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Glucose transport and metabolism in rat renal proximal tubules: multicomponent effects of insulin

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Glucose transport and metabolism, and the effect of insulin thereon, was studied using suspensions of rat renal tubules enriched in the proximal component. (1) [U-^{14}C]Glucose oxidation is a saturable process (K_m 3.1 ± 0.2 mM; V_{max} 14 ± 0.2 μmole $^{14}CO_2$ formed/g tissue protein per h.) Glucose oxidation and [^{14}C]lactate formation from glucose are inhibited in part by phlorizin and phloretin: the data suggest that the rate-limiting entry of glucose into the cell metabolic pool occurs by both the Na-glucose cotransport system (at the brush border) and the equilibrating, phloretin-sensitive system (at the basal-lateral membrane). Raising external glucose from 5 to 30 mM markedly increases aerobic and anaerobic lactate formation. Gluconeogenesis from lactate is not affected by variations of glucose concentrations. (2) 24 h after streptozotocin administration, aerobic lactate formation is enhanced, as is the uptake of methyl α-D-glucoside by the tubules, while anaerobic glycolysis is depressed. Streptozotocin treatment (ST) increases both the K_m and V_{max} of glucose oxidation; gluconeogenesis and lactate oxidation are not affected. The effect of streptozotocin treatment on lactate formation are abolished by 1 mU/ml insulin. (3) Streptozotocin treatment increases tissue hexokinase activity, decreases glucose-6-phosphatase, but has no significant effect on fructose-1,6-diphosphatase, phospho*enol*pyruvate carboxykinase and pyruvate dehydrogenase. The data demonstrate fast streptozotocin-induced changes in cellular enzymes of carbohydrate metabolism. (4) The enhancing effect of streptozotocin on methyl α-glucoside uptake is transient: 8 days after administration of the agent, no significant difference from controls is found. (5) It is concluded that under the given experimental conditions insulin enhances the equilibrating glucose entry by the phloretin-sensitive pathway at the basal-lateral membrane, and transiently inhibits the Na-glucose cotransport system.

Introduction

There is now concensus concerning the steps in glucose absorption by renal proximal tubules: In the luminal (brush border) membrane, glucose is transported into the cells by a secondarily active, phlorizin-sensitive Na-glucose cotransport system. The driving force for this system is the operation of the ($Na^+ + K^+$)-ATPase (at the basal-lateral cell face) which produces the sink for the electrogenic downhill flux of Na^+ associated with the uptake of glucose (against its chemical gradient). At the basal-lateral face, glucose is assumed to exit from the cells into the peritubular space by an equilibrating transport system. As to specificity and effects of inhibitors, the latter transport system is considered to be similar to, or identical with, that found also in non-polar cells, i.e. adipocytes, red blood cells, muscle, etc. (see, for example, Ref. 1). In renal cells incubated in the

presence of methyl α-D-glucoside, the non-metabolizable substrate of the Na-glucose cotransport system, the concentration profile of this sugar is in accordance with above view [2]: there is a sizable cellular accumulation of this saccharide at the brush-border membrane. The analysis of glucose transport proved to be more complex since this sugar undergoes rather rapid cellular metabolism. The analysis of the concentration profile of glucose across the renal tubular cells lead some authors [3,4] to postulate an additional 'glucose pump' at the basal-lateral cell face, directed from the cells to the peritubular fluid. Unfortunately, a non-metabolizable glucose analog selectively specific for the equilibrating transport system at the basal-lateral cell membrane is not available.

The present study was undertaken with the aim of examining whether the scattered reports on insulin effects on renal carbohydrate metabolism [5,6] might be amenable to an analysis of the extent to which the two distinct transport processes involve an insulin-controlled step. This direction was emphasized by several considerations. (1) In non-polar cells, e.g. in adipocytes [7] the equilibrating glucose transport system is greatly enhanced by insulin. (2) Clinical and in vitro studies (see Ref. 8) reported a stimulating effect of insulin on Na-fluxes across epithelial cells; such an effect might enhance the entry of sugars at the brush-border membrane of renal tubular cells. (3) There is considerable diversity in reports as to specific effects of insulin (and/or conditions related to insulin action, such as alloxan- or streptozotocin-induced hyperglycemia) on glucose metabolism in renal cortical tissue [5,6].

We report here data obtained on glucose transport and metabolism in a simple preparation of renal proximal tubules. Renal tubules from control rats were compared with preparations from (a) animals treated for 24 h with streptozotocin, and showing massive hyperglycemia; (b) rats treated for 10 min by an intravenous administration of alloxan; under these conditions the breakdown of insulin in the membrane of renal cortical cells is greatly inhibited [5]. It will be shown here that the above experimental conditions, as well as additions of insulin to the tissue preparation, produce effects on glucose metabolism which indicate that the hormone influences both sugar transport processes.

Methods

Sprague-Dawley male rats, 100–140 g body weight, were used. Unless otherwise stated, hyperglycemic rats were obtained by an i.v. injection of streptozotocin (60 mg/kg wt. in 0.1 M citrate buffer, pH 4.5) as described by Gertz and Haugaard [9]. Buffer alone was administered to control animals. Both control and experimental animals were fed ad libitum for 24 h prior to the start of the experiment. Animals with plasma glucose levels of 16 mM or higher were used. The above procedure was chosen in the light of evidence that 24 h after administration of streptozotocin, no toxic effects on several parameters of renal function were found [10–12] and the animals closely resemble diabetic (hypoinsulinemic) conditions. At that stage plasma insulin has not yet fallen below that in healthy controls while pancreatic insulin is practically absent [10,12]. In a group of experiments, the effect of alloxan-induced diabetes (see Ref. 5) on glucose transport and metabolism was also tested: alloxan (20 mg/100 g) in saline was injected intravenously; the animals were sacrificed 10 min later.

Suspensions of purified renal proximal tubules in Krebs-Henseleit [13] bicarbonate saline (KBS) buffer were prepared by the method of Vinay et al. [14]. It was then found that a simplified procedure could be used, differing from that of the above authors only in the care taken for separating by slicing of the cortical tissue from the external medulla: The decapsulated kidneys were cut lengthwise and cortical slices (approx. thickness 0.4 mm) were cut free-hand [15]. Slices of 3 rats were usually pooled, weighed and incubated aerobically (5% CO_2 + 95% O_2) at 37°C for 40–50 min in KBS buffer (10 ml per 1–2 g tissue) containing 2 mg/ml collagenase, and 4 mg/ml hyaluronidase. The suspension was strained through gauze, the tubules were spun off at 4°C at $100 \times g$ for 1 min, washed twice with fresh KBS buffer, and the isolated tubules were resuspended in saline (3.5-times the original weight of the slices). 0.2 ml of this suspension plus 2 ml KBS buffer containing the appropriate metabolic substrates were then incubated in siliconized flasks in a shaker water bath at 37°C for 60 min. The final suspension contained approx. 2 mg tissue pro-

tein/ml (determined according to Lowry et al. [16]). Unless otherwise stated, the incubating salines contained 5 mM D-glucose and 2 mM L-lactate in order to assure a steady state approximating physiological conditions for the measurement of metabolic parameters. Aerobic incubation: 37°C, 5% CO_2 in O_2; anaerobic conditions: 37°C, 5% CO_2 in N_2; the presence of a stick of yellow phosphorus in the center well of the incubation flask assured absorption of traces of O_2.

Attempts to purify the above suspension (F_T) further by centrifugation on a Percoll (Pharmacia, Uppsala, Sweden) density gradient (cf. Ref. 14) yielded only one clearly defined tissue band. The 'crude' suspension (F_T) corresponded microscopically (95% proximal convoluted tubules; courtesy of Dr. B. Atkinson, Dept. of Pathology, Univ. of Pennsylvania), in density and in metabolic parameters to the purified fraction F_4 of renal proximal tubules of the above authors, and thus represents a preparation enriched in proximal tubules.

Measurements of metabolic parameters. The oxidation of D-[U-^{14}C]glucose (0.1 μCi)/ml) was determined by the conventional procedure of trapping $^{14}CO_2$ (see, for example, Ref. 17). Glucose utilization was followed by using D-[5-^{3}H]glucose (0.2 μCi/ml) as substrate [18]. After incubation, the tissue was quickly spun off, and 0.2 ml of the supernatant was injected through a rubber cap on filter paper in the center well of a conical flask; 0.2 g dry $CaCl_2$ at the bottom of the flask served as absorbent for formed ^3H_2O. After absorption of all $H_2O + {}^3H_2O$ (overnight), the $CaCl_2$ was dissolved in 1.0 ml H_2O and was taken for determination of radioactivity. Measurements of aerobic lactate formation and gluconeogenesis: After incubation, a portion of the tissue suspension (0.5 ml) was placed into a conical tube containing 2.0 ml of H_2O at 100°C and the tube was maintained in a boiling water bath for 10 min, thus stopping metabolism. Sugar phosphates were removed from this extract by the $ZnSO_4$-$Ba(OH)_2$ procedure [19]. Glucose and lactate in the supernatant were then separated quantitatively by ion-exchange chromatography on a Dowex AG 1-X8 column in Cl^--form (approx. 50 mm long). Portions of the effluent were used for the determination of the activity of [^{14}C]glucose (formed from L-[U-^{14}C]lactate) or of [^{14}C]lactate formed from glucose. The efficiency of the procedure for lactate and glucose determination was checked by enzymic analysis of the substrates. Measurement of methyl α-D-glucoside uptake: Suspensions of tubules were incubated in saline containing 1 mM methyl α-D-[U-^{14}C]glucoside (0.1 μCi/ml) but devoid of glucose or lactate in order to avoid conditions where the Na-glucose cotransport system would be competitively inhibited. After incubation a portion of the suspension was spun off for 60 s in an Eppendorf 3200 centrifuge, the sedimented pellet was washed with ice-cold KBS buffer containing 0.5 mM phlorizin + 0.5 mM phloretin; in this way the efflux of cellular sugar was prevented while extracellular sugar was eliminated. The sedimented tubules were then suspended in 0.6 ml saline, 0.5 ml of the suspension was placed in 0.5 ml of boiling H_2O for 10 min in order to complete the extraction of methyl α-D-glucoside, and the supernatant was taken for counting. All values are expressed in μmol formed, used or taken up per g tissue protein [15]. Mean values ($\pm$ S.E.) are given.

Radioactivity of samples were measured by counting 1.5 ml aqueous samples plus 5 ml scintillation fluid Formula 963 (New England Nuclear Corp.) in a Packard scintillation spectrometer Model 3320.

Table I compares parameters of glucose metabolism in the enriched (F_T) tubule suspension with that further purified by the Percoll density gradient centrifugation (F_4, cf. Ref. 4). It will be seen that within the limits of experimental error the values for glucose oxidation, lactate formation and methyl α-D-glucoside uptake were identical in the crude (F_T) and purified (F_4) tubule suspensions, providing evidence that the present preparation F_T represents a suspension enriched in renal proximal tubules. The significantly lower value for gluconeogenesis in F_4, compared to F_T, may indicate impairment of this metabolic process by the centrifugation on the Percoll gradient; removal of contaminating fragments of distal nepron segment should be expected to increase the specific gluconeogenetic activity [3]. Glucose metabolism (i.e. glucose oxidation, glucose utilization and lactate formation) was practically stable during the 60 min. of incubation (compare also Ref. 14), and the values given therefore approximate rates; how-

TABLE I

SOME METABOLIC PARAMETERS OF RAT RENAL PROXIMAL TUBULES

Tissue suspensions before (F_T) and after (F_4) separation on a Percoll density gradient were compared. Incubation: 60 min at 37°C, 5%CO_2 + 95% O_2, KBS buffer with 5 mM glucose and 2 mM lactate as substrates. For the measurement of 1 mM methyl α-[^{14}C]glucoside uptake, no glucose or lactate were present in the medium. Mean values ± S.E., all in μmol/g protein.

	F_T	F_4	Number of measurements
[5-^{3}H]Glucose utilization (μmol ^3H_2O formed)	68.6 ± 1.8	66.9 ± 1.6	8–10
[U-^{14}C]Glucose oxidation (μmol $^{14}CO_2$ formed)	15.3 ± 0.8	13.7 ± 0.5	6–10
L-Lactate formation (μmol formed)	134.8 ± 7.4	124.6 ± 7.6	5
Gluconeogenesis (μmol glucose formed)	108.8 ± 1.2	78.4 ± 0.6	5
Methyl α-glucoside uptake (μmol taken up)	11.8 ± 0.9	13.1 ± 0.7	5

ever, this does not apply to methyl glucoside uptake.

Enzyme assays. These assays were carried out on perfused renal cortex [14], to assure sufficient amounts of tissue for the analyses. The cortices were sliced, and homogenized in media given for individual enzymic assays: hexokinase (EC 2.7.1.1), [20]; glucose-6-phosphatase (EC 3.1.3.9), [21]; fructose-1,6-diphosphatase (EC 3.1.3.11), [22]; pyruvate dehydrogenase complex (1.2.4.1), [23]; phospho*enol*pyruvate carboxykinase (EC 4.1.1.32), [24,25]; for the assay of the activity of this enzyme, the tissue was homogenized in a medium containing 0.25 M sucrose, 5 mM Tris-HCl (pH 7.4) and 1 mM mercaptoethanol, and the cytosolic fraction was analyzed. The data are reported in units given by the respective references, normalized per g tissue protein/min.

Data analysis. For each experiment, the individual analytical values were averaged and the S.E. was assessed. The significance of difference between controls and experimental values was evaluated by Student's *t*-test. Mean values of several experiments (± S.E.) are also given.

Materials. Collagenase I was obtained from Worthington Biochem. Corp., Freehold, NJ; other enzymes were purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN. Insulin was a gift from Eli Lilly Co., Indianapolis, IN. Streptozotocin was obtain from Sigma Chemical Co., St. Louis, MO. Labelled sugars were obtained in part from New England Nuclear Corp., Boston, MA, and Amersham Corp., Arlington Heights, IL. All other reagents were commercial products of the highest available purity.

Results

Parameters of glucose metabolism in renal proximal tubules

Table I presented data on pertinent parameters of carbohydrate metabolism in suspensions enriched in rat renal proximal tubules. While in individual experiments the measured values of metabolic parameters were consistent, as indicated by the relatively low S.E. (see data below), there was some variation in values obtained in separate experiments; in particular, L-lactate formation from glucose proved to vary considerably. The mean value was 127 ± 14 μmol/g tissue protein per h (20 experiments) with a range of 50–200 μmol. Such variations may reflect some differences between groups of animals. The mean values for lactate formation (above) and of gluconeogenesis from lactate (115 ± 15 μmol/g protein per h, seven experiments) were comparable to those reported by Janssens et al. [26] when expressed in the same units, using the conversion factor of 0.8 g protein per g dry wt. of tubules.

Glucose oxidation was in part (30–50%) inhibited by selective agents known to interfere, directly or indirectly, with the active Na-glucose cotransport system at the brush border membrane of tubule cells, i.e. 0.5 mM phlorizin and 0.5 mM ouabain (Table II). Phlorizin and ouabain also significantly inhibited ($P < 0.02$) aerobic L-lactate formation from glucose by 37 ± 12% (three experiments). Such data are taken to indicate that in the given preparation only some 30–50% of glucose entering the cellular metabolic pool involves the phlorizin-sensitive Na-glucose cotransport system; the major portion of glucose entering the metabolic pool is insensitive to phlorizin and ouabain, and appears to enter the cells by the (equilibrating) phloretin-sensitive transport system at the

TABLE II

EFFECT OF PHLORIZIN AND OUABAIN ON GLUCOSE OXIDATION AND LACTATE FORMATION IN RENAL PROXIMAL TUBULES

Suspensions of renal proximal tubules were incubated 60 min at 37°C without (control) or with 0.5 mM phlorizin or ouabain (experimental). Mean values of $^{14}CO_2$ or L-lactate formed (μmol/g tissue protein) ± S.E. (n = 4–6).

	Inhibitor	Control	Experimental
Glucose oxidation	Phlorizin	21.0 ± 1.1	8.7 ± 0.4
	Ouabain	16.0 ± 0.3	11.9 ± 0.6
Lactate formation	Phlorizin	120.4 ± 9	85.5 ± 6
	Ouabain	111.7 ± 5	49.7 ± 5.5

basal-lateral cell face. This conclusion is consistent with data on the sidedness of glucose transport in flounder renal tubules [27], results of studies in vivo [28] and in renal slices [29], as well as in renal epithelial cells grown in vitro [30].

Effect of glucose concentration on substrate metabolism

Glucose oxidation is a saturable process: A double-reciprocal plot (Fig. 1) showed a linear relationship, with an apparent K_m of 3.1 ± 0.2 mM and a V_{max} of 14 ± 0.2 μmol CO_2/g tissue protein per h, corresponding to a value of 3.4 μmol/g tissue per h. The value of the apparent K_m was two to three orders of magnitude higher than that of the first intracellular metabolic step for glucose, i.e., the K_m for D-glucose of 10^{-5}–10^{-6} mM for the predominant renal hexokinases, type I and some type III (cf. Ref. 31).

Under a variety of experimental conditions the cellular concentration of free glucose in renal cortical tissue appears to be lower than in the plasma [4], and own unpublished data; see, however, Ref. 32. It may therefore be surmised that glucose entry into the cells represents the rate-limiting step for its subsequent metabolism at least at the physiological glucose concentration of 5 mM, and hence the K_m for glucose oxidation may reflect that for glucose transport. The linearity of the plot in Fig. 1 suggests the involvement of only one transport component, in contrast to the data on the effect of some inhibitors (Table II). This apparent discrepancy may indicate either only minor differences in the K_m values of the

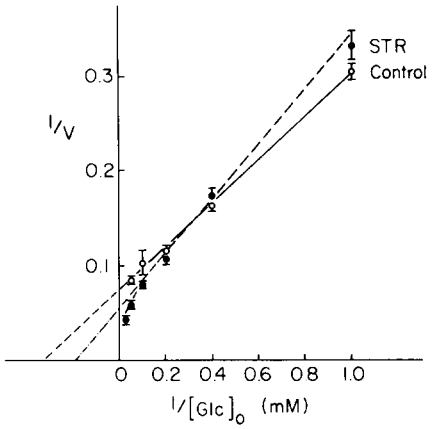

Fig. 1. Effect of glucose concentration on $^{14}CO_2$ formation from [^{14}C]glucose by renal proximal tubules of healthy and streptozotocin-treated rats. (STR) Suspensions of tubules were incubated 60 min at 37°C (5% CO_2, 95% O_2) in media containing 2 mM lactate and varying (0–30 mM) concentrations of [U-^{14}C]glucose. ○, controls; ●, streptozotocin-treated rats. Mean values ± S.E. (in μmol $^{14}CO_2$ formed/g tissue protein) are given. Each point is the mean of at least six analyses, and for most points, the mean of data from two or three experiments.

respective transport pathways, or the quantitatively minor contribution of the Na-dependent transport system. Raising the glucose concentration in the saline also greatly enhanced aerobic and anaerobic lactate formation (see Table IV). On the other hand, variations of the saline glucose concentration (0–30 mM) had little effect on gluconeogenesis from lactate. In this respect, renal gluconeogenesis differs from that in the liver [33].

TABLE II

EFFECTS OF STREPTOZOTOCIN TREATMENT ON GLUCOSE METABOLISM IN RENAL PROXIMAL TUBULES

Suspensions of tubules were prepared from healthy (controls) and streptozotocin-treated (experimental) rats of the same group. Mean values of the metabolic parameters (all in μmol/g tissue protein in 60 min) are given, ± S.E. (number of measurements in parentheses). Significance of differences: ** $P < 0.01$; n.s., non-significant.

	Controls	Experimental	
Glucose oxidation	8.8 ± 0.3 (30)	9.4 ± 0.4 (9)	n.s.
Gluconeogenesis	99 ± 2.3 (24)	92 ± 2.2 (16)	n.s.
L-Lactate formation	67 ± 3.7 (6)	90 ± 2.8 (6)	**
L-Lactate oxidation	199 ± 2 (4)	165 ± 7 (4)	n.s.

Effect of streptozotocin treatment on glucose transport and metabolism in renal proximal tubules

Of the four metabolic parameters for glucose studied, streptozotocin treatment produced significant changes ($P < 0.01$) only on aerobic L-lactate formation (Table III). The mean increase in lactate formation was 47 ± 8% of the controls (20 experiments). The effect of streptozotocin treatment on glucose oxidation proved to be complex: When carying glucose concentrations, a significant effect was seen (Fig. 1). However, as opposed to the linear double-reciprocal plot for the controls, the results reflected a multicomponent effect of streptozotocin treatment, i.e. for this process glucose oxidation was enhanced at substrate concentrations higher than the apparent K_m whereas at 1 mM a slight decrease was found. Extrapolating the apparently linear portion of the Lineweaver-Burk plot between 1 and 10 mM glucose, the apparent K_m was 6.3 ± 0.17 mM, and the V_{max} was 21.5 ± 0.3 μmol/g tissue protein per h; both these values differ significantly from those in the controls. It would follow that streptozotocin treatment alters at least two separate parameters of glucose metabolism, i.e. glucose oxidation and lactate formation. The absence of an effect of streptozotocin treatment on gluconeogenesis and lactate oxidation suggests that as opposed to glucose transport into the cells, lactate transport was not affected. In renal cortical slices, alloxan treatment increased gluconeogenesis [6]; while these data may appear to be at variance with the results reported above for streptozotocin treatment, it has to be borne in mind that the experimental conditions in both sets of experiments differ.

TABLE IV

LACTATE FORMATION IN RENAL PROXIMAL TUBULES OF HEALTHY AND STREPTOZOTOCIN-TREATED RATS: EFFECT OF GLUCOSE CONCENTRATION

Suspensions of tubules were prepared from healthy (controls) and streptozotocin-treated (experimental) rats of the same group. Mean values (five or six measurements) of aerobic and anaerobic L-lactate formation in 60 min (μmol per g tissue protein)±S.E. in salines containing 5 or 30 mM D-glucose.

	Glucose (mM)	Controls	Experimental
Aerobic	5	100± 5	200± 6
	30	351± 7	934±30
Anaerobic	5	438± 9	225±10
	30	2004±56	739±18

TABLE V

ANAEROBIC LACTATE FORMATION IN RENAL PROXIMAL TUBULES OF HEALTHY AND STREPTOZOTOCIN-TREATED RATS: EFFECT OF PHLORETIN

Suspensions of tubules from healthy (controls) and streptozotocin-treated (experimental) rats were incubated anaerobically for 60 min in saline containing 5 mM glucose. Mean values are given for L-lactate formed (μmol/g protein)±S.E. ($n = 5$ or 6), without and in the presence of 0.5 mM phloretin.

	Lactate formed (μmol/g protein)	
	No inhibitor	Phloretin
Control	230±5	165±12
Experimental	150±4	75± 5

The multicomponent effect of streptozotocin treatment on glucose metabolism in renal proximal tubules was borne out by a more detailed study of L-lactate formation from glucose. The enhancing effect of streptozotocin on aerobic lactate formation was seen also when the medium glucose concentration was raised from 5 to 30 mM, i.e. the level found in the plasma of streptozotocin-treated rats (Table III). The anaerobic lactate formation at 5 and 30 mM glucose was several fold higher than that under oxidative conditions, reflecting the pronounced Pasteur effect in the tissue [30]. The fact that both aerobic and anaerobic lactate formation were enhanced by increasing glucose concentration from 5 to 30 mM suggests the possibility that glucose entry is not the only rate-limiting step for the subsequent cellular metabolism of the substrate. In striking contrast to the enhancing effect of streptozotocin treatment on aerobic lactate formation, under anaerobic conditions such treatment produces a marked depression of glycolysis. Again, this effect was seen at both 5 and 30 mM medium glucose. The anaerobic lactate formation was phloretin-sensitive (Table V), as might be inferred from the properties of the equilibrating glucose transport system [1].

It is generally recognized that the streptozotocin-induced hyperglycemia is produced by a de-

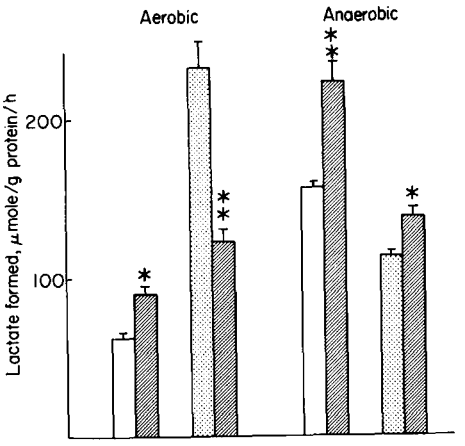

Fig. 2. Insulin effect of lactate formation by renal proximal tubules. Suspensions of renal tubules from healthy rats (controls) and streptozotocin-treated rats of the same group were used. Man values of lactate formed (μmol/g tissue protein in 60 min) ± S.E., 5–11 measurements. Bars: open, controls; stippled, streptozotocin-treated; cross-hatched, insulin added to incubation medium, 1 mU/ml. Significance: ** $P < 0.01$, * $P < 0.05$ vs. appropriate controls.

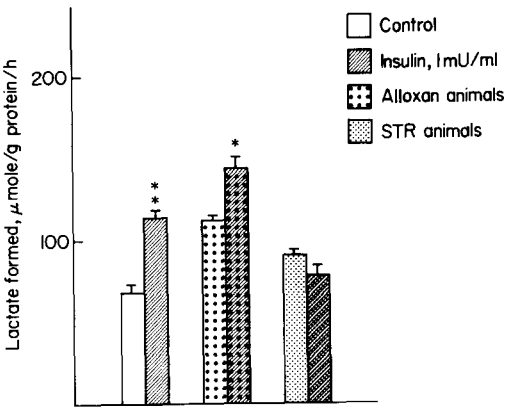

Fig. 3. Lactate formation from glucose in renal tubules from alloxan- and streptozotoxin-treated rats; effect of insulin. Mean values of lactate formed (μmol/g tissue protein in 60 min), ± S.E., 5–6 measurements. Bars: Open, control; dotted, alloxan-treated; stippled, streptozotocin-treated; cross-hatched, insulin added to incubation medium, 1 mU/ml. Significance: ** $P < 0.01$, * $P < 0.05$ vs. appropriate controls.

crease in insulin formation and hence a gradual decrease in the plasma level of the hormone [11,12]. Fig. 2 shows that the changes in lactate formation by the renal tubules produced by streptozotocin treatment can be reversed by the addition of 1 mU of insulin per ml incubating medium; hence, the observed data directly reflect a cellular effect of insulin.

The decrease of anaerobic lactate formation in renal tubules of streptozotocin-treated rats is consistent with the concensus that insulin stimulates the equilibrating glucose transport system in various cells [7]. A decrease of available insulin in streptozotocin-treated animals would be expected to reduce the (phlorizin- and ouabain-insensitive) glucose entry at the basal-lateral membrane and hence decrease the subsequent cellular metabolism of the substrate.

Additional evidence in favor of a direct involvement of an insulin-stimulated process in the streptozocin-induced changes in lactate formation was sought by testing the effect of insulin on lactate formation in alloxan-treated rats. The degradation of insulin in the membrane of renal cells [5,35] is blocked by a short treatment of the animals with alloxan [5]; under these conditions, increased aerobic lactate formation was seen (Fig. 3), implying an increased entry of glucose into the cells; no significant changes in glucose oxidation were found, confirming previous observations [5]. Insulin stimulated lactate formation in the controls and in tubules of alloxan-treated rats (Fig. 3); thus, tubules from alloxan- and streptozotocin-treated rats differ in their responses to the hormone. These experiments support the view that insulin is directly involved in the facilitated entry (at the basal-lateral cell face) of glucose into the metabolic pool. Data not given here in detail showed that alloxan treatment, and/or the presence of insulin (1 mU/ml) in the incubating medium had no significant effect on gluconeogenesis, and glucose or lactate oxidation.

The above results provide cogent evidence in favor of the view that insulin is involved in the streptozotocin- and alloxan-induced effects on lactate formation from glucose by renal proximal tubules. However, the results do not suggest a rationale for the anomalous effect of streptozotocin on the enhancement of aerobic lactate formation (Fig. 2).

Streptozotocin effect on enzymes of carbohydrate metabolism in renal cortical tubules

Diabetes produces significant changes in the

cellular level of some enzymes of carbohydrate metabolism in a variety of cells [36]. In particular, alloxan-induced hyperglycemia resulted in increased levels in renal cortex of hexokinase, but also of enzymes of the pentose phosphate pathway [37]. As opposed to the 28 days required to demonstrate the alloxan-induced changes, streptozotocin hyperglycemia is fully developed within 24 h. The effect of streptozotocin treatment on the levels of several enzymes of carbohydrate metabolism was therefore examined.

The values in Table VI in the renal cortex for the controls are comparable to those reported by Vinay et al. [14] for purified proximal tubules; the only exception was the somewhat higher level of hexokinase in our preparation. As compared to the controls, streptozotocin treatment produced a significant increase in tissue hexokinase (50%), and also a decrease in glucose-6-phosphatase; these changes attest to significant adaptive changes as an early result of streptozotocin treatment. An increase in hexokinase may be relevant under conditions when increased amounts of glucose are entering the cells at elevated plasma levels of glucose. The increased aerobic and anaerobic lactate formation at 30 mM glucose (Table VI) is consistent with this view.

TABLE VI

EFFECT OF STREPTOZOTOCIN TREATMENT ON SOME ENZYMES OF CARBOHYDRATE METABOLISM IN RENAL CORTEX

Renal cortex was homogenized and the component enzymes were assayed (see Methods). All data are means ± S.E. of multiple analyses in several animals (n in parentheses), and are expressed per g protein × min.

	Control	Experimental
Hexokinase (μmol NADPH formed)	13 ± 0.7 (6)	19 ± 1.2 (9)
Glucoses-6-phosphatase (μmol P_i formed)	94 ± 3 (9)	59 ± 4 (12)
Fructose-1,6-diphosphatase (μmol NADPH formed)	37 ± 0.5 (16)	29 ± 0.5 (13)
Phospho*enol*pyruvate carboxykinase (μmol PEP formed)	24 ± 1 (12)	31 ± 3 (8)
Pyruvate dehydrogenase (μmol CO_2 formed from pyruvate)	30 ± 1 (12)	28 ± 1 (12)

Streptozotocin effect on the Na-glucose cotransport system

The stimulation of aerobic lactate formation by streptozotocin treatment of the rats raised the possibility of an activation of glucose entry into the cellular metabolic pool by the Na-glucose cotransport system at the brush border membrane of renal tubular cells [1]. 1 mM methyl α-D-glucoside was employed as a non-metabolizable glucose analog. In order to avoid possible competition for the transport system by glucose formed from lactate, no other substrate was added to the media. Fig. 4 shows the results of one such experiment.

The tubules took up the sugar rapidly, and within 20 min a cellular level of 25 μmol/g protein was reached. This corresponds to a cellular accumulation ratio of 5–6, similar to that found in slices [2]. The tubules contained 3.23 ± 0.05 kg H_2O/kg dry wt. The preparation did not maintain the maximal accumulation of methyl α-glucoside; after 15 min, some of the accumulated sugar was gradually lost. This property did not stem from an absence of metabolic substrate; an identical time curve was obtained when 5 mM sodium acetate was present in the medium. Control experiments

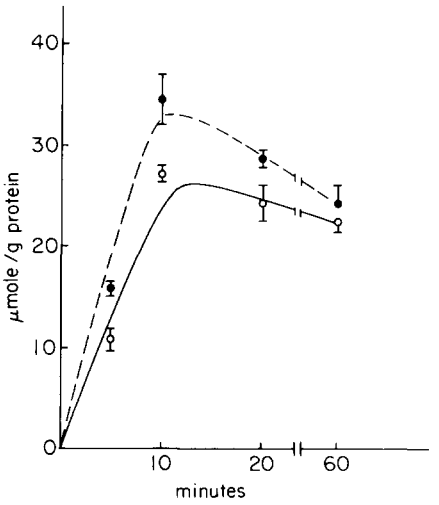

Fig. 4. Uptake of methyl α-D-glucoside by renal proximal tubules of control and streptozotocin-treated rats. Suspensions of tubules were incubated aerobically at 37°C in salines containing 1 mM methyl D-[U-^{14}C]glucoside. ○, Controls; ●, streptozotocin-treated rats (24 h). Each point is the mean (± S.E.) of 5–6 determinations.

showed that methyl α-D-glucoside uptake by the tubules displayed the usual properties of the Na-glucose cotransport system, i.e., major inhibitions by 0.5 mM ouabain (74%) and 0.5 mM phlorizin (83%) [38]. Compared with controls the transport system for methyl α-D-glucoside was significantly stimulated in tubules of streptozotocin-treated rats. This result suggests a plausible explanation of the observed enhancement of aerobic lactate formation from glucose by streptozotocin treatment. However, since insulin stimulates transepithelial Na flux (cf. Ref. 8), streptozotocin-induced hyperglycemia might then be expected to decrease transepithelial Na flux, and hence also decrease the Na-dependent glucose transport system. The above results are not consistent in this respect. No effects of streptozotocin treatment on tissue water, Na^+ and K^+ in the renal proximal tubules were seen (details not given here).

The apparent discrepancy was resolved when examining the time-course of the effect of streptozotocin treatment on methyl glucoside uptake (Fig. 5). As compared with controls, the enhancement of methyl α-glucoside uptake by streptozotoxin treatment seen after 24 h can not be detected 8 days after administration of the agent. Hence, the observed stimulation of the Na-glucose cotransport system is transient, and reflects adaptive changes reported above for some other cellular enzymes (Table VI). It should be noted that 8 days after streptozotocin treatment, the uptake of microvillous membrane vesicles to display a decrease in methyl glucoside uptake, as might be predicted for an impairment of the Na-glucose cotransport system by streptozotocin-induced hypoinsulinemia (El Seifi, S., Freibert, J.M. and Sacktor, B., personal communication).

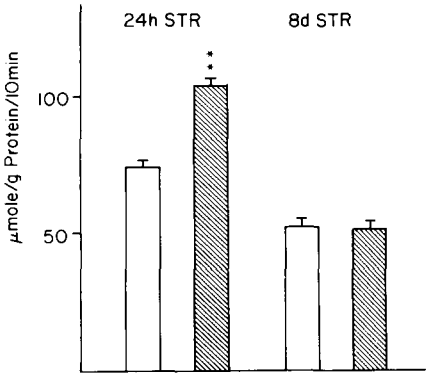

Fig. 5. Effect of duration of streptozotocin treatment on methyl α-D-glucoside uptake by renal proximal tubules. Rats were treated with streptozotocin for 24 h (see Methods) or 8 days [36] prior to killing the animals and preparation of renal proximal tubules. Incubation with 1 mM methyl α-D-[^{14}C]glucoside for 10 min. Mean values (±S.E.) of two experiments (each point four animals, 9–10 analyses). ** $P < 0.01$.

Discussion

An analysis of the data presented above hinges on the view that glucose entry into renal tubular cells at the brush border and the basal-lateral membrane limits the rate of the subsequent glucose metabolism within the cellular pool. This view is substantiated by experimental evidence: (a) Both glucose oxidation to CO_2, and L-lactate formation, were inhibited in part by phlorizin and ouabain (Table II), implicating the Na-glucose cotransport system at the brush border membrane (cf. Ref. 1). (b) The K_m of the saturable glucose oxidation (Fig. 1) was at least one order of magnitude higher than the K_m values for glucose found for the two prevalent renal hexokinases, types I and III [27]. (c) Phloretin (and cytochalasin b), inhibitors known to interact with the equilibrating glucose-transport (at the basal-lateral face of renal tubular cells), inhibited lactate formation from glucose (Table V). (d) Although no attempt was made to carry out a full analysis of the concentration dependence of lactate formation from glucose, the data in Table IV demonstrate that the reactions of the glycolytic cycle are not limiting the rate of glucose metabolism. The fact that the above transport inhibitors only partially inhibited glucose metabolism indicates that under the given experimental conditions glucose enters the renal tubular cells at comparative rates by both transport pathways, as also shown in the LLC-PK_1 cells [30]. The above view predicts that the intracellular concentration of free glucose, being determined by the respective rates of sugar entry, exit and intracellular metabolism, could be lower than that in the medium. Such prediction is consistent with most observations [3,4], but contrasts with the report of Tune and Burg [32].

Hyperglycemia produced by 24 h streptozotocin treatment of rats affected glucose oxidation and lactate formation by renal proximal tubules, but had no significant effect on gluconeogenesis or lactate oxidation (Fig. 1, Table III). The use of varying experimental conditions then permitted to localize the streptozotocin effect on both glucose transport processes, viz: (a) Under anaerobic conditions, streptozotocin treatment inhibited lactate formation from glucose (Table IV, Fig. 2). Under these conditions the Na-glucose cotransport is inoperative [38] due to the absence of the required electrochemical gradient of Na^+ at the membrane; this observation locates the effect at the equilibrating glucose transport system (at the basal-lateral membrane). (b) On the other hand, streptozotocin treatment actually stimulated aerobic lactate formation (Tables III, Fig. 2) and the active uptake of methyl α-D-glucoside (Fig. 5). These observations localize the streptozotocin effect on the Na-glucose cotransport system (in the microvillous membrane). Fig. 6 summarizes schematically the pertinent conclusions. The fact that insulin added to the incubation medium abolished the above effects of streptozotocin provides direct indications for an involvement of insulin in the respective transport processes. The direct insulin effect in vitro represents a cogent argument against the possibility that the reported results may reflect an adaptation to hyperglycemia [39].

It is suggested that insulin stimulates glucose entry at the basal-lateral cell face by a mechanism similar to that visualized for insulin effects on e.g. adipocytes [7], and in the first 24 h stimulates the Na-glucose cotransport system by an unknown mechanism (e.g. changes of cell pH [40,8]). The data on the effect of alloxan treatment (Fig. 3) are consistent with such view: As compared to controls, alloxan produced a significant increase in lactate formation. Under these conditions Mahler and Szabo [5] found an increased glucose utilization, and explained this by the demonstrated decrease in insulin degradation by renal cells. The additional increase in lactate formation on addition of insulin then reflects the hormonal stimulation of glucose entry into the cellular metabolic pool. The above suggestion does not exclude the possibility of insulin acting not only on the respective glucose transport processes, but also directly

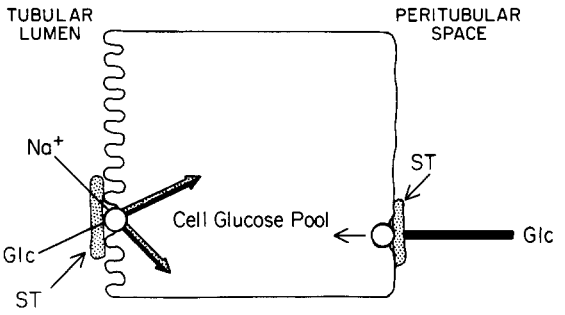

Fig. 6. Scheme of effects of streptozotocin treatment (ST) on the respectively glucose transport pathways.

on intracellular metabolism of the substrate [40,41].

The observed enhancement of the Na-glucose cotransport system in renal tubules by 24 h streptozotocin treatment (Fig. 4) was at first difficult to reconcile with evidence showing that insulin actually stimulates the $(Na^+ + K^+)$-ATPase [8]: hence, an inhibition of methyl α-glucosidase uptake might be expected. Fig. 5 shows that the increase in methyl α-glucoside uptake is transient; 8 days after administration of streptozotocin, no effect of the treatment on methyl α-glucoside uptake could be detected, and at that stage, glucose uptake by vesicles of microvillous membranes is decreased [39]. This set of observations demonstrates that an analysis of streptozotocin-induced changes in renal metabolism has to consider also possible alterations of intracellular enzymic activities. Table IV presents evidence that within 24 h after streptozotocin administration the activity of two key enzymes of glucose metabolism has significantly changed; such relatively fast changes in enzymes involved in renal carbohydrate metabolism have also been observed after partial hepatectomy [42]. These findings therefore detract from the usefulness of streptozotocin as a tool for the investigation of insulin effects on transport and metabolism.

Acknowledgements

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