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PECULIARITIES OF THE Na^+/D-GLUCOSE COTRANSPORT SYSTEM IN *NECTURUS* RENAL TUBULES

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The effects of D-glucose addition to a glucose-free luminal perfusate were investigated in the proximal tubule of *Necturus* kidney, by electrophysiological techniques. The main findings are: (1) In the presence of sodium, D-glucose produces 10.5 mV ± 1.1 (S.E.) depolarization. (2) Phlorizin reduces the magnitude of this response to 2.1 ± 0.1 mV. (3) The glucose-evoked depolarization, ΔV_G, does not alter the intracellular K^+ activity nor is it affected by peritubular addition of ouabain. (4) Isosmotic reduction of Na^+ concentration in luminal perfusate from 95 to 2 mmol/l (choline or Li^+ substituting for Na^+) does not change the magnitude of ΔV_G; complete removal of sodium from the lumen lowers the value of ΔV_G (3.2 ± 0.2 mV) but the response is not abolished. This observation suggests that the D-glucose carrier of renal tubules in *Necturus* is poorly specific with regard to the cotransported cation species.

Introduction

Several studies have established that glucose is transported across the brush border membrane of renal and intestinal epithelia, primarily via a specific Na^+/D-glucose cotransport mechanism. Evidence in favor of Na^+/D-glucose cotransport in proximal tubule has been provided by both electrophysiological techniques applied on the kidney in vivo [1–4] and by experiments performed in vitro on purified brush border membrane vesicles [5–7]. However, the responses obtained by these studies are not strictly identical in mammalian and amphibian preparations. For example, phlorizin completely abolishes the glucose evoked depolarization across the apical membrane of the rat kidney [4], but it is only partly effective when added in a glucose-containing perfusate in newt kidney [1]. Although such discrepancies may be accounted for by interspecies differences, they raise also the question of the specificity of the Na^+/D-glucose cotransport system in amphibia. In this paper we wish to report some observations suggesting that cations other than sodium may cross the cotransport pathway of the brush border membrane in association with glucose, in the proximal tubule of *Necturus*. In addition, a few other aspects of the Na^+/D-glucose cotransport in *Necturus* are briefly considered.

Methods

All experiments were performed in the proximal tubule of the in vivo *Necturus* kidney. The methods for anesthesia, dissection of the animals, preparation of the kidney, initiation of intraluminal perfusion (occasionally associated with peritubular capillary perfusion) by one or two double-barreled micropipette(s), and recording of the cell membrane potential, V_{bl}, have been described in detail

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

elsewhere [8–10]. V_{bl} readings were considered acceptable if stable within ±1 mV for at least 2 min.

Solutions

Several artificial solutions were used to perfuse the lumen of studied tubules. The composition of six glucose-free artificial perfusates is listed in Table I. Solution A represents the 'control' Ringer perfusate. Sodium concentration was reduced in solutions B through F, by isosmotic replacement of part or the whole Na^+ content by Li^+ or choline. Nominally Na^+-free solutions were introduced in the lumen of studied tubules through Na^+-free capillary glass tubing (K. Hilgenberg, 3509 Malsfeld, F.R.G.). The solutions appearing in Table I were used as such or supplemented with D-glucose; glucose concentration was (in mmol/l) 11.0, 2.2, 0.45 or 0.22. In some experiments, phlorizin (10^{-4} mol/l) or ouabain (10^{-5} mol/l) was added to one of the solutions of Table I.

Experimental protocol

In general, a double barreled micropipette was introduced in the lumen of a tubule. Its two channels were respectively filled with (i) one of the six glucose-free solutions listed in Table I, and (ii) a paired electrolyte solution, containing in addition glucose. A conventional microelectrode was inserted in the epithelial layer and the effects of glucose addition to and removal from the tubular perfusate on V_{bl} were studied, by allowing the glucose-containing and glucose-free solutions to flow alternatively in the lumen. All perfusates were collected downstream by a single micropipette [10]. In a few experiments the effects of several glucose concentrations were tested at a single site, by using a triple-barreled or two double-barreled intraluminal micropipettes. In other experiments ouabain or a glucose-free solution was added in the peritubular circulation.

Intracellular K^+ activity (α^i_K)

The effects of glucose addition into a luminal glucose-free perfusate on α^i_K were studied by means of double-barreled selective K^+ microelectrodes. The technique for construction of these electrodes and its limitations have been described recently elsewhere [11]. Results are given as mean ±S.E.

Results

Luminal addition of glucose in a physiologic Ringer solution

The addition of D-glucose (11 mmol/l) into a Na^+-containing glucose-free solution resulted in 10.5 ± 1.1 mV depolarization. This change was reversible and essentially reproducible (Fig. 1). The magnitude of the glucose-elicited depolarization, ΔV_G, did not correlate with the value of V_{bl} recorded prior to the addition of glucose in the perfusate (Fig. 2). A similar independence of the voltage response on V_{bl} was reported in endoderm cells from *Xenopus* embryo over the range of −90 to −50 mV [12]; by contrast, in rat kidney, the magnitude of ΔV_G decreased as V_{bl} was lowered [4]. Further analysis of our data failed to provide significant correlation between ΔV_G and the dis-

TABLE I

COMPOSITION OF THE PERFUSING SOLUTIONS IN mmol/l

	A	B	C	D	E	F
NaCl	82.0	–	–	–	–	–
KCl	3.0	3.0	3.0	3.0	3.0	2.0
$CaCl_2$	1.8	1.8	1.8	1.8	1.8	1.8
$MgCl_2$	1.0	1.0	1.0	1.0	1.0	1.0
$NaHCO_3$	13.0	13.0	13.0	4.0	1.0	–
Hepes/NaOH	4.0/1.0	4.0/1.0	4.0/1.0	4.0/1.0	4.0/1.0	–
LiCl	–	82.0	–	–	–	–
Choline Cl	–	–	82.0	82.0	82.0	82.0
Choline HCO_3	–	–	–	9.0	12.0	13.0
Hepes/KOH	–	–	–	–	–	4.0/1.0

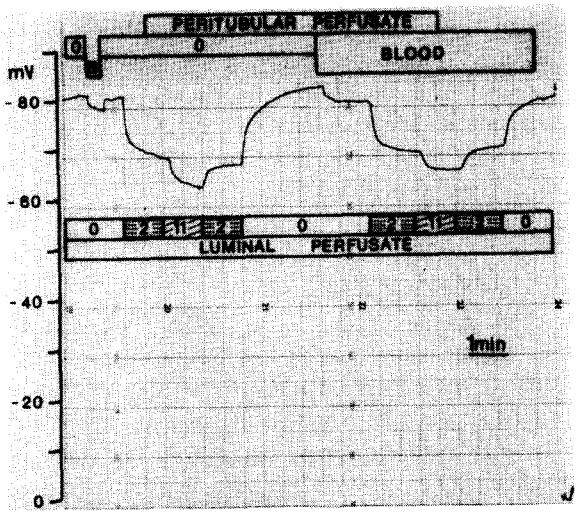

Fig. 1. Reproduction of a recording illustrating the effects of luminal addition of glucose on V_{bl}. Cell membrane potential is plotted (ordinate) as a function of time (abscissa). In this particular experiment a single-barreled micropipette containing a physiologic, glucose-free, Ringer solution (labelled 0) was initially inserted in a peritubular vessel. Subsequently, a triple-barreled luminal micropipette was used to perfuse sequentially the lumen with a glucose-free perfusate (mark 0), then the same solution containing in addition D-glucose 2.2 mmol/l (dots) or 11 mmol/l (hatches). It is seen that luminal addition of glucose in a glucose-free perfusate elicits slightly larger depolarization when the peritubular perfusate is glucose-free than in the presence of blood (B) in peritubular circulation. Similar observations were reported in rat proximal tubule and were accounted for by changes in the glucose concentration gradient across the brush-border membrane [4]. All perfusates were collected downstream. Na^+ concentration was in all cases 95 mmol/l.

tance separating the puncture site from either the glomerulus or the perfusion pipette.

The effect of various glucose concentrations (luminal perfusate) on ΔV_G are illustrated in Fig. 3.

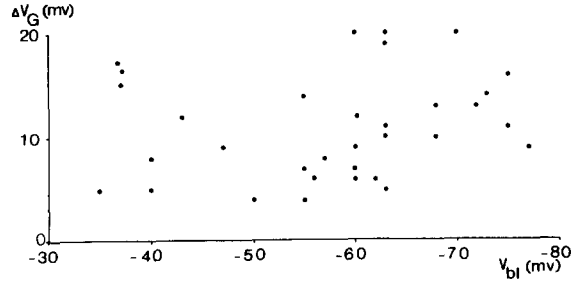

Fig. 2. The magnitude ΔV_G (resulting from addition of glucose, 11 mmol/l in a physiologic Ringer solution) is plotted in ordinate, as a function of V_{bl} in abscissa.

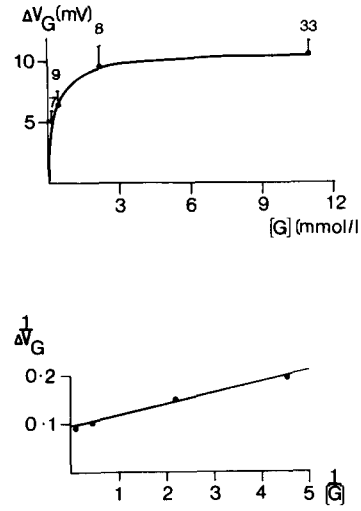

Fig. 3. (Top) Relationship between the concentration of glucose in luminal perfusion fluid ([G]) and the resulting change in cell membrane potential, ΔV_G. The solid line was drawn by eye. Vertical bars and numbers on top indicate S.E. and number of observations, respectively. The average cell membrane potentials of the four series of experiments illustrated from left to right were (mV): -62.4 ± 3.6, -61.3 ± 3.4, -58.5 ± 3.0 and -63.5 ± 1.6, respectively. (Bottom) Lineweaver-Burk plot (same data). The regression line, calculated by least squares, is described by the equation $y = 0.095\ x + 0.0228$.

As seen, the relationship between luminal glucose concentration and ΔV_G is consistent with Michaelis-Menten kinetics. The apparent K_m for glucose, calculated from the slope of Fig. 3, bottom, is 4.17 mmol/l. This figure is higher than other K_m values for glucose in renal tubules: 0.1 mmol/l [13], 0.7–1.1 mmol/l [4], 1.15 mmol/l [1], 1.8 mmol/l [14] and 2.6 mmol/l [3]. By contrast, in brush border membrane vesicle preparations endowed with two transport systems, the K_m of the low affinity system was 6 mM [15] and 43 mM [14].

Luminal addition of glucose in solutions of lowered Na^+ concentration

Fig. 4 shows that the stepwise withdrawal of sodium from the luminal perfusate did not affect the magnitude of the glucose elicited depolarization: no substantial reduction of ΔV_G was observed as Na^+ concentration was lowered from 95 to 2 mmol/l. The complete removal of sodium from the luminal fluid was attended by a significant decline of ΔV_G to 3.2 ± 0.2 mV; yet, the

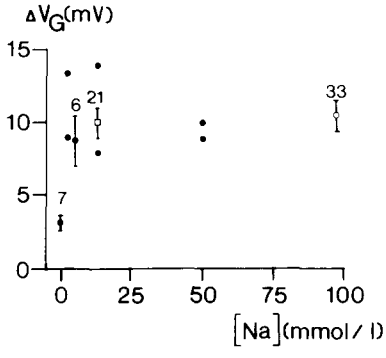

Fig. 4. The magnitude of depolarization elicited by D-glucose addition in luminal perfusate (ΔV_G) is plotted (ordinate) as a function of Na^+ concentration (abscissa). Paired perfusing solutions were used in all cases, glucose-free vs. glucose-containing, 11 mmol/l. Sodium was isosmotically replaced by choline (dots) or lithium (square). The value of ΔV_G, obtained in the presence of physiologic Na^+ concentration (open circle), is represented for comparison. Single dots are single impalements. Vertical bars and numbers as in Fig. 3. The mean cell membrane potential (mV ± S.E.) was -66.0 ± 4.2 in Na^+-free solutions, -65.8 ± 3.6 in the presence of 5 mmol/l Na^+90 mmol/l choline and -48.8 ± 3.2 in the Li^+ series.

response was not abolished even in this highly unphysiologic situation.

Effect of inhibitors

Addition of phlorizin (10^{-4} mol/l) to the glucose-containing solution (11 mmol/l) reduced the magnitude of ΔV_G from 10.5 to 2.3 ± 0.1 mV ($n = 3$), in *Necturus* as in newt kidney [1]. Similarly, the passage from a choline-containing solution (solution C) to a solution of similar composition supplemented with glucose (11 mmol/l) and phlorizin (10^{-4} mol/l) produced 2.1 ± 0.1 mV depolarization ($n = 3$). The inhibitory effect of phlorizin on ΔV_G appears to be independent of the main cation carried by the cotransport system.

The presence of ouabain in the peritubular perfusate failed to alter the ΔV_G response. The depolarizing effect of glucose was measured, first, in the absence of ouabain, then the peritubular capillaries were perfused with an ouabain-containing solution for 1–2 min and glucose was added anew in the luminal perfusate during peritubular ouabain application. In four such paired experiments, ΔV_G was 8.8 ± 0.6 mV before ouabain application and 8.7 ± 0.6 mV after addition of the inhibitor. This difference was not statistically significant. The ouabain experiments suggest that ΔV_G arises solely as a result of Na^+/D-glucose cotransport, without interference from concomitant development of electrogenic Na^+ transport stimulation.

Effect of luminal glucose on α^i_K

The average value of α^i_K, 60.8 ± 4.4 mmol/l ($n = 9$), measured during luminal perfusion with a glucose-free Ringer solution, is similar to that reported in a recent work on the proximal tubule of *Necturus* kidney, in vivo, 58 mmol/l [11]. Addition of glucose (11 mmol/l) in the luminal perfusate failed to alter α^i_K. Exposure of the apical side of the cell to the glucose-containing solution lasted from 30 s to 2 min in this series of experiments and the average ΔV_G was 11.3 ± 1.5 mV. Analogously, Na^+/alanine cotransport did not change the intracellular K^+ content in rat hepatocytes [16].

Discussion

In view of the abundant experimental background on coupled transport systems, the description of the D-glucose cotransport mechanism in *Necturus* kidney constitutes a trivial observation. The reason that prompted us to report on this issue is that several peculiarities of this amphibian preparation raise a number of interesting questions.

Lack of dependence of ΔV_G on V_{bl} and/or tubular inhomogeneity?

The driving force for uphill transport of D-glucose is the transmembrane sodium electrochemical potential difference not the Na^+ concentration difference alone [4]. Experiments with brush-border vesicles are consistent with this view too [6,13]. The reported insensibility of ΔV_G with regard to V_{bl} in this paper constitutes only an apparent deviation from this rule. However, the observation that ΔV_G does not vary as Na^+ concentration is lowered from 95 to 2 mmol/l suggests that the Na^+/D-glucose carrier is already saturated when the sodium concentration is as low as 2 mmol/l. Therefore, variations of V_{bl} between

-35 and -75 mV in the presence of 95 mmol/l Na^+ would not be expected to have a noticeable effect of the rate of glucose-mediated Na^+ current. In addition tubular inhomogeneity may account for the scatter of the data of Fig. 2 and obscure a borderline effect of V_{bl} on ΔV_G. Tubular inhomogeneity is a common feature in mammalian kidney. Present and previous [17] observations in *Necturus* suggest that tubular inhomogeneity is more pronounced in amphibian preparations than in mammalian proximal tubule.

Glucose cotransport in Na^+-free media

The strict dependence of Na^+/organic substrate cotransport systems on the presence of sodium in rat kidney has been recently demonstrated [4]. By contrast, several reports on intestinal epithelia indicate that organic substrate-evoked depolarizations may be observed after complete removal of sodium from the luminal bathing fluid [18–20]. For example amino acids and oligopeptides elicit substantial depolarization (> 10 mV) in the small intestine of *Necturus* even after 30 min exposure to Na^+-free solutions (choline substituting for Na^+) [20]. The presence of sodium does not appear to be an absolute requirement for the operation of every glucose cotransport system. In the present study, the removal of sodium from the luminal fluid preceded the determination of glucose effects on V_{bl} by 2 to 10 min. The possibility of sodium back-leak into the lumen and its subsequent recycling into the cell is unlikely, in view of the high luminal perfusion rates used in our experiments [10].

Na^+/D-glucose cotransport and transepithelial Na^+ transport

It is generally believed that Na^+-organic substrate cotransport across brush border membranes of epithelial tissues enhances Na^+ influx into the cell, thereby increasing intracellular sodium activity, α^i_{Na}, which in turn stimulates the rate of basolateral Na^+ extrusion out of the cell. In *Necturus* proximal tubule the initiation of Na^+/D-glucose cotransport did not affect α^i_K. Assuming that the introduction of glucose in the lumen does not change in the intracellular cation content (i.e. that the sum $\alpha^i_K + \alpha^i_{Na}$ is constant), we must infer that α^i_{Na} remains also stable even though the Na^+/D-glucose cotransport rate increases. Similar conclusions were reached in intestinal cells, where amino acid uptake failed to increase α^i_{Na} for periods extending over 10 min or more (it actually decreased it slightly) [21]. Thus, although cell depolarization in response to Na^+/organic substrate cotransport corresponds to a transient cationic (sodium) current from lumen to cell, this initial Na^+ influx does not increase α^i_{Na}: the opening of the Na^+/D-glucose conductive pathway leaves α^i_{Na} unaffected because its effects are counterbalanced by a decrease of the driving force for Na^+ entry (cell depolarization). Moreover, the rate of transepithelial Na^+ transport (determined by short circuit current) in response to luminal introduction of organic substrates appears to increase in a progressive manner (extending over several minutes) [22]. The link between the quasi-instantaneous change of cell membrane potential and the resulting, delayed, increase of transepithelial transport rate has not been clearly established. If α^i_{Na} does not fulfill the role generally assigned to it, it may be speculated that other mechanisms account for the adaptation of basolateral Na^+ transport, to changes of Na^+/organic substrate cotransport at the apical membrane.

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