Access of reabsorbed glucose to renal lymph[1]

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Summary. Measurements of the glucose to mannitol tracer concentration ratios in renal venous blood and renal lymph of rats supported the hypothesis that reabsorbed glucose may have direct access to renal lymph by passage through the interstitium.

Recent morphological investigations have contributed to the clarification of the structural relationship between the renal cortical interstitium and the lymphatic system. Thus, under normal conditions the volume of the interstitium has been measured by morphometric techniques and was found to constitute approximately 4–7% of the renal cortex[2–4]. The interstitial space appears as variably narrow slits, and at some other places as wider gaps between tubules and peritubular blood capillaries. Lymphatic vessels have been seen to originate regularly in the loose connective tissue around the interlobular blood vessels[5] and in the vicinity of glomeruli[6]. The morphological evidence thus suggests that the interstitium in the renal cortex through which the bulk of tubular reabsorbate flows, may be separated by a considerable distance from the initial lymph capillaries. Functionally, it has been generally assumed that renal lymph is derived from the cortical interstitium. However, it has not as yet been determined whether those interstitial spaces through which tubular reabsorbate flows do contribute to renal lymph.

The experiments described here were aimed at the finding of a constituent of tubular reabsorbate, specifically of reabsorbed glucose, in renal lymph. The results suggest that the concentration of glucose in renal hilar lymph is higher than in renal venous plasma, and that the source of the excess glucose in renal lymph is the tubular reabsorbate.

Methods. Sprague-Dawley rats, weighing between 180 and 250 g, were anesthetized with Inactin, 100 mg/kg b.wt. The femural blood vessels on both sides, the left ureter and a left renal hilar lymphatic vessel were cannulated as described earlier[7]. Blood from the left renal vein was collected by a non-occluding PE-10 catheter. A mixture of ^{14}C-glucose and ^{3}H-mannitol tracers was administered to the rats in constant i.v. infusion. After a near stabilization of the concentration ratio (R) of ^{14}C-glucose/^{3}H-mannitol in arterial plasma serial samples of this ratio in arterial plasma urine, lymph and renal venous plasma were determined (R_{ap}, R_u, R_l and R_{vp}, respectively). Because the molecular weights and structures of glucose and mannitol are nearly identical, it was assumed that in the kidney both these tracers are affected equally by diffusion and convective transport. This assumption was confirmed experimentally after the administration of phlorizin (40 mg/kg): When the tubular reabsorption of glucose was blocked by phlorizin, there was no difference between the ^{14}C-glucose/^{3}H-mannitol ratios in arterial and renal venous plasma, urine and renal lymph, i.e. $R_{ap} \approx R_{vp} \approx R_l \approx R_u$.

Results. The results are illustrated in the table. Because R_{ap}-values in different experiments depended only on the arbitrary proportions of both tracers in the infused mixture, R_{ap} was taken as unity and R_{vp} and R_l were normalized to the arterial ratio in each experiment. The figures in the table were calculated as follows: within each animal for each kind of sample an average was calculated, and then these figures were averaged over animals (n=8). The standard deviations shown reflect the animal-to-animal variability. For higher discrimination by statistical analysis paired comparisons were made within each animal. The table shows that R_{vp} exceeded R_{ap} by approximately 21%. The higher ratio in renal venous plasma signified that the renal venous concentration of mannitol was lower than the arterial since the concentrations of glucose tended to be equal in arterial and renal venous plasma. The lower mannitol concentration in renal venous plasma indicated renal extraction and urinary excretion of mannitol; the figure shown corresponds to an approximately 20% filtration fraction. The urine contained a relatively high concentration of mannitol and only traces of glucose; thus R_u was nearly zero.

Glucose-^{14}C/mannitol-^{3}H ratios (mean ± SD; n = 8)

Arterial plasma	1.000 (all values normalized)
Venous plasma	1.213 ± 0.160
Renal hilar lymph	1.280 ± 0.132

The table shows also that R_l was slightly higher than R_{vp}. The statistical comparison of the venous and hilar lymph ratios was carried out within each animal by means of both the t-test and the Mann-Whitney U-test. The p-values obtained by using the Mann-Whitney test were combined according to the statistical method of Pearson-s P_λ[8]. The combined p for comparing the over-all R_l and R_{vp} values turned out to be less than 0.01. (The combination of p-values derived from the t-tests led to an even lesser probability.) These results indicated that in a quasi steady state condition the glucose to mannitol ratio in hilar lymph tended to be slightly higher than in renal venous plasma.

As noted above, this was not the case after the administration of phlorizin when R_l was equal to R_{vp} (and also to R_{ap} and R_u). Therefore, in the absence of phlorizin, the difference between R_l and R_{vp} was dependent on the tubular reabsorption of glucose.

Discussion. In the normal kidney under steady state conditions the interstitial concentration of glucose must exceed the intravascular plasma concentration since this gradient is the driving force for the net entry of glucose into the peritubular capillaries from the interstitium. Our finding of $R_l > R_{vp}$ was in agreement with this premise. It shows a tendency of lymph to equilibrate with interstitial fluid containing tubular reabsorbate, leading to a high R_l. Although downstream a subsequent diffusion between intrarenal lymphatics and small veins cannot be disregarded, the results indicate that this subsequent diffusion does not fully dissipate the difference between R_l und R_{vp}.

These experiments specifically suggest that renal lymph contains reabsorbed glucose. In general they support the hypothesis that renal lymph carries information from such parts of the renal cortical interstitium through which reabsorbate flows from the tubules into the peritubular capillaries.

Peterson et al.[9] recently reported that in the renal capsular lymph of dogs the glucose concentration is higher than in simultaneously sampled renal venous plasma. They interpreted their finding in the context of gluconeogenesis by the renal cortical tissues. Our experimental finding showing that such a difference exists under conditions of steady infusion of tracer glucose, raises the possibility that the

result of Peterson et al. obtained in dogs can also be ascribed to a direct access of reabsorbed glucose to renal capsular lymph.

1 Acknowledgment. This research was supported by grant No. AM-17093 of the USPHS.

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Cholinergic mechanisms in the production of focal cortical slow waves[1]

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Summary. Microiontophoretic application of scopolamine and atropine usually induced or increased focal cortical slow waves of under 3 Hz and abolished or decreased focal fast waves of over 6 Hz whereas acetylcholine iontophoresis and electrical stimulation of the mesencephalic reticular formation had the opposite effect, suggesting that focal cortical slow waves may be due to the interruption of cholinergic input from the reticular formation.

The mechanisms which produce focal slow waves in the EEG are poorly understood. Because focal slow waves appear at the site of lesions which destroy white matter underlying the cortex, it has been suggested that these slow waves are due to partial cortical deafferentation[3,4]. The observation that systemic administration of atropine, a blocker of muscarinic cholinergic receptors, induces generalized slow waves[5-7] has led to the suggestion that slow waves may result from an interruption of normal cholinergic input to the cortex[8]. Since slow waves can be suppressed by electrical stimulation of the midbrain reticular formation (MRF)[9] which projects to the cortex at least in part through cholinergic fibers[10], one may ask whether focal slow waves may be due to the interruption of cholinergic input from the MRF to the cerebral cortex. This idea finds some support by experiments showing that injection of atropine into cortical arteries causes local slow waves which are reduced by stimulation of the MRF[11]. Because systemic applications of atropine cannot determine the site of drug action and because topical application of atropine to the cortical surface causes local spikes[12], we attempted to answer this question by studying the effects of microiontophoretic application of acetylcholine (ACh) and its antagonists on the local cortical EEG and by comparing these effects with that of MRF stimulation.

22 cats were prepared under inhalation anesthesia with a mixture of oxygen, nitrous oxide and halothane. The spinal cord was transected at C1 and the animal was ventilated with an air-oxygen mixture. Wound margins and pressure points were infiltrated with local anesthetic. Blood pressure, heart rate, temperature and CO_2 level were monitored. Eight-barreled micropipettes with tip diameters of 3–5 µm and with protrusions of the central recording tip of up to 60 µm[13] were used. The recording barrel contained potassium citrate (2 M, pH 7), the surrounding barrels contained scopolamine hydrobromide (1 M, pH 5.5), atropine sulfate (0.5 M, pH 7.3), ACh chloride (1 M, pH 4.3) and sodium chloride (3 M, pH 7). Drugs were expelled for periods of 32–64 sec with currents of usually 5×10^{-8} A and retained with currents of opposite polarity up to 1×10^{-8} A between applications. A bipolar stimulating electrode was inserted into the MRF using a stereotaxic atlas[14] and a frame of David Kopf Instruments (A 3 to 4, L 3 to 5, D 0 to −2). The location of the stimulus electrode was verified postmortem.

The micropipette was inserted into the pericruciate cortex. The EEG was recorded from tip positions at depths of up to 2 mm for at least 1 min before, during and after microiontophoretic drug applications. The EEG was also recorded during periods of intermittent stimulation of the MRF with electrical pulses of 0.01 msec and up to 25 V, delivered in trains of 30 stimuli at 300 Hz; the trains were repeated every 2–5 sec. Recordings were stored on magnetic tape and later filtered to eliminate neuronal action

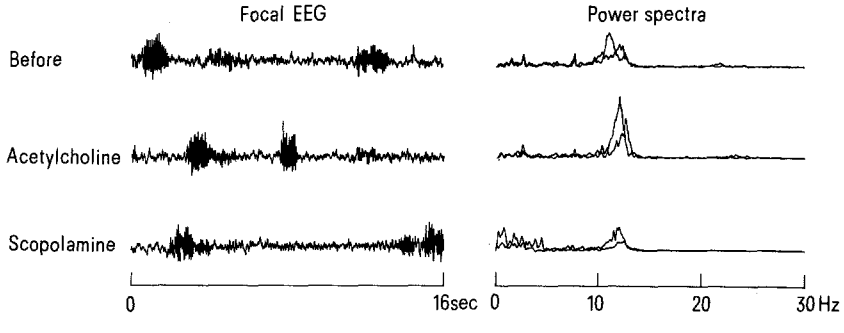

Figure 1. Left: Focal EEG at the micropipette tip during 16 sec of recording before drug application, during iontophoretic application of acetylcholine (5×10^{-8}A) and of scopolamine (5×10^{-8}A). Right: Power spectra computed for 32-sec epochs of EEG recording; 2 power spectra are superimposed for each condition.