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The role of intracellular cAMP in renal gluconeogenesis in view of differential action of various cAMP analogues☆

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Abstract

Effects of various cAMP analogues on gluconeogenesis in isolated rabbit kidney tubules have been investigated. In contrast to N^6,2′-*O*-dibutyryladenosine-3′,5′-cyclic monophosphate (db-cAMP) and cAMP, which accelerate renal gluconeogenesis, 8-bromoadenosine-3′,5′-cyclic monophosphate (Br-cAMP) and 8-(4-chlorophenylthio)-cAMP (pCPT-cAMP) inhibit glucose production. Stimulatory action of cAMP and db-cAMP may be evoked by butyrate and purinergic agonists generated during their extracellular and intracellular metabolism resulting in an increase in flux through fructose-1,6-bisphosphatase and in consequence acceleration of the rate of glucose formation. On the contrary, Br-cAMP is poorly metabolized in renal tubules and induces a fall of flux through glyceraldehyde-3-phosphate dehydrogenase. The contribution of putative extracellular cAMP receptors to the inhibitory Br-cAMP action is doubtful in view of a decline of glucose formation in renal tubules grown in the primary culture supplemented with forskolin. The presented data indicate that in contrast to hepatocytes, in kidney-cortex tubules an increased intracellular cAMP level results in an inhibition of glucose production.

Keywords: Rabbit; Renal tubules; Gluconeogenesis; cAMP; Br-cAMP; db-cAMP; Glyceraldehyde-3-phosphate dehydrogenase

The view that gluconeogenesis in liver is the main source of glucose in vertebrates has recently been loosing its credibility due to the accumulation of data, indicating that de novo renal gluconeogenesis may be equal to that in liver [1]. Thus, glucose production in kidneys is an important factor in the regulation of glucose metabolism of the whole body, having profound consequences for physiology and biomedicine.

In hepatocytes glucagon- and adrenaline-evoked rise in intracellular cAMP, stimulates gluconeogenesis via PKA,[1] that controls the opposing activities of the bifunctional enzyme PFK-2/FBPase-2, resulting in changes in fructose-2,6-bisphosphate concentration, believed to determine the rates of both glycolysis and gluconeogenesis [2]. However, discussion still persists concerning the relative contributions of both cAMP concentration and calcium transients to the acute hormonal regulation of glucose production in hepatocytes, as elevation of cAMP in these cells causes a rise in the intracellular calcium level [3]. The authors argue that calcium transients are decisive in the hormonal regulation of glucose output via activation of Krebs cycle, respiratory chain, and pyruvate carboxylase. Various modes of cross talk between the pathways employing cAMP and calcium transients have been reviewed by Bygrave and Roberts [4].

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[1] *Abb.reviations used:* db-cAMP, N^6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate; Br-cAMP, 8-bromoadenosine 3′,5′-cyclic monophosphate; pCPT-cAMP, 8-(4-chlorophenyltio)-cAMP; FBPase, fructose-1,6-bisphosphatase; PKA, protein kinase A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PFK-2/FBPase-2, phosphofructokinase-2/fructose bisphosphatase-2.

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Some data suggest that mechanisms regulating glucose production in kidneys and liver differ significantly, so the action of cAMP in kidneys may be different than that in liver. This is supported by following observations: (i) glucagon, which stimulates glucose production in hepatocytes due to cAMP increase and calcium transients, does not enhance the rate of glucose release from kidneys [5], (ii) glucose formation in liver and kidneys responds differently to some hypoglycemic agents [6], and (iii) stimulation of gluconeogenesis in renal tubules by purinergic receptor agonists and melatonin results from a fall in intracellular cAMP [7,8]. On the other hand, various authors have published data contradictory to the latter observations, showing stimulation of glucose production in renal tubules upon a rise in cAMP content [9–11]. To our knowledge, however, they all have used either extracellularly added db-cAMP or cAMP as research tools. Moreover, some investigators postulate the existence of putative cAMP receptors in tubular brush border membranes [12]. This phenomenon might further complicate the interpretation of the results obtained with the use of extracellularly added cAMP analogues.

The aim of the present investigation was to study glucose production in renal tubules in the presence of exogenous cAMP and its various analogues to elucidate the role of cAMP in the regulation of renal gluconeogenesis.

Materials and methods

Chemicals

Enzymes and nicotinamide adenine dinucleotides were obtained from Roche (Mannheim, Germany). HPLC solvents were purchased from Merck (Darmstadt, Germany) and were of either gradient or HPLC grade. Other chemicals were from Sigma and were of highest purity available. Silicon oils AR20 and AR2000 for tubule separation from the reaction medium were generous gifts of Brenntag, Polska.

Animals, preparation and incubation of kidney-cortex tubules

Male white Termond rabbits were used throughout. Animals were maintained on standard rabbit chow with free access to water and food. In some experiments, alloxan-diabetes was induced by a single injection of alloxan (150 mg/kg body weight) [13]. Only those alloxan treated animals that displayed blood glucose concentration in excess to 300 mg/100 ml 3 days after treatment were considered diabetic. All animal used procedures were approved by the First Warsaw Local Commission for the Ethics of Experimentation on Animals. Kidney-cortex tubules from male rabbits (2–3 kg body weight) starved for 48 h were isolated as described previously [14]. The tubules were suspended in the Krebs–Ringer bicarbonate buffer and immediately used for incubation.

Isolated kidney-cortex tubules (about 10 mg d.w.) were incubated at 37 °C in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) in 25 ml plastic Erlenmeyer flasks under the atmosphere of 95% O_2 + 5% CO_2 in the presence of substrates and effectors as indicated in legends to table and figures. For measurement of the total production of glucose in renal tubule suspension, the reaction was stopped after 60 min of incubation by the addition of 35% perchloric acid (0.1 volume of tubule suspension). Both intracellular and extracellular levels of metabolites were measured in 1 ml samples withdrawn from the reaction medium after 60 min of incubation and centrifuged through the silicon oil mixture into 12% perchloric acid solution as described previously [15].

Isolation of kidney-cortex tubules for primary cultures

Kidney-cortex tubules for primary culture were isolated and cultured as described by Derlacz et al. [16].

Analytical methods

Intracellular gluconeogenic intermediates were quantified enzymatically [17] either fluorometrically or spectrophotometrically, by determining changes in NAD(P)H absorbance at 340 nm or fluorescence at 465 nm (excitation 340 nm) due to coenzyme's consumption/production in the followed reactions. Total glucose was measured by the method described by Bergmeyer and Bernt with the use of glucose oxidase, peroxidase, and *o*-dianisidin [17]. Glycogen content in rabbit kidney cortex was determined by measuring glucose produced during glycogen hydrolysis in excised kidney-cortex employing the method described by Sprangers et al. [18]. cAMP analogues and their metabolites were determined by HPLC with UV detection as described by Teerlink et al. [19], using C-18 reversed-phase column from Beckman: Ultrasphere, 5 μm spherical particle 80 Å pore, 15 cm × 4.6 mm. Linear gradient of buffer B: water/acetonitrile/methanol (50:25:25, V:V:V) from 1 to 35% in buffer A: 0.2 M KH_2PO_4, pH 5.0, set with KOH was developed within 6 min. Absorbance at 210 nm was measured with the UV/Vis detector. Instrument used was Beckman System Gold encompassing two 110B solvent delivery modules. The method was slightly modified for the separation of db-cAMP, as buffer A was changed to 0.2 M sodium acetate, pH 5.0, set with glacial acetic acid, the gradient was run linearly from 1B to 80% within 7 min and the UV/Vis detector operated at 260 nm. The detected compounds were quantified by comparison of peak areas on chromatogram produced during the sample analysis and those generated during separation of appropriate standards.