Inner medullary lactate production and accumulation: a vasa recta model

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THIS STUDY EXPLORES the possibility that countercurrent recycling of lactate produced continually in the renal inner medulla (IM) by anaerobic glycolysis might lead to an axial gradient of interstitial osmoles. By serving as “external osmoles” (i.e., external to the tubular fluid), such osmoles could contribute to the single effect for countercurrent multiplication in the IM.

The extent to which a metabolically produced external solute can accumulate an axial interstitial (and vasa recta) concentration gradient by countercurrent exchange will depend on at least four factors: its rate of production within the medulla, its descending vasa recta permeability (a high value favors recycling), inner medullary blood flow (IMBF) (low IMBF favors decreased transport across vasa recta). This study explores the possibility that countercurrent recycling of lactate produced continually in the renal inner medulla (IM) by anaerobic glycolysis might lead to an axial gradient of interstitial osmoles. By serving as “external osmoles” (i.e., external to the tubular fluid), such osmoles could contribute to the single effect for countercurrent multiplication in the IM.

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1 I am not speaking here of the intracellular osmoles that protect IM cells from high external urea and salt concentrations.
accumulation by minimizing washout; Ref. 53), and volume uptake from the nephron system. It has long been known that energy for the buildup of the IM osmotic gradient could theoretically be supplied not only by recycling of urea and NaCl (the crux of the “passive hypothesis”; Refs. 30 and 52) but alternatively (or in addition) by “external,” or interstitial, osmoles (24, 31, 37, 46), that is, solutes present in interstitial fluid but not in tubular fluid of the loop of Henle or collecting duct. Using a detailed three-dimensional model of rat medulla to estimate the amount of such external osmoles needed to boost concentrating ability, we calculated (55) that 100 mosmol/kgH2O of interstitial solutes would considerably increase the axial IM osmotic gradient. The literature has remained mute as to the identity or possible source of such hypothetical interstitial osmoles, which would have to be produced continuously within the IM and recycled downward by the vasa recta to accumulate significantly toward the papilla.

I recently proposed (54) that IM glycolysis may contribute to such a pool of external osmoles, since it is known that in the relatively hypoxic (49) IM, a large fraction of the energy for cell metabolism is supplied by anaerobic glycolysis (32, 47), which yields two lactate molecules (and two protons) for each glucose molecule; that is, anaerobic glycolysis produces net osmoles as a matter of course. Anecdotally, this phenomenon is the reason for corneal swelling under hypoxic conditions (27).

Having suggested that IM metabolic osmole production may contribute to the IM single effect for urine concentration, one immediately wonders how the effect would depend on the animal’s diuretic state. It seems unlikely that cell metabolism in the IM, which is just housekeeping since there is no known active epithelial transport in this region except in the collecting ducts, should vary with the organism’s water balance; that is, there is no reason to suppose a priori that IM glycolytic rate should vary as a function of the animal’s salt and water balance, so its involvement in the concentrating mechanism appears hard to rationalize. However, studies of the IM microcirculation (e.g., 19, 35, 36, 50, 62) have shown that the IM blood supply may vary in response to perturbations of the organism’s water balance, being reduced under antidiuretic conditions. Also, it is clear from earlier modeling studies (see review by Stephenson (53)) that net flow rate through a countercurrent exchange system determines the extent to which intrinsic osmole production will build up an axial gradient, with low flow being favorable to steeper gradients.

The crux of my conjecture is thus: first, that intrinsic metabolic osmole production may be sufficiently high and IM blood flow sufficiently low, especially in antidiuresis, to result in significant osmole accumulation by countercurrent exchange, and second, that this could contribute importantly to the urinary concentrating mechanism. The present work addresses the first of these.

The present study uses a mathematical model of inner medullary vasa recta (IMVR), first, to discover conditions under which lactate produced by IM anaerobic glycolysis might accumulate a significant axial concentration gradient and, second, to propose experimental tests of this idea. Using a conservative estimate of IM glycolytic lactate production, based on glycolytic rates measured in the kidney and in other tissues, I calculate projected axial IM gradients of lactate and glucose for a range of assumed vasa recta permeabilities to lactate and glucose. I also briefly treat the case of species with different percentages of long loops reaching all the way to the papillary tip. I find it plausible that lactate accumulation could be considerable in the deep IM, though volume uptake from the nephrons (resulting in part from the metabolic osmole production) will limit the tendency. It remains for one to apply this idea in medullary models incorporating the nephrons and collecting ducts explicitly to see how this extratubular metabolic osmole production might affect salt and urea recycling and, thus, the IM osmotic gradient.

MODEL DESCRIPTION

Since the nephron is essentially impermeable to small sugars, glucose and lactate are distributed only among the interstitium, the microcirculation, and of course the cytoplasm of IM cells (i.e., interstitial cells, epithelial cells, and cells of the capillary walls). If, in addition, we consider only the steady state, we can estimate IM recycling and accumulation of glucose and lactate using a simple model of IMVR, lumping the interstitium with ascending vasa recta (AVR) and excluding nephrons and collecting ducts. The important parameter with respect to the question of a single effect for the IM axial osmotic gradient is the interstitial concentration of supposed external osmoles, but interstitial concentrations are hard to handle both experimentally and theoretically. I thus limit this modeling study to consideration of steady-state glucose and lactate flows in descending vasa recta (DVR) and AVR, with a conservative range of estimated glycolytic rate within the IM, assumed here not to depend on diuretic state. The “AVR” represent the interstitium as well as the ascending vasa recta. Previous models specifically investigating vasa recta flow and exchange (14, 15, 34, 56) addressed the relative importance of hydrostatic vs. osmotic pressure, protein oncotic force, dissimilarity between DVR and AVR, likely roles of water channels and urea transporters, importance of varying number of vessels with medullary depth, and other issues. Compared with those earlier models, the present study adopts a simpler model, since it addresses the simpler issue of accumulation by recycling of solutes excluded from the nephrons.

Mass balance constraints require that total glucose consumption and lactate production in all cells within a slice of medulla at a given depth x must, in the steady state (and independent of considerations of reduced numbers of vessels with depth), equal the net differ-
ence of vasa recta glucose and lactate outflows and inflows through the slice, so by restricting the analysis to the steady state, one sidesteps the difficulty of estimating glucose and lactate distributions between cell cytoplasm and interstitium. As stated above, I make the further assumption that AVR concentrations are equal to interstitial concentrations. This model thus says nothing about intracellular glucose or lactate concentrations. Figure 1 schematically depicts the model.

Since in vivo flows and concentrations are unknown at the OM/IM border, I set up the baseline case according to predictions of our recent calculations with a three-dimensional model of the whole medulla (54, 57).

Anatomy. DVR and AVR are assumed to diminish exponentially in number along the IM toward the tip of the papilla according to the same relation as in our earlier models and in conformity with reported rat anatomy (28), i.e.

$$N(x) = N(0)e^{-k_{sh}x}$$  \hspace{1cm} (1)

with $k_{sh} = 1.213$ mm$^{-1}$. Thus, compared with the number of tubes entering the IM, the fraction of tubes reaching the papillary tip is $1/128$ for $L = 4$ mm at the tip and $x = 0$ at OM/IM border.

Other species have different proportions of tubes and vessels extending to the tip. I investigate possible implications of this by running simulations with different values of $k_{sh}$.

The number of entering DVR, $N(0)$, was nominally set at 128, so the simulation represents behavior of a system that gives a single DVR at the papillary tip. Qualitatively, the model behavior is in fact independent of the numerical value chosen for this parameter, depending instead on the loop distribution, determined by $k_{sh}$. Everything is scaled to this assumption, in particular, glycolytic glucose consumption (and lactate production) is expressed as percent of glucose inflow, and DVR volume reabsorption and net volume uptake into AVR from nephrons and collecting duct are expressed as percent of DVR inflow. By this strategy, the model can represent kidneys containing any number of vasa recta simply by varying the medullary length and/or the factor describing the exponential decrease of their number with depth ($k_{sh}$).

Inflows. Baseline volume flow into DVR is set to 3.75 nl·min$^{-1}$·tube$^{-1}$, the DVR flow at the OM/IM border in (57). The glucose and lactate concentrations entering the IM DVR are set at 10 and 2 mM, respectively, i.e., $c_{GLU}(0) = 10$ mM, $c_{LAC}(0) = 2$ mM.

Mass balance considerations. Conservation of matter in the steady state requires that in any slice of IM extending from depth $x_1$ to depth $x_2$, with $x_2 > x_1$, we must have, taking glucose flows as an example, the following: (rate of glucose entry into the slice) − (rate of glucose exit from the slice) = (rate of glucose conversion to lactate within the slice), i.e., accounting for both DVR and AVR flows through the slice and noting that ascending flows carry a negative sign

$$\left(F_{GLU}^{DVR}(x_1) - F_{GLU}^{AVR}(x_2)\right) - \left(F_{GLU}^{DVR}(x_2) - F_{GLU}^{AVR}(x_1)\right) = \int_{x_1}^{x_2} J_{gly}(x) \, dx$$  \hspace{1cm} (2)

For lactate balance, since each converted glucose molecule yields two lactate molecules, we have the following: (rate of lactate exit from slice) − (rate of lactate

![Fig. 1. Schematic representation of the steady-state model of IMVR. Dotted walls for AVR indicate that it represents interstitium as well as AVR. See Glossary for complete description of abbreviations.]
entry into slice) = \( 2 \times \) (rate of glucose conversion to lactate within slice), i.e.

\[
\frac{F_{\text{DVR}}^\text{LAC}(x_2) - F_{\text{AVR}}^\text{LAC}(x_1)}{2} - \frac{F_{\text{DVR}}^\text{LAC}(x_1) - F_{\text{AVR}}^\text{LAC}(x_2)}{2} = \int_{x_1}^{x_2} J_{\text{gly}}(x) \, dx
\]  

Integrating all the way to the tip from any point \( x \), and given the continuity condition at the papillary tip, namely

\[
F_{\text{DVR}}^i(L) = -F_{\text{AVR}}^i(L), \text{ for } i = \text{volume, glucose, or lactate}
\]

we have net glucose consumption from any depth \( x \) to the tip equal to

\[
F_{\text{DVR}}^\text{GLU}(x) + F_{\text{AVR}}^\text{GLU}(x) = \int_x^L J_{\text{gly}} \, dx
\]

and net lactate production from \( x \) to the tip is

\[
-(F_{\text{DVR}}^\text{LAC}(x) + F_{\text{AVR}}^\text{LAC}(x))/2 = \int_x^L J_{\text{gly}} \, dx
\]

In particular, for the IM as a whole

\[
F_{\text{DVR}}^\text{GLU}(0) + F_{\text{AVR}}^\text{GLU}(0) = \frac{F_{\text{DVR}}^\text{LAC}(0) + F_{\text{AVR}}^\text{LAC}(0)}{2} = \int_0^L J_{\text{gly}} \, dx
\]

From this, it is straightforward to show that the maximum rate of osmole addition to the IM from anaerobic glycolysis, if all the glucose were converted to lactate, in which case \( F_{\text{GLU}}^\text{AVR}(0) = 0 \), would equal the rate of entry of glucose into the IM in the DVR. The actual IM rate of glycolysis must be only a fraction of this maximum.

Prediction of the gradients of glucose and lactate concentration along the vasa recta is the purpose of what follows.

**Estimate of glycolytic rate.** Although extraction of a consensus estimate for IM glycolytic rate from the literature is subject to the usual difficulties, the following two facts seem clear: the IM is relatively hypoxic [\( \text{PO}_2 \sim 10 \) Torr (33)], and anaerobic glycolysis supplies a substantial part of the energy budget, with lactate clearly being produced in the IM, whereas it is consumed in the cortex (3), although complete dependence of IM on anaerobic glycolysis is a myth (10). In what follows here, I attempt a best, albeit conservative, estimate of fractional IM glucose consumption.

Quoting from Ross and Guder (47, p. 393): “The high rate of glycolysis observed in [rat] papillary slices corresponds to the highest local activity of hexokinase. It may be calculated that this enzyme activity, expressed in terms of dry weight of tissue, exceeds by a factor of 2 the maximum rate of glycolysis which has been recorded, i.e., 800–1,000 \( \mu \text{mol/hr/g dry weight} \) lactate production under anaerobic conditions.” Their table II shows about 100 ng dry weight/mm tubule, so this converts to 1.67 pmol/min \(^{-1}\)-mm of tubule \(^{-1}\), which I consider an extreme upper limit on glucose consumption.

To compare glucose consumption with its delivery rate, I consider a minimal group, served by each DVR, to correspond roughly to seven tubule equivalents, as follows: DVR, two AVR, long descending limbs (LAL), IM collecting ducts (IMCD), plus some interstitial cells. I thus estimate maximal glycolytic rate of a minimal group as roughly seven times that of one tubule, or 11.7 pmol/min \(^{-1}\)-mm \(^{-1}\). This need must be met by the input from one DVR: given my base case assumptions of 10 mM glucose and 3.75 nI/min inflow rate to DVR entering the IM, the glucose supply for each group is 37.5 pmol/min. This rough estimate of maximal metabolic glucose consumption thus implies that all entering glucose would be consumed in a group of length 4 mm, the IM length of the longest loops in a rat kidney. However, in the rat kidney, most long loops turn back before reaching the papillary tip. Integration over the whole depth of IM using our assumed loop distribution for rat kidney (Eq. 1, above) predicts a maximal total IM consumption of 25% of the entering glucose. This equals the estimate made by Ruiz-Guinazu et al. (48).

An alternative approach to estimation of IM glycolytic rate is to estimate IM energy requirements. A minimal estimate (it ignores basic cell metabolism needs) can be based on measurements of total IMCD sodium transport, since IMCD are the only demonstrated site of IM active transepithelial transport. These measurements were recently summarized in association with a model of IMCD transport (table 4 of Ref. 58). Taking a conservative estimate of reabsorption of, say, 2% of filtered \( \text{Na}^+ \) from the IMCD and glomerular filtration rate (GFR) of 500 \( \mu \text{l/min} \) gives

\[
\frac{500 \, \mu \text{l}}{\text{min}} \times \frac{0.140 \, \mu \text{mol Na}^+}{\mu \text{l}} \times 0.02 = 1.4 \, \mu \text{mol Na}^+ \text{ min}^{-1}
\]

If anaerobic glycolysis were the only source of ATP (but see below), and given transport of 3 \( \text{Na}^+ \) per ATP and production of 2 ATPs per glucose, then this would amount to consumption of \( \sim 240 \) nmol glucose/min, or 1.6 \( \mu \text{mol min}^{-1} \cdot \text{g wet wt}^{-1} \) of IM, if the IM represents 10% of a 1.5-g rat kidney. This is higher than direct measurements of lactate production in rabbit medulla (32), which reported values of 0.2 \( \mu \text{mol min}^{-1} \cdot \text{g wet tissue}^{-1} \) in the presence of oxygen and 0.4 \( \mu \text{mol min}^{-1} \cdot \text{g wet tissue}^{-1} \) under \( \text{N}_2 \) perfusion, but it is much lower than measured anaerobic lactate production in guinea pig medulla, namely 6.2 \( \mu \text{mol min}^{-1} \cdot \text{g wet tissue}^{-1} \) (from Refs. 18 and 13, cited in Ref. 10). We can compare these consumption rates to an estimate of IM glucose delivery rate as follows. Estimates of papillary plasma flow (PPF) in antidiuretic rats range from 0.3–0.5 \( \text{ml min}^{-1} \cdot \text{g wet tis-}
GLYCOLYSIS AND THE URINE CONCENTRATING MECHANISM

If glucose concentration in blood entering the IM is 10 μmol/ml (i.e., 10 mM), then glucose delivery to IM is around 3 to 5 μmol·min⁻¹·g wet tissue⁻¹, or double to triple the above estimate of minimal expenditure. Thus it would appear that glucose (via anaerobic glycolysis) could in principle suffice as the IM energy supply. Nonetheless, despite the relative hypoxia of the IM, anaerobic glycolysis is in fact only responsible for perhaps one-third of the energy supply (6).

Based on these considerations, I adopt a baseline glucose consumption of 20% of IM delivery and investigate a wider range of values in the sensitivity studies reported below.

System equations. The model treats steady-state flows and exchanges of volume, glucose, and lactate along DVR and AVR. The interstitium and cells (epithelial and interstitial) are assimilated with AVR. I assume that glucose consumed by cellular glycolysis is supplied from AVR and that the resulting lactate is recovered into AVR/interstitium. Also included is net volume reabsorption into the AVR from LDL and IMCD, designated as \( J_{\text{AVR}}(x) \).

The system is subject to the continuity condition at the papillary tip (Eq. 4) and is described by the following system of six differential equations:

\[
\frac{dF_{\text{DVR}}(x)}{dx} = -J_i(x) - k_{sh}F_{\text{DVR}}(x) \\
\frac{dF_{\text{GLU}}(x)}{dx} = J_{\text{GLU}}(x) - k_{sh}F_{\text{DVR}}(x) \\
\frac{dF_{\text{LAC}}(x)}{dx} = J_{\text{LAC}}(x) - k_{sh}F_{\text{LAC}}(x) \\
\frac{dF_{\text{AVR}}(x)}{dx} = J_i(x) + k_{sh}F_{\text{DVR}}(x) + J_{\text{AVR}}(x) \\
\frac{dF_{\text{GLU}}(x)}{dx} = J_{\text{GLU}}(x) + k_{sh}F_{\text{GLU}}(x) - J_{\text{GLU}}(x) \\
\frac{dF_{\text{LAC}}(x)}{dx} = J_{\text{LAC}}(x) + k_{sh}F_{\text{LAC}}(x) + 2J_{\text{LAC}}(x)
\]

where \( k_{sh}F_{\text{DVR}}(x) \) is shunt transfer of \( i \) from DVR to AVR at depth \( x \) (59). \( J_i(x) \) terms are the diffusion transfers (pmol·min⁻¹·mm⁻¹) and osmotic volume flux (nl·min⁻¹·mm⁻¹) at \( x \) from all DVR to AVR. Treating the capillary walls as a single barrier (i.e., no distinction here between transcellular and paracellular transport), we have for glucose and lactate fluxes

\[
J_{\text{GLU}}(x) = N(x)P_{\text{GLU}}\left( c_{\text{GLU}}^{\text{DVR}} - c_{\text{GLU}}^{\text{AVR}} \right) \\
+ (1 - \sigma_{\text{GLU}})J_i(x) \frac{c_{\text{DVR}}^{\text{GLU}} + c_{\text{AVR}}^{\text{GLU}}}{2} \\
J_{\text{LAC}}(x) = N(x)P_{\text{LAC}}\left( c_{\text{LAC}}^{\text{DVR}} - c_{\text{LAC}}^{\text{AVR}} \right) \\
+ (1 - \sigma_{\text{LAC}})J_i(x) \frac{c_{\text{DVR}}^{\text{LAC}} + c_{\text{AVR}}^{\text{LAC}}}{2}
\]

where \( N(x) \) is the number of DVR at depth \( x \) (Eq. 1), \( P_{\text{GLU}} \) and \( P_{\text{LAC}} \) are permeabilities to glucose and lactate, \( \sigma_{\text{GLU}} \) and \( \sigma_{\text{LAC}} \) are reflection coefficients (both set at 0.5 here), and concentrations of solute \( j \) in tube \( i \) are

\[
c_i(x) = \frac{F_i(x)}{F_i'(x)}
\]

Since volume flux along the DVR (\( J_{\text{GLU}}(x) \)) depends on forces not represented in this model, it cannot be calculated explicitly here. \( J_{\text{GLU}}(x) \) is thus taken to be an explicit fraction (30% as baseline value) of entering flow, distributed over the length of the IM in proportion to the number of DVR at each depth. Simulations were run for various fractional volume fluxes.

To avoid unrealistic glycolytic glucose consumption in the event that glucose concentration falls locally to zero in the course of numerical analysis, I describe glycolytic rate simply with a first-degree Michaelis-Menten equation, saturable as a function of AVR glucose concentration, setting \( K_m \) very low (0.1 mM) and \( V_{\text{max}} \) equal to estimated local glycolytic rate

\[
J_{\text{GLY}}(x) = N(x) V_{\text{max}} c_{\text{GLU}}^{\text{AVR}}(x) K_m + c_{\text{GLU}}^{\text{AVR}}(x)
\]

Thus glycolytic rate will be virtually equal to \( V_{\text{max}} \) except for extremely low glucose concentrations. In practice, for exploration of model behavior, I wanted to specify \( V_{\text{max}} \) values that would result in specified fractions of total glucose consumption (rather than specifying glycolytic rates per unit tissue volume or per mm of medullary depth). To this end, assuming \( K_m c_{\text{GLU}}^{\text{AVR}}(x) \) for all \( x \), substituting from Eq. 1 for \( N(x) \), and integrating over the whole IM, total glucose consumption is

\[
J_{\text{GLY}}^{\text{TOT}} = V_{\text{max}} c_{\text{GLU}}^{\text{AVR}}(0) \int_0^L e^{-k_{sh}x} \, dx
\]

\[
= V_{\text{max}} c_{\text{GLU}}^{\text{AVR}}(0) \frac{1 - e^{-k_{sh}L}}{k_{sh}}
\]

where \( L \) is the total length of the IM. Solving this for \( V_{\text{max}} \) and expressing \( J_{\text{GLY}}^{\text{TOT}} \) as a fraction \( \text{GlyFract} \) of total baseline glucose delivery, \( P_{\text{GLU}}^{\text{DVR}}(0) \), we obtain

\[
V_{\text{max}} = \frac{k_{sh}J_{\text{GLY}}^{\text{TOT}}}{N(0)(1 - e^{-k_{sh}L})}
\]

\[
= \frac{k_{sh}}{N(0)(1 - e^{-k_{sh}L})} \left( \text{GlyFract} \cdot P_{\text{GLU}}^{\text{DVR}}(0) \right)
\]

In like manner, \( J_{\text{AVR}}(x) \), the volume reabsorbed from LDL and IMCD, was distributed in proportion to the number of DVR (assumed equal to the number of LDL) at each depth

\[
J_{\text{AVR}}^{\text{TOT}} = V_{\text{max}} \cdot F_{\text{AVR}}^\text{DVR}(0)
\]

By analogy with the treatment of the glycolytic \( V_{\text{max}} \), I express total IM volume absorption as a proportion of entering blood flow, i.e., \( J_{\text{AVR}}^{\text{TOT}} = \text{VolFract} \cdot F_{\text{AVR}}^\text{DVR}(0) \),
and following the development of Eq. 13, we obtain an expression for \( k_v \):

\[
k_v = \frac{k_{sh}}{N(0)(1 - e^{-k_{sh}z}) (VolFract \cdot F^DVR(0))}
\]  

(15)

Parameter values. Table 1 gives baseline parameter values (in dimensions of most literature reports as well as in the dimensions used for the present model).

In the absence of measurements of DVR permeabilities to lactate and glucose (\( P_{LAC} \) and \( P_{GLU} \)), I set the baseline value for \( P_{LAC} \) at \( 100 \times 10^{-5} \) cm/s, which is midway between the measured DVR permeabilities to NaCl and urea used in our recent simulations of the whole rat medulla (57). During the simulations, it became immediately necessary to reduce the glucose permeability well below this value to avoid convergence problems due to near zero glucose concentrations. Baseline \( P_{GLU} \) was thus set 25-fold lower than \( P_{LAC} \). Whether this requirement reflects reality remains to be seen; certainly such low permeability for glucose across the vasa recta wall contradicts the general assumption that such vessels are very leaky to small solutes. Around these baseline values, simulations were run over a wide range of \( P_{LAC} \) and \( P_{GLU} \) values. It is interesting in this context to cite Kean et al. (26): “It should be pointed out that anaerobic metabolism of the renal medulla in vivo, although a satisfactory explanation for the metabolism by which energy is made available despite probable deprivation of oxygen, still poses the significant problem of substrate delivery to the tissue for this type of metabolism. If oxygen exchanges across the limbs of the vasa recta, thereby depriving the deeper portions of the medulla, why does not glucose also exchange in a similar manner?”

\( J_{DVR}^{ABS} \) baseline value is set at 30% of flow into IM DVR. This is the value reported by two studies in antidiuretic rats based on measurement of vasa recta protein concentration at the base and tip of the papilla (38, 63). However, values as high as 117% have been reported (21). This is a crucial parameter for lactate accumulation, as the results below show, but proper investigation must be done in a model of the full medulla that includes not only vasa recta but also nephrons and collecting ducts.

Numerical solution. I programmed Mathematica to solve this nonlinear system of ordinary differential equations by simple shooting, which also amounts to multidimensional Newton-Raphson (43). Briefly, the system of six equations and six unknowns (Eq. 8) was solved subject to three initial conditions (flows into DVR) at \( x = 0 \) and three boundary conditions at \( L \), where \( L \) is the depth at the papillary tip. The three boundary conditions are continuity relations on the flows at the hairpin turn, normalized by the entering flow rate

\[
\frac{F_{DVR}^i(L) + F_{AVR}^i(L)}{F_{DVR}^i(0)} = \text{score}
\]

(16)

where \( i \) is volume, glucose, or lactate. Ideally, \( \text{score} \) equals zero. Using the initial conditions for the three DVR inflows \( F_{DVR}^i(0) \) and guesses for the three AVR outflows \( F_{AVR}^i(0) \), the equations were integrated from 0 to \( L \) (Mathematica’s function NDsolve, which switches automatically between a non-stiff Adams method and a stiff Gear method, based on LSODE). Using Mathematica’s LinearSolve function (which uses LU decomposition), the error vector, \( \text{score} \), from Eq. 16 is used with a numerically determined Jacobian matrix, \( JAC \), to solve for a corrections vector, \( s \), to the guesses for \( F_{AVR}^i(0) \) according to

\[
JAC \cdot s = -\text{score}
\]

(17)

Improved guesses for AVR flows at \( x = 0 \) are obtained by adding the corrections vector \( s \) to the previous guesses. The system is again integrated from \( x = 0 \) to \( L \), and \( \text{score} \) is recalculated. This cycle is repeated until the maximum of \( \text{score} \) is \( \leq 10^{-5} \). Typically only two or three iterations were needed to reach a solution. As a post hoc check on the quality of the solutions, mass balance at each depth is verified based on conservation of glucose equivalents, i.e., according to

\[
\left( F_{DVR}^G(x) + \frac{F_{LVR}^D(x)}{2} \right) + \left( F_{AVR}^G(x) + \frac{F_{LVR}^D(x)}{2} \right) = 0
\]

(18)

The maximum of this sum was typically less than \( 10^{-15} \) pmol \( \cdot \) min\(^{-1} \) \cdot mm\(^{-1} \).

RESULTS

Logically, the main factors influencing IM accumulation of metabolically produced lactate should be: 1) the rate of glycolysis; 2) \( P_{LAC} \), the lactate permeability of DVR, which should be sufficiently high to efficiently recycle lactate to the papilla instead of having it carried out in the AVR; 3) blood flow into the IM, which should be low to favor accumulation instead of wash-out, especially if glycolytic rate is insensitive to the animal’s water balance state; and 4) the amount of volume absorption from the nephrons and collecting ducts, which will tend to dilute the interstitial lactate

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Table 1. Baseline parameter values and initial conditions

<table>
<thead>
<tr>
<th>&quot;Baseline&quot; Permeabilities and Constants</th>
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<tbody>
<tr>
<td>( P_{LAC} ) = ( 100 \times 10^{-5} ) cm/s = 33.93 nL ( \cdot ) min(^{-1} ) \cdot mm(^{-1} ) tube (for radius = 9 ( \mu )m)</td>
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<tr>
<td>( P_{GLU} ) = ( 4 \times 10^{-5} ) cm/s</td>
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<tr>
<td>( \alpha_{GLU} = \alpha_{LAC} = 0.5 )</td>
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<tr>
<td>( k_{sh} = 1.213 ) mm(^{-1} )</td>
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<tr>
<td>( N(0) = 128 ) (arbitrary)</td>
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<tr>
<td>Glycolytic rate parameters</td>
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<tr>
<td>( V_{max} ) is calculated to obtain a desired fractional glucose consumption</td>
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<td>( K_m = 0.1 ) mM</td>
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<th>Initial Conditions</th>
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<tr>
<td>( F_{DVR}^i(0) = 3.75 ) nL ( \cdot ) min(^{-1} ) \cdot DVR(^{-1} )</td>
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<tr>
<td>( c_{GLU}(0) = 10 ) mM</td>
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<tr>
<td>( c_{LAC}(0) = 2 ) mM</td>
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DVR, descending vasa recta.
and increase the washout rate by increasing AVR flow rate.

Glycolytic rate. Figure 2 shows baseline model behavior for overall glycolytic glucose consumption of 0 to 40% by steps of 5%. The volume flow profiles (Fig. 2, A and B) show the 30% net increase of volume flow due to assumed uptake from the nephrons and IMCD and the 30% fall of single-vessel DVR flow due to the assumed baseline rate of DVR volume flux. Note that volume flows here are not coupled to glucose and lactate concentrations (see MODEL DESCRIPTION, above). As glycolytic rate increases, glucose concentration falls and lactate rises. Note that the fall of glucose concentration even for glycolytic rate of zero is due to dilution by incoming volume from the nephrons ($J^\text{V}_{\text{ABS}}$ in the model equations).

Volume absorption from nephrons. The effect of varying $J^\text{V}_{\text{ABS}}$ is shown in Fig. 3 for absorption rates from 10 to 90% of DVR inflow. This fluid absorption is seen to significantly reduce the accumulation of a lactate gradient. As an indication that this range spans physiological values, it can be easily shown that IMCD volume reabsorption of 1 or 2% of GFR corresponds to 25–50% of PPF, assuming a filtration fraction of 25% and PPF equal to 1% of renal blood flow.

Reduction of IMBF. Figure 4 shows the effects of reducing IMBF, under the assumption that tissue glucose consumption is not affected by the change of blood flow. Lactate accumulation is seen to dramatically increase as IMBF falls to one-half its baseline value. The predicted lactate profiles clearly suggest that IMBF may play an important role in the extent of lactate accumulation.

Lactate permeability. The effect of increasing $P_{\text{LAC}}$ from 0 to its baseline value in steps of 10% is shown in Fig. 5. As is clear from the tendency seen in the lactate profiles, higher values lead to no further improvement of lactate accumulation. That is, given the baseline assumptions, there is no reason to postulate specialized lactate transport systems that would increase its effective permeability above the values measured in IMDVR for sodium and urea. On the other hand, lactate permeability would have to be less than half that of sodium and urea before its efficient recycling would be compromised.

Glucose permeability. As mentioned above, it was necessary in the present simulations to drastically reduce glucose permeability to assure its delivery to the deep medulla. That is, higher values resulted in glucose shunting, much as outer medullary oxygen
shunting leads to hypoxia in the IM. Figure 6 illustrates this behavior.

$J_v$ along DVR. Since this model does not include solutes other than glucose and lactate and also ignores hydrostatic and oncotic pressures, volume flux along the DVR could only be treated by assuming some ad hoc profile. The results shown in Fig. 7, where volume loss along the DVR was varied from 0 to 40% of delivery rate, indicate that this is not a crucial parameter with respect to glucose or lactate profiles. Simulations

Fig. 4. Effect of reducing inner medullary blood flow (IMBF) on glucose ($c_{GLU}$) and lactate concentrations ($c_{LAC}$). IMBF was reduced from 100% to 50% of its baseline value. Absolute glucose consumption was held constant. Bottom: glucose and lactate concentrations at the papillary tip (mM) vs. IMBF (expressed as percentage of baseline IMBF). Top: glucose and lactate profiles from $x/L = 0$ to 1.0.

Fig. 5. Effect of lactate permeability ($P_{LAC}$) on glucose ($c_{GLU}$) and lactate concentrations ($c_{LAC}$). $P_{LAC}$ was increased from 0 to 100% of baseline value by steps of 10%. Other parameters are at baseline values (except $P_{GLU} = P_{LAC}/25$). Bottom: lactate concentrations at papillary tip vs. $P_{LAC}$ (expressed as percentage of baseline value). Top: glucose and lactate profiles from $x/L = 0$ to 1.0.
Fig. 6. Effect of glucose permeability on glucose and lactate profiles. Bottom: glucose concentrations at papillary tip vs. $P_{GLU}$ (expressed as fraction of baseline $P_{LAC}$). $P_{GLU}$ was varied from 0.01–0.085 × baseline $P_{LAC}$. All other parameters were held at baseline values, with glucose consumption at 20% of delivery rate. Top: glucose and lactate profiles from $x/L = 0$ to 1.0.

Percentage of early returns. All simulations up to here have assumed a conic papilla like that of the rat, with only a small fraction (1/128) of IM tubes reaching the papillary tip. Many species of rodents have longer papillae with a greater proportion of tubes extending to the region near the tip. In Psammomys, for example, about 40% of nephrons enter the IM and nearly all of these run essentially the whole length of the very long papilla. In such papillae, since metabolic rate must be proportional to the amount of tissue, it seems reasonable to assume that glycolytic lactate production must consume a greater fraction of entering glucose. Also, one would anticipate that, with a longer papilla, lactate accumulation by countercurrent recycling would be favored. To get some idea how well the present model reflects this hunch, I ran a series of simulations with decreasing values of $k_{sh}$, the factor for exponential decrease of the number of vessels. As a starting point, I chose a $V_{max}$ giving 20% glucose consumption for baseline $k_{sh}$ value (this percentage increases as the proportion of long vessels increases). Figure 8 (A and B) shows the resulting profiles for various values of $k_{sh}$.

Fig. 7. Effect of fractional DVR volume loss. Volume flux out of DVRs was varied from 0 to 40% of entering flow rate. As a function of normalized medullary depth, top shows total (left) and single-vessel (right) volume flows (expressed as fraction of entering flow), and bottom shows profiles of glucose and lactate concentrations.
It is seen that lactate accumulation is favored by an increased proportion of long loops. Table 2 gives, for each value of $k_{sh}$, the percentage of long loops attaining the papillary tip and the corresponding percentage of delivered glucose converted to lactate.

Alternatively, one may ask how glucose and lactate profiles would differ in kidneys having identical overall glucose consumption but different loop distributions. Figure 8C shows the results of such a simulation in which glucose consumption was held constant at 20% of delivery rate. The main effect one sees is better delivery of glucose to the papilla when more vessels extend deeper. Lactate profiles are unaffected by the changing loop distribution.

Reflection coefficients. To test the sensitivity to assumed reflection coefficient values, I varied $s_{GLU}$ and $s_{LAC}$ independently from 0 to 1.0. Figure 9 shows the results. The glucose profile is seen to depend somewhat on $s_{GLU}$, but the lactate profile is essentially independent of $s_{LAC}$, mainly because baseline lactate permeability is sufficiently high to assure nearly identical DVR and AVR lactate concentrations, i.e., solvent drag of lactate is negligible compared with lactate diffusion.

**DISCUSSION**

Our earlier modelling study (55) suggested that 100 mosmol/kgH$_2$O of external osmoles would suffice to significantly boost the concentrating mechanism. Jen and Stephenson (24) suggested that an even smaller amount, around 20 mosmol/kgH$_2$O, might suffice. The present results suggest that IM lactate production could conceivably furnish substantial levels of such external osmoles but that lactate accumulation to concentrations as high as 100 mM seems unlikely. To my knowledge, Dell and Winters (12), working with dog kidney, did the only experimental study aimed at evaluation of a possible IM gradient of lactate concentration. In their thoughtful study, they not only demonstrated a corticomedullary lactate gradient (lactate concentration doubling from base to tip in normal and diuretic dogs) but also attributed it to “countercurrent exchange between afferent and efferent limbs of the vasa recta” and speculated that the most likely source was anaerobic glycolysis. More thorough theoretical evaluation of this possibility will necessitate its inclusion in a model of the full medulla, including flows not only of glucose and lactate but also of urea and NaCl in both nephrons and blood vessels, to account explicitly for enhanced volume absorption from descending limbs and collecting ducts, which will in turn affect the recycling of salt and urea among nephrons and vessels.
To put things in scale, it is useful to compare total IM glucose delivery to the amount of urea dumped into the papilla from IMCD. Based on numbers from our three-dimensional models (54, 57), the present assumed delivery of glucose to the IM amounts (in osmolar equivalents) to about 9% of the filtered load of urea (FLu). If about 40% of FLu is dumped into the interstitium from the IMCD, conversion of 20% of total delivered glucose to lactate would represent generation in the IM of only about 4% as many lactate osmoles as urea osmoles. Nonetheless, it must be kept in mind that the sugars exert their full osmotic force across nephron walls (intratubular concentration near zero and tubular reflection coefficient of 1.0), whereas urea is highly permeable (thus only a small concentration difference), so the relative contribution of each milliosmole of interstitial lactate to an “external osmole” single effect will be much greater than a milliosmole of interstitial urea.

Acid production. In addition to lactate, anaerobic glycolysis also produces protons, one for each lactate molecule, so it is natural to wonder whether the steady IM glycolysis poses an acid-base problem. I suggest that it does not, since the small production of protons would presumably be buffered immediately by ambient HCO₃⁻ or ammonia. Even if papillary lactate accumulation by recycling is increased during progressive onset of antidiuresis by reduction of blood flow or by increased DVR lactate permeability, this will presumably have no effect on the rate of local production of protons and lactate.

This does, however, raise the issue of lactate and proton exit from cells in the papilla, whose surroundings may have high lactate concentration. If the cells are equipped with one-to-one coupled lactate-proton transporters (MCT family of transport proteins (44)), then high external concentrations imply high cellular concentrations (and acidity?) as well, since these transporters are passive and reversible. This issue warrants investigation.

Experimental tests. In the end, of course, the relevance or irrelevance of metabolically produced osmoles to the concentrating mechanism is an experimental question. To my knowledge, only one attempt has been made to measure papillary glucose and lactate concentrations. In 1961, Ruiz-Guinazu et al. (48) enzymatically measured glucose and lactate concentrations in micropuncture samples of vasa recta blood collected at the tip of golden hamster papillae. They found glucose concentration diminished by about one-third and lactate concentration doubled compared with arterial blood (aorta), but to obtain sufficient sample volume they had to collect for up to 30 min. Perhaps because of perturbations due to the long collection times, osmolality in their vasa recta samples was only about threefold plasma osmolality (based on their freezing-point depression measurements). These data thus support IM lactate production from glucose, but the values seem too low to contribute significantly to the single effect. It would be interesting to repeat these measurements (in frankly antidiuretic animals), since there are now micro-enzymatic methods for measuring glucose and lactate in nanoliter samples.

Besides this technically demanding measurement of glucose and lactate concentrations in papillary interstitium, it will be relevant to search for specialized transport paths favoring lactate transport. These should be located in plasma membranes of interstitial cells and on basolateral membranes of epithelial cells of the nephrons and collecting ducts. Obvious first candidates are members of the recently cloned family
of monocarboxylate transporters (MCT1, MCT2, . . .) (44), which serve in other tissues to couple lactate and proton exit from glycolyzing cells (e.g., 9, 17, 23, 29, 39, 41, 45, 60). Although some of these have been reported in kidney tissue, their precise locations and regulation have not yet been studied.

It will also be interesting to look further into the correlation of IMBF with urinary concentrating ability. Although some studies have shown that IMBF is reduced by almost 50% in anti- diuresis (4), others showed only slight sensitivity of urinary concentrating ability to medullary blood flow (11).

Conclusion. The picture that emerges here is that IM glycolytic lactate production in the range of reported values is probably sufficiently high and vasa recta recycling sufficiently efficient to result in an osmotically significant corticomedullary lactate gradient. The extent to which this external osmole production amplifies concentrating ability remains to be explored in full medullary models. It is hoped that the present work will serve as a guide for such studies as well as a stimulus for experimental tests of this idea.

I conclude with a few more general remarks. The countercurrent arrangement of nephrons and blood supply was an evolutionary innovation that permitted animals to move into arid ecological niches (20, 51, 61), but we see now that it also posed problems for the supply of nutrients for the cells in the deep medulla. First, plasma skimming and shunting of O2 from descending vessels to the avid salt pumps of the outer medullary thick ascending limbs lead to hypoxia below a certain depth (42, 49), necessitating reliance on anaerobic glycolysis for metabolic maintenance of cells deeper in the medulla (32). By nature’s serendipity, this may have provided an alternative single effect (the proposition in the present work), namely, a source of metabolically produced osmoles, thereby favoring continued lengthening of the papilla for progressively more concentrated urine. That said, the suggestion must not be taken too narrowly, since the relationship across species among papillary length, percentage of long loops, and number of nephrons per unit body weight is far from straightforward (2). Nonetheless, for very long papillae (as in certain desert species) or for high IM metabolic rates, I do predict that DVR glucose permeability must be low in order not to starve the deepest regions by glucose shunting.

This suggested role in the concentrating mechanism for metabolically produced osmoles suggests a nuanced interpretation of the role of urea. In omnivores like the rat, and in carnivores [such as cat and dog, both with 100% long loops (2)] maintenance of urea balance and maintenance of water balance are arguably intimately linked, in the manner suggested by the classic passive hypothesis (according to which, the dumping of a relatively small amount of urea from the terminal collecting ducts serves efficiently as external osmoles in the drastically reduced volume of the rat papilla) and in relation to dietary protein intake (1). However, most of the desert rodents that have been studied eat a low-protein diet of dry seeds (Mongolian gerbil, jerboa, pocket mouse, spiny mouse) or succulent plants (Psammomys) and have very long papillae in which the number of IM nephrons and vessels remains nearly constant almost to the tip. Although these species can concentrate their urine better than the rat, at least one study showed that papillary urea concentration is not correlated with urine osmolality (22). Even in the rat, it has been reported (5) that NaCl concentration was not correlated with urine osmolalities above 1,500 mosmol/kgH2O. It is thus tempting to speculate that although the kidneys of species that elaborate the most concentrated urine are somewhat paradoxical in the context of the classic hypothesis, their long, thick papillae, with presumably correspondingly abundant cell metabolism throughout their length, would appear to be consistent with an important single effect for metabolic osmoles such as lactate. Relevant to this point is the observation, pointed out by Beuchat (7, 8) in her exhaustive review of the relationships between kidney size, metabolic rate, and urinary concentrating ability, that small animals have a higher mass-specific metabolic rate than do larger animals, although the relationship varies among individual organs.

As a final thought, I suggest that if metabolic osmole production does turn out to participate importantly in the urinary concentrating mechanism (through regulation of IMBF or by some other means), then it also adds another mode of separation of renal regulation of water balance, salt balance, and urea balance.

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REFERENCES


