0014-4754/96/060554-04$1.50 + 0.20

Differences in membrane ion transport between hepatocytes from the periportal and the pericentral areas of the liver lobule

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Received 7 December 1994; received after revision 24 August 1995; accepted 26 September 1995

Abstract. We studied the Na^+/K^+ pump, Na^+/K^+ ATPase activity, and oxygen consumption (QO_2) in hepatocytes isolated from the periportal (PH) and pericentral (CH) regions of the liver lobule, to provide an insight into the functional properties of these cells. Na^+/K^+ pump activity was determined using $^{86}Rb^+$ (a functional analog of K^+) and ouabain, a specific inhibitor of this transport system. Our results indicate the the Na^+/K^+ pump and Na^+/K^+ ATPase activity are significantly lower in CH than in PH, although basal ouabain-sensitive (OS) QO_2 was negligible in both of these cell preparations. However, $OSQO_2$ was significantly lower in CH than in PH when the Na^+/K^+ pump was activated using the ionophore nystatin in a Na^+-containing medium. These results indicate that the differences in membrane ion transport exist between hepatocytes from different locations of the liver lobule.
Key words. Ion transport; hepatocytes; liver.

Differences in structure and function have been found between hepatocytes located in different regions of the liver lobules. Morphometric studies have shown a greater number of lysosomes and peroxisomes in the cells located closer to the central vein (pericentral hepatocytes) than in those located closer to the portal vein (periportal hepatocytes)[1]. Similarly, mitochondria in pericentral hepatocytes are more numerous but smaller in size than the mitochondria in the periportal hepatocytes[1]. The activities of various key enzymes also show significant differences. For example, alanine amino transferase activity shows a 1.89 ratio between periportal and pericentral cells while glutamate dehydrogenase activity shows a periportal to pericentral ratio of 0.71. Key enzymes of glucose uptake are localized predominantly in the pericentral cells while those of glucose release are situated preferentially in the periportal cells. Oxidative enzyme activity is also higher in the periportal than in the pericentral cells of the liver lobule[2]. These differences are assumed to be related, at least in part, to the gradients in concetration of oxygen, metabolites an/or hormones present in the sinusoid[2–5]. It is known that hormones such as insulin, glucagon, and epinephrine are degraded during passage through the liver and, therefore, hormone concentration gradients are formed between periportal and pericentral areas of the liver lobule. Further, the rate of destruction for some of these hormones is different, and the hormone ratios change. The rate of hepatic insulin degradation appears to be lower than that of glucagon. The pericentral region is, therefore, under the influence of a higher insulin/glucagon ratio than the periportal zone[2].

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The activity of the Na^+/K^+ pump, one of the most important transport systems of cells, is reportedly, regulated by various hormones such as insulin, glucagon, epinephrine, and the thyroid hormones[6–8]. Since, as stated above, the concentration of these hormones in the sinusoidal blood changes from the periportal to the pericentral zones[2], it is possible that Na^+/K^+ pump activity is different in periportal and in pericentral hepatocytes. Evidence on Na^+/K^+ pump activity in these types of cellular perparations has been lacking. To provide this basic information we have studied the activity of this transport system in isolated pericentral and periportal hepatocytes, by evaluating $^{86}Rb^+$ transport, Na^+/K^+ ATPase activity, and oxygen consumption. Ouabain was used as a specific inhibitor of this transport system.

Materials and methods

Materials. Collagenase (type IV), digitonin, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), nystatin, and ouabain were purchased from Sigma Chemical Co. (St. Louis, Mo). Other chemicals were reagent grade and were also purchased from Sigma Chemical Co. $^{22}Na^+$ and $^{86}Rb^+$ were obtained from New England Nuclear (Boston, Ma).

Methods. Adult female Wistar rats (250–300 g body weight) were anesthetized with sodium pentobarbital (40 mg/kg) and hepatocytes from the periportal and pericentral areas of the liver were isolated following a digitonin-collagenase perfusion method[9–11]. This technique is based on region-specific tissue destruction and produces suspensions of hepatocytes enriched in cells from the periportal or pericentral areas of the liver

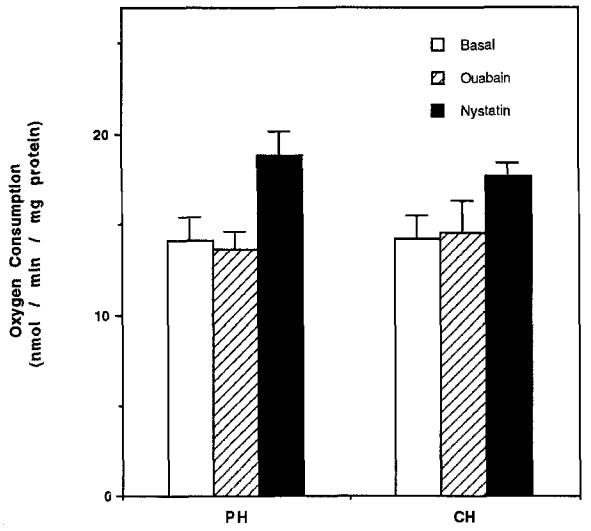

Figure 1. Oxygen consumption of isolated periportal (PH) and pericentral (CH) hepatocytes under basal conditions and in the presence of 1 mM ouabain and 60 μM nystatin. Height of the columns represents mean values of 5 observations in PH and 4 observations in CH. Bars indicate 1 standard error. Basal oxygen consumption was not different between PH and CH and was not changed by ouabain. Addition of nystatin significantly ($p < 0.05$) increased oxygen consumption.

sinusoid. The degree of enrichment was tested by comparing the activity of the enzyme glutamate dehydrogenase in the isolated cells, following a procedure previously described[12].

Oxygen consumption was measured in cells suspended in Krebs–Henseleit buffer (120 mM NaCl, 1 mM KH_2PO_4, 1.2 mM $MgSO_4$, 3 mM $CaCl_2$, 24 mM HCO_3^-, 12.5 mM HEPES, pH 7.4) at 37 °C using a Clark oxygen electrode (Yellow Spring Instruments). Approximately 1.5 million cells were added to 3 ml of buffer that was equilibrated with 95% O_2 and 5% CO_2. Basal O_2 consumption was calculated from the rate of O_2 disappearance from the buffer for 4 to 5 min. At the end of this time ouabain (1 mM) or nystatin (60 μM) was added and O_2 consumption measured as described. In the cases in which nystatin was added after the determination of basal oxygen consumption, this was followed by the addition of ouabain.

Under the conditions described, O_2 consumption was linear during the course of the measurements (no more than 20 min) and at no time did O_2 concentration appear to be limiting. The difference between basal O_2 consumption and that after the addition of ouabain was taken as the O_2 consumption corresponding to Na^+/K^+ ATPase. Oxygen consumption of isolated hepatocytes was measured in the presence of 60 μM nystatin to induce maximal activity of the Na^+/K^+ ATPase[13]. The difference between oxygen consumption in the presence of nystatin and in the presence of nystatin plus ouabain was taken to represent the maximal oxygen consumption due to Na^+/K^+ ATPase activity.

Plasma membranes were isolated as described by Lesko et al.[14] and the ATPase activity of the isolated membranes assayed at 37 °C in a medium containing (in mM) Tris-HCl 50 (pH 7.5), EDTA 1, 5 $MgCl_2$, NaCl 100, KCl 10, and ATP 20. The reaction was started with the addition of the isolated membrane preparation and stopped by the addition of 10% ice-cold trichloroacetic acid. The amount of inorganic phosphate produced was measured by the colorimetric assay of Fiske and SubbaRow at 660 nm[15]. In order to estimate Na^+/K^+ ATPase activity, determinations were performed in the presence and absence of ouabain (10 mM). The Na^+/K^+ ATPase activity was calculated by subtracting from the total ATPase activity the one obtained in a K^+-free medium in the presence of ouabain.

Na^+/K^+ pump activity was also estimated from measurements of $^{86}Rb^{-+}$ uptake. Briefly, hepatocytes were incubated in Krebs–Henseleit buffer with and without ouabain (10 mM) for 10 min at 37 °C. The influx was started by the addition of hepatocytes to eppendorf tubes containing Krebs–Henseleit buffer with $^{86}Rb^+$ (1.5 μCi $^{86}Rb^+$/ml). Aliquots were then taken at 0, 30 and 60 s and spun immediately at 13,600 × g. These times were chosen on the basis of previous experiments in which it was determined that $^{86}Rb^+$ uptake was linear for up to 5 min. The pellets were washed 3 times with a cold 0.1 M $MgCl_2$ solution and radioactivity counted in a gamma counter (Beckman). The difference between total $^{86}Rb^+$ uptake and that in the presence of ouabain was considered to be an estimate of K^+ uptake by the Na^+/K^+ pump. All these procedures were done in triplicate and the data are expressed as nmol/hr/mg of protein. Details of this procedure are described elsewhere[6].

Na^+ influx was estimated using a similar procedure to that used in the $^{86}Rb^+$ uptake experiments with the exception that Na^+ concentration in the buffer was reduced to 50 mM. This was done to increase the specific activity of $^{22}Na^+$ (13 μCi/ml). Hepatocytes were incubated with ouabain (2 mM) to inhibit active Na^+ transport. The cells were added to the medium containing $^{22}Na^+$ and samples were taken at 0, 30, 60 and 90 s and spun immediately at 13,600 × g. It was previously found that Na^+ uptake was linear during this time interval. The data are expressed as nmol/hr/mg protein. Details of this procedure are described elsewhere[6].

Results and discussion

The periportal and pericentral hepatocytes were comparable in gross morphological characteristics (size, shape and general appearance) as observed by light microscopy. Viability of the isolated periportal and pericentral cells, estimated by the capacity to exclude a 0.1% trypan blue solution, was similar and typically between 85 and 94%. Activity of glutamate dehydrogenase, an enzyme known to have different activities in different

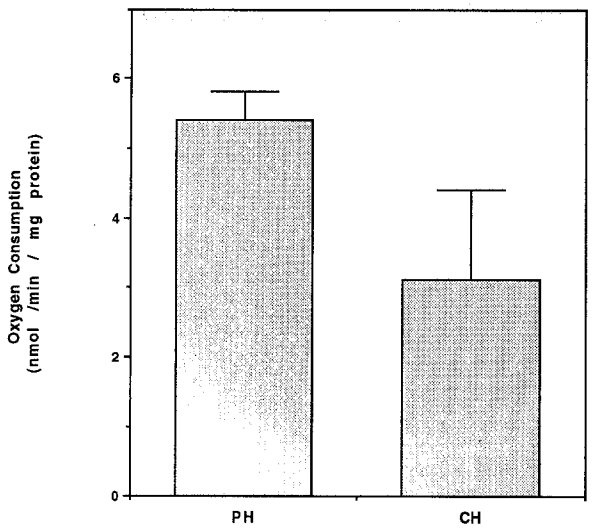

Figure 2. Ouabain-sensitive oxygen consumption of periportal (PH) and pericentral (CH) hepatocytes after nystatin treatment. Height of the columns represent mean values of 5 and 3 observations in PH and CH respectively. Bars indicate 1 standard error. Oxygen consumption was measured in isolated PH and CH in the presence of 60 μM nystatin and after the addition of 1 mM ouabain. Ouabain significantly reduced ($p < 0.05$) oxygen consumption in both cell preparations. However, the inhibition of oxygen consumption by ouabain was significantly greater in PH than in CH ($p < 0.05$).

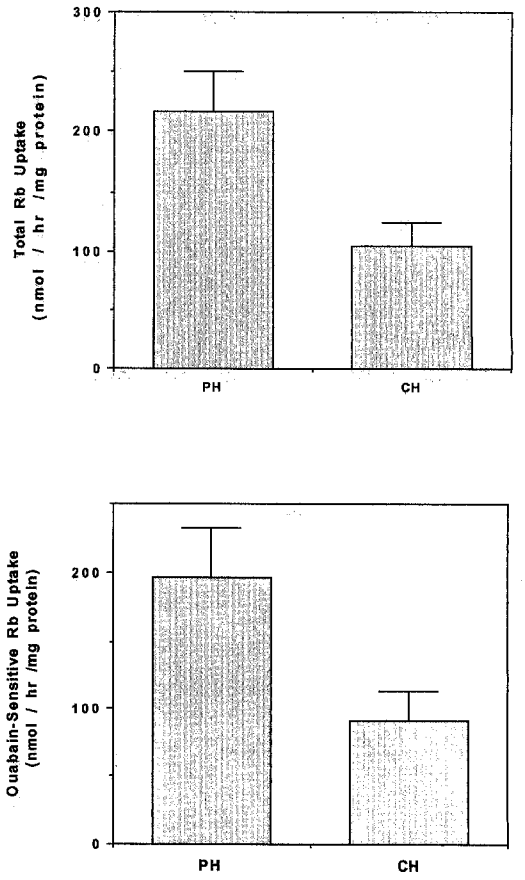

Figure 3. Total (upper panel) and ouabain-sensitive (lower panel) $^{86}Rb^+$ uptake by isolated periportal (PH) and pericentral (CH) hepatocytes. Height of the columns represents mean values of 3 observations. Bars indicate 1 standard error. Total and ouabain-sensitive $^{86}Rb^+$ uptake was significantly ($p < 0.05$) greater in PH than in CH.

parts of the liver lobule[5], was 3 times greater in the isolated pericentral than in the isolated periportal cells (2288 ± 48 vs 682 ± 238 nmol of NAD reduced/min/mg protein). In another set of experiments in which the same cell isolation procedure was used, we found that the activity of alanine amino transferase was 1.7 times greater in the periportal than in the pericentral cells (517 ± 40 vs 310 ± 28 nmol of NADH oxidized/min/mg protein). This agrees with previous reports on the activity of this enzyme in periportal and pericentral hepatocytes[5]. Thus, it would seem that the procedure did produce two types of suspensions of hepatocytes, one rich in periportal and the other rich in pericentral hepatocytes.

Basal oxygen consumption was similar between periportal and pericentral hepatocytes and was not affected by 1 mM ouabain in either cell type (fig. 1). This agrees with previous observations[16–19], suggesting that in hepatic tissue or in isolated hepatocytes the proportion of the basal respiratory rate that is sensitive to ouabain is small and, many times, undetectable. Since intracellular Na^+ might not be at saturating concentrations for maximal pump activity, the effect of nystatin on oxygen consumption was evaluated. As expected, addition of nystatin resulted in a significant increase in oxygen consumption in both groups of cells (fig. 1). Under this condition, addition of ouabain produced a decrease in oxygen consumption that was greater in periportal than in pericentral cells (fig. 2). This would indicate a greater pump capacity in periportal cells than in the pericentral cells. Further, the reduction in oxygen consumption by ouabain in the nystatin-treated cells is of relevance since it demonstrates that when the Na^+/K^+ pump is maximally activated, ouabain, in the concentration and under the conditions used, is capable of reducing oxygen consumption. Nobes et al.[19] also found that 1 mM ouabain rapidly inhibits Na^+/K^+ ATPase and the proportion of oxygen consumption attributable to this enzyme.

Results from $^{86}Rb^+$ uptake experiments (fig. 3) indicate that, under basal conditions, active transport of $^{86}Rb^+$ is present in both cell types even though, under these conditions, no ouabain-sensitive oxygen consumption could be detected (fig. 1). Estimated oxygen consumption due to basal $^{86}Rb^+$ uptake, assuming a P/O ratio of 3 and an K^+/ATP ratio of 2, is 0.5 nmol/min/mg protein. This small amount is not easy to detect and probably explains the observed lack of effect of ouabain on oxygen consumption by the cells. Our results also show that both total and ouabain-sensitive $^{86}Rb^+$ up-

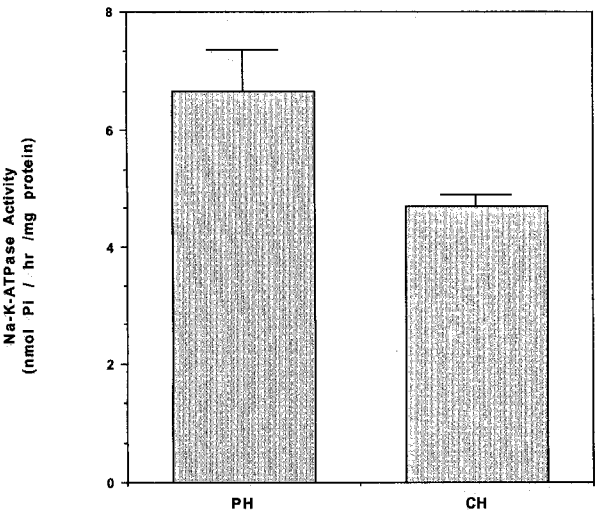

Figure 4. Na^+/K^+-ATPase activity in isolated membranes from periportal (PH) and pericentral (CH) hepatocytes. Height of the columns represents mean values of 3 observations. Bars indicate 1 standard error. Na^+/K^+-ATPase activity was significantly ($p < 0.05$) higher in PH than in CH.

take (fig. 3), and Na^+/K^+-ATPase activity (fig. 4) are higher in periportal than in pericentral hepatocytes. The Na^+/K^+-ATPase results support those of ouabain-sensitive oxygen consumption in the presence of nystatin (fig. 2) and are further evidence that the capacity of the Na^+/K^+ pump is higher in the periportal than in the pericentral hepatocytes. Further, the results of ouabain-sensitive $^{86}Rb^+$ uptake indicate that Na^+/K^+ pump acitivty is also higher in the periportal than in the pericentral hepatocytes.

To correlate the transport properties of the hepatocyte membrane with the basal membrane permeability to sodium, the entry of this cation was determined in hepatocytes treated with ouabian using $^{22}Na^{+6}$. In periportal hepatocytes, sodium entry was approximately 150 ± 30 ($n = 4$) nmol/hr/mg protein while it was 120 ± 21 ($n = 4$) nmol/hr/mg protein in the pericentral hepatocytes. Thus, basal $^{22}Na^+$ entry in pericentral hepatocytes is about 20% lower than that observed in periportal hepatocytes. Although the difference did not reach statistical significance, the tendency correlates with the higher $^{86}Rb^+$-uptake in the periportal cells.

The results of this study, concerning differences in the Na^+/K^+ pump activity in pericentral and periportal hepatocytes, are in line with available evidence indicating other differences in structure, function and biochemistry between these two populations of hepatocytes. The significance of our findings is not clear, but quite probably, some of the differences observed represent functional adaptations to normal changes in the composition of the blood bathing them. A lower Na^+ permeability and Na^+/K^+-ATPase activity of the pericentral hepatocytes is likely to result in reduced energy demands to maintain the ionic gradients across the cell membrane. This functional adaptation could be significant for cells that are exposed to lower oxygen concentrations[3]. Finally, it seems clear that, although studies with heterogeneous populations provide important information on hepatic function, they lack relevant data on the relationship between structure and function of the liver.

Acknowledgements. This work was supported in part by NIH grants GM-08224 and RR-03051.

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