH of 7.5–7.8. Preincubation at 0° served to deplete the tissue slices of intracellular potassium. Thereafter the slices were blotted between filterpaper, and were transferred to 3 ml of the above medium containing 0.5 mC $^{86}Rb^+$ per l ($^{86}RbCl$, specific activity > 50 mC/g Rb, was obtained from Philips Duphar, Amsterdam), with or without ouabain (10^{-3} M). The Rb^+ concentration in the medium was in all cases less than 0.5 mM. After 30 min incubation at 37° in air with gentle shaking the slices were filtered on filterpaper with suction, rinsed briefly (1–2 sec) in non-radioactive medium, blotted between filterpaper, weighed and subsequently counted in a Philips PW 4119 scintillation detector mounted on a Philips PW 4251 counter.

The determinations with and without ouabain were each carried out in triplicate. A correction was made for superficially adhering radioactive medium by subjecting some slices to immediate filtration after addition of the radioactive medium. The values, obtained for these slices, were subtracted from those for the slices incubated for 30 min at 37°. After expression of the radioactivity in counts/min per μl tissue water (assuming an average dry weight percentage of 20 %), the T/M ratios (counts/min per μl tissue water divided by counts/min per μl medium after incubation) were calculated for the slices incubated with and for those incubated without ouabain. The relative decrease in the T/M ratio (Δ T/M) upon addition of ouabain was used as a measure of the ouabain-sensitive $^{86}Rb^+$ uptake rate. There was no significant difference in dry weight percentage between slices incubated with or without ouabain for 30 min at 37°.

For determination of the passive permeability for $^{86}Rb^+$ slices of normal and regenerating livers were preincubated for 1 h at 37° in air in the above potassium-free balanced salt solution in the presence of 7.5 mC $^{86}Rb^+$/l. Thereafter the slices were transferred to the same medium, now without $^{86}Rb^+$, but containing 10^{-3} M ouabain. After 5, 10 and 20 min incubation at 37° in air, 50-μl samples were taken from the medium and counted as described above. After completing the incubation the slices were isolated by filtration and likewise counted. The amount of radioactivity, taken up by a slice during preincubation was calculated by adding the radioactivities in the slice and in the medium at the end of the experiment. Next, the percent efflux could be calculated after 5, 10 and 20 min, respectively. All determinations were carried out in triplicate.

For determination of the passive permeability for $^{22}Na^+$ slices of normal and regenerating livers were incubated at 37° in air in a balanced salt solution of the following composition (mmoles per l): Na^+ 146, K^+ 3, Ca^{2+} 1, Mg^{2+} 1, Cl^- 144, HCO_3^- 6, HPO_4^{2-} 1.5, $H_2PO_4^-$ 0.15, glucose 5.6 at pH 7.5–7.8. The medium contained in addition 0.125 mC $^{22}Na^+$ per l ($^{22}NaCl$ was purchased from Philips Duphar, Amsterdam). 10^{-3} M ouabain was present in the incubation medium to prevent an active transport of $^{22}Na^+$ out of the slice. After 0, 5, 10 and 20 min incubation slices were filtered on filterpaper with suction, rinsed briefly in non-radioactive medium, blotted between filterpaper, weighed and the amount of radioactivity assayed as described above. All determinations were carried out in triplicate. The counts obtained for the non-incubated slices were subtracted from those for the slices incubated for 5, 10 and 20 min. In the same way as above T/M ratios were calculated after 5, 10 and 20 min incubation.

RESULTS

Fig. 1 shows the change in absolute (Na^+-K^+)-ATPase activity in the liver after regeneration, expressed as percent increase in activity, compared with the part of the same liver removed during hepatectomy acting as control. The enzyme activity showed already an increase at 1 day after partial hepatectomy. Maximal increase of activity was observed after 3 to 6 days of regeneration. No explanation can be offered for the low value at 5 days. After 6 days the activity decreased to normal values. Mg^{2+}-ATPase activity was not significantly changed during regeneration except for an increase of 18% ($P = 0.0074$) at 6 days. When animals were subjected to sham-operations in which the abdomen was incised and the liver fingered as in real operations, but no hepatectomy was performed, they showed no significant change in (Na^+-K^+)-ATPase activity 3 days after the sham-operation.

In determining $^{86}Rb^+$ uptake rate an incubation period of 30 min was used in routine experiments, because the increase in T/M ratio was found to be linear up to this time (Fig. 2). The changes in ouabain-sensitive $^{86}Rb^+$ uptake rate in the regenerating liver, expressed as percent increase, relative to liver tissue removed from the same animal at hepatectomy, are also plotted in Fig. 1. It can be seen that 2 days after partial hepatectomy the ouabain-sensitive $^{86}Rb^+$ uptake rate in the liver had increased by about 50%. Thereupon the uptake rate slowly decreased, until after 6–7 days nearly normal values were reached. Animals subjected to sham-operations

showed 3 days after operation a $^{86}Rb^+$ uptake rate, which was not significantly different from that in animals before operation.

From the results shown in Fig. 1 it may be concluded that both the (Na^+-K^+)-activated ATPase activity and the ouabain-sensitive $^{86}Rb^+$ uptake rate were increased in the regenerating liver. Considering the variation in results from different animals,

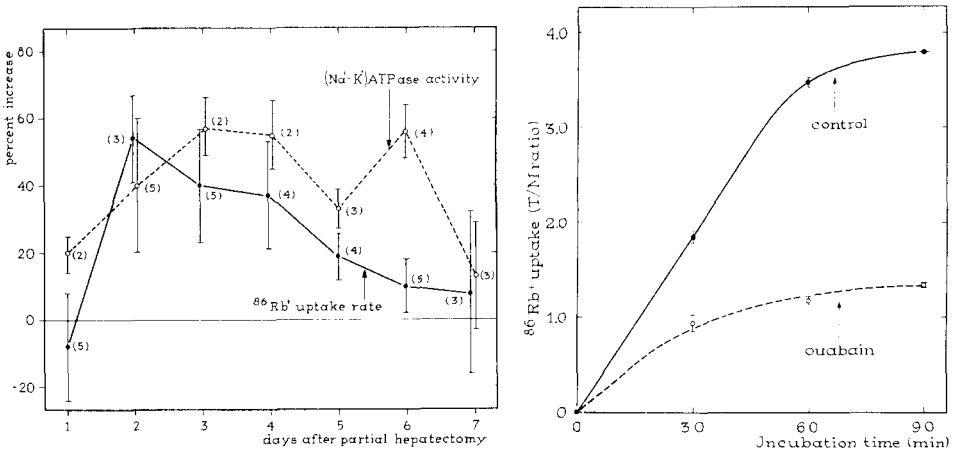

Fig. 1. Relative increase of (Na^+-K^+)-activated ATPase activity and of ouabain-sensitive $^{86}Rb^+$ uptake rate in rat liver after partial hepatectomy. ○---○, percent increase in (Na^+-K^+)-activated ATPase activity on dry weight basis, relative to liver tissue removed from same animal at hepatectomy. ●—●, percent increase after hepatectomy in ouabain-sensitive part of T/M for $^{86}Rb^+$, relative to liver tissue removed from same animal at hepatectomy. Each point represents mean of three observations on each of 2–3 animals (number of animals in parentheses) with standard errors (ranges in case of two animals).

Fig. 2. Rate of uptake of $^{86}Rb^+$ by rat liver slices with and without 10^{-3} M ouabain. Each point represents mean of duplicate observations.

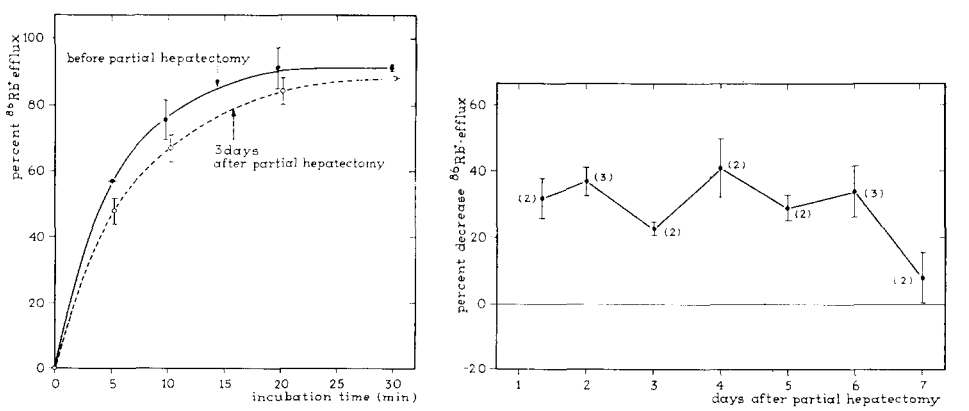

Fig. 3. Passive $^{86}Rb^+$ efflux from rat liver slices before and 3 days after partial hepatectomy. Each point represents mean of three observations with standard errors.

Fig. 4. Relative decrease of passive transport of $^{86}Rb^+$ in rat liver after partial hepatectomy. ●—●, relative change of the percent $^{86}Rb^+$ efflux after 5 min incubation, compared with mean value before operation. Each point represents mean of three observations on each of 2–3 animals (number of animals in parentheses) with standard errors (ranges in case of two animals).

a reasonable agreement between both parameters was obtained. There was a significant difference only at 6 days after hepatectomy. At that time also the only increase in Mg^{2+}-ATPase activity was found.

The passive permeability for Rb^+ was determined by measuring the $^{86}Rb^+$ efflux during 5 min incubation in non-radioactive medium with slices previously loaded with $^{86}Rb^+$. Up to this time a linear increase in $^{86}Rb^+$ efflux was observed (Fig. 3). In Fig. 4 the relative change (compared with the mean value before operation) of the percent efflux after 5 min incubation has been plotted against the time after partial hepatectomy. Clearly, the $^{86}Rb^+$ efflux, and thus the passive permeability for Rb^+ were decreased from 1 to 6 days after partial hepatectomy.

The passive permeability for Na^+ was estimated by determining the $^{22}Na^+$ uptake by slices of normal and regenerating livers in a $^{22}Na^+$-containing incubation medium, while the active transport processes were inhibited by the presence of 10^{-3} M ouabain. By calculating the T/M ratios after 5 and 10 min of incubation, the changes in passive permeability for Na^+ could be determined. Comparing the results from 10 experimental animals after 1, 2, 3, 4, 5 and 6 days of regeneration and those from 9 control animals, no significant change in passive Na^+-transport was found (either at 5 or 10 min incubation).

DISCUSSION

The results reported here prove that the cation pump activity in regenerating rat liver was indeed increased, as shown in the simultaneous increases in (Na^+-K^+)-activated ATPase activity and ouabain-sensitive $^{86}Rb^+$ uptake rate. Previously it was shown[11] that Rb^+ may be substituted for K^+ in studies of active cation transport in rat liver. The effects on both parameters were maximal between 2 and 6 days after partial hepatectomy, in agreement with the findings of HUMPHREY[3] for the changes in cation levels. Since HUMPHREY had suggested that decreases in the passive permeabilities for both cations might explain the changes in cation levels in regenerating rat liver, permeabilities for $^{86}Rb^+$ and $^{22}Na^+$ were also determined. It was found that the passive permeability, at least for $^{86}Rb^+$, was indeed decreased. No significant change in permeability for $^{22}Na^+$ could be detected.

Since an increase in cation pump activity could occur as an adaptive process in cells, in which the cation levels are disturbed by an increased permeability for these ions, it was a somewhat unexpected result or our studies, that in the case of the regenerating rat liver an increase in cation pump activity was accompanied by a decrease in permeability for Rb^+. The effects of these two changes would tend to reinforce each other. As to the significance of the increased cation pump activity, one is tempted to think of the coupling demonstrated in various tissues of the cation pump with active uptake of sugars (CSÁKY[14,15], CURRAN[16]), amino acids (CSÁKY[14,15], BITTNER AND HEINZ[17], FOX *et al.*[18], CURRAN[16]) and other substances (CSÁKY[14,15]). The active uptake of these substances would need to be increased in order to support the accelerated mitotic rate during regeneration of the rat liver. However, it is unlikely that the increased cation pump activity could be of much importance for the increased metabolic requirement, since the peak of mitosis occurs at about 28 h after operation (HARKNESS[19], BUCHER[20]), which is much earlier than the increase of pump activity observed in our study. Since the increase of weight is maximal in the first two days

after partial hepatectomy[19], it is equally unlikely that the increase of cation pump activity is simply correlated with the compensatory growth of the residual liver. In addition we could not demonstrate an ouabain-sensitive active uptake of ^{14}C-labeled amino acids (L-phenylalanine and the non-metabolizable amino acid cycloleucine) and sugars (the non-metabolizable sugar 3-*O*-methylglucose) in rat liver slices.

Another possibility might be that the activity of the cation pump would be connected with bile secretion. LEONG, PESSOTTI AND BRAUER[21] found in regenerating rat liver a transient increase in bile flow rate per unit weight of liver, which reached a maximum of 53 % above the preregenerative level at 3 days after partial hepatectomy and returned to normal after 14 days. The extent of the maximal increase and the time at which it was first reached agree rather well with our findings for the cation pump system. BARTÓK *et al.*[22] observed by means of histochemical staining techniques an increased ATPase activity in the cell membranes of the bile canaliculi of the regenerating rat liver, beginning 1 day after partial hepatectomy and becoming maximal after 4 days. It is understandable that the liver after reduction to one-third of its original volume still has to produce sufficient bile to maintain digestion. This would impose an increased burden on the remaining one-third of the liver. Preliminary experiments in our laboratory clearly demonstrated that bile secretion in rats is inhibitable by ouabain injected into the portal vein, suggesting that bile secretion depends on $(Na^+\text{-}K^+)$-ATPase cation pump activity. Hence the increased cation pump activity would help to keep the total bile production at the physiologically required level.

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