Gluconeogenesis from glutamine and lactate in the isolated human renal proximal tubule: longitudinal heterogeneity and lack of response to adrenaline

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Recent studies *in vivo* have suggested that, in humans in the postabsorptive state, the kidneys contribute a significant fraction of systemic gluconeogenesis, and that the stimulation of renal gluconeogenesis may fully explain the increase in systemic gluconeogenesis during adrenaline infusion. Given the potential importance of human renal gluconeogenesis in various physiological and pathophysiological situations, we have conducted a study *in vitro* to further characterize this metabolic process and its regulation. For this, successive segments (S1, S2 and S3) of human proximal tubules were dissected and incubated with physiological concentrations of glutamine or lactate, two potential gluconeogenic substrates that are taken up by the human kidney *in vivo*, and glucose production was measured. The effects of adrenaline, noradrenaline and cAMP, a well established stimulator of gluconeogenesis in animal kidney tubules, were also studied in suspensions of human renal proximal tubules.

The results indicate that the three successive segments have about the same capacity to synthesize glucose from glutamine; by contrast, the S2 and S3 segments synthesize more glucose from lactate than the S1 segment. In the S2 and S3 segments, lactate appears to be a better gluconeogenic precursor than glutamine. The addition of cAMP, but not of adrenaline or noradrenaline, led to the stimulation of gluconeogenesis from lactate and glutamine by human proximal tubules. These results indicate that, in the human kidney *in vivo*, lactate might be the main gluconeogenic precursor, and that the stimulation of renal gluconeogenesis observed *in vivo* upon adrenaline infusion may result from an indirect action on the renal proximal tubule.

Key words: cAMP, catecholamines, glucose synthesis, kidney, metabolic heterogeneity.

INTRODUCTION

It is well established that, on a gram-for-gram basis, the kidney can synthesize glucose at rates several times higher than those observed in the liver [1]. Despite this, renal gluconeogenesis in humans under normal or pathological conditions was considered until the mid-1990s to have little physiological or pathophysiological importance when compared with hepatic gluconeogenesis; this was due to the fact that arteriovenous difference measurements revealed that the kidneys of postabsorptive subjects show little or no net take-up or release of glucose [2–5]. Only one study demonstrated that the kidneys of obese patients undergoing prolonged fasting released almost as much glucose as the liver [6]; small net renal outputs of glucose were also observed in certain subjects under conditions of metabolic acidosis [7].

However, more recently, the combination of arteriovenous difference measurements with tracer techniques has allowed the demonstration that, in humans, the synthesis of glucose by the kidney in the postabsorptive state may account for between 5% and 25% of the total glucose formed by gluconeogenesis in the body [8–10]. It has also been demonstrated that human renal glucose synthesis *in vivo* is stimulated by a 60 h fast [9], adrenaline [8,11], hypoglycaemia [12,13] and diabetes mellitus [14], is inhibited by insulin [10,12], and is unaffected by glucagon [15]. These results underline not only the potential importance of human renal gluconeogenesis in various pathophysiological states in which glucose homeostasis is disturbed, but also the

need for a thorough characterization of renal gluconeogenesis in humans.

Since the initial work of Benoy and Elliott [16], who demonstrated that the kidney has the capacity to synthesize glucose from non-carbohydrate precursors, many studies, carried out mainly *in vitro*, have been devoted to the characterization of renal gluconeogenesis (see [1,17,18] for reviews). On the basis of animal data, it is now well established that, along the nephron, the functional unit of the kidney, only the proximal tubule is capable of synthesizing glucose, as it is the only nephron segment containing the key gluconeogenic enzymes [19]. Our knowledge of human renal gluconeogenesis *in vitro* is restricted to the demonstration that suspensions of isolated proximal tubules or isolated kidney cortex slices synthesize glucose from a variety of precursors, including lactate, pyruvate, glutamine, glutamate and alanine [20–25]. However, on the basis of results obtained in various animal species, it is well established that the proximal tubule is metabolically heterogeneous, and suspensions of proximal tubules contain three distinct proximal nephron segments (S1, S2 and S3) whose individual contributions to glucose synthesis under various experimental conditions may be very different.

Therefore, in a first step towards better characterization of human renal gluconeogenesis, we have conducted a study in which we have microdissected single segments (S1, S2 and S3) of the human proximal tubule. These single segments were incubated in the presence of a physiological concentration of either lactate

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or glutamine, two major substrates known to be taken up by the human kidney *in vivo* [6,8–10,14,16,18,19,26,27]. In these experiments, glucose, one of the products of lactate and glutamine metabolism, was measured in the picomolar range with a microanalytical method involving amplification by enzymic cycling. In view of the *in vivo* demonstration that adrenaline stimulates renal gluconeogenesis [8,11], we also wanted to test in the present study if such a stimulation occurs in the human proximal tubule *in vitro*. For this, dose–effect experiments were performed with suspensions of human proximal tubules using adrenaline, noradrenaline and cAMP as potential agonists.

EXPERIMENTAL

Preparation of segments of human renal proximal tubules

The study received ethical committee approval, and informed consent was obtained from patients. Pieces of fresh normal kidney were obtained from the uninvolved pole of kidneys removed for neoplasm from 18 h-fasted adult patients. Only tissue from kidneys of patients without concomitant renal or non-renal diseases was used. The age of the patients (eight men and three women) was 58 ± 3 years (mean $\pm$ S.E.M.). Thin slices were cut with a Stadie–Riggs microtome and used to prepare tissue pieces from both the cortex and the outer stripe of the outer medulla. These pieces of cortex and medulla were incubated in Krebs–Henseleit medium containing 0.02 % (w/v) BSA (fraction V, fatty acid-free; Sigma) and collagenase ($2 \text{ mg} \cdot \text{ml}^{-1}$; Roche) in an atmosphere of 95 % $\text{O}_2/5$ % CO_2 for 60 min. At the end of the incubation, the tissue pieces were rinsed with collagenase-free dissection solution that consisted of Krebs–Henseleit buffer and 10 mM Hepes, both at pH 7.4. This solution was gassed with 95 % $\text{O}_2/5$ % CO_2 before addition of 0.02 % (w/v) BSA.

The microdissection of nephron segments was carried out at 4 °C with thin needles under stereomicroscopic observation at 25–40 $\times$ magnification. The S1 and S2 segments of the proximal tubule were dissected from the cortical tissue pieces, and the S3 segments were dissected from the outer medulla tissue pieces. The S1 segments comprise the beginning and middle portions of the convoluted part. The S2 segments comprise the final portions of the convoluted part and the beginning portions of the straight part. In our study, only the straight part was microdissected. Because of the limited quantity of tissue in most specimens, no attention could be paid to the superficial or juxta-medullary origin of the nephron segments microdissected. After isolation, each microdissected segment was aspirated with 2 μl of dissection medium and transferred on to a small disc of dry BSA on a microscope slide, which was cooled to 4–6 °C by a thermostatic system fixed on the plate of a microscope. The tubule length was measured with an ocular micrometer and the help of hair-tip tools [28] used to uncoil the tubules. Thereafter the tubules were transferred back into 50 μl of dissection medium in individual wells of a microplate, and kept at 4 °C until incubation. For each experiment, the number of nephrons microdissected and incubated was between 10 and 20 for S1, S2 or S3.

Incubations and glucose measurements

Thermowell polycarbonate microplates were utilized for the incubations. Strips of 24 wells were cut off. Each tubule segment was drawn along with 3 μl of dissection medium into the tip of a Drummond variable-stroke piston pipette. The tubule segments and fluid were deposited from the pipette into the individual cavities of the microplate. Then 5 μl of Krebs–Henseleit medium (pH 7.4) containing 10 mM Hepes (pH 7.4) in the presence or

absence of either L-lactate (Roche) or L-glutamine (Sigma) was added to each droplet containing the tubules. The final lactate (1 mM) and glutamine (0.75 mM) concentrations chosen were in the upper physiological range. Then the microplate was placed inside a small waterproof box, which was immersed in a thermostatically controlled water bath at 37 °C. In order to prevent evaporation, two sheets of filter paper soaked in distilled water were placed in the incubation box. An atmosphere of 95 % $\text{O}_2/5$ % CO_2 saturated with water at 37 °C was maintained inside the incubation box throughout each experiment. This allowed us to limit fluid evaporation to only 0.3–0.4 μl out of a total volume of 8 μl of incubation medium after 90 min of incubation at 37 °C. At both zero time and the end of the incubations, 20 μl of 10 mM NaOH was added to each sample used subsequently for measurement of glucose production. Glucose standards were incubated in the same way. The specific methods employed for measuring glucose in the concentration range 10–200 pmol were those described by Passonneau and Lowry [28]. These take advantage of the enzymic cycling method developed by Chi et al. [29], which allows an accurate analysis of metabolites in the picomolar range.

Briefly, 10 μl of sample was added to 20 μl of reagent containing 150 mM Tris/HCl, pH 8.1, 2 mM MgCl_2 , 0.8 mM ATP, 0.8 mM dithiothreitol, 0.2 mM NADP^+ , 1 unit/ml hexokinase and 0.2 unit/ml glucose-6-phosphate dehydrogenase, for conversion of NADP^+ into an equimolar amount of reduced NADPH. After 20 min of reaction, the total 30 μl volume of reaction mixture was incubated with 50 μl of 0.2 M NaOH at 95 °C for 60 min to destroy the excess NADP^+ .

This step was followed by an enzymic cycling procedure used to measure the NADPH produced by the glucose-6-phosphate dehydrogenase reaction [29]. It should be noted that the above general procedure was used when the amounts of glucose expected to be found were in the range 40–160 pmol. An adaptation of this procedure was required when the expected amounts of glucose were lower, i.e. in zero-time samples or after incubation of the tubules in the absence of substrate. This adaptation consisted mainly of a decrease in the volume of solution used in each step. Standard solutions of glucose were processed in the same way as the tubule samples.

Preparation and incubation of suspensions of human renal proximal tubules

Suspensions of human proximal tubules were prepared as described previously [20]. Incubations were performed at 37 °C in a shaking water bath in 10 ml hermetically stoppered Erlenmeyer flasks in an atmosphere of 95 % $\text{O}_2/5$ % CO_2 . The tubular fragments obtained were incubated for 60 min in 1 ml of Krebs–Henseleit medium with 1 mM L-lactate or 0.75 mM L-glutamine in the absence or the presence of various concentrations of adrenaline (bitartrate salt; Sigma), noradrenaline (Sigma) or cAMP (sodium salt; Sigma). In all experiments, each experimental condition was carried out in duplicate. Incubations were terminated by adding HClO_4 (2 %, v/v; final concentration) to each flask. In each experiment, zero-time flasks were prepared by adding HClO_4 before the tubules. The denatured proteins were removed by centrifugation (4000 g for 5 min at 4 °C) and the supernatant was neutralized with 20 % (w/v) KOH and kept on ice until glucose was assayed.

Expression of results and statistical analysis

Values of glucose accumulation after incubation of microdissected segments are expressed in pmol per unit time per mm

of proximal tubular segment. Statistical differences in the rates of glucose accumulation among the three proximal tubular segments studied or, for a given segment, between the two substrates (lactate and glutamine) were analysed by ANOVA followed by the Newman–Keul post-hoc test. These tests allowed us to take into account, in the statistical analysis of our results, not only the inter-individual variability of glucose accumulation, but also the intra-individual variability of glucose accumulation measured in 10–20 S1, S2 or S3 segments in each experiment.

Values of glucose production after incubation of suspensions of proximal tubules with lactate or glutamine are expressed in μmol of glucose produced/h per g of protein. The results were analysed by Student's *t* test for paired data, comparing values obtained in the absence with those in the presence of adrenaline, noradrenaline or cAMP.

RESULTS

Distribution of gluconeogenesis from lactate and glutamine along the human proximal tubule

Figure 1(A) shows that, when lactate or glutamine was the substrate at a physiological concentration, the S3 segments of human renal proximal tubules synthesized glucose in a linear manner with time over a 60 min incubation period. As shown clearly in Figure 1(B), the production of glucose in the presence of either 0.75 mM glutamine or 1 mM lactate as the substrate was linear with the length of the S3 segments. Such linearities were also observed with the S1 and S2 segments (results not shown).

Figure 2 shows the production of glucose when S1, S2 and S3 segments of proximal tubules dissected from the kidneys of 11 subjects were incubated for 60 min in the presence of either 0.75 mM glutamine (Figure 2A) or 1 mM lactate (Figure 2B) as substrate. This Figure reveals that the production of glucose from glutamine and from lactate was very variable from one subject to another. In general, the production of glucose from lactate in the S1 segment tended to be lower than that in the S2 and S3 segments.

Table 1 presents the mean values and the statistical evaluation of the results obtained with kidney tissue from these 11 subjects.

With glutamine as substrate and after 60 min of incubation, glucose production in the S1 segment was not statistically different from that in the S2 and S3 segments. In contrast, the amount of glucose formed in the presence of lactate was statistically lower in the S1 than in the S2 and S3 segments; as shown in Table 1, glucose production with lactate as substrate in the S1 segment was at least 2-fold lower than that in the S2 and S3 segments. Note that the amount of glucose formed in the presence of lactate as substrate in the S2 segment was not statistically different from that in the S3 segment.

The data of Table 1 also reveal that, in the S1 segment, the production of glucose in the presence of lactate was not different from that in the presence of glutamine. In contrast, the production of glucose in the presence of lactate was approx. 2-fold higher than that in the presence of glutamine in the S2 and S3 segments.

In order to test if the three segments synthesized glucose from endogenous substrates, they were incubated for 60 min in the absence of lactate or glutamine. In the S1, S2 and S3 segments, the amounts of glucose found at time zero (the beginning of the incubation period) were 7.0 ± 0.7 , 9.1 ± 2.6 and 10.5 ± 2.7 $\text{pmol} \cdot \text{mm}^{-1}$ respectively; after 60 min of incubation the corresponding values were 6.9 ± 0.7 , 9.9 ± 1.2 and 10.7 ± 4.7 $\text{pmol} \cdot \text{mm}^{-1}$ respectively ($n = 7$ subjects). This clearly indicates that the three segments did not produce glucose from endogenous substrates.

Effects of adrenaline, noradrenaline and cAMP on gluconeogenesis from lactate and glutamine in suspensions of human renal proximal tubules

The data of Table 2 show that, at concentrations known to cause stimulation of glucose synthesis from lactate in isolated rat kidney cortex tubules or slices [30–33], both adrenaline and noradrenaline failed to do so in isolated human kidney tubules. No stimulation of glucose synthesis was observed with lower concentrations (10 nM–10 μM) or higher concentrations (1 mM) of these adrenergic agonists ($n = 3$; results not shown).

Given these surprising results, we tested the reactivity of our human tubules to the effect of cAMP, another well established stimulator of renal glucose synthesis in isolated animal kidney cortex preparations [30,31,33–36]. As shown in Table 2, at

Figure 1 Glucose production by S3 segments of human renal proximal tubules

(A) Time course of glucose production from 1 mM lactate and 0.75 mM glutamine by S3 segments of human proximal tubules. Values are means $\pm$ S.E.M. for 10–20 segments at each time point in a representative experiment. (B) Glucose production from 1 mM lactate and 0.75 mM glutamine as a function of the length of S3 segments of human proximal tubules. Values shown are from a representative experiment. Each point corresponds to one tubular segment; the incubation lasted 60 min.

Figure 2 Glucose production from 0.75 mM glutamine (A) and 1 mM lactate (B) by different proximal tubular segments (S1, S2 and S3) from 11 subjects

Values are means $\pm$ S.E.M. of 10–20 segments of each type in each experiment.

Table 1 Glucose synthesis in human proximal tubular segments (S1, S2 and S3) after 60 min of incubation with glutamine or lactate

Results are reported as means $\pm$ S.E.M. for 11 experiments (subjects) in the presence of glutamine or lactate. The total numbers of tubular segments are shown in parentheses. The significance of differences was calculated by ANOVA, followed by the Newman–Keul post-hoc test: * $P < 0.05$ for differences between the glutamine and the lactate groups in the same segments; † $P < 0.05$ between S1 and S2 and ‡ $P < 0.05$ between S1 and S3 within the same substrate group.

Segment	Glucose synthesis ($\text{pmol} \cdot \text{h}^{-1} \cdot \text{mm}^{-1}$)	
	Glutamine (0.75 mM)	Lactate (1 mM)
S1	15.4 ± 4.2 ($n = 158$)	$21.0 \pm 5.4^{\dagger\ddagger}$ ($n = 131$)
S2	$22.0 \pm 4.1^*$ ($n = 148$)	43.7 ± 7.0 ($n = 138$)
S3	$27.5 \pm 4.6^*$ ($n = 130$)	57.2 ± 7.2 ($n = 143$)

Table 2 Effects of cAMP, adrenaline and noradrenaline on glucose synthesis from 1 mM lactate in suspensions of isolated human renal proximal tubules

Values are means $\pm$ S.E.M. for $n = 5$ (cAMP) or $n = 4$ (adrenaline/noradrenaline) experiments; all conditions were performed in duplicate in each experiment. Significance of differences: * $P < 0.05$ compared with control (Student's t test for paired data).

Agonist concn (μM)	Glucose synthesis ($\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}$ of protein $^{-1}$)		
	cAMP	Adrenaline	Noradrenaline
0	53.6 ± 15.0	51.5 ± 20.7	61.1 ± 20.2
10	$61.8 \pm 16.2^*$	—	—
100	$76.8 \pm 18.5^*$	48.3 ± 20.6	57.1 ± 22.9
1000	58.7 ± 16.9	—	—

concentrations of 10 and 100 μM (but not 1000 μM), cAMP stimulated mean glucose production from lactate by 15% and 43% respectively.

Table 3 Effects of cAMP and adrenaline on glucose synthesis from 0.75 mM glutamine in suspensions of isolated human renal proximal tubules

Values are means $\pm$ S.E.M. for $n = 3$ (cAMP) or $n = 4$ (adrenaline) experiments; all conditions were performed in duplicate in each experiment. Significance of differences: * $P < 0.05$ compared with control (Student's t test for paired data).

Agonist concn (μM)	Glucose synthesis ($\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}$ of protein $^{-1}$)	
	cAMP	Adrenaline
0	19.3 ± 5.3	24.6 ± 7.0
10	23.0 ± 6.7	—
100	$35.7 \pm 12.2^*$	23.4 ± 6.7
1000	$33.8 \pm 8.5^*$	—

In the presence of concentrations of 100 and 1000 μM cAMP, mean glucose synthesis from 0.75 mM glutamine in isolated human proximal tubules was increased by 85% and 75% respectively (Table 3). In contrast, no stimulation of glucose synthesis was observed in the presence of 100 μM adrenaline (Table 3).

DISCUSSION

Characteristics of gluconeogenesis from glutamine and lactate along the human renal proximal tubule

To our knowledge, the present study is the first in which glucose synthesis has been measured in freshly microdissected human nephron segments. It shows that the three segments (S1, S2 and S3) of the proximal tubule can synthesize glucose from lactate and glutamine at physiological concentrations. For all segments, the linearity of glucose synthesis with time and amount of renal tissue (segment length) indicates that these segments were metabolically viable.

The absence of glucose synthesis from endogenous substrates (in the absence of glutamine and lactate) is in agreement with the

very low glycogen content of the proximal tubule in normal subjects [37]. It also suggests that the microdissected single nephron segments we used did not degrade their proteins and use the resulting amino acids to synthesize glucose; again, this suggests that they were in a good metabolic state.

It is noteworthy that the large inter-individual variability in the rate of gluconeogenesis observed with microdissected human proximal segments is in agreement with results observed with suspensions of human proximal tubules [20,22,25] (see also Tables 2 and 3). It is also in accordance with observations *in vivo* [7,10].

It is interesting to emphasize that our single microdissected human nephron segments synthesized more glucose from physiological concentrations of lactate and glutamine than reported previously for single microdissected rabbit nephron segments in the presence of 5–10 mM lactate, pyruvate, 2-oxoglutarate or glutamate [38–40]. In addition, only the S1 segment of the rat nephron synthesized more glucose from 2–10 mM glutamine, pyruvate or 2-oxoglutarate [36–41] than did our human nephron segments from 1 mM lactate or 0.75 mM glutamine.

The present study establishes that the human proximal tubule has the same capacity to synthesize glucose from a physiological concentration of glutamine along its entire length. Thus it differs from the rat nephron, in which glucose synthesis from 2 mM glutamine and 2 mM pyruvate has been shown to decrease progressively from the S1 to the S2 and the S3 segments [41]. Our data also provide the first demonstration that the S2 and S3 segments of the human proximal tubule synthesize more glucose from a physiological concentration of lactate than the S1 segment. In this respect, the human proximal tubule resembles the rabbit proximal tubule, in which the pars recta has been shown to synthesize more glucose from lactate or pyruvate than the convoluted tubule [38,39]. Taken together, these observations clearly indicate that it is difficult to extrapolate results concerning the metabolic heterogeneity of the nephron obtained in a given animal species to humans.

Our results imply that all the enzymes involved in glucose synthesis from lactate and glutamine are active along the entire length of the human proximal tubule. These include lactate dehydrogenase, pyruvate carboxylase, mitochondrial and cytosolic aspartate aminotransferases, the enzymes of the gluconeogenic pathway from oxaloacetate to glucose, including three key gluconeogenic enzymes (phosphoenolpyruvate carboxykinase, fructose bisphosphatase and glucose-6-phosphatase), glutaminase, glutamate dehydrogenase, the enzymes involved in the conversion of 2-oxoglutarate into malate, and the cytosolic malate dehydrogenase. To our knowledge, the activity of none of these enzymes has so far been determined along the human proximal tubule; only the activities of lactate dehydrogenase, glutamate dehydrogenase, malate dehydrogenase and aspartate aminotransferase have been measured in the proximal tubule [42]. The use of immunohistochemical techniques has also allowed to demonstrate the presence of glucose-6-phosphatase activity in the human proximal tubule [43]. Thus, given our ignorance of the metabolic heterogeneity of the human proximal tubule, the lower production of glucose from lactate in the S1 segment than in the S2 and S3 segments cannot be attributed at the present time to the rate-limiting influence of one or several of these enzymes or of the lactate transporters.

The mechanisms responsible for the differences in gluconeogenic rates with lactate and glutamine in the S2 and S3 segments cannot be identified from the data of the present study. In this respect, it is, however, of interest to note that, at equimolar concentrations (1 mM), the rate of lactate utilization by suspensions of human renal proximal tubules was found to be

approx. 3-fold higher than that of glutamine utilization [20,22]. Under these conditions, the rate of glucose synthesis from lactate was approx. 2-fold higher than that from glutamine [20,22], which is in agreement with the rates of glucose synthesis observed in the single S2 and S3 segments measured in the present study. This means that the amount of oxaloacetate made available for glucose synthesis was also about 2-fold greater with lactate than with glutamine. Whether this difference is related to the rate-limiting influence of the glutamine transporters or of enzymes involved in the pathway from glutamine to cytosolic oxaloacetate deserves further study.

Failure of adrenaline to stimulate gluconeogenesis from lactate and glutamine in suspensions of isolated human renal proximal tubules

Given the well accepted view that adrenaline and noradrenaline stimulate gluconeogenesis from lactate or pyruvate [30–33] and from glutamine or 2-oxoglutarate [30,31,44] in isolated rat kidney cortex slices or tubules via α -adrenergic receptors [31,45,46], it may appear at first sight surprising that similar observations were not made with isolated human proximal tubules in the present study. Careful examination of the experimental conditions used by other authors reveal that these stimulatory effects were observed with low, unphysiological external calcium concentrations [30,32], but not with a physiological calcium concentration (2.5 mM) [30] similar to that used in the present study. We believe that, as in the present study, it is important to test the potential effects of catecholamines in the presence of physiologically relevant external calcium concentrations. Under these conditions, neither physiological (in the picomolar to nanomolar range) nor pharmacological (in the micromolar range) concentrations of either adrenaline or noradrenaline stimulated glucose synthesis from lactate or glutamine in isolated human renal proximal tubules (see Tables 2 and 3 and the Results section).

Considering that our tubules were metabolically viable (see above), it was important to check that they would respond to another well established stimulator of renal gluconeogenesis in rat kidney cortex slices or tubules [30,31,33–36]. In this respect, it should be emphasized that their sensitivity to the stimulatory effect of 100 μ M cAMP on gluconeogenesis from lactate or glutamine was in the same range as that observed with isolated rat kidney tubules by other authors [33–35,47]. It should also be mentioned here that, as in isolated rat kidney tubules [34], no stimulation of glucose synthesis from lactate occurred in human kidney tubules upon the addition of 1 mM cAMP (see Table 2).

Other possibilities deserving of further study that might explain the lack of effect of adrenaline are that: (i) the adrenergic receptors were damaged by the proteases contained in the collagenase solutions used to prepare our tubules; (ii) the human renal proximal tubule does not contain adrenergic receptors; or (iii) alternatively, stimulation of the latter does not lead to an increase in glucose synthesis. In accordance with the two latter possibilities are the observations that: (i) isoprenaline (isoproterenol) stimulates the accumulation of cAMP without a concomitant increase in glucose synthesis from pyruvate in isolated rat tubules [35]; (ii) cAMP accumulation is stimulated by isoprenaline in rat [44], but not rabbit [48], kidney tubules; and (iii) high concentrations (0.01 and 0.1 mM) of isoprenaline were found to stimulate the adenylate cyclase activity of whole human kidney cortex homogenates by only approx. 20% [49]. As the exact nephron segment on which isoprenaline acted cannot be determined from the latter study, it is conceivable that segments other than the proximal tubule responded to this β -adrenergic agonist.

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